National Institute on Drug Abuse

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Problems of Drug Dependence 1991: Proceeding of the 53rd Annual Scientific Meeting

The Committee on Problems of Drug Dependence, Inc.

119

U.S. Department 0f Health and Human Services • Public Health Service • Alcohol. Drug Abuse, and Mental Health Administration

Problems of Drug Dependence 1991

Proceeding of the 53rd Annual Scientific Meeting The Committee on Problems of Drug Dependence, Inc.

Editors:

Louis Harris, Ph.D.

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ANNUAL REPORTS

Welcome and Current Status of CPDD

L.S. Harris

I would like to welcome you all to the 53rd Annual Meeting of the Committee on Problems of Drug Dependence. We have an exciting program for you. The quantity and quality of the presentations, posters and symposia are high. Mary Jeanne Kreek and the Program Committee are to be congratulated on putting together an outstanding program.

We are especially pleased that the International Cannabinoid Study Group (ICSG) and the International Study Group Investigating Drugs as Reinforcers (ISGIDAR) are holding their Satellite Meetings with us at The Breakers. The National Institute on Drug Abuse will also be holding three workshops throughout the week. We look forward to these informative and instructional sessions and urge your attendance at these important meetings.

This meeting marks the end of my term as Chairman of CPDD. I will be succeeded by Keith Killam and the Chairman-elect is Tom Crowley. Joe Brady remains as Treasurer and Marty Adler as Executive Officer.

This has been a very productive and exciting year for CPDD. We have officially become a Collaborating Center of the World Health Organization. We are in the final stages of negotiating with Elsevier to become associated with the journal, DRUG AND ALCOHOL DEPENDENCE. Bob Balster and Chris Johanson are to be congratulated for ably carrying on the delicate negotiations of this matter.

I would like to thank all of you for your letters to Congress on behalf of the NIDA and NIAAA research budgets. Unfortunately, this was not very successful since the House Appropriations Committee cut the NIDA research budget by $14 million. I urge all of you to

contact members of the Senate Appropriations Committee to have this cut restored. As you are probably aware, there is a strong move to separate services from research at ADAMHA and to move the Institutes to the NIH. CPDD will be working hard to assure that these changes, when and if they occur, will be to the best advantage of the treatment, prevention, and research communities. We will be asking you to help in this effort.

To bring you up-to-date on the progress toward a membership organization, the CPDD Board voted to amend the by-laws to convert us to a membership organization. The organization will consist of Fellows, Regular Members and Associate Members made up of scientists and other professionals working in the field of substance abuse. There will be a nomination and credentialing process. Detailed procedures and information will be published and distributed as soon as they have been officially finalized.

A great debt of thanks is owed to the Rules Committee, Chuck Gorodetzsky, Mary Jeanne Kreek and Jim Kulikowski, with a special thanks to David Marshall for his legal advice.

The Committee on Problems of Drug Dependence will officially change its name to "College on Problems of Drug Dependence" after an amendment to the CPDD Articles of Incorporation. This will become effective immediately.

Finally, a new initiative was instituted this year to develop increased relationships between CPDD and the international community. Arthur Falek chairs this committee. He is working with Dr. Richard Lindblad, who has responsibility for foreign affairs at NIDA. They are now in the process of setting up a meeting with scientists in Eastern Europe. This promises to be the start of a successful program in setting up mechanisms to aid our colleagues in the international community in drug-abuse research, prevention and treatment efforts. My appreciation to Arthur, Bob Balster and David Musto for their hard work in this new endeavor.

CPDD continues to be a leading and growing organization in the academic and scientific research community. As it becomes a membership organization, I see a continuous and steady growth potential ahead. Thank you for contributing to a satisfying and exciting year.

AFFILIATION; Virginia Commonwealth University, Medical College of Virginia, Richmond, VA

The National Institute on Drug Abuse 1991: New Faces, Responsibilities, and Opportunities

C.R. Schuster

NIDA is alive and well and will continue as an Institute to sponsor research on the incidence and prevalence of illicit drug use, and the causes and consequences of drug abuse. Based on this knowledge, NIDA will continue to develop and evaluate improved prevention and treatment interventions. If legislation currently being developed is passed, the three research Institutes of ADAMHA (NIDA, NIAAA, NIMH), will return as separate institutes to the NIH with the enthusiastic endorsement of the NIH Director. The service components of ADAMHA, that is, the Office of Substance Abuse Prevention (OSAP) and the Office of Treatment Improvement (OTI) will remain at ADAMHA. You can be assured that NIDA will continue to foster linkages with these service organizations to guarantee that we learn from them regarding the service problems that demand research and to assure that effective prevention and treatment interventions are adopted by practitioners.

There have been many new developments since I was last able to address the Committee so I would like to give you an update on the new faces, new responsibilities and new opportunities at NIDA.

NIDA has grown considerably over the past year in a number of respects. Our FY 91 budget - a total of $416 million, represents an increase of $36 million from last year. Our staff now totals 467, and oversees the approximately 1,200 grants and contracts that NIDA currently sponsors. In addition, The Administration's FY 92 proposed allocation for NIDA represents a substantial increase. At the present time, Congress has not finalized the appropriations process but I am confident that NIDA will see continued growth.

NIDA's primary effort continues to be funding investigator-initiated research in several broad categories. This year, for example, allocations for our research grants were divided in the following way: $68

million, basic biomedical; $53 million, neuro-behavioral; $43 million, prevention; $63 million, treatment; and $15 million for epidemiology. The sum for all areas totaled $242 million.

Organizational Structure

NIDA's formal structure comprises three interdependent elements, working together to achieve our overall mission. Staff offices lend support to the Office of the Director in a variety of management functions; program divisions oversee NIDA's broad portfolio of research grants, contracts and interagency agreements; and our internationally acclaimed intramural research facility, the Addiction Research Center (ARC), located in Baltimore, MD, conducts research which complements our extramural programs.

Heading up the three staff offices, five program divisions and the ARC at NIDA, are a good many new faces, and in a few cases, faces familiar to NIDA have taken on new and different roles.

We are delighted to have Ms. Eleanor Friedenberg, formerly with the National Institute of Mental Health, as our new office of Extramural Program Review (OEPR) Director. This office coordinates and conducts the scientific review of all grant and contract applications. We are equally fortunate to have recruited the talents of Ms. Laura Rosenthal from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) to lead our Office of Planning and Resource Management (OPRM) in formulating and tracking our Congressional budget requests, as well as in most operational management services within the Institute. To head our third staff office we are pleased to have drafted a seasoned senior NIDA staffer, Dr. Marvin Snyder, who formerly served as the Division of Preclinical Research's Director for many years. The Office of Policy and External Affairs (OPEA) conducts strategic planning, analysis, and evaluation of *our* research, sponsors the training of new scientists in the drug abuse field, and manages our publications and technology transfer activities.

A number of newcomers have joined the ranks of our research program Division Directors, while some already overseeing programs have taken on additional challenges in key areas of interest to the Institute. Our Division of Clinical Research's (DCR) Acting Director, Dr. Harry Haverkos, has recently appended a new title to his *many* existing duties, as Associate Director for AIDS Research. The primary areas of focus for DCR, as in the past, center on the behavioral pharmacology of abused

substances, the effectiveness of existing drug abuse treatment therapies, and the assessment of new and promising ones. A program focused on medical health problems closely associated with drug abuse has rapidly evolved into a new branch within DCR - the Clinical Medicine Branch.

The transfer of our prevention research program, formerly housed in DCR, took place this year to the Division of Epidemiology and Prevention Research (DEPR). Dr. Zili Amsel, the extremely capable head of the Prevention Research Branch not only has moved to this new division but agreed to lead the group as Acting Division Director. She is doing a marvelous job of overseeing all of the many activities of this Division including their epidemiological research program to examine the incidence and prevalence of drug abuse in certain populations. The data obtained both from the extramural funded research and the intramural field studies we conduct aids NIDA immeasurably in directing its research priorities.

We have also found a superbly qualified new Director for our Division of Preclinical Research (DPR) in Dr. James Dingell, formerly with the National Heart, Lung, and Blood Institute for many years, as well as having been a neuropsychopharmacology researcher at Vanderbilt. DPR continues to explore the mechanisms underlying drug abuse and new methodologies to test the abuse potential of new compounds. Although a good many staff members formerly with DPR have transferred to help staff up our new Medications Development Division, we will soon be filling those positions left vacant, particularly in the area of Research Technology.

Our new Division of Applied Research (DAR) Director, Dr. Joseph Autry, although new to NIDA, has been with ADAMHA in a variety of key research and policy positions for many years. The knowledge and expertise he has brought to this position makes him uniquely qualified to direct a program as diverse as this one is. A research program focusing on drugs in the workplace, our community-based AIDS outreach demonstration projects, as well as intramural research efforts centered on financing and services research all fall under DAR's purview. The Economic Cost of Alcohol, Drug Abuse and Mental Health report sponsored by the Financing and Services Research group and released earlier this year estimated the cost of drug abuse in 1988 to have been a staggering $58.3 billion.

DAR also has a number of programmatic responsibilities including development and oversight of the National Laboratory Certification Program (NLCP), which sets

stringent Scientific and technical standards for labs performing Federal employee drug testing. As of July, 1991, NIDA has certified 75 labs and another 25 are in various stages of the certification process. As you may have heard, our system was put to the test this year and came through with flying colors due primarily to the well thought out safeguards built into the program. When a number of false positive results for meth-amphetamine were detected in the specimens of several transportation workers taking large amounts of ephedrine, it was the Medical Review Officer (MRO) who Put all the evidence into proper perspective. Fortunately, another system safeguard requiring the saving of positive samples for possible retesting, enabled closure on the status of these specimens and allowed NIDA to act quickly in conducting an in-depth investigation into the cause of the erroneous test results.

Our newest division, Medications Development, has been rapidly expanding under the competent direction of Dr. Charles Grudzinskas, formerly with Lederle Laboratories for two decades. Dr. Grudzinskas has been ably leading this high priority program in its search for new and improved pharmacotherapies aimed at treating drug addiction. To this end, INDs have been, or are in the process of being filed for a number of drugs to treat opiate addiction - LAAM, buprenorphine and depot naltrexone. A considerable portion of NIDA's research budget for this year was invested in this key research area thus strengthening our commitment to improving drug abuse treatment and helping to curtail the spread of AIDS.

Last, but by no means least, is our oldest division and the world's leading drug abuse research center - the ARC. This year we formally appointed Dr. Roy Pickens as Director. For some time now he has been providing excellent leadership to this group of qualified staff, currently numbering 113, in their investigations into the causes, treatment and prevention of drug abuse and addiction; the behavioral mechanisms of addiction; and the addictive potential of new drugs.

While our organizational structure as I have outlined, neatly compartmentalizes many of our activities in a very manageable way, such a breakdown does not accurately reflect the scope, nor fully encompass the complexities, of the research program challenges confronting NIDA. our approach to the problems presented by those issues that are "crosscutting" has been to establish task forces or program management committees, providing us with a matrix management system. This system, we hope, will give us the

capability of addressing issues which transcend the administrative structure of NIDA in a more effective manner. The program management committees now forming at NIDA are in the following high priority areas that involve two or more NIDA divisions: Neurosciences, Maternal-Paternal-Fetal Drug Effects, Medications Development, Epidemiology and Data Systems, AIDS, Services Research, and Training.

Our Neurosciences program, headed by Dr. James Dingell will focus NIDA's involvement in the "Decade of the Brain" activities, including study of the long term neurotoxicity of drug use, the neurobiological effects of maternal and fetal drug use, the mechanisms underlying neurobiological vulnerability to drug abuse, and the neurobiological basis for the rewarding consequences of drugs.

Dr. Loretta Finnegan, formerly Associate Director of the Office of Treatment Improvement (OTI) has become NIDA's special expert on women's issues and will guide our Maternal-Paternal-Fetal Drug Effects program committee as they explore the epidemiology of drug exposed infants, the treatment of drug abusing women of childbearing age, and the teratogenic, mutagenic, and environmental effects of drugs. Among the specific activities already underway are the National Pregnancy and Health Survey - a study of the prevalence of substance abuse during pregnancy in a nationally representative sample of women and of two important outcome indicators of the overall health of their infants; an interagency agreement with NICHD/NIDA/OTI/ACF which will study the development of low birth weight infants exposed to drugs in utero; and our "Perinatal 20" research demonstrations reflecting the diversity in available treatment approaches, settings, and populations, which seek to identify effective treatment approaches for drug abusing pregnant and postpartum women as well as for women of childbearing age in general.

Our Medications Development program, like its structural division counterpart, aims, under the expert leadership of Dr. Charles Grudzinskas, to develop pharmacotherapies to treat drug addiction. This program's activities cover the gamut from synthesizing new chemicals, screening in animals, to developing linkages with pharmaceutical firms in an effort to further our mutual goals, and conducting clinical trials that test potential medications in humans. The establishment of a separate task force in this area allows us to expand our horizons on the issues addressed by incorporating relevant research from other

NIDA programs as well as other government components involved in similar pursuits.

Our Epidemiology and Data Systems program is an essential part of our overall research program. With Dr. Zili Amsel acting as chair, this group will focus on research on the nature and extent of drug abuse in the population as a whole (National Household Survey on Drug Abuse) as well as in several important subpopulations (National High School Senior Survey; National Pregnancy and Health Survey). New and improved methods for obtaining accurate trends in drug use and its medical consequences will be examined. Currently NIDA's Drug Abuse Warning Network (DAWN) system is being modified to reflect a national probability sample of hospitals rather than only reporting from those in select metropolitan areas, thus allowing projections to the entire country. Under the auspices of this program management committee, the efficacy of various drug abuse prevention strategies and the biological and behavioral factors underlying vulnerability to drug abuse will also be assessed.

Currently, our estimates tell us that IV drug use is related to 29% of cases of AIDS reported. Efforts directed at the improvement of drug abuse treatment in this population and in the development of alternative HIV control measures comprise the agenda for our AIDS program management committee, led by Dr. Harry Haverkos. Our National AIDS Demonstration Research grants being conducted at numerous sites nationwide are providing a wealth of information on community based interventions for preventing the spread of AIDS.

The economic cost of drug abuse treatment, as in other areas of health care, is a significant impediment to its overall success. NIDA's Services Research program, guided by Dr. Joseph Autry, has been set up to address ways of assessing the capacity of the drug abuse treatment system, the cost effectiveness and efficiency of treatment and/or services, and treatment funding, reimbursement and cost containment issues.

As in many areas of scientific inquiry these days, there is a shrinking pool of trained and qualified drug abuse researchers to carry on the work in this field burgeoning with opportunities and needs. NIDA's firm commitment to the expansion of the numbers of researchers in this area through the training of new scientists is evidenced by its formation of a task force specifically for this purpose. Headed by Dr. Marvin Snyder, this group will look for ways to strengthen and foster interest in our existing training mechanisms which include training programs for

researchers, health professionals, special populations and HIV and substance abuse researchers, and will continue to search for ways of drawing new scientists into the fold. This year, with a training budget totalling $6.8 million, NIDA sponsored 283 trainees and we firmly intend to increase both of these figures in the coming years.

New Research Opportunities

Our grantees will be interested to learn of the many new opportunities for funding reflected in the new program announcements we have issued this year. A Cooperative Agreement for AIDS Community-Based Outreach/ Intervention Research (DA 90-02) will build on grant support provided since 1987 through our community outreach demonstration projects. During this phase, NIDA will support both new research initiatives and the refinement of existing outreach and intervention strategies to prevent the spread of AIDS. The Behavioral Consequences of Long Term Use of Abused Drugs (PA 90-10) encourages studies which describe behavioral deficits which may persist after prolonged drug abuse. In concert with a number of other Public Health Service components including NIMH and several of the NIH institutes, NIDA has issued an announcement encouraging the study of Children with HIV Infection and AIDS (PA 90-15). Although the PHS has already issued a number of announcements related to the study of AIDS, this one will complement these in highlighting specific areas with respect to affected infants and children as well as their families and caregivers. NIDA has reiterated its commitment to Research on the Prevalence and Impact of Drug Use in the Workplace (PA 90-19) by refining and reissuing its 1987 announcement this year. Since current statistics indicate that the majority of our nation's current drug users are employed, the worksite is an important locus for drug prevention/reduction programs.

Clearly, NIDA and its research programs continue to thrive and the opportunities available seem almost boundless. It is fortunate that we have been the recipients of the increased resources essential to build upon these opportunities to stimulate our successful research efforts. I firmly believe that NIDA will, at the least, receive the kind of stable support in the future which will allow us to maintain and build upon these accomplishments. I encourage all of you to continue to share the challenges and the many rewards that this meaningful research holds.

AFFILIATION: NATIONAL INSTITUTE OF DRUG ABUSE, ROCKVILLE, MD 20857

Biomedical Research and Scientific Citizenship: The Price of Progress

F.K. Goodwin

Introduction

The subtitle of my presentation, "The Price of Progress," has two meanings. The first is paradoxical: our progress, evident in the stunning successes of biomedical science, is itself at the root of some of our problems. The second is that we must acknowledge that an effective response to contemporary anti-science challenges has a price, in the form of needed changes in the culture of science.

Let me first address the paradoxical meaning. On the one hand, the scientific establishment continues to grow and to produce truly stunning achievements in all of its realms. One illustrative area of activity is that of the neurosciences. Fifteen years ago, when I joined the Society for Neuroscience, it had 300 members, and now there are well over 15,000. Information about the workings of the brain is accumulating logarithmically; the great bulk of what we know today has been learned in the last two decades. And, it is fair to say that the public's awareness of our society's increasing dependence on the yield of science and technology is keeping pace with advances in the biomedical sciences.

But now, the paradox: we also face an unmistakable increase in the public's uneasiness about biomedical science. Secretary of Health and Human Services Dr. Louis Sullivan has characterized these negative attitudes as focusing on the four 'C's: that is, biomedical research is too costly, too corrupt, too cruel, and too closed. Public support of biomedical research remains broad, but it is not as deep as it once was, and the impact of anti-science fringe groups is intensifying.

When the anti-science poison started to hit very hard a decade ago--particularly in the form of the animal rights movement--many of my colleagues at the NIH didn't understand the problem. Many could not believe that the assumption they had grown up with in the '50s and '60s--that biomedical research is a noble endeavor reflecting the best instincts of advanced civilizations--was being questioned, indeed was being challenged as immoral at its core.

Our abject failure to respond effectively to the animal rights challenge is a paradigm for our problems, and is symptomatic of the difficulty that the biomedical community faces

in shaking off its complacency and adjusting itself to a very different ambiance, Not only Carl biomedical science can no longer take for granted its place in the sun, but we must fight to win it back.

While public opinion surveys and focus groups conducted by ADAMHA indicate that the public is well aware of, and appreciates the yield of science, the same cannot be said of the process of science. The public's lack of understanding of the process of science makes us vulnerable. It is imperative that we in the scientific enterprise take the lead in efforts to enhance public scientific literacy. If we wait for someone else to take the initiative, we will deserve the erosion of support for science that certainly will continue and likely will accelerate. If you look at the attacks on science in their various forms--for example, distortions by the environmental movements anti-science fringe of the concept of "risk" and estimates of risk, the fight against genetic engineering, animal rights--you see that each cleverly exploits the public's ignorance about the process of science--an ignorance that we have allowed to develop.

What's worrisome is that today's attacks on science reflect a contemporary expression of long-term trends, the roots of which were set down over a quarter century ago. "Quick fixes" won't remedy the problem. We need to accept that we are in for a long-term struggle requiring a fundamental reorientation of directions and priorities.

This need is strikingly evident when you consider that on one meaningful index--civilian R&D as a percent of Gross National Product--the position of the United States has shifted over the past 25 years, from ranking first among the industrial powers to last. West Germany and Japan now stand where we did before the Kennedy assassination set oft the "troubled" sixties, and we've shifted to where they were then. Ominously, that shift from first to last reflects our decreasing willingness, as a society, to invest in improving our future.

Such megatrends notwithstanding, people tend to think that biomedical research has fared relatively well in this era. And while it has compared to some areas, the ratio of investment in research to total health care cost, has shown a similar pattern of decline. Between 1970 and 1986, U.S. health research funding from all sources as a percent of total expenditures for health care dropped from 2.6 percent to 1.6 percent.

Clearly, the calculation of biomedical research costs as a percent of health care is a complex one, and can be misleading. Health care, which now accounts for 11 percent of GNP, has expanded faster than any other facet of the economy, and much of that expansion is a function of new, research-generated health care technologies. Also, the question of funding for biomedical science is inherently more political than many other science enterprises, and thus long-term trends can be disguised by ups and downs. Still, parallels can be seen between our performance in the biomedical research arena and performance in the larger arena of research and development. That is, in biomedical research, too, the trend is irrefutable; our position today among industrialized nations is essentially the reverse of what is was some 25 years ago.

While it is heartening that President Bush has designated science education as one of his top objectives for the 1990s we need to remember that, at present, tests of science literacy and proficiency among high school students consistently find U.S. students to rank dead last--12th out of 12 countries in the ratings most recently compiled by the National Science Board.

Within an overall dismal picture, U.S. performance in the life sciences is worst of all. We would do well to consider that one explanation for the somewhat better performance of our

students in the physical sciences may be the longstanding direct involvement of NASA in science education activities. For many years, the space agency has invested approximately $75 million annually in science education and outreach targeted to primary and secondary level students. Until recently, by contrast, the Public Health Service had essentially no activities focused on pre-college life sciences education. That's changing. Today, ADAMHA and NIH are coordinating an array of initiatives that range from school-based programs to educational efforts conducted through the public media. Also, at the request of Secretary Sullivan, I represent the Department on the Federal Coordinating Council on Science, Engineering, and Technology.

Even as government and academia is gearing up a program in science education, the antivivisectionist movement is doing as much as it possibly can to promote life science illiteracy in the schools. Of the estimated $50 million the movement is targeting for its "stop animal research" campaign, a substantial portion is being spent in the schools in a very aggressive campaign to turn kids off to the biological sciences before they even get started.

To understand our current dilemma we must appreciate both the fundamental societal trends in the last quarter century and the evolution of internal problems within science's own house.

What are these broad societal trends? Since the early- to mid-'60s--the advent of the "troubled '60s that I referred to--we've seen an approximate doubling of the rates for a variety of social ills: depression in the young; suicide in the young; crimes of violence, including domestic violence; drug abuse; and divorce. In parallel with the reversal of our international ranking on the R&D/GNP ratio, we've seen an increasing tendency for businesses to invest in the short-term, to think primarily about the next quarter's profits, rather than the long-term health of the firm. The decline in our educational system has been parallelled by a decline in our physical infrastructure--roads and bridges and the like.

It comes as no surprise that our competitive position in the world has slipped badly, and we must ask ourselves why. This is a country that, coming out of World War II, implemented the Marshall Plan, enjoyed a prolonged period of prosperity, and had enormous confidence in itself and in its ability to improve the future. What happened to so dramatically change a whole generation of Americans?

My own view is that the Kennedy assassination in '63, and the two assassinations that followed--of Robert Kennedy and Martin Luther King--were far more profoundly traumatic than we allowed ourselves to realize at the time.

We've learned from behavioral research that when Type A individuals--hard-driving competitive personality types--are faced with grief, they deny direct feeling of loss; instead, they put themselves even more aggressively into whatever it is they're doing. It seems to me that this overcompensation reaction is one way to understand the relentless and massive escalation in Viet Nam and our governments simultaneous escalation on the domestic front under the banner of "The Great Society." In effect, in the post-assassination period, our government turned up the burners on both the domestic and foreign fronts. Living through that period in Washington, one could palpate the growing cynicism about the capacity of the government to solve problems. Indeed, as the government rushed into seemingly mindless activity on all fronts at once--with enormous new bureaucracies springing up across government, while popular support for the war rapidly evaporated--the net effect was to begin to call into question the post-World War II consensus that government could solve problems,

Other characteristics of grief reactions include a loss of confidence in the future, and a turning inward. If a society has had its confidence in the future stolen, a societal shift from long-term to short-term goals should not be unexpected; people are not sure the future is manageable or can be improved.

As these psychological reactions played out across society, we saw behaviors and reactions that became self-fulfilling, We in science, as part of the intellectual leadership of this country, need to reflect on such general long-term trends and commit ourselves to being part of the solution.

More specific to the status of the scientific enterprise was a post-Vietnam evolution of mistrust of institutions that was massively reinforced by Watergate. Watergate marked the advent of the "institutionalization of mistrust," and, particularly in Washington, the birth of what has become a massive and powerful "mistrust industry." The two years following Watergate brought substantially increased enrollment in schools of journalism; many of these new journalists were cynical and suspicious, and, indeed, had chosen journalism to find new Watergates. Members of the post-Watergate generation now hold influential positions in the media; they determine what's on the evening news and how it is presented; and they determine what's in the headlines.

Also relevant was the very large increase in the size of congressional staffs during the '70s. Most of the increase could be accounted for by increased oversight functions rather than the drafting of legislation per se. Congressional oversight is a necessary part of our system of government based on checks and balances between the legislative and executive branches. But the post-Watergate mood spawned a type of oversight that in some cases seems to be based on mistrust. Here, again, because the investigative process itself can insinuate problems, one sees a self-fulfilling aspect; the public says, "See, we need that and we'll have to have even more of it." In the executive branch, this attitude played out in the proliferation of inspectors general staffs and an apparently permanent horde of special prosecutors.

A related development, I would suggest, is the erosion of what used to be a consensual process in medicine and science. Our traditional consensual process has gradually yielded to an adversarial process as we've turned more and more to the legal System to solve problems. Legalistic formulations can become a substitute for a moral compass.

We've also seen a growing moral and cultural relativism in our schools, a trend eloquently described by Allen Bloom in The Closing of the American Mind. In a studious attempt to avoid anything that might involve moral judgement, our educational establishment has fallen back on the notion that everything is relative. I would submit to you that absolute relativism is a very slippery slope for science. In the final analysis, science depends on a commitment to seeking truth, and implicit in that commitment are some absolutes: that the scientific method can yield knowledge about nature; that theories can be confirmed or rejected on the basis of discernable facts; and that knowledge is superior to opinion or sentiment.

I was stunned by this cultural and moral relativism when I recently encountered a young protestor at an animal rights rally at the NIH. She listened politely to me as I explained the importance of the research and its potential contributions to health. After appearing to listen carefully to my elaboration of information that was totally new to her--she clearly had no understanding of science or medicine--she said, "Well, doctor, you're entitled to your opinion and I'm entitled to mine." In that one sentence, she had reduced a huge gap of information and understanding to a matter of opinion.

I focus on the animal rights movement because it serves as a paradigm for a whole new generation of anti-intellectual attacks on science. The failure of academia, government leadership, and industry to respond adequately does not portend well for all of science--with or without animals--unless we can learn from past mistakes.

Academia's--and, here, I include the government research community's--preoccupation with short-term crisis solving and the parallel tendency to turn inward is self-destructive. When the animal rights attacks began more than a decade ago, we responded defensively, even apologetically. Our primary strategy seemed to consist of repetition of the "three R's"--reduction, refinement, and replacement. No wonder that large segments of the public came to believe that maybe the animal rights movement was on to something. Maybe there really was something wrong with the use of animals in research--after all, the research community was certainly emphasizing how diligently it was working to eliminate it just as soon as possible!

Actually, the 3 R's in themselves are not unreasonable, but that should be a secondary message--after we've established both the vital importance of animal research for human health and the the distorted values and moral decadence implicit in the animal rights philosophy. By ignoring the fundamental moral premise of their argument--that animals and humans share the same rights--we gave them a "free pass" around their most vulnerable point: That when they elevate animals to the level of humans, they degrade humans to the level of animals.

We allowed that philosophy and the accompanying agenda, which was driving the animal rights takeover of the animal welfare movement, to go unchallenged. All we did was say, "No, research is not inherently cruel. Yes, research is important to human health." And we didn't even do that very well. Focus groups and public opinion surveys conducted under ADAMHA auspices indicate that a substantial portion of the public did take our response--especially the 3 R's--as an apology; our defensive reaction reinforced the power of the animal rights movement.

Those of you who like history are no doubt aware of the rather sorry performance of much of the academic community in Germany in the '30s. When one is faced with a truly radical movement, action is required, and most of us in science and scholarship aren't particularly action-oriented; we are more comfortable with intellectual analyses. Indeed, the academic research community has generated quite a few very scholarly, probing, thoughtful, and balanced analyses of the animal rights issues, but these alone will not win this fight.

On the broader anti-science front, we in the scientific community have allowed Jeremy Rifkin and his colleagues to get away with absolute nonsense about risk assessment. Not only has there been very little in the way of effective response from the scientific establishment, but we've naively cast some of our own internal scientific arguments in terms that make them more readily adaptable by the anti-science movement. All too often, when scientists discuss problems associated with extrapolating from animal carcinogenicity testing to human risk--and discuss this in the media--these well-meaning scientists seem blissfully unaware of how to do this without giving ammunition to the anti-science movement.

We've also seen growing political attention to scientific misconduct and to conflict of interest issues. These are so often lumped together that there is a dangerous tendency on Capitol Hill to treat conflict of interest as if it were a subset of misconduct. We must make clear that conflict of interest issues relate to the process in which science legitimately interacts with corporate America to fulfill the legitimate goals of the Nation,

as articulated, for example in the Stevenson-Weidler Technology Transfer Act, a law enacted to assure that scientific discoveries get beyond the lab into the marketplace.

Our free enterprise system presupposes that it is legitimate for scientists to be rewarded when their work leads to a potential product; in fact, financial incentives may increase the likelihood that useful products will be developed, thereby serving an important public good. If the mere existence of any financial incentive is considered likely to corrupt scientists' objectivity, we should just forget about the rapid translation of new information into clinical or other applications. While we need disclosure as well as mechanisms for independent review of financial arrangements, these need not stifle either the process or the rewards of science.

Another issue that warrants our attention, and one which I alluded to earlier, concerns the tendency of many in the academic research community, when faced with an ethical issue, to turn to lawyers to solve it for us. Lawyers do what lawyers are trained to do: think in legalistic, often adversarial, terms. As one who tries to understand human behavior, I have come to think that the more one relies on legalistic formulations, the more confused one's own moral compass can become. One starts thinking in terms of what's legal--what's within the rules--rather than what is necessarily right. Indeed, one might argue that the more rules there are, the more favorable is the environment for psychopaths, given that psychopaths are especially adept at getting around rules!

Another often overlooked casualty of overly legislated morality is its erosive effect on a sense of "citizenship" by the majority--good people assuming responsibility for their behavior. Resentments that breed in an environment of mistrust can tend to make the mistrust self-fulfilling. In other words, systems which imply that corruptibility is the rule may be eroding the very attitudes that keep it in check.

Yielding to the "mistrust industry" can lead to self-inflicted wounds. This would be the case, for example, if the scientific enterprise were to develop a conflict of interest policy that goes beyond what we really believe, simply to preempt the possibility of something worse being imposed from an external source such as the Congress.

I do not happen to believe that preemptive concessions which go beyond one's own convictions are helpful. In my view, this approach simply ratchets up the bargaining; the outside force is reinforced in believing that they must have been on the right track and should push even further. My point is not to denigrate the process of compromise, or political give-and-take, but only to emphasize that we should not compromise prematurely.

The debate in 1990 within the Public Health Service on the initial conflict of interest guidelines anticipated the vigorous response from the scientific community; clearly this response has produced a far superior document. We now have the support of Secretary Sullivan and Assistant Secretary for Health Mason for guidelines that put the primary responsibility where it belongs--on the universities. Basic responsibility for ensuring that the integrity of the scientific enterprise is protected has been assigned to the universities, which I see as preferable to having government prescribe in detail exactly how that integrity is to be accomplished in each and every setting and situation.

Finally, the paradoxical "price" of progress involves issues that are internal to the scientific enterprise. All of us who do research, or who manage science programs, are forced to deal with the effects of a massive explosion in information that is a function of our astounding success in technology development. We are inundated by the task of keeping up with the information in our fields. That means we all have less time for tending to our

infrastructure--less time for scholarship, for leadership, for mentorship, and for public science education, including what's happening in our primary and secondary schools.

One wonders how many of the problems that are confronting us today might be handled more effectively if all of us weren't so busy keeping up with the data, keeping up with publishing, with competing for grants. As it is, we simply don't have very many scientists available to defend the infrastructure at a critical time.

Somehow, we have to find ways to enhance the incentives for what I call "scientific citizenship.'! If we don't make it possible for more working scientists to act as good citizens of our community, trends that already are a matter of concern surely will worsen. In our current culture of science, however, people who explain their own research in the popular media run the risk of being seen as self-promoting, and, thus, may be stigmatized by colleagues. While pure self-aggrandizement is unacceptable, the scientific enterprise could certainly be more creative in finding ways to promote involvement in public education by scientists who happen to be gifted communicators. The scientific establishment might develop major awards for contributions in this arena. With the plethora of awards for scientific achievement, certainly we could afford a few for scientific citizenship.

We also must do more to stem the dehumanization of medicine by technology, a process that is helped along by reimbursement formulae which are geared to techniques and procedures rather than to a clinician's time. One outgrowth of the dehumanized, technology-driven hospital is patient dissatisfaction; this often translates into frustration with biomedical science.

This is a reimbursement problem, but its origins--advances in science--suggest that we must view reimbursement as related to the scientific infrastructure. This is certainly true in clinical research. But in any event, patients understandably feel cheated when the doctor spends only a few minutes with them, and the doctor may well miss what's really going on. At present, only one in three patients who present with major depressive symptoms are properly diagnosed by primary care physicians. Major psychiatric problems (which so frequently underlie a host of non-specific medical complaints or are associated with substance abuse problems) are especially vulnerable to being missed because proper detection takes time--a commodity for which reimbursement is clearly inadequate.

Fortunately, the relative value based scales currently being developed and implemented by HCFA suggest that the reimbursement bias that favors procedural over cognitive interventions may be waning. The new Agency for Health Care Policy and Research within the Public Health Service anticipates that its science-based guidelines will be useful to HCFA in its development of reimbursement formulae, and I anticipate that the process will produce a shift toward a more equitable balance in reimbursement for clinician time vis-a-via procedures and tests. In my view, that's a healthy shift.

What are ADAMHA and NIH are planning to do in response to the various impediments to science literacy, obstacles to public support of science, and threats to the vitality of science? For science education purposes, ADAMHA will spend about $3.2 million in fiscal year 1991, and we hope to see a comparable investment from NIH. The centerpiece of our program is the Science Education Partnership Award (SEPA), which we are introducing to enable scientists to link with colleagues in the primary and secondary schools around innovative science education projects. The SEPA awards should make it possible for a working scientist with a special interest in science education to devote part-time to such efforts without a negative impact on his or her research. Other ADAMHA science education initiatives will focus on special media projects, minority

initiatives, and linkages between substance abuse prevention and science education.

We also need to persist in an aggressive response to the core of the anti-science movement, especially antivivisectionism, or the animal rights movement. All of us--and I include the pharmaceutical industry, whose own long-term economic health is at stake--need to do a great deal more. We are enjoined in a major struggle for the hearts and minds of Americans--especially our young people. Right now, we are far behind, because for nearly a decade we buried our heads in the sand. The effort to stop animal research is outspending us by a 50 to 1 ratio, and if we don't correct that, the rightness of our cause will matter little.

We need an effective coalition that includes government, the professsional organizations, universities, industry, and patient groups, If we don't cement these linkages and all get on the same page around crucial issues of common concern, the stunning progress of biomedical research will slow, and opportunities to improve the Nation's health as we enter the next century will not be realized.

AFFILIATION: Alcohol, Drug Abuse and Mental Health Administration
U.S. Department of Health and Human Services

Introduction of the Nathan B. Eddy Award Co-Recipients

E.L. Way

It is my pleasure to introduce the co-recipients of the Nathan B. Eddy Award which is given annually for distinguished contributions in drug dependence. Although both awardees are well-known for their individual research accomplishments, the Award Committee deemed that their joint efforts in opioid receptor research have made a major impact on understanding the fundamental mechanisms involved in the development of tolerance and physical dependence.

Dr. Akira Takemori received the A.B. from the University of California at Berkeley, the M.S. from the University of California at San Francisco and the Ph.D. from the University of Wisconsin. He taught at SUNY, Syracuse before going to Minnesota where he rose rapidly through the ranks, becoming full professor in 1969. His pioneering research activities in the sixties and seventies provided pharmacologic evidence for the existence of multiple opioid receptors. By application of pA2 concepts for studying opiate agonist and antagonist interaction in vivo as well as in vitro, Takemori and associates demonstrated, that different opioid receptors were responsible for inducing analgesia, respiratory depression, gastrointestinal transit and hyperthermia. They also concluded that opiate antinociception is mediated on two different types of receptors which are now known respectively as the mu and the kappa receptors. Subsequent studies, based on interactions between met- and leu- enkaphalin with opiates, led them to postulate the existence of opioid receptors with coupled mu and delta sites. Another extremely important contribution by Takemori was the demonstration that increased sensitivity to naloxone could be an indicator for the development of opiate tolerance. This finding led to studies by others showing increased sensitivity for naloxone during physical dependence development, thus providing compelling circumstantial evidence for a close link between tolerance and physical dependence. Such experiments were performed at least a decade prior to

the characterization of opioid binding sites and the isolation of the opiopeptins (the enkephalins, endorphins and dynorphins).

Dr. Philip Portoghese received his B.S. and M.S. at Columbia University and the Ph.D. at Wisconsin. He was a post-doctoral at Kansas and in 1961 was appointed assistant professor at Minnesota where he was promoted subsequently to full professorship in 1969. His early research centered on the stereochemistry and conformation of opiate agonists and more recently on the design of selective opioid receptor antagonists. His contributions in these areas have been recognized by awards from national pharmaceutical and chemical societies. The structure and configuration studies in 1965 by Portoghese and associates led them to postulate the existence of multiple opioid receptors and multiple modes of binding to such receptors by different classes of opioid ligands. In designing affinity labels for opioid receptors, a number of opioid antagonists were synthesized that followed predictions with respect to activity and have become classic pharmacologic tools.

The close collaboration between Takemori and Portoghese have enabled the development of several opioid antagonists which, by virtue of their highly selectivity for various opioid receptor types, provided definitive proof for the existence of multiple opioid receptors.

The first useful affinity label for opioid receptors was B-chlornaltrexamine (ß-CNA) a universal non-equilibrium antagonist. Next, ß-furnaltrexamine (ß-FNA) , a highly selective nonequilibrium mu antagonist was introduced and this property has been widely exploited to identify mu effects of various opioid agonists or to localize their sites of action. As an example of the latter application, B-FNA was used to demonstrate that development of physical dependence on mu agonists could occur at both supraspinal and spinal loci. In a similar vein, norbinaltorphimine (nor-BNI, the selective kappa agonist, and naltrindole (NTI), the delta antagonist, were synthesized and utilized subsequently by many laboratories as tools for characterization of opioid actions and sites. More recently, NTB, the benzofuran analog of NTI was prepared and used to identify delta opioid subtypes in vivo as well as in vitro. Finally, selective blockade of opioid receptors with various antagonists reveal that mu, kappa and delta receptors are all involved in opiate tolerance and physical dependence development with the mu receptors having the major role with delta and kappa receptors playing modulatory roles.

Like most academicians, Takemori and Portoghese have been role models as teachers and have developed many students who have made their own research contributions. Moreover, their professional activities identify them as leading scientific statesmen. Both have served the government on numerous review committees and as consultants to help formulate policy. Aki is a former member of the Executive Committee of CPDD and President-Elect of ASPET. Phil is the timeless Editor-in-Chief of the Journal of Medicinal Chemistry. Their strong dependency on opiate research early evidenced by the fact that both have been hooked for over a quarter of a century.

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Opioid Pharmacology a' la mode

A.E. Takemori

I wish to thank the Award Committee of CPDD for honoring me with this award. Receiving the Nathan B. Eddy Award means more to me than any other award because it is given for research done in a particular field in which I have devoted almost my entire academic career of nearly four decades. In fact, if one counts from the beginning of my graduate training with Professor E. Leong Way, it would be, almost to the day, forty years. The title of my talk seems somewhat peculiar but I am using the literal translation of a la mode which is "according to the fashion". I believe that my research career has been shaped by current scientific fashions of the day. When I was finishing my doctoral studies, the fashion of that period was biochemical pharmacology. That is one of the reasons why I chose to do postdoctoral studies at the Institute for Enzyme Research at a time when postdoctoral training by pharmacology graduates was not common.

During my last two years of graduate studies with Dr. G.J. Mannering at the University of Wisconsin, Julius Axelrod at NM put forth a hypothesis that the hepatic enzymes that N-demethylate opioid drugs could be used as models for opioid receptors in the development of tolerance. To support this hypothesis, he showed that when rats are treated chronically with several opioid analgesics, the capacity of the hepatic enzymes to N-demethylate the analgesics are markedly attenuated. He explained that this was analogous to the development of tolerance in that both the hepatic sites for demethylation and the opioid receptor sites become "inactivated" with continual exposure to the opioids. If this postulate was correct, we reasoned that optical isomers of the morphinan-type opioids should be differentially N-demethylated because it was known that the levo isomers were active analgesics whereas the dextro isomers were inactive. Using three pairs of optical isomers, including levorphanol and dextrorphan, we demonstrated that both l- and d-isomers were N-demethylated by hepatic enzymes with equal ease. In addition, we showed that dextrallorphan, the inactive opioid antagonist, inhibited N-demethylation of opioids just as well as the active antagonist, levallorphan. In confirmation of Axelrod's findings, chronic administration of the active l-isomer opioids resulted in an attenuation of the N-demethylase activity. However, a more serious objection to the postulate was the fact that the chronic administration of the inactive d-isomers did not alter N-demethylase activity even though both isomers are equally N-demethylated. These results demonstrated clearly that the hepatic N-demethylase system could not be used as proper models for opioid receptors in the phenomenon of opiate tolerance.

One of the perks to come out of the above study was the fact that when the 3-position of either the morphine- or morphinan-type analgesics was muzzled by substitution, the N-demethylation activity was increased by about 7-fold. Such a compound was ethylmorphine which we introduced in 1958, and today it is one of the most often, if not the most often, used substrate for the P_{450} mixed function oxidase system.

When I first arrived at SUNY, Syracuse in 1959 and received my first NIH grant, I began to study the individual enzymes responsible for the formation of morphine glucuronide, the major metabolic product of morphine in man. The enzymic activities of UDP-glucose pyrophosphorylase, UDP-glucose dehydrogenase, UDP-glucuronyl transferase and nucleoside diphosphokinase were examined in livers of rats that were treated chronically with either saline or morphine. The activity of UDP-glucuronyl transferase was shown to be decreased by the chronic treatment of morphine. This caused a rate limiting step and the overall formation of glucuronides was decreased in rats treated with morphine. Thus, this metabolic alteration could not contribute to the development of morphine tolerance.

One of the techniques I learned at the Enzyme Institute was the use of the Warburg constant volume respirometer, with which one can measure the oxygen uptake or respiratory rate of tissue slices, minces, homogenates or mitochondria. I initiated a study in which the respiratory rate of cerebral cortical slices from control and morphine-treated rats was examined. The basal respiratory rate was not altered by morphine in vitro but when the oxygen uptake was stimulated by KCl, morphine did inhibit the KCl-stimulated respiratory rate. As the animals received daily doses of morphine, the cortical slices adapted to the depressant effect of morphine so that by the fifth or sixth day of morphine treatment, the KCl-stimulated respiratory rate of the cortical slices were no longer inhibited by morphine in vitro. After abrupt cessation of the daily morphine injections, the sensitivity of the cortical slices to morphine returned gradually to normal within a week. The report of this work in 1960 was one of the first biochemical demonstrations that neural tissues from a morphine-tolerant animal also was adapted to the depressant effect of morphine in vitro. The morphine-adapted cortical slices of morphine-tolerant rats were shown to be cross-adapted to the depressant effects of methadone and meperidine but not to those of other depressants such as pentobarbital or ethanol. Thus, this adaptation observed in cortical slices was selective to the opiate-type class.

In keeping with the dispositional theme, one of my students, Joe Wang, was studying the transport of morphine in the choroid plexus, in vitro and found a carrier-mediated transport system for morphine. The question now was whether the transport of morphine was directed out of the CNS or into it. To answer this question, the technique of cercbroventricular perfusion in rabbits was utilized in which either a constant blood level of morphine was maintained by i.v. infusion, or a constant concentration of morphine was kept in the perfusing artificial CSF. The results showed that the active transport of morphine was in the direction of blood to CSF. Knowing that morphine has some difficulty entering the brain, here was a situation whereby morphine could be rapidly transported to the CNS and to its receptor sites. However, this transport system was not altered by the chronic treatment of morphine, and thus changes in this transport system could not account for the development of tolerance.

In the early ‘60’s, I read a paper by Brian Cox which caused me to change the course of my research activities to a more physiological approach. The paper described the use of the pA_2 concept, for the first time, in vivo and I was

particularly inspired because Prof. Schild who devised the pA_2 concept had personally sanctioned the in vivo work . We introduced the apparent pA_2 concept (pA_2 in vivo) as a pharmacological constant. We came under heavy criticisms by suggesting a constant in vivo but the credibility of a constant is whether or not it can be reproduced in other laboratories. For example, the pA_2 of naloxone to antagonize the antinociceptive effect of morphine is around 7.0 in mice. This figure had been reproduced many times by several graduate and postdoctoral students in my laboratory. I believe the credibility of this constant is assured because in the last two decades, laboratories from around the country (WI, CA, NY, IN, MI) and abroad (Hungary, Australia, Japan, England, Germany, Italy, Spain, India) have replicated the pA_2 of about 7 for morphine-naloxone not only in mice but in rats and monkeys using various antinociceptive assays.

One of the first studies using the apparent pA_2 concept was by my student Steve Smits, who examined the antagonism of the antinociceptive activity of several opioid analgesics and mixed agonist-antagonist analgesics. In 1966, Blumberg's group reported that the then relatively new opioid antagonist, naloxone, had the capacity to antagonize the antinociceptive effects of mixed agonist-antagonist analgesics as well as the pure agonist analgesics. Thus it was convenient for us to use naloxone as a tool to examine the apparent pA_2 values of the two groups of analgesics. The apparent pA_2 values for morphine, methadone and levorphanol with naloxone were all around 7.0, whereas those for nalorphine, pentazocine and cyclazocine were about 6.3 The significant difference in these values led us to report in 1969 that the two groups of drugs may be interacting at different receptor sites to produce antinociception. We did not coin a name for these receptors at that time, but of course, they are now known as μ and kappa opioid receptor agonists after the receptor classifications by Martin in 1976.

The increased sensitivity of animals to naloxone has been suggested to be a sensitive indicator of the development of morphine tolerance. This sensitivity is manifested in a significant increase in the apparent pA_2 of morphine-naloxone. Thus, one of my postdoctorates, Dr. Cankat Tulunay, and my collaborator in Japan, Dr. Tetsuo Oka, have been able to show that even a pretreatment of one dose of morphine in mice will significantly shift the apparent pA_2 for morphine-naloxone so that the animals become approximately twice as sensitive to the antagonistic effect of naloxone. This sensitivity to naloxone is enhanced several-fold more by chronic administration of morphine, by S.C. implanted morphine pellets. If one monitors the development of tolerance to the antinociceptive effect of morphine, one finds that the increased sensitivity to naloxone can be detected long before the antinociceptive tolerance

Further use of the apparent pA_2 concept was to characterize the receptors mediating different pharmacological effects of opioids. Dr. Tulunay, along with my graduate student, Kip McGilliard, studied various central effects of morphine and found, using naloxone, that the apparent pA_2 values for antinociception, respiratory depression and hypertheirmia significantly differed from each other. Thus, we suggested at the INRC meeting in 1976, that these pharmacologic effects of morphine were mediated probably by interacting at different opioid receptors.

In the late '70's, two observations from our laboratory had a major influence in guiding our research efforts. One was by my postdoctorate, Dr. Ichiro Yano, who showed using slow releasing preparations as well as multiple injections of morphine and naloxone, that chronic tolerance and dependence was a continuance of acute tolerance and dependence. He found that, if during the blockade of

morphine tolerance by naloxone, the opioid receptors were exposed to morphine alone even for only a few hours, some degree of tolerance and dependence would develop. The complete blockage of the development of morphine tolerance and dependence required the complete blockade of the receptors by naloxone continuously during the time that morphine is in the receptor environment. Thus in our laboratory, we have made acute tolerance and dependence that is induced by a single injection of morphine, a model for chronic tolerance and dependence that is usually produced by s.c. implanted morphine pellets for several days.

The second observation is one that was made by my student, Jeff Vaught, who shared that leu- and met-enkephalin produced differential effects on the activity of morphine. Whereas leu-enkephalin potentiated morphine-induced analgesia, tolerance and dependence, met-enkephalin did not. The same differential effects were observed in the guinea pig ileal longitudinal muscle preparation. This was the first study involving the interaction of these newly found pentapeptides with opiates and is the basis for the currently postulated μ and delta opioid receptor coupling or complex.

Even after initiating our work with apparent pA_2 values, we knew that characterizing opioid receptors in this manner required a high amount of animals and effort. Thus in the mid-60's, I began a collaboration with Phil Portoghese, at our College of Pharmacy, who had similar thoughts as mine on multiple opioid receptors. If we could come up with highly selective probes for the different opioid receptor types, then we could obviate the apparent pA_2 method and test directly whether or not a certain receptor type is involved in mediating certain effects of opioids. This task was not an easy one and it took us about 12 years before the first useful, selective affinity label, ß-chlonaltrexamine (ß-CNA) was reported in 1978. My student, Tom Caruso, was very instrumental in characterizing this antagonist pharmacologically and biochemically. ß-CNA is a nitrogen mustard derivative of naltrexone. It was highly selective for opioid receptors and did not interact with any other known receptors, e.g. adrenergic, cholinergic, prostaglandin, etc. Thus, ß-CNA was useful for some studies in which blockage of all types of opioid receptors was required but did not satisfy our goal of identifying specific receptor types.

Two years after the announcement of ß-CNA, we reported on another affinity label, ß-funaltrexamine (ß-FNA) which was highly selective for the μ-type opioid receptors. ß-FNA is the fumaramate methyl ester derivative of naltrexone that possessed not only irreversible opioid receptor antagonist properties, but reversible opioid receptor agonist properties as well, in vitro and in vivo. By apparent pA_2 analysis, my postdoctorate, Dr. Susan Ward, was able to demonstrate that the reversible antagonist activity was mediated through interaction with μ opioid receptors. Thus with one compound, we were able to demonstrate that it interacted differently with two different types of opioid receptors. Additionally, the fumaramate methyl ester derivative of oxymorphone, ß-FOA, turned out to be a completely reversible μ opioid receptor agonist with no evidence of alkylation. This suggested that μ opioid agonists and antagonists may be interacting at different sites. Protection studies using ß-FNA and an array of agonists and antagonists have provided further evidence for the differential sites of agonists and antagonists, at least in the μ opioid system in the guinea pig ileum preparation.

Now that we had a highly selective, irreversible JL opioid antagonist, one was no able to test directly the involvement of μ opioid receptors in various pharmacologic and physiologic effects. I have documented in my talk, numerous studies in which

ß-FNA was utilized but space limits my quoting or listing them here. One important question that was addressed was the role played by μ opioid receptors in the development of opioid tolerance and dependence. My student, Gary DeLander, demonstrated that in rats, i.t. treatment with ß-CNA can markedly attenuate the development of tolerance and dependence to parenterally administered morphine showing the importance of spinal μ opioid receptors in these adaptive processes. With the collaboration of Bill Dewey and Mario Aceto at the Medical College of Virginia, we demonstrated in an i.p. morphine infusion model in rats that with appropriate doses of ß-FNA, the development of physical dependence on morphine can be completely suppressed. In addition, in morphine-dependent monkeys, ß-FNA caused a prolonged withdrawal syndrome which persisted in spite of additional morphine injections. This observation corroborated our findings in vitro that ß-FNA alkylates μ opioid receptors irreversibly. These findings indicated that μ opioid receptors play a major role in the development of opioid tolerance and physical dependence.

In 1985, we reported on the first selective kappa opioid receptor antagonist, TENA, which is a bivalent ligand and possessed the highest known selectivity for kappa opioid receptors at that time. It was much more selective than the putative kappa opioid receptor antagonists, MR 2266 and WIN 44,441. We did not go on to develop TENA because it was difficult to synthesize and also we soon discovered binaltorphimine (BNI) and nor-binaltorphimine (nor-BNI) which were even more superior than TENA as highly selective kappa opioid receptor antagonists. In opioid receptor binding assays, both BNI and nor-BNI possessed Ki values in the sub-nM range and high selectivity for kappa receptors. The selectivity was particularly high for nor-BNI which had >150-fold selectivity for kappa over μ and delta receptors. In vivo the selectivity profile was the same, i.e., pretreatment with nor-BNI either i.c.v. or s.c. inhibited the antinociceptive activity of kappa opioid receptor agonists, ethylketazocine and U-50,488H. at doses that did not alter the antinociceptive activity of μ opioid receptor agonists, morphine and DAMGO, or the delta opioid receptor agonist, DPDPE. Again, space constraint does not permit me to list all the studies which have employed nor-BNI to investigate directly the involvement of kappa opioid receptors in various opioid effects. Some highlights are that N_20-, CRF- and parturition-induced analgesia, diuresis and regulation of dopamine neurons all appear to specifically involve kappa opioid receptors.

More recently in 1988, we reported on a highly potent and selective delta opioid receptor antagonist, naltrindole (NTI) and a year later on its affinity label derivative, 5'-naltrindole isothiocyanate (5'NTII). NTI, together with its benzofuran derivative, NTB, constituted the most potent delta opioid receptor ligand available with Ki values in the pM range in opioid binding assays. These antagonists had very high selectivity for delta receptors and NTB particularly, possessed delta receptor selectivity of about 1,500 and 12,000 over μ and kappa receptors, respectively. In vivo, both NTI and NTB administered either i.c.v. or s.c. inhibited the antinociceptive activity of DSLET at doses that did not affect the antinociceptive activity of either morphine or U-50,488H.

More interestingly, when my student, Dr. Mehmet Sofuoglu, tested NTB against various delta opioid receptor agonists, NTB antagonized the antinociceptive activity of DSLET but not that of DPDPE, a more delta-selective agonist than DSLET. This differential antagonism was even more apparent when the peptide agonists were administered i.t. Unexpectedly, the antinociceptive effect of DADLE, an agonist with least selectivity for delta receptors among the three peptides, also was

not altered by NTB. These findings suggested the possibility of delta opioid receptor subtypes. Further evidence for this suggestion came from a study done in collaboration with Frank Porreca at Arizona. Using 5'-NTII and DALCE (a peptide antagonist that irreversibly interacts with delta opioid receptors), it was shown that 5-NTII antagonized the antinociceptive activity of deltorphin II (a highly selective delta opioid receptor agonist) but not that of DPDPE; whereas, DALCE antagonized the antinociceptive effect of DPDPE but not that of deltorphin II. Also, DSLET was shown to be differentially antagonized similarly to deltorphin II. These results fortified the suggestion of sub-types of delta opioid receptors where DPDPE and DADLE interacted at one site and DSLET and deltorphin II at a different site. Lastly, in tolerance studies in which mice were made acutely tolerant to the antinociceptive effects of DSLET and DPDPE, the animals displayed absolutely no cross-tolerance. I believe these are some of the strongest functional evidences for the existence of delta opioid sub-types.

Again, the use of NTI to study directly the involvement of delta opioid receptors in certain pharmacologic and physiologic effects are too numerous to list. However, I wish to comment on one aspect of the use of NTI and 5-NTII in which my postdoctorate, Dr. Essam Abdelhamid, was involved. That is the question of the role played by delta opioid receptors in the development of opioid tolerance and physical dependence. Pretreatment with NT1 or 5'-NTII in the acute or chronic model of morphine tolerance/dependence, caused a marked attenuation and in the case of 5'-NTII a complete suppression of morphine tolerance and dependence. In contrast to the blockage of μ opioid receptors which blocks all actions of morphine including antinociception, tolerance and dependence, selective blockage of delta opioid receptors by either NTI or 5'-NTII blocked the development of tolerance and dependence without inhibiting the antinociception mediated through μ opioid receptors. This situation was one of the main goals of Nathan B. Eddy when he started this whole business over sixty years ago, namely to find a strong analgesic without the liability of the development of tolerance and dependence. I believe that we are getting very close to this situation.

In summary, we have developed for the opioid field, highly selective antagonists, ß-FNA for μ, nor-BNI for kappa and NTI and NTB for delta opioid receptors with which one can now directly test for specific involvement of opioid receptor types in the pharmacologic or physiologic effect being studied.

Finally, I wish to thank my graduate students and postdoctoral fellows who did the actual work described in this talk and to acknowledge especially my last three laboratory technicians, Joan Naeseth, Masako Ikeda and Mary (Schwartz) Lunzer who did a tremendous amount of work in characterizing these antagonists. Joan has been with me for 19 years and Mary for 10 years, and this continuity has made them responsible for helping me train my many graduate students. I also must apologize to some of my students whose work I could not include in this talk because of the time constraint. Especially relevant in this regard are the numerous studies involving neuropeptide-opiate interactions, neurotransmitter-opiate interactions, drug-opiate interactions and opiate biotransformation.

As a last comment, I wish to now use a la mode as it is used today, that is, "the ice cream topping on a pie". Winning the Nathan B. Eddy Award certainly puts an ice cream topping on my opioid pharmacology career.

Thank you all very much.

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Chemical Tools in Opioid Research

P.S. Portoghese

It's an honor which I appreciate deeply to be among the list of notable recipients of this prestigious award. I am particularly touched because Dr. Nathan B. Eddy was responsible for stimulating my interest in the opioid area while I was a graduate student at the University of Wisconsin in the Department of Medicinal Chemistry in 1960. My major advisor, Professor Edward E. Smissman, suggested that I present a seminar topic unrelated to my research. My research was concerned with the synthesis of podophyllotoxin and its analogues which are anti-cancer agents. I took his suggestion very seriously, because I decided that my topic should be unrelated not only to podophyllotoxin, but to anti-cancer agents in general.

In my journal browsing for possible topics, I came upon Dr. Nathan B. Eddy's sixth Lister memorial address, which he delivered in Edinburgh in 1959.1 His presentation so intrigued me that I decided that my seminar topic would be on the relationship between structure and biological activity of analgesics. My interest in opioids grew, and by the time I joined the medicinal chemistry (then pharmaceutical chemistry) faculty at the University of Minnesota in 1961 as an assistant professor, I already had prepared an NIH grant application to support research to investigate opioid ligand-receptor interactions using stereochemically defined compounds as tools. I was intrigued by the fact that molecules with quite different geometries possessed comparable analgesic activity. Moreover, the enhancement of potency by a phenolic hydroxy group in the benzomorphans but not in the phenylmorphans,1 and the different effects of N-substituents on potency in these two different series suggested to me that these ligands were not interacting with putative "analgesic receptors" in the same way, Also, if one compared how a change of the N-substituent of normorphine and of normeperidine affects analgesic potency, it was apparent that there was no correlation between the two series. What was particularly noteworthy was the report that

ally1 normeperidine was as potent as meperidine in mice, while nalorphine was an antagonist.2 One explanation for such a structure-activity relationship was that the N-substituted normeperidines and normorphines either interact with different recognition sites or they interact with a single recognition site in different ways so that their N-substituent contributes differently.3

Since the studies by Beckett and Casy4 had demonstrated that the more potent enantiomers of methadone, thiambutene and related compounds possessed identical absolute configurations, we decided to investigate additional compounds having a chiral center in common with methadone. The first series we investigated were the basic anilides.5 To our surprise, we found that the chiral center of the more potent isomers in this series had an absolute configuration opposite that of methadone.6 Further, other stereochemical studies also showed a lack of correlation with methadone.7

At that point it was clear to me that a lock-and-key model could not explain the structure-activity relationship for opioid ligands, and that the reported results could best be explained in terms of a complex system containing multiple receptors and multiple modes of binding to a single receptor. Although I had proposed what I considered to be a plausible concept,7,8 I didn't know how to distinguish between these possibilities.

This led me to propose an approach using affinity labels, an area that was pioneered by the famous medicinal chemist B. R. Baker.9 If the reactive group of the affinity label is sufficiently selective, there should be a double recognition process leading to covalent bond formation. In other words, there would be an amplification of the recognition process. This I referred to as "recognition amplification."10 The recognition amplification is derived from the second recognition step which is a function of the proximity and chemical selectivity of the electrophilic group with a receptor-based nucleophile. Thus, if you have different receptor types and the distribution of the nucleophiles on these receptor types is different, one might be able to alkylate one subpopulation in the presence of others.

Demonstrating a convincing case for this concept was tedious. We started this project about 1967, and Aki Takemori and I decided to collaborate, since Aki also was of the opinion that there might be multiple populations of receptors through his <u>in vivo</u> PA2 studies.11

Our first Paper published in this area was on electrophilic anileridine analogues.12 These initial studies were not terribly successful. Since we thought more potent compounds were necessary, we then went on to investigate the morphinan series which afforded unspectacular results.13 One of the uncertainties was that we didn't know what to expect. The tacit assumption was that an affinity label derived from an agonist would inactivate the receptor and would act as an irreversible antagonist. We were not correct in that assumption because the nitrogen mustard derivative of oxymorphone, which we named chloroxymorphamine (COA), behaved as an irreversible opioid agonist in the guinea pig ileum.14 These results were unexpected, because the literature pointed to the irreversible antagonism of adrenergic and cholinergic receptors by electrophilic ligands. This study led us to the conclusion that the Paton concept 15 was not tenable because receptor dissociation of covalently bound COA was not possible. You may recall that the Paton concept stated that agonists dissociate from receptors faster than antagonists.

The next step in this research was obvious. What happens if we have an antagonist derived affinity label? Would it turn out to be an irreversible antagonist? This time we attached a nitrogen mustard group to naltrexone, a potent reversible antagonist. This afforded ß-chlornaltrexamine (ß-CNA), which is an extremely potent **α**-non-equilibrium antagonist.16 What is useful about ß-CNA is that it is active both in vitro and in vivo, and it can block antinociception for prolonged periods of time.17 ß-CNA, having a very reactive electrophilic group, is a promiscuous ligand with respect to opioid receptors. It blocks all opioid receptor types because of this high reactivity. We would have to install a more chemically selective electrophilic group to obtain a selective ligand. These studies led to the synthesis of ß-funaltrexamine (ß-FNA), which is a highly selective nonequilibrium μ opioid antagonist.18 The rationale for the design of ß-FNA was based on the high chemical selectivity of an **α,ß**-unsaturated carbonyl moiety for a sulfhydryl group that was implicated as a receptor-based nucleophile.19

Why does an affinity label derived from oxymorphone behave as an agonist, while an identical modification of naltrexone affords an agonist? One answer might lie in the possibility that there are different recognition sites on the opioid receptor system for agonists and antagonists. In an effort to test this idea, we made ß-FOA which is the agonist counterpart of ß-FNA. What we discovered was that ß-FOA did not act irreversibly on the guinea pig ileum preparation.20 In fact, ß-FOA could not protect the guinea Pig ileum against

irreversible blockage by ß-FNA even though it was found to be a reversible agonist. That suggested the possibility of two different recognition sites, but there are other explanations that are also consistent with these results. I might add that we envisioned these recognition sites to be allosterically coupled. It remains to be seen if this is indeed the case.

Just to jump ahead to the present, I would just like to call your attention to the non-equilibrium δ antagonist, naltrindole-5'-isothiocyanate, which we reported *on* a year ago.21 Quite recently this compound has been used to sort out different 6 opioid receptor subtypes in vivo.22

Some new concepts concerning the design of selective reversible opioid antagonists led to the synthesis of bivalent ligands. Bivalent ligands are defined broadly as molecules that contain two discrete recognition units linked through a spacer.23 The idea behind this concept was that if you have multiple recognition sites on the same opioid receptor system, different opioid receptor types might have these sites located different distances from one another, or in different orientations with respect to one another. This would add an additional dimension to the structure-activity relationship, in that varying the spacer length between the two pharmacophores could modulate the selectivity of a ligand. Thus, if you have, for example, two different receptor types and your recognition sites are different distances from one another, it may be possible to bridge the neighboring recognition sites in *one* receptor type but not in the other.

We had published our first paper on this project in 1982, in which we introduced the bivalent ligand concept.24 Our studies demonstrated that the structure-activity relationship at κ receptors differed from that at μ receptors as the spacer length was varied.23 As the spacer became shorter, the κ selectivity increased. Out of these studies, we obtained the first κ selective opioid antagonist known as TENA.25 TENA was a fairly potent κ antagonist and selective, but not nearly as selective as norBNI.26 NorBNI was an extension of our work in this area, as we had found that the short spacers enhanced selectivity. NorBNI contains the ultimate short spacer, a pyrrole ring which locks the two pharmacophores rigidly with respect to one another. This compound is the most highly selective κ opioid receptor antagonist.

In addition to the bivalent ligand approach, we investigated the "messageaddress" concept that was proposed by Schwyzer.27 Essentially Schwyzer proposed that a class of peptide hormones, which he termed

"sychnologic," are composed of a message sequence of amino acids and an address sequence. The message sequence is involved in signal transduction while the address sequence is not involved in transduction, but adds only to the affinity. If there are multiple receptor types, then each receptor type has a unique address which confers affinity to the different ligands. This affinity presumably is due to complementarity between the ligand and the receptor.

In an effort to test this concept, an opioid peptide "address" was attached to an oxymorphone molecule through a spacer.28 The tyramine moiety of oxymorphone was considered the message. Phe-Leu or Phe-Leu-Arg-Arg-Ile-OMe derived from leucine enkephalin or from a truncated dynorphin were postulated to be the δ and κ addresses, respectively. These compounds were Indeed δ selective and κ selective, whereas the unsubstituted compound was μ selective. Although the selectivity was not high, this gave us confidence in this approach to design non-peptides which would be highly selective using address mimics.

Leucine enkephalin can be divided into a message (Tyr), a spacer (GlyGly), and an address (Phe-Leu). The aromatic ring of phenylalanine was considered to be an important part of the address.23 Since we were considering the design of antagonists, we assumed that the message subsite on the opioid receptor recognition site recognizes the tyramine moiety of both agonists and antagonists. In the design of the δ antagonist, naltrindole (NTI), the C ring of the morphinan ring system and pyrrole moiety constitute the spacer, and the benzene portion of the indole moiety is considered to be the address. Naltrindole29 was the first non-peptide δ antagonist and has a thousand times greater affinity30 than the 6 antagonist that is an enkephalin analogue (ICI174864). The benzofuran analogue of naltrindole, NTB, has been recently reported to selectively antagonize 6-selective agonists, suggesting the presence of δ receptor subtypes.31

Although there is evidence in the literature for a bivalent ligand bridging two recognition sites,32 we realized after our success with naltrindole that the reason why that norBNI is a selective κ antagonist is not related to its bridging two different receptors. It appears that it derives its selectivity by a message-address mechanism that involves the second basic nitrogen in norBNI.33 This basic nitrogen may be simulating the Arg7 of dynorphin by interacting with the putative address subsite of the κ receptor,

Finally, I'd like to acknowledge the graduate students, postdoctorals and others, over 70 in all, who have

worked in my lab. I owe a great debt to this group, who over the past 30 years was responsible for the research we conducted. Also, I would like to acknowledge my wife, Chris. Without her support and encouragement I would not be standing here today. Also, one needs a patron to support research activities; my patron for the past 30 years has been the Public Health Service, and more specifically, I wish to acknowledge the financial support from NIH and from NIDA. Finally, a proper environment is important for productive research. The University of Minnesota was instrumental in providing this environment and of course this includes not only research facilities but also colleagues.

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Recent Studies on a Mu Opioid Binding Protein Purified from Bovine Striatal Membranes

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We have previously reported the purification to homogeneity of an opioid binding protein (OBP) from bovine striatal membranes and its characterization by its ability to bind opioid antagonists saturably, reversibly, and with high affinity (Gioannini et. al. 1985). That OBP is a mu binding opioid protein is supported by the following data. OBP is isolated on an affinity matrix that contains a ligand that binds mu receptors preferentially. Binding by opioid antagonists is displaced by the mu selective ligand DAGO. OBP binds the mu specific antagonist ^{3}H-cyprodime (Schmidhammer et al., 1989). At saturating concentrations of ligand, OBP binds as much cyprodime as bremazocine., suggesting that all of the bremazocine binding to OBP is to mu sites. Finally, the molecular weight of OBP (65 kDa) is the same as that observed when ^{125}I-beta endorphin is crosslinked to mu opioid binding sites (specifically blocked by DAGO and other mu ligands) in tissues and a cell line.

The presence of disulfide bridges (-S-S-) that contribute to the secondary structure of OBP is indicated by the difference in mobility of OBP in polyacrylamide gel electrophoresis under non-reducing vs. reducing conditions (Gioannini et al., 1985). Treatment of OBP with increasing concentrations of the reducing reagent dithiothreitol (DTT) produced a stepwise shift from an apparent molecular weight of 53 kDa to 65 kDa. The importance of -S-S- bonds not only to secondary structure but also to function is reflected in the sensitivity of opioid ligand binding to treatment with DTT. The major opioid receptor types differed in their sensitivity to DTT, as follows: mu>delta>>kappa, with kappa sites being virtually resistant to even very high concentrations of DTT (Gioannini et al., 1989). The inhibition produced by DTT is reversible, was observed to a much lesser degree with antagonist ligands, and was due to a reduction in affinity (increase in K_D) rather than in the number of receptor sites (B_{max}).

The availability of pure OBP has allowed us to attempt determination of a part of its primary sequence. Direct sequencing of OBP proved unsuccessful, indicating that OBP is an N-terminally blocked protein and must be fragmented to obtain peptides for amino acid sequencing. We have, in collaboration with Dr. Catherine Strader (Merck, Sharp, and Dohme Co., Rahway, NJ), obtained the amino acid sequence of two peptide fragments, 20 amino acids and 13 amino acids in length, respectively. The fragments were generated by chemical cleavage of OBP with CNBr followed by isolation of the peptides on reverse phase HPLC. The amino acid sequences which resulted were not found in data bases of known protein sequences. Polyclonal antibodies have been generated against sequences derived from these peptide fragments, in collaboration with Drs. Huda Akil, Lawrence Taylor and Stanley Watson at the University of Michigan. The interaction of these antibodies with purified OBP, cell lines and bovine brain regions are discussed below. The peptide fragments, and the antibodies generated against them are indicated in Table 1.

Table 1. Antibodies senerated against peptide sequences derived from purified OBP.

ANTIBODY	FRAGMENT
165, 166, 6639	N-terminal 12 amino acids of Peptide 1
163	C-terminal 7 amino acids of Peptide 1
161, 162	N-terminal 10 amino acids of Peptide 2

All of the antibodies were able to immunoprecipitate a major portion of ^{125}I-labelled OBP incubated with the antibody. The amount precipitated after correction for background ranged from 40 % for the weakest antibody (Ab 161) to over 60% with the strongest, Ab162 and 165 at a dilution of 1:200. Background of the assay, i.e., radioactivity precipitated by non-specific antisera, accounted for 5-7% of added radioactivity. Ab 162 and Ab 165 can immunoprecipitate 30% of ^{125}I-OBP even at a 1:1000 dilution. The protein precipitated by these antibodies was dissociated from the complex and subjected to SDS polyacrylamide gel electrophoresis. Autoradiography of the gel showed a radioactive band with an apparent Mr = 65 kDa, i.e., the molecular weight of OBP.

Sequential treatment of OBP with antibodies 162 and 165, which are derived from the two different peptides, indi. cated that initial treatment with either antibody re-

moved all immunoprecipitable antigen, i.e., no further immunoprecipitation occurred with the second antibody. These results confirm that the two peptides, against which antibodies were generated, are derived from the same protein.

The interaction of OBP with the antipeptide antibodies was also examined in immunoblots. An immunoreactive protein corresponding to a molecular weight of 65 kDa was detected with each antibody examined. The strongest signal was produced with antibodies against the N-terminal end of Peptide 1 (Ab 165, 166, 6639) at a 1:100 dilution. The reaction with OBP can be blocked by preincubation of the antisera with 100 uM peptide. No signal was detected when OBP was immunoblotted against non-immune serum. It is noteworthy that no signal was detected unless OBP was reduced with DTT. Apparently, the presence of the disulfide bridge(s) precludes accessibility of the antibody to the epitope.

The strength of the signal against immunoreactive protein (Mr = 65 kDa) seen in immunoblots with both digitonin and CHAPS soluble extract of bovine striatal membranes prompted an examination of cell lines and bovine brain tissues with the antipeptide antibodies. Immunoblots of SDS solubilized bovine tissues with the various antibodies indicated the presence of immunoreactive protein (Mr = 65 kDa) whose signal could be blocked by preincubation of antisera with the appropriate peptide (50-lOOμM). The tissues which reacted with antibody, in addition to striatum, were frontal cortex, hippocampus, and thalamus, all regions known to have moderate to high levels of mu opioid receptors. Pons and white matter produced no or a barely detectable response which correlates with their very low levels of opioid receptors. The sensitivity of Ab165, which produces the strongest signal, is evidenced by its ability at a dilution of 1:100 to detect mu opioid binding material equivalent to 0.001% of the 30-50 μg of protein loaded per sample (300-500 pg of OBP) in an immunoblot.

Immunoblots with the cell line SK-N-SH, which contains predominantly mu binding sites, produced a strong positive reaction at a position comparable to that seen with purified OBP and brain tissue (65 kDa). The immunoreactive protein detected in NG-108-15 cells, a cell line that is reported to contain only delta receptors, migrated to a position slightly lower than that seen in the SK-N-SH cells or with OBP (apparent Mr of ca 58 kDa). Two negative controls (HELA cells and C6 cells) produced no detectable response. In both cell lines, the response may be blocked by pre-incubation of the serum with 100uM concentration of peptide. The detection of a response with the NG-108-15 cells suggests

crossreactivity of the antibodies with the delta receptor or the presence of small amounts of mu receptors, not detectable by binding assays.

The ability of the antipeptide antibodies to react with native receptors was investigated by examining the effect on opioid ligand binding and by evaluating the extent to which active receptors can be removed by immunoprecipitation. None of the antibodies inhibit binding of opioid ligands to either membrane-bound or soluble receptors. No depletion of receptors was detected in the supernatants after immunoprecipitation with any of the antibodies. We conclude that the antibodies recognize only denatured receptors. Not unexpectedly, the short amino acid sequences to which the antibodies were made may not be accessible to the antibody in the native receptor or may assume a secondary structure not recognized by the antibody.

Biochemical and physiological evidence indicate that all 3 major types of opioid receptors, mu, delta, and kappa, negatively modulate adenylate cyclase and are therefore coupled to guanine nucleotide regulatory proteins (G-proteins). This suggests that opioid receptors belong to the large family of receptors for hormones, neurotransmitters, and peptides that effect signal transmission by activating a G-protein. Analysis of the amino acid sequences of a large number of proteins of this class, has revealed some significant structural features common to all members of this family. The most striking feature is the presence of 7 hydrophobic domains thought to span the cell membrane. The following results further support the hypothesis that OBP belongs to this class of G-coupled proteins.

Antibodies generated against membrane-associated rhodopsin as well as against five specific amino acid sequences in rhodopsin were generously supplied to us by Drs. Ellen Weiss (Univ. of N. Carolina, Chapel Hill, NC) and Gary Johnson (Natl. Jewish Center for Immunology and Respiratory Medicine, Denver, CO). In immunoblots against OBP, 2 antibodies, one against membrane associated rhodopsin and one against a sequence in the carboxyl terminal end (CT_1), reacted strongly, while an antibody against the 1-2 loop (first cytoplasmic loop between transmembrane domains 1 and 2) reacted weakly. Weiss et. al.(1987) had previously studied the interaction of purified beta adrenergic receptor from 849 lymphoma cells with this same series of antibodies under identical reaction conditions. The pattern of reactivity of these antibodies with the beta-adrenergic receptor and OBP was identical.

To verify that rhodopsin antibodies recognize the same protein as the peptide antibodies, OBP was immunoprecipitated by Ab165. An immunoblot of the proteins remaining in the supernatant after immunoprecipitation showed a diminution of the signal with the rhodopsin antibody CT_1 relative to a control supernatant from "immunoprecipitation" with normal rabbit serum (NRS). The protein immunoprecipitated with Ab165 was eluted from the antigen-antibody complex and examined in an immunoblot against CT_1. A positive signal was observed at Mr = 65 kDa, whereas no signal was detected with the NRS control. This experiment indicates that the protein recognized by the rhodopsin antibodies is the same as that recognized by OBP-derived peptide antibodies. These results indicate that the three proteins, bovine rhodopsin, S49 lymphoma beta adrenergic receptor, and OBP share common epitopes. Since there seems to be little amino acid sequence homology in the areas used for antibody production (at least between rhodopsin and the beta-adrenerqic receptor), structural features, perhaps at the level of secondary and/or structure, along with very limited amino acid homology, may be responsible for the immunological cross-reactivity. The results represent evidence, beyond that previously obtained for G-protein coupling, that opioid binding sites are members of the family of G-protein coupled receptors and are likely to show the typical structure of these proteins, when their complete amino acid sequence becomes known.

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Effects of Endogenous Calcium Regulating Peptides on Opiate Actions

S.L. Welch, F. Smith, M. Saxen and W. L. Dewey

An expansive literature exists implicating the role of calcium in the actions of opiates. Our recent work summarized herein is directed toward a determination of the role of the endogenous calcium-regulating peptides in the antinociceptive effects of the opiates in both the central nervous system and at peripheral sites. Early work by Hano et al. (14) showed that intracisternal Ca^{++} administration would antagonize morphine, and conversely, the use of the Ca^{++} chelator EGTA would potentiate morphine. Their early work was confirmed by Kakunaga et al. (31), Harris et al. (23), Guerrero-Munoz and Fearon (12) and Vocci, et al. (3). Harris et al. (15) and Vocci et al.(3) showed that ionophores (X-537A or A23187), which facilitate Ca^{++} uptake by the cell, enhanced antagonistic effects of low concentrations of Ca^{++} on antinociceptive responses to morphine. Since the activity of ionophores is largely that of increasing intracellular calcium, it was postulated that the antagonism of morphine occurred due to alterations in intracellular events by Ca^{++}(5). Later work by Chapman and Way (6) demonstrated that Ca^{++}, Mn^{++}, and Mg^{++} would antagonize ß-endorphin- and methionine-enkephalin-induced antinociception, an effect enhanced by ionophore A23187 and blocked by the Ca^{++} chelator EGTA. Thus, the alteration of antinociceptive responses to morphine by Ca^{++} also occurred with the endogenous opioids that morphine mimics. In vitro demonstrations of the effects of opiates on the Ca^{++} content of brain regions or synaptosomes supported the view that acute administration of opiates produced depletion of Ca^{++} in vivo (16). Morphine also decreased Ca^{++} binding to synaptic membranes and synaptic vesicles (17). ß-endorphin was similar to morphine in its alterations of Ca^{++} fluxes (18). Thus, opioid peptides appeared to be modulated by Ca^{++} and to modulate Ca^{++} in a manner similar to morphine.

Our work (27) has shown that calcium injected intrathecally produces intrinsic antinociceptive effects, and potentiates and extends the antinociceptive effects of morphine. Up until this time, most research has dealt with evaluation of the effects of calcium injected intraventricularly. Our intriguing finding indicates a possible important differential regulation of calcium by morphine in the brain versus in the spinal cord. The role of the endogenous calcium-modulating peptides in this differential regulation has been the subject of current investigations in our laboratory.

The calcium-regulating peptides calcitonin (CT) and calcitonin gene-related peptide (CGRP) have been shown to modulate calcium concentrations in the periphery. The peripheral activity of CT involves the facilitation of calcium sequestration into bone with subsequent creation of a hypocalcemic state in serum (29). The most potent form of CT in lowering serum Ca^{++} levels is the fish hormone, salmon calcitonin (sCT) (13). The conservation evolutionarily of sCT is evidenced by the recent discovery of sCT-like peptides in humans (36). The function of these peptides remains unknown. The ability of CT to bind selectively to those areas of the brain such as the brainstem, midbrain and periaquaductal gray matter that involve pain transmission and processing (8) led investigators to postulate a role for calcitonin in antinociception. Previous investigations clearly indicate that sCT produces analgesic effects (2,3,8,37). These effects most likely occur via both opiate and non-opiate mechanisms. The hypothesis of opiate interaction is based on the ability of sCT to produce antinociception by site injection to the periaquaductal gray matter, an area of high opiate receptor density (8), the ability of naloxone to reverse sCT-induced antinociception (37), and the ability of sCT to modulate opiate antinociception (37,38) which correlates to its modulation of Ca^{++} uptake in vitro . Other lines of evidence for a CT/opiate interaction include the following findings: 1) High serum CT levels are present in heroin addicts (34). 2) Morphine antinociception is potentiated by acute human CT pretreatment (subcutaneously-administered, s.c.) in the tail-pinch test, and attenuated by 14-day pretreatment of mice with human CT (44). 3) sCT and morphine (both intraventricularly-administered, icv.) act synergistically to produce antinociception in the rabbit tooth-pulp test (2).

CGRP is a novel peptide product of the gene which encodes for calcitonin (CT) (1). The binding of CGRP in the brain occurs in areas such as the brainstem and midbrain which are important in pain perception and neuronal transmission (10,ll). CGRP is one of the most abundant peptides in the spinal cord and is especially high in the dorsal horn of the spinal cord (10,ll). CGRP produces several effects in the spinal cord which are the opposite of those produced by opiates. CGRP co-localizes and is co-released with Substance P, a major nociceptive transmitter in spinal afferents (9,19). CGRP also enhances Substance P concentrations spinally, possibly by enhancing release (30) and decreasing degradation of Substance P (25). Opiates, on the other hand, decrease the release of Substance P (26). CGRP enhances the nociceptive effects of i.t. Substance P (4). Opiates, on the other hand, decrease spinal nociception (43). The abundance of CGRP in spinal neurons may indicate that its role is one of a general neuromodulator which is responsible for "fine-tuning" of neurotransmission.

Our work has shown that both sCT and CGRP produce biphasic modulation of calcium uptake in isolated synaotosomes from mouse brain. These biphasic effects of CGRP and CT in vitro correlate temporally in vivo to initial antagonism of and then enhancement of morphine-induced antinociception by icv. sCT and CGRP (39,40). We have also shown that CGRP i.t. blocks' the antinociceptive effects of intrathecally-administered morphine, calcium and the calcium ionophore, A23 187. CGRP produces a parallel shift to the right of the dose-response curves of i.t. calcium and A23187, indicating that the CGRP/Ca^{++} and CGRP/A23187 interactions occur at similar sites (41). These data, along with those of other investigators using dorsal root ganglia in culture (31) indicate that CGRP enhances calcium uptake or influx in spinal afferent neurons. Recent work has shown that intrathecal administration of antibodies to CGRP produces antinociception in rats (34), while intrathecal CGRP itself facilitates nociception and produces slight

hyperalgesic effects (4). These data substantiate the hypothesis that CGRP is a modulator of spinal nociception.

Previous work from our laboratory has shown that sCT and CGRP modulate the in vivo and in vitro effects of acute morphine on antinociception and $^{45}Ca^{++}$ uptake, respectively (37-41). Several lines of evidence have led us to hypothesize that CGRP and/or CT may be involved in the production of tolerance:

1) Both peptides modulate the acute effects of morphine (2,3,39).
2) Both peptides modulate calcium uptake by brain synaptosomes (39).
3) Intrathecal CGRP appears to attenuate opiate antinociception in vivo via modulation of spinal calcium.(41).
4) The highest binding of sCT in the brain (11) and CGRP in the dorsal horn of the spinal cord (33) coincides with the major sites of opiate binding and activity.
5 The highest quantities of CGRP in the brain are found in the locus coeruleus (35). Interestingly, it has recently been reported that the locus coeruleus is the site of up-regulation of adenylate cyclase activity upon chronic morphine administration. This effect of chronic morphine on adenylate cyclase has been proposed to be a mechanism of tolerance production (7).
6) CGRP both enhances sympathetic outflow from the brain and enhances the release of Substance P spinally (30) effects which have been shown to be related to enhanced influx of calcium by CGRP. Both enhanced sympathetic tone and enhanced release of Substance P occur during withdrawal from chronic opiates.
7) CGRP co-localizes with dynorphin in spinal neurons (9).
8) CT levels in the plasma of heroin addicts are high (34). Two-week pretreatment of rats with sCT attenuates the antinociceptive effects of morphine (44). CT administration results in the release of beta-endorphin from the pituitary (24).
9) CT release is evoked by changes in Ca^{++} levels peripherally (28). Both peptides are homeostatic modulators of serum Ca^{++} levels. It is unlikely that their role in the central nervous system differs from that in the periphery.
10) Both CGRP and CT have been shown to modulate cyclic-AMP levels (21,22). Since opiates, CGRP, and CT modulate adenylate cyclase, CT and CGRP may alter opiate tolerance by altering cyclic- AMP production in neurons.
11) In the mouse paw, incision leads to hyperalgesic effects using the mouse paw withdrawal test (32). In this system, the level of CGRP decreases as antinociceptive effects increase. This is further evidence of the role of CGRP in pain in peripheral systems.

The role of calcium-regulating hormones in opiate antinociception can be summarized by the following model. We hypothesize that the release of endogenous opioids act on an adjacent Substance P- containing neuron to decrease intracellular calcium, decrease c-AMP, and/or enhance potassium efflux. These biochemical events lead to a decrease in the release of Substance P and antinociception results. In addition, we hypothesize that administration of CGRP, or endogenous CGRP, releases Substance P and counters the effects of the opioids by increasing intracellular calcium and/or releasing Substance P. This model summarizes but one of the possible interactions between the endogenous calcium-regulating peptides and opioid peptides. This model summarizes known neural connections in the spinal cord, however, the neuronal pathways involved in the

interaction between the opioids and CGRP or sCT in the brain or other spinal sites has not yet been determined.

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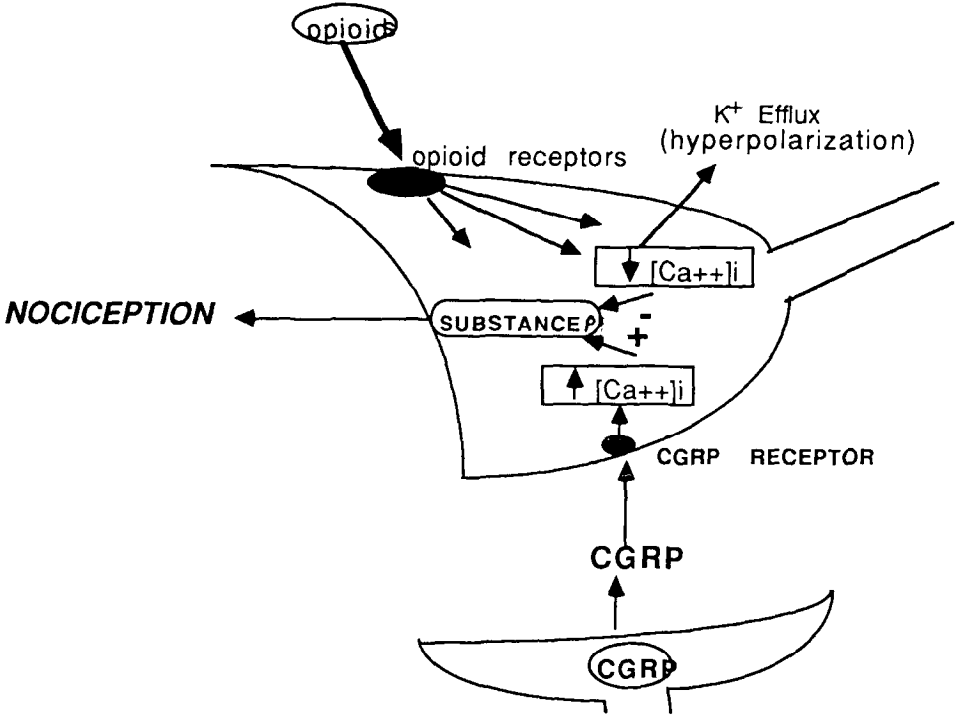
PROPOSED MODEL OF THE BLOCKADE OPIOID - INDUCED ANTINOCICEPTION CGRP IN THE SPINAL CORD



Effects of Opiates, Opioid Antagonists and Cocaine on the Endogenous Opioid System: Clinical and Laboratory Studies

M.J. Kreek

The very exciting, independent and original reports in 1973 of Snyder, Simon and Terenius which constituted the final discovery and delineation of specific opiate receptors, after early conceptualization and experimental work in the late 1960's and early 1970's by Dole, Martin, Goldstein and others, led to the discovery of the endogenous opioids. First, Kosterlitz and Hughes isolated and defined the structure and action of met- and leu-enkephalin. This was followed by the work of Terenius, Goldstein and many others, who discovered beta-endorphin, dynorphin, and other related endogenous opioid peptides. Many other workers have contributed to the research findings which have now defined three subtypes of opioid receptors, mu, delta and kappa, each with the possibility of more than one subtype. Research findings by classical biochemical and also molecular biological techniques have defined three distinct classes of endogenous opioids, each now characterized by the identification and cloning of three separate genes and three distinct single gene products,i.e.,three peptides which in turn are processed and converted into many different endogenous opioid peptides which are active at one or more of the specific opioid receptor types. We had hoped that, after the predicted finding of specific opioid receptors and the hypothesized endogenous opioids, we might very soon have the answer for the biochemical or metabolic basis of mechanisms underlying opiate addiction. Many years later, we are still attempting to determine what indeed may be the role of the endogenous opioid system in the addictive diseases.

Our research group, working with heroin addicts and former heroin addicts in methadone maintenance or drug-free treatment in basic clinical research studies, has ruled out an earlier hypothesis that opiate addiction might be a disorder of "endorphin deficiency". Our work and that of others has also eliminated the possibility that opiate addiction is associated with excess production of "endorphins" with "end organ" or receptor failure to respond. However, work from our basic clinical and related laboratory research using animal models has suggested that abnormal levels, negative and positive feedback control mechanisms, and patterns of release of at least one endogenous opioid, beta-endorphin, as well as potentially other endogenous opioid peptides, and possibly also abnormal activation of one or

more subtypes of opiate receptors are involved in the pathophysiology of opiate addiction and possibly also cocaine dependency. Also our data suggest that abnormal responsivity to stress, as manifested by abnormalities in the endogenous opioid system, may be intrinsically involved in relapse to opiate and possibly also cocaine use in the abstinent former addict, and thus in the perpetuation of addiction. In contrast, our studies have shown that steady dose long-term methadone maintenance treatment of former heroin addicts permits normalization of at least one stress responsive neuroendocrine axis involving the endogenous opioids, that is, the hypothalamic-pituitary-adrenal axis.

In parallel with this work concerning opiate addiction and cocaine dependency are the many studies addressing the question of what are the roles of each type of the endogenous opioid receptors in normal mammalian physiology as well as pathology. We certainly know that it would have been more convenient if the three classes of opioid peptides each bound only to one type of opioid receptors, but this is not the case. Investigators are still seeking the answers as to what may be the receptor subtype activity of each endogenous ligand and also what may be the physiological roles of activation of each of the endogenous opioid receptor subtypes. It is very important to stress that there may be profound species and strain differences in the physiological effects of the endogenous opioids, which may parallel the profound differences in the opioid receptor subtype and ligand differences. (See Table 1) Most species and strains of mammals seem to possess all three classes of endogenous opioids and possibly all subtypes of opioid receptors, but with very different receptor densities in specific regions, as well as possibly different receptor affinities.

TABLE 1.

ACUTE NEUROENDOCRINE EFFECTS OF OPIATES IN BATS VERSUS HUMANS

RAT	HUMAN
↑ ACTH	↓ ACTH
↑ BETA ENDORPHIN	↓ BETA ENDORPHIN
↑ CORTICOSTERONE	↓ CORTISOL OR NO CHANGE; FLATTENED DIURNAL VARIATION
↓ LH	↓ LH
↓ TESTOSTERONE	↓ TESTOSTERONE
↑ PROLACTIN (OPIOID ANTOAGONIST NALOXONE IN RAT: ↓ PROLACTIN)	↑ PROLACTIN (OPIOID ANTAGONIST NALOXONE IN HUMANS: ↑ ACTH ↑ BETA ENDORPHIN ↑ CORTISOL ↑ PULSATILE LH; NO EFFECT ON PROLACTIN)

The situation is even more complicated, and there are fewer approaches for experimental study which one can use to determine the role of the endogenous opioid system in human physiology and also in human pathology. We have had the opportunity to study prospectively groups of heroin addicts as they entered methadone maintenance treatment and remained in treatment for several years thereafter. We have now followed some of these former addicts in treatment for up to 27 years. By observing these methadone maintained patients very carefully, we have been able to

determine such indices as the rate at which tolerance develops to each of the various specific opiate effects. By this experimental approach, we have been able to tease out what may be the controlling or dominating roles of exogenous opiates, as contrasted with simply modulating roles of these agents, which bind primarily with mu, but also possibly with delta or kappa receptor subtypes. These prospective clinical studies in this way have also provided us and others with clues as to the possible roles of the endogenous opioids in normal human physiology. Also when narcotic withdrawal is observed in a controlled setting of slow methadone dose reduction and elimination, or when opiate withdrawal is precipitated and observed in a controlled setting, in either chronic pain patients receiving opiates in treatment on a chronic basis, or in addicts, we and others again have had the opportunity to see in which of the physiological systems may the endogenous opioids be predominantly involved.

To further conduct studies in human volunteers or patients with specific disorders of various types which may involve abnormalities, primarily excessive activity, of the endogenous opioid system, use of specific opiate antagonists has been our major experimental approach. Unlike the situation which pertains to studies using animal models and in vitro systems, for which there are now available a large number of increasingly selective ligands, both agonists and antagonists, for research in humans, we still have a very limited number of opioid antagonist compounds which have been approved for introduction into man. Nevertheless, much has been learned over the past 15 years regarding the possible role of excessive activity of the endogenous opioid system in several human pathological conditions, as well as about the role of the endogenous opioids in normal physiology. (See Table 2)

TABLE 2.

SOME HUMAN PATHOLOGICAL CONDITIONS IN WHICH EXCESSIVE ACTIVITY OF THE ENDOGENOUS OPIOID SYSTEM HAS BEEN IMPLICATED

1) SECONDARY AMENORRHEA RELATED TO EXCESSIVE EXERCISE OR STRESS
2) MALE HYPOGONADISM WITH DELAYED ONSET OF PUBERTY
3) GASTROINTESTINAL DYSMOTILITY DISORDERS RESULTING IN CHRONIC CONSTIPATION
4) PRURITUS ASSOCIATED WITH PRIMARY BILIARY CIRRHOSIS
5) PRURITUS ASSOCIATED WITH SPECIFIC DERMATOLOGICAL DISORDERS
6) INTERSTITIAL CYSTITIS
7) ENDOTOXIC OR HYPOVOLEMIC SHOCK
8) NECROTIZING ENCEPHALOMYELOPATHY
9) ACUTE AND CHRONIC SEQUELAE OF STROKE
10) ACUTE AND CHRONIC SEQUELAE OF HEAD INJURY
11) ACUTE AND CHRONIC SEQUEULE OF SPINAL CORD INJURY
12) SUDDEN INFANT DEATH SYNDROME
13)? SPECIFIC ARTHRITIC AND COLLAGEN VASCULAR DISORDERS
14)? SPECIFIC NEUROLOGICAL AND NEURONUSCDIAR DISORDERS
15)? SPECIFIC SUBTYPES OF OBESITY
16)? OTHER SPECIFIC ENDOCRINE DISORDERS (eg. HYPERPROLACTINEMIA)

These pathological conditions in which excessive activity of the endogenous opioid system has been implicated cannot be detailed

in this very brief review. Our laboratory has concentrated on studies of the endogenous opioid system in five areas: neuroendocrine, hepatobiliary, gastroenterological, immunological and in the addictive diseases. Our work has been conducted in human subjects, as well as in the rat, guinea pig, and mouse and is referenced herein along with a few references from other workers underscoring the variations in findings concerning opioid effects in different species.

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The Continuing Interrelationship of CPDD and NIDDK

A.E. Jacobson and K.C. Rice

Perhaps as a result of the numerous changes in the organizational names of the College on Problems of Drug Dependence (CPDD) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), few scientists are knowledgeable about the close relationship which has existed between CPDD and NIDDK, and the link which still binds them. NIDDK is not known for its support of research in the field of drug abuse, and many of the members of NIDDK are not aware of the CPDD. NIDDK, in fact, is heir to that part of the National Institute of Health which contained scientists who were appointed in 1929 by the Committee on Drug Addiction of the Division of Medical Sciences (DMS, of the National Research Council (NRC), National Academy of Sciences). These scientists were chosen to initiate chemical and pharmacological research on drugs subject to abuse. The Committee on Drug Addiction was the primordial committee from which the contemporary CPDD traces its existence.

In 1919, a New York City committee noted that there were about 100,000 people addicted to narcotics and cocaine in the United States (May and Jacobson 1989). A few years later, a Committee on Drug Addictions was created in the Bureau of Social Hygiene in New York to address this problem. After reassessment in 1928, the Bureau offered to fund a committee in DMS, NRC, for the scientific investigation of narcotic drugs. A Temporary Advisory Committee on Drug Addiction was formed by DMS in 1929, and the four members of this committee decided to establish a Committee on Drug Addiction in DMS (White 1941, Eddy 1973). The DMS Chairman, Dr. William Charles White, served as the first Chairman of that Temporary Advisory Committee. Among the initial members of the Temporary Committee were two scientists who came from the National Institute (singular) of Health, Dr. Claude S. Hudson, a carbohydrate chemist, and Dr. Carl Voegtlin, a pharmacologist and Director of the National Cancer Institute. An additional seven men (including the Asst. Surgeon General, Dr. Lawrence Kolb, and the Commissioner on Narcotics, Harry J. Anslinger) joined the Temporary Committee soon thereafter, and these eleven men sewed for the next 12 years. With the beginning of World War II, this committee terminated and a smaller Committee on Drug Addiction was formed in 1941 to act as an advisory group to the U. S. Public Health Service during the war. Drs. Lyndon F. Small and Nathan B. Eddy sewed on this Advisory Committee after the war, and these scientists came from the NIH. They were among the progenitors of the contemporary Laboratory of Medicinal Chemistry (LMC) in NIDDK, as well as the contemporary CPDD (May and Jacobson 1989).

The remarkable decisions made during the 1929-1941 tenure of the Committee on Drug Addiction were clearly instrumental in determining the future path of CPDD and some of the contemporary research inclinations of

LMC. Among the present goals of the contemporary CPDD are: (1) to nurture, promote and carry out abuse liability research and testing, at clinical and preclinical levels; (2) to advise public and private sectors, nationally and internationally; (3) and to sponsor an annual scientific meeting in fields related to drug abuse and chemical dependence. The policies developed by the Committee on Drug Addiction in 1929 were not too dissimilar. These were to: (1) synthesize analgesics without addiction as replacements for addictive drugs; (2) study the effects of these compounds on animals and to test them in human therapy; (3) educate, by preparation of lay and scientific press monographs (White 1941). The 1929 Committee created 4 groups, synthesis, animal pharmacology, human pharmacology, and education, and the Committee's research efforts in chemistry and, to a lesser extent pharmacology, were carried out at NIH for many years. The broad drug testing program of the Committee has been coordinated at NIH for the last 50 years, from 1941 in Bethesda to the present time.

In 1929 the Committee initiated a search for a research chemist who could lead the synthetic effort on new analgesics. Dr. Small, who had studied under a NRC fellowship in the Munich laboratory of Professor Heinrich Wieland, was chosen by the Committee on Drug Addiction of the NRC to establish a Drug Addiction Laboratory in the University of Virginia in 1929. This laboratory was founded as the chemical arm of the Committee. Dr. Small and his colleagues undertook the task of modifying- the morphine molecule and synthesizing morphine-like substances based on the phenanthrene nucleus. The NIDDK Laboratory of Medicinal Chemistry's current research program on opioids can trace its origin to the work of this small group of researchers from Virginia (figure 1).

In June 1939, the Committee researchers moved to the NIH facilities at 25th and E Streets in Washington, D.C. The impetus for their relocation came from Dr. White, still Chairman of the Committee on Drug Addiction, and the U.S. Surgeon General Thomas Parran, who encouraged Dr. Smalls group to come to NIH when funding for their analgesic research at the University of Virginia was discontinued. After two years "downtown," the University of Virginia researchers set up shop on NIH's Bethesda campus under Dr. William H. Sebrell, Chief of the Division of Chemotherapy. Dr. Small and his colleagues, including Dr. Erich Mosettig, moved to Building 4, and Dr. Eddy moved to Building 2. Dr. Mosettig was brought to Virginia by Dr. Small; he was an expert in alkaloid synthesis, having been private assistant to Prof. Ernst Spath in Vienna (White 1941).

Dr. Eddy came to NIH from the University of Michigan. He was appointed by the Committee on Drug Addiction, DMS, in 1930 to lead the pharmacological unit which evaluated drugs synthesized by Dr. Small's group of chemists. This unit was the second component of the Committee's efforts to fully investigate analgesics. Clinical evaluation, the third element of the exploration, was initiated a few years later in Kansas, and then in Lexington, Kentucky.

Dr. Everette L. May joined the chemistry group in December 1941. Other members of the initial group at NIH were Dr. Lewis J. Sargent, Dr. Edward M. Fry, Dr. Charles I. Wright, and Theodore D. Perrine. Thus, in 1941, the intramural analgesic research program was established at the National Institute of Health in Bethesda, Maryland. Dr. Small became a member of CDAN in 1945, and Dr. Eddy served as Secretary of the Committee, a position which he held for many years. The chemical work of Drs. Small and

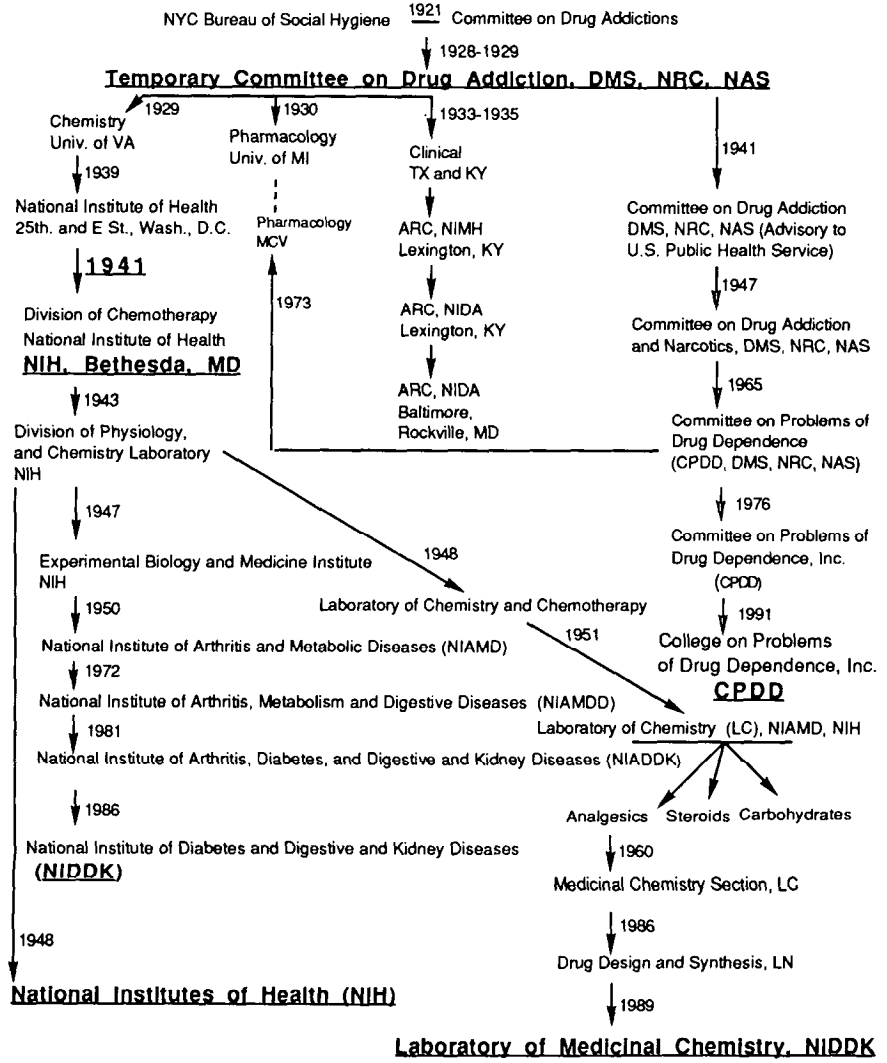

Figure 1. Evolution of the Laboratory of Medicinal Chemistry, the NIDDK, NIH, and the CPDD.

Mosettig, and of Dr. Alfred Burger, an eminent medicinal chemist who remained at the University of Virginia, combined with the pharmacological results of Dr. Eddy and others, and the clinical work by, for example, Drs. Walter L. Treadway, Clifton K. Himmelsbach, Lawrence Kolb, and Lyndon E. Lee, Jr., were published as a book of collected reprints (White 1941). This book served as the final report to the NRC, in May 1941, from Dr. White, Chairman of the Committee on Drug Addiction, In that compilation, Drs. Eddy, Small and Mosettig summarized the years of efforts in the following

way: “The systematic scientific program has resulted in the accumulation of much affirmative data on two major problems: First, the quantitative dissociation of the complex morphine effect on the living organism by chemical modification of the morphine molecule; and second, the development of compounds with definite analgesic action by suitable chemical additions to simple nuclei.”

During World War II Dr. Eddy encouraged the continuation of clinical research on analgesics at the U.S. Public Health Service Hospital in Lexington, Kentucky. The research division there was administratively separate from the remainder of the hospital and was called the Addiction Research Center of the National Institute of Mental Health. Clinical research at the Addiction Research Center was, for many years, an integral part of the evaluation of new analgesics, many of which were obtained from NIH chemists. The Addiction Research Center eventually evolved into the research division of the National Institute on Drug Abuse (NIDA) in the Alcohol, Drug Abuse, and Mental Health Administration. The close relationships that now exist between research groups in NIDA and NIDDK were founded on that early collaborative work.

In the 194Os, several name changes were implemented at NIH. In 1943, the Division of Chemotherapy was renamed the Division of Physiology. In 1947, this division and others, including the Chemistry Laboratory (formerly called the Division of Chemistry) with the renowned carbohydrate chemist Dr. C. S. Hudson as Chief, became part of the Experimental Biology and Medicine Institute, a new institute in NIH. Ten months later, in October 1948, the laboratories in the Institute were reorganized. The Chemistry Laboratory became the Laboratory of Chemistry and Chemotherapy with Dr. Hudson as its Chief and Dr. Small as the Assistant Chief, and *in* 1948, the National Institute of Health became the National Institutes of Health. In 1951, Dr. Small succeeded Dr. Hudson as Chief of the Laboratory of Chemistry (LC). This lab was the direct descendent of the Laboratory of Chemistry and Chemotherapy. Three sections were created, one on analgesics headed by Dr. Eddy, one on carbohydrates headed by Dr. Hewitt Fletcher, and one on steroids headed by Dr. Mosettig. With the death of Dr. Small in 1957, Dr. Bernhard Witkop became Chief of the LC. Dr. May changed the name of the Analgesics Section to the Medicinal Chemistry Section in 1960 when he became Section Chief in LC following Dr. Eddy’s retirement. In 1977, Dr. Arnold Brossi became Chief of the Medicinal Chemistry Section, LC, after Dr. May retired from NIH to begin his work at the Medical College of Virginia, Virginia Commonwealth University. Subsequently, Drs. Kenner C. Rice and Arthur E. Jacobson, the permanent staff in the Medicinal Chemistry Section, formed the Section on Drug Design and Synthesis in the Laboratory of Neurochemistry (Dr. Phil Skolnick, Chief), with Dr. Rice as *Section* Chief. In 1989, the Section on Drug Design and Synthesis became the contemporary Laboratory of Medicinal Chemistry (LMC) with Dr. Rice as Laboratory Chief. At least 4 of the original small group of researchers who came to NIH, Drs. Eddy, May, Sargent and Small, were intimately involved with the affairs of the CPDD. Those who represent the contemporary LMC in NIDDK, Drs. Jacobson and Rice, have continued that relationship.

Beginning in 1950, the Experimental Biology and Medicine Institute went through four name changes. First, it became the National Institute of Arthritis and Metabolic Diseases; then in 1972, it became the National Institute of Arthritis, Metabolism, and Digestive Diseases; in 1981, it became the National Institute of Arthritis, Diabetes, and Digestive and Kidney

Diseases; and finally, in 1986, the Institute became known as the National Institute of Diabetes and Digestive and Kidney Diseases, its name today.

In 1947, the DMS formed a new committee to deal with drug abuse associated problems, the Committee on Drug Addiction and Narcotics (CDAN), and it succeeded the 1941-1946 Advisory Committee on Drug Addiction. Dr. Eddy was appointed Secretary, and Dr. Small became one of the initial eight members of CDAN. At that time, both Dr. Eddy and Dr. Small were in the Experimental Biology and Medicine Institute of NIH. CDAN's name changed to the Committee on Problems of Drug Dependence (CPDD) in 1965. The CPDD remained within the DMS, NRC, until 1976, when it became independent, guided by a Board of Directors and sponsored by a number of diverse major scientific organizations, such as the American Chemical Society, and the American Medical Association. Each sponsoring organization nominated a member to the Board of the CPDD. The CPDD is now an incorporated, non-profit, scientific organization that is independent of the federal government and the pharmaceutical industry. It is a World Health Organization Collaborating Center for research and training in the field of drug dependence and has permanent liaison relations with several governmental bodies. The CPDD is currently undergoing a transition and reorganization to a membership organization, enabling its members to have a voice on issues relating to drug abuse, and in 1991 its name was changed to the College on Problems of Drug Dependence.

The CPDD and NIDDK have been intertwined for more than half-a-century, and links still exist between them. One of the major functions of the CPDD has been abuse liability evaluation, and research on new analgesics, stimulants and depressants at the preclinical level. The research efforts of a considerable number of scientists in a consortium of laboratories in five U.S. universities and NIDDK, all of whom work partially under the auspices of the CPDD, have enabled progress to be made in methodological development, and new strategies to be devised for drug evaluation and testing. The coordinators of this function have all been NIH scientists, initially Dr. Eddy, then Dr. May and now Dr. Jacobson. Also, Dr. Rice is a member of the Board of Directors of the CPDD, representing the American Chemical Society, and Dr. Jacobson served on the Board from 1975 to 1981.

ACKNOWLEDGEMENT
We gratefully acknowledge the assistance of Dr. Victoria A. Harden (Director, NIH Historical Office and DeWitt Stetten Jr. Museum of Medical Research), Mr. Peter B. Hirtle (Curator of Modern Manuscripts, History of Medicine Division, National Library of Medicine, NIH), Dr. Everette L. May (Professor of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University), and Dr. Martin W. Adler (Executive Officer, CPDD, and Professor of Pharmacology, Temple University School of Medicine).

REFERENCES - available upon request.

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Potent Analgesia, Respiratory Depression, Dependence, Abuse Liability-Clue to Separability

A.H. Newman, K.C. Rice and A.E. Jacobson

The formation of the Committee on Drug Addiction of the National Research Council, in 1929, marked the beginning of a concerted effort toward solving the drug abuse problem through chemical, pharmacological and clinical research. These early committee members recognized that synthesis of novel drugs based on the morphine structure could result in pharmacologically modified agents which would be useful therapeutically but would potentially not have the abuse liability of morphine. Dr. Reid Hunt said.

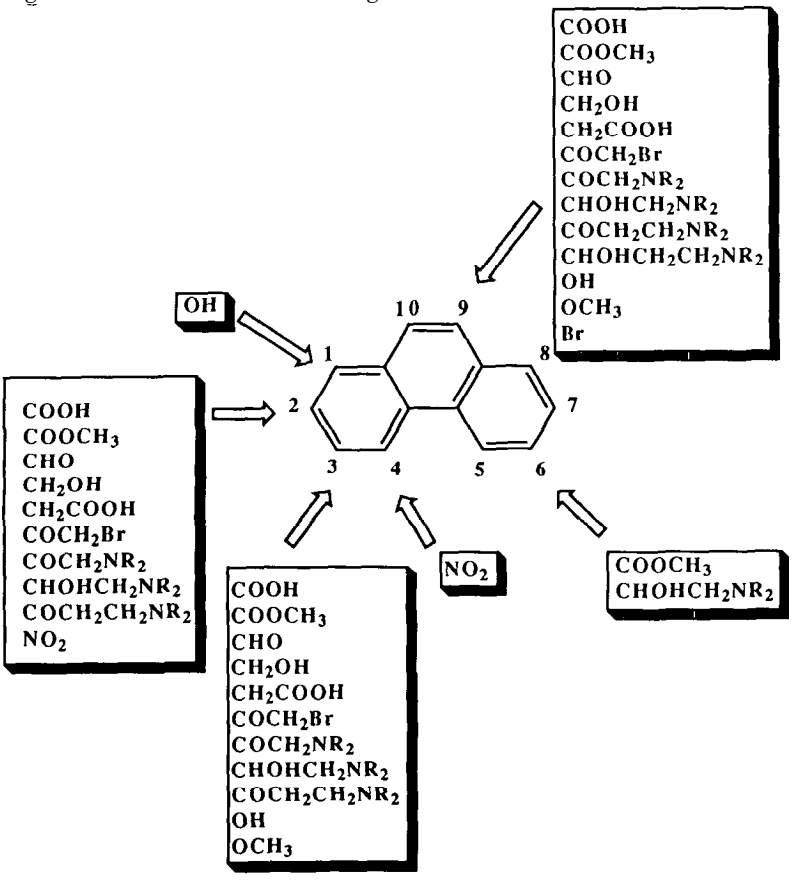
> "A thorough study of the morphine molecule might show a possibility of separating the analgesic form from the habit forming property...work along these lines would involve cooperation between the highest type of organic chemists and pharmacologists." (Hunt 1929, xxi)

To this end, Dr. Lyndon F. Small, of the University of Virginia was appointed to this monumental task. Dr. Small immediately sought the help of another alkaloid chemist, Dr. Erich Mosettig and together they synthesized a large number and variety of new drugs based on the morphine molecule and phenanthrene. In these early days of drug design and synthesis, the term "medicinal chemistry" did not exist. And yet these chemists and their colleagues, systematically modified the opium alkaloids in such a way as to determine several structural features that were responsible for certain pharmacological effects. In this manner, many new structure-activity relationships for the morphine-like drugs were established. Furthermore, the productivity of these chemists and their successes are even more impressive when one reflects on the total lack of modern technologies that we now have at our easy access, to chemically characterize our synthetic agents. The comparison of physical properties, combustion analyses and other simple analytical techniques were all that these chemists had and much of their "drug design" was first serendipitous and second dependent on being able to compare their new structure, or a

derivative thereof, to a known compound that had already been characterized. The internal hazard with this method, as illustrated repeatedly throughout this early work was that either the new compounds were not comparable to compounds already structurally characterized in the literature or these previously described structures were discovered to be incorrect. Despite these conditions, Small and Mosettig prepared a large number of synthetic analogs of phenathrene and morphine which were characterized and prepared in large enough quantities for in-depth pharmacological evaluation.

CHEMISTRY

Figure 1. Phenanthrene Analogs



Phenanthrene Analogs

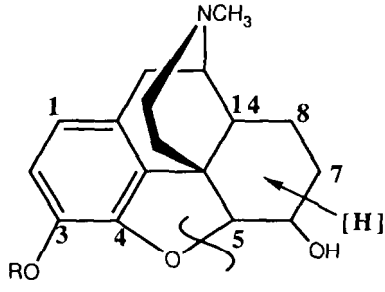
Dr. Mosettig began his synthetic studies on the simple phenanthrene nucleus. This compound was chosen since

phenanthrene and its derivatives were common isolated products in many degradative studies used for structural elucidation of the morphine alkaloids. Chemical modification of this nucleus seemed to be an appropriate beginning to determining structural features necessary for analgesia. Most of the chemical modifications were made at positions 2, 3 and 9 by Mosettig and colleagues and can be summarized in Figure 1.

Morphine Analogs

Dr. Small took on the morphine molecule itself. His approach was somewhat different than Mosettig's, in that his molecule was substantially more complicated and thus adding substituents in various parts of the molecule was not the most rational approach to chemical modification. Furthermore, some morphine derivatives had been synthesized previously and therefore a more systematic approach was taken to determine which structural features were responsible for certain effects of these drugs. Small's approach can be categorized into five major areas 1) desoxycodeine studies where the 4,5-epoxy bridged compounds were compared to the open 4-phenolic analogs, 2) C-ring reduction studies of codeine and its iso-, pseudo-, and allopseudo-isomers. 3) Grignard alkylation of the C-ring, 4) 3-phenolic alkyl ethers and 5) miscellaneous syntheses of some phenyl ring-substituted, 14-hydroxy, and N-methylated quaternary analogs, as illustrated in Figure 2.

Figure 2. Systematic modifications of the morphine molecule.



PHARMACOLOGY

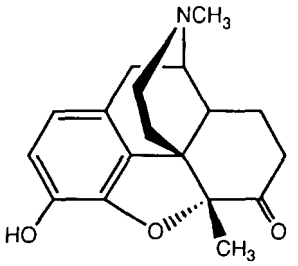
All of the compounds prepared in the Cobb Chemical Laboratory by Small and Mosettig were pharmacologically evaluated by Dr. Nathan B. Eddy, at the University of Michigan. Dr. Eddy tested all compounds for 1) toxicity in white mice and young rabbits, 2) analgesic action in cats, 3) respiratory effects in rabbits, 4) general depression in rabbits, and 5) gastrointestinal motility in rabbits. Furthermore, heart rate and body temperatures were compared and observations were made for convulsions, emetic effects and general behavior. Later, Dr. Eddy established evaluation procedures for tolerance and addiction in dogs, cats and monkeys.

Structure Activity Relationships

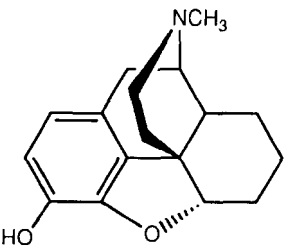
In general, the phenanthrene derivatives produced depression, muscular weakness and incoordination with the 3-substituted analogs being more potent than the 2 or 9-substituted analogs. Di-substitution generally decreased all activities with the exceptions of some 3,4-disubstituted analogs which were potent analgesics. However, the most promising analgesics in the phenanthrene series were found to be too toxic for therapeutic utility.

In the morphine series, 3-O-alkyl ethers were less potent as analgesics than their phenolic counterparts, cleavage of the 4,5-epoxide bridge reduced analgesic activity, alkyl-substitution in the 5 position resulted in increased analgesia as well as the dissociation of other effects. Alkylating the alcohol function in the 6-position increased analgesia, toxicity and convulsant effects, generally, saturating the C-ring led to an increase in analgesia and alkylating the 17-N to a quaternary compound eliminated CNS activity and increased toxicity. The most promising compounds to result from this work were desomorphine and metapon whose structures can be seen in Figure 3. Desomorphine was generally more potent in all activities tested than morphine. It demonstrated a rapid onset and a brief duration of action. A low addiction liability in cats, dogs and monkeys showed promise, however, later human studies showed a rapid onset of addiction. Metapon was found to be twice as potent an analgesic as morphine in humans, with equal duration of action. No emesis or respiratory depression was observed and a brief duration of dependence satisfaction was suggestive of a low addiction liability.

Figure 3. Chemical structures of Metapon and Desomorphine



METAPON



DESOMORPHINE

CLINICAL STUDIES

Since animal studies showed promise for the synthetic drugs desomorphine and metapon, they were evaluated clinically as therapeutic pain relievers. Their addictive liability was assessed individually by Drs. C. K. Himmelsbach and Lyndon Lee and their

conclusions were that while desomorphine showed no outstanding advantages over morphine due to its brevity of action and early development of tolerance and addiction, metapon was determined to be an excellent analgesic for the control of chronic pain. It was shown that tolerance and development of dependence was less rapid than with morphine and there were fewer side effects with this drug. However, in the mid-1950's production and distribution of metapon was ended.

CONCLUSIONS

This decade of medicinal chemistry on the morphine and phenathrene molecules marked the first major concerted effort toward the separation of pharmacological activities by chemical modification. Thousands of related compounds have since been prepared based on these early studies and vastly improved analgesics have resulted. The chemistry and pharmacology developed in these early years was remarkably sophisticated and complete, especially considering the time and tools with which these scientists worked. An appropriate conclusion to this era of research can be summarized in a quote by Dr. William C. White, Chairman of the Committee on Drug Addiction.

> "In the course of man's life many things are begun and some are finished. But work which entails fundamental research is never ended even though an era comes to an end. Those who have participated in the work of the Committee on Drug Addiction of the National Research Council are hopeful that the foundation that has been laid may prove of value not only to contemporary research but to posterity." (White, 1941,xv)

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All reference material came from the National Research Council Report of Committee on Drug Addiction, 1929-1941 and Collected Reprints.

AFFILIATIONS

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Leads to Current Therapy, to Opioid Receptor Subtypes and to Sigma Receptors

E.L. May

Following the World War II hiatus and a six-year effort on the malaria program at NM, during which time pethidine, methadone, isomethadone and 3-hydroxy-N-methylmorphinan had been developed in Germany and Switzerland, attention was again directed to the opioid scene. Our mission still was to provide analgesics that would relieve moderate to severe pain at safe doses and would cause minimal or no dependence and tolerance development. A side issue was the synthesis of potential antitubercular agents following Selman Waxman's discovery of streptomycin.

Because methadone and isomethadone, developed in Germany, resembled morphine pharmacologically, operations on these molecules seemed like a worthy project to Erich Mosettig and Nathan Eddy, my earliest mentors in that order. Accordingly, reduction of the carbonyl group of these isomeric compounds, with one chiral center, was effected in ways that produced both possible diastereomers of each antipode as well as of each racemate (Scheme I). The alcohols so obtained were invariably of lower antinociceptive potency than the parent ketones. However, O-acetylation restored, in every instance, activity to a level equal to or greater than that of the ketone from which it was derived. In all, 24 compounds, methadols and isomethadols and their O-acetyl derivatives were prepared and tested in the CPDD program. One of these, levo-alphaacetylmethadol (LAAM), has, for many years, been under investigation as a substitute for methadone in maintenance therapy. It is about three times more potent than methadone and has a longer duration of action.

While the chemistry of this project was being completed, the Korean war erupted and our charge in the Laboratory of Chemistry was altered somewhat. We were now exhorted to discover adequate, totally synthetic substitutes for morphine and codeine because of the threat to opium supply lines (Chart I). Accordingly, our "sights" were leveled at 3-hydroxy-N-methylmorphinan, an indirect result of one of the earliest attempts at the total synthesis of morphine by a German Chemist, Rudolph Grewe. This compound (the racemate called racemorphan, the levo-isomer levorphanol) was synthesized by Grewe and Swiss-Hoffman-LaRoche Chemists, Schnider and associates. Although it lacks the allylic alcohol system and the oxygen bridge of morphine, levorphanol is three to five times more potent than morphine, analgesically, with no greater, perhaps less side effects at equi-analgesic doses. My simplistic reasoning was that still simpler, smaller molecules might elicit similar or improved pharmacologic action if structural features believed at that time to be essential for strong, central, pain-relieving properties were retained. Stated tersely, these features are: a phenyl group and a tertiary-aminoethyl system attached to the same quaternary carbon, the phenyl nucleus probably needing a m-hydroxy

group. The structure at the right, in Chart I, generically called a 6,7-benzomorphan by J. A. Barltrop of England, meets these criteria.

Three (mental) dissections of levorphanol that leave inviolate the just - stated concepts are in Chart II. The first, elimination of the 9,10-carbon bridge of the octahydrophenanthrene system and relocation of nitrogen attachment from what had been position 9 to 8, generated the so-called phenylmorphans to be discussed shortly. The other two involve excision of two (6 and 7) or three (6. 7 and 8) carbons of hydroaromatic ring C. The resulting carbon vestiges may become methyl or higher alkyl by satisfying the unsaturation left with H or C_nH_{2n+1}. respectively.

Returning to the phenylmorphans, a relatively simple synthesis is shown in brief in Scheme II. The resulting racemate, 5-m-hydroxyphenyl-2-methylmorphan (IVb) was indeed morphine-like in almost every respect. Optical resolution resulted in a favorable separation of deleterious from desired effects (Chart III). The (1S,5R), (+)-enantiomer (absolute configuration determined by Dr. Todd Cochran, Duquesne University) is a typical mu agonist in vivo and in vitro, slightly more potent than morphine antinociceptively (mice), somewhat less potent in dependence liability (monkeys and rats) and in vitro. The (-), (lR,5S) antipode is comparable to morphine antinociceptively (mice) but will not support morphine dependence in monkeys or rats, in fact, exacerbates abstinence symptoms in monkeys. It has relatively weak, mu-binding properties and would be an interesting study in man.

Attempts to prepare antagonists from racemic or (+)-I by replacing methyl with the standard groups - allyl, propyl, cyclopropylmethyl gave only weaker agonists with no more than a hint of antagonist property. Introduction of a 9-methyl substituent did, however, produce a mild agonist-antagonist, a little less potent than nalorphine and one relatively pure antagonist when the radical on N was methyl.

Before leaving the phenylmorphans you may be interested in what happens when the octahydrophenanthrene moiety is restored. (Scheme III) The resulting isomeric morphinan (VIIc) with nitrogen closure at position 8 rather than 9 as in racemorphan is almost devoid of antinociceptive activity. The sequence for its synthesis is shown in Scheme III as is its degradation to the same octahydrophenanthrene (VI) as that obtained from 3-hydroxy-N-methylmorphinan (Vc) proving identical stereochemistry at the concerned chiral centers.

As for structures resulting from deletion of carbons 6 and 7 of racemorphan, several 5,9-dialkyl-2'-hydroxy-2-methyl-6,7-benzomotrphans were obtained from appropriate 3,4-dialkyl pyridines converted to precursors as shown in Scheme IV. Both possible racemates, initially designated a (VII) and ß (IX) were produced from these precursors in a ratio of about 10:1 (Scheme V). The lesser ß-compounds were much more potent as mu agonists. NMR measurements along with reaction rates with methyl iodide proved that the 9-alkyl groups were axial for the predominant a-isomer, equatorial for the ß with the hydroaromatic ring as reference point. Scheme VI depicts the various routes used to synthesize the 5-monoalkyl as well as the 5,9-dialkyl-6,7-benzomorphans. Initially phenylacetonitrile and/or ß-tetralone and analogs were used for the 5-alkyl compounds. Later, ß-tetralones and the aforementioned 2-

benzyltetrahydropyridines served as intermediates for the mono and dialkyl compounds.

Once again optical resolution effected a favorable separation of pharmacological actions. Invariably, the (-)-isomer of the a-series, whose absolute stereochemistry has been determined in several laboratories to be 1R, 5R, 9R [identical to that of morphine and the (-) morphinans at the three common centers of chirality] were 2-3 times more potent than the racemates and morphine and would not support morphine dependence in rhesus monkeys. In fact, all shown in Table I and a few others, exacerbated and/or precipitated withdrawal symptoms. However, they bind to mu receptors or sub-receptors except in the guinea-pig ileum where they are again similar to nalorphine. In two cases of human study (5,9dimethyl and 5,9-diethyl, (-)metazocine and etazocine, respectively) they were at least as good as morphine in pain relief but caused somewhat less tolerance and dependence production. The (+)-isomers, in equal surprise, were, in all but one instance {(+)-metazocine}, codeine-like antinociceptively and in the morphine-dependent monkey. 5-Monoalkyl compounds, the result of excision of carbons 6,7 and 8, were somewhat less potent than corresponding 5,9-dialkyl homologs.

In Chart IV are given data obtained for 6,7-benzomorphans with a tertiary rather than quatenary carbon (position 5). As is evident, these non-quaternary carbon structures (2,4 and 6) are about half to 1/10 as potent antinociceptively as their quatemary-carbon counterparts (1,3,5). And, even as the racemates, they are nalorphine-like (agonist-antagonists) in the morphine- dependent monkey. Thus, in these rigid structures, antinociceptive activity is not abolished, in fact reduced only two- to ten-fold in going from a quaternary to a tertiary carbon in contrast to the less rigid molecules such as pethidine, ketobemidone and methadone. The fairly complex schemes devised for these non-quaternary-carbon benzomorphans are due to the talents, skill and patience of Arthur Jacobson, and three, Japanese visiting scientists.

N-Substitution (Chart V) in the racemic a-benzomorphan series produced results similar, with respect to antinociceptive potency, to those observed with morphine and the morphinans. Phenethyl for methyl increased potency 6-10 fold without, however, a corresponding increase in physical dependence capacity in monkeys where there is, a 25-50 fold difference favoring the N-phenethyl-benzomorphan. Carryover to man was by no means quantitative, although the compound in question, phenazocine, is orally and parenterally effective for deep pain with relatively minimal harmful effects, including abuse liability and those on circulation and respiration. In N-alkyl substitution, mixed agonist-antagonists were obtained from N-ethyl to N-butyl inclusive. However, N-pentyl to N-octyl-N-normetazocines were potent mu agonists. (more about this later).

The first typical antagonist of the 6,7-benzomorphan series to gain attention was synthesized by Dr. Maxwell Gordon and his associates at The Smith Kline & French Laboratories about 1960. This compound, racemic α-N-allyl-N-normetazocine, (lower right, R = allyl) now well-known as SKF 10047 was considered the prototypical sigma agonist as a result of the brilliant research of Dr. William Martin at Lexington, Kentucky on multiple opioid receptors.
Subsequent separation of this racemate into its enantiomers and studies that ensued, demonstrated that the (-)-isomer is a strong mu antagonist, the (+)-antipode a non-opioid sigma agonist which binds to PCP sites. It is similar to

PCP in discriminative stimulus properties as determined by Brady and Balster, et. al.

In 1964 Archer, Harris and associates at Sterling-Winthrop published a scholarly study on N-substitution in the benzomorphan series which heightened interest in agonist-antagonists and led to the development of pentazocine and cyclazocine. This research, no doubt, provided at least part of the stimulus for the ultimate marketing of buprenorphine, butorphanol and nalbuphine. Pentazocine, incidentally, was the first agonist-antagonist to be used as an analgesic in clinical practice and is still a Schedule IV compound. Cyclazocine is a strong agonist-antagonist and has been a good research tool.

A CPDD program on N-alkyl substitution in the henzomorphan series is now in its final stages (Chart VI). It consists of the preparation and extensive testing in vitro and in vivo of 2-H- to 2-octyl- (inclusive) 2'-hydroxy-5,9a-dimethyl-6,7-benzmorphans {(-) and (+)-enantiomers}. Chemical, animal and some in vitro work is being done at The Medical College of Virginia, most in vitro studies at NIH and The University of Michigan.

In vivo in the minus series, N-ethyl to N-Butyl-N-normetazocines are mild agonist-antagonists as stated before and N-methyl, pentyl, hexyl, heptyl and octyl-N-normetazocines are morphine-like (in potency) antincoceiptively which (excepting N-pentyl) are poor supporters of morphine dependence in monkeys. In vitro studies were performed as indicated in Chart VI.

The antinociceptive and narcotic antagonist activity of these levo-isomers could not be attributed to any single opioid receptor subtype and agonist vs antagonist activity could not be differentiated by opioid receptor subtype. The active agonist or antagonist compounds were those which interacted with both mu and kappa receptors. Little selectivity was observed, there was at most a three-fold difference between displacement at the mu and kappa receptors. They were less active at the delta receptors.

As for the (+)-enantiomers, the (+)-N-methyl had significant effects on PCP binding sites and N-butyl. pentyl. heptyl and octyl were exceptionally potent at sigma receptors; the heptyl and octyl homologs are among the most potent sigma ligands yet discovered. Furthermore, there is a good separation between interaction with sigma and PCP sites ranging from about 200 fold for the (+)-N-butyl to 900 fold for N-pentyl. Thus, these compounds are of potential interest for future in vivo work on the physiological function of sigma receptors and the distinction between the sigma 1 and 2 subtypes of receptors.

Finally, attempts (Table II) to prepare antagonists at NIH from the agonist (strong analgesic) ketobemidone (4-m-hydroxyphenyl-4-ketoethyl-1-methylpiperidine) by replacement of methyl on nitrogen with allyl, cyclopropylmethyl, ethyl, propyl or butyl resulted only in producing weak to relatively strong mu-like agonists without antagonist effects. However, N-pentyl-N-norketobemidone is a morphine-like antinociceptive agent in mice with atypical properties of antagonism in the morphine-dependent monkey. N-hexyl- and N-heptylnorketobemidones are between ketobemidone and pethidine in antinociceptive potency and are also moderately potent antagonists in monkeys. N-Octyl to N-decyl are relatively inert. For the homologous N-alkyl compounds (N-methyl to Ndecyl), there is a statistically significant correlation

of antinociceptive activity (hot plate and Nilsen tests) and capacity to bind to mouse-brain homogenates as determined by Pert and Snyder.

In closing, I acknowledge, with many thanks, the strong support and collaboration of several talented visiting scientists, and past and present colleagues and friends at NIH, particularly Arthur Jacobson, Ken Rice, Marienna Mattson, Werner Klee and Dick Streaty. I thank heartily also, especially Lou Harris who provided me, during the last 14 years, a second scientific home at MCV, Bill Dewey, Mario Aceto, Ed Bowman, Billy Martin, Bob Balster and John Rosecrans along with their effective supporting groups and four postdoctoral fellows, Drs. Uwaydah, Vincek, Awaya and Zenk. Bill Glassco, at present a postdoctoral fellow and graduate student, Brian Thomas, both in the Department of Pharmacology and Toxicology, MCV, are also due my gratitude. And last, but far from least, Joyce Pye, Sussie Robinson and Laura Johnson, Dr. Harris's cooperative, effective office staff, deserve my heartfelt thanks.

AFFILIATION: **Department of Pharmacology and Toxicology**
Medical College of Virginia
Virginia Commonwealth Univerisity
Richmond, VA 23298-0613

CHART I

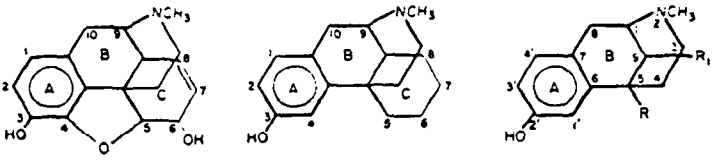


CHART II

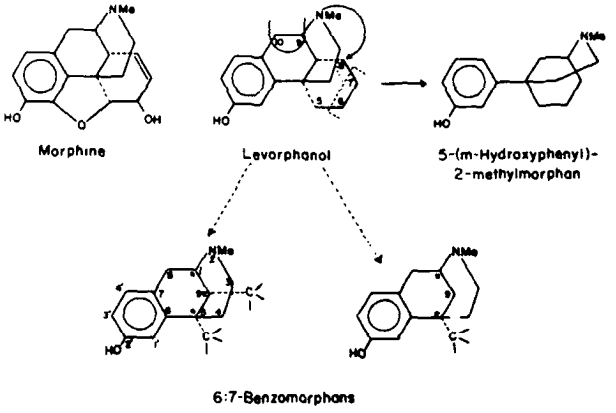
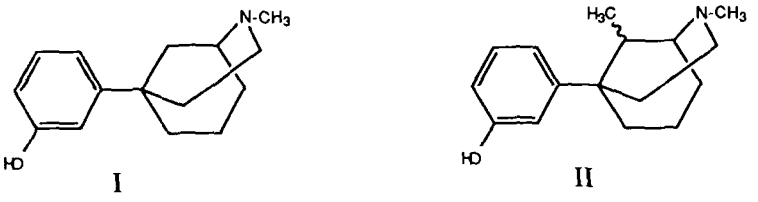


CHART III



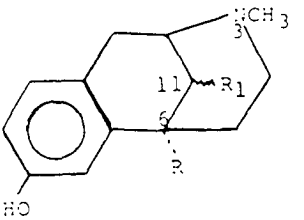
(-)- I, morphine-like in potency in HP, PPQ and TF antinociceptive tests, will not support morphine dependence in monkeys or rats. Exacerbates monkey abstinence Symptoms.

(+)-I, Morphine-like in all respects.

(+)-II, pore antagonist, 1/80 as potent as naloxone when Me is a for the cyclohexane ring.

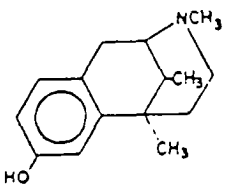
(-)-II, mixed agonist-antagonist, half as potent as nalorphine.

CHART IV

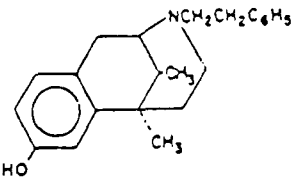


1.	R=Me, R_1=H	ED_{50} 3.2	Mg/Kg
2.	R=R_1=H	4.5	
3.	R=Me, R_1-93-Me	1.2	
4.	R=H, R_1=93-Me	4.3	
5.	R=CH_3, R_1=93-Me	0.1	
6.	R-H, R_1-93-Me	1.1	

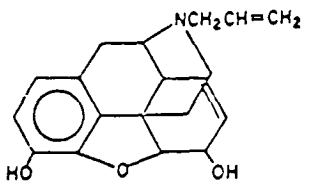
CHART V



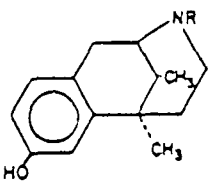
Metazocine



Phenazocine
(Prinadol, Narphen)



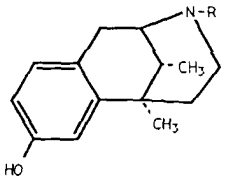
Nalorphine



R = CH_2Δ-Cyclazocine

R = $CH_2CH=CMe_2$-Pentazocine

CHART VI



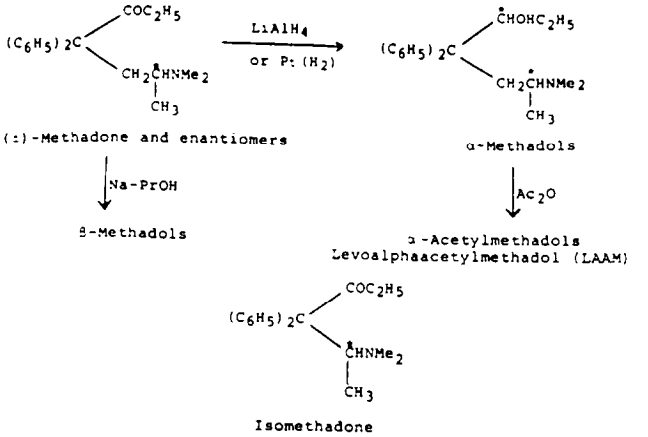
(-) and (+), R = H - Cg_8H_{17}

Sigma Binding- GP Brain (Jacobson-Mattson)

TCP and DAMGO (B. R. Martin, *et al.,* MCV)

Mu. Kappa and Delta Binding, and Mouse Vas Deferens work (Woods, Medzihradsky, Smith)

SCHEME I



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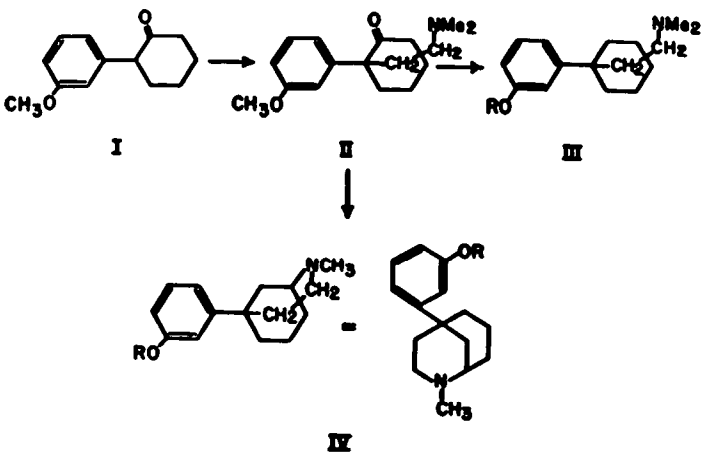
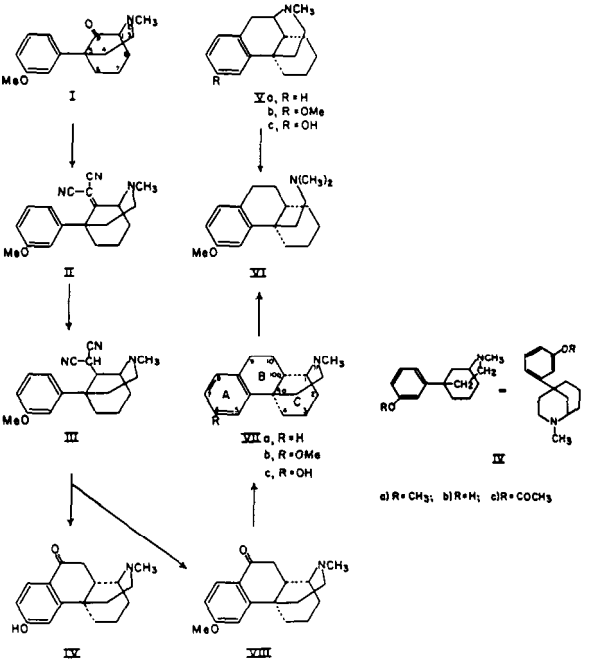
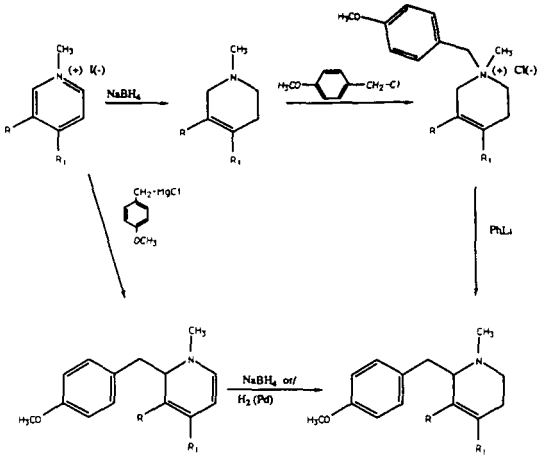


a) R = CH_3; b) R = H; c) R = $COCH_3$

SCHEME III

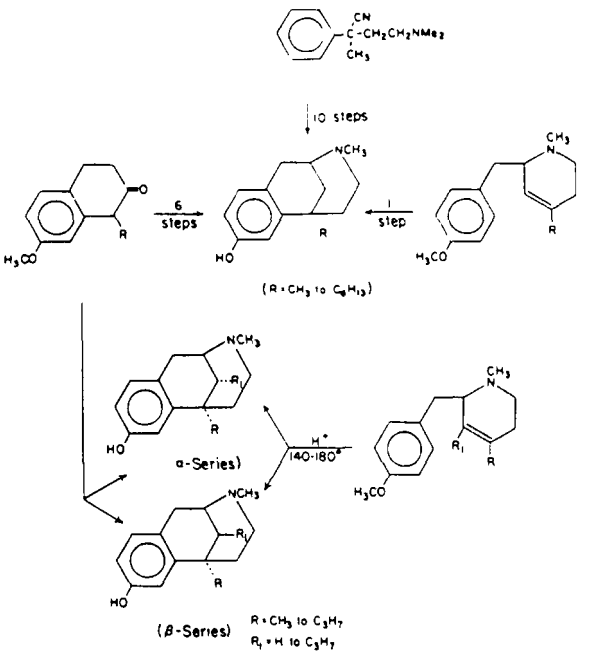


SCHEME IV



R = H to C_3H_7, R_1 = H to C_6H_{13}

SCHEME V



SCHEME VI

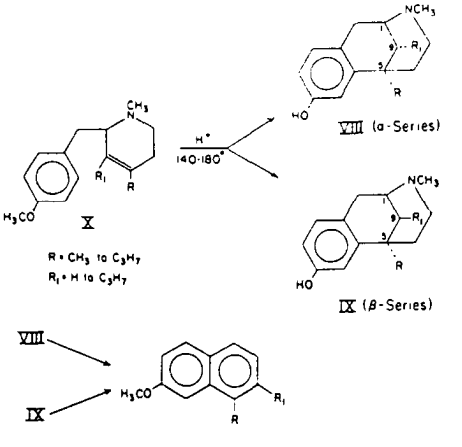
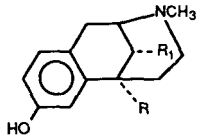
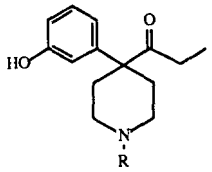


Table 1. Analgesic Activity, Physical Dependence Capacity, and Antagonist Potency of Some Benzomorphan Enantiomers



R	R_1	Enantiomer	ED_{50} mg/kg	PDC	Antagonistic Potency
Me	Me	(-)	0.6	No	1/50-1/30 Nalorphine
		(+)	Inactive	No	No
Et	Et	(-)	1.2	No	1/10 Nalorphine
		(+)	7.5	Intermediate	No
Pr	Me	(-)	0.8	No	1/5 Nalorphine
		(+)	12.3	High	No
Et	H	(-)		No	1/40-1/20 Nalorphine
		(+)	2 %	Low	No
Me	H	(-)	1.8	No	1/50 Nalorphine
		(+)	22.9	Very low	No
	Morphine		1.2	High	No
	Codeine		1.5	Intermediate	No

Table 2



R = $-CH_2CH{=}CH_2$, $-CH_2-$  , -Et, -Bu,

-Pent, -Hex, -Hept, -Oct. -Non, -Dec.

Compd	R	Hot-plate analgesic μM ED50	Inhibition of [^{3}H]Naloxone binding(1nM), ED50,nM No sodium	100 mM sodium	Ratio of +NaCl/-NaCl
1	Methyl	2.1 (1.4-2.8)	7-10	70	7-10
2	Ethyl	67.2 (52.0-87.0)	400	1500-2000	3.8-5
3	Propyl	16.0 (13.2-19.1)	200	800-1000	4-5
4	Butyl	4.6 (3.8-5.9)	50	600-700	12-14
5	Pentyl	0.78 (0.62-1.0)	8	30	3.8
6	Hexyl	7.5(5.5-10.3)	20	40	2
7	Heptyl	9.0 (7.0-11.6)	20	40-50	2-2.5
8	Octyl	26.5 (20.2-34.9)	200	200	1
9	Nonyl	Inactive	700	700	1
10	Decyl	Inactive	500	600-700	1.2-1.5

State-of-the-Art Analgesics from the Agonist-Antagonist Concept

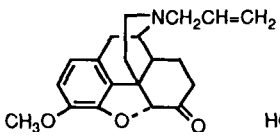
S. Archer

Nalorphine, N-allylnormorphine, is the prototypical agonist-antagonist. It was synthesized in 1941 (McCawley et al., 1941) and was used clinically as an antidote for opiate overdoses.

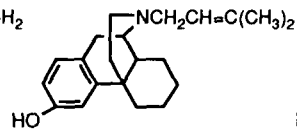
In 1954, Lasagna and Beecher (Lasagna and Beecher 1954) examined the clinical analgesic effectiveness of nalorphine and nalorphine-morphine combinations to answer the following questions: (1) does nalorphine antagonize the analgesic effects of morphine?; (2) can a morphine-nalorphine combination be devised which will reduce the side effects and the development of tolerance and physical dependence to this opiate?; (3) is nalorphine an analgesic when given alone?

The major conclusions of this paper were: (1) at doses of 10mg/70kg and 15mg/70kg nalorphine produced significant analgesia frequently accompanied by unpleasant side effects which rendered the drug unsuitable for use as an analgesic; (2) combinations of either 2mg or 5 mg of nalorphine with 10mg of morphine produced analgesia indistinguishable from that produced by morphine alone. This was the first time that nalorphine was demonstrated to have analgesic effects in any mammalian species.

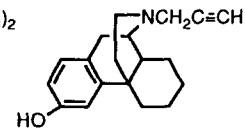
Keats and Telford (Keats and Telford 1956) confirmed these observations and evaluated the analgesic effects of several putative antagonists supplied by Dr. Nathan B. Eddy. These compounds were tested under their NIH code numbers and their structures are shown below.



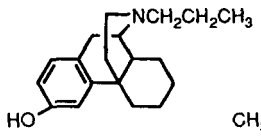
NIH 7305



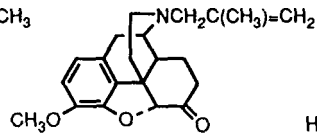
NIH 7446



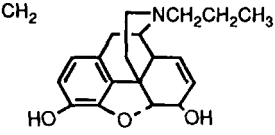
NM 6045



NM 6076



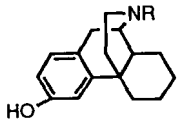
NIH 7796



NIH 5704

NIH 7446 and NM 6045 proved to have analgesic activity in man but the latter produced side effects similar to those produced by nalorphine. During the course of the investigation it was reported that NIH 7446 was antagonist without antagonist properties. Thus it was not surprising that no dysphoric effects were reported for this drug.

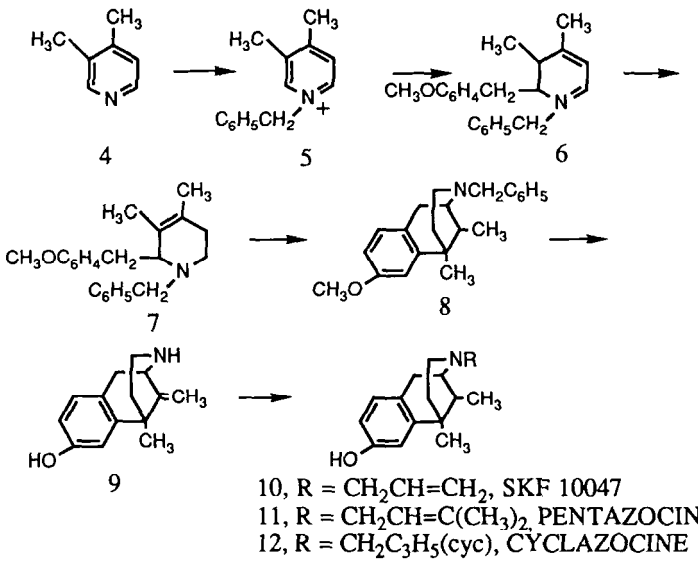
May and his colleagues (May and Fry 1957, May and Ager 1959, Ager and May 1960, May and Kugita 1961, May et al., 1961a) prepared a series of benzomorphans by adapting the Grewe-type synthesis used by Schnider (Schnider et al., 1950) to prepare levorphan,**1**, and levallorphan,**2.**



1, R = CH_3, LEVORPHAN
2, R = $CH_2CH{=}CH_2$. LEVALLORPHAN
3, R = $CH_2C_3H_5$(cyc), CYCLORPHAN

A slight modification is shown in Scheme 1. (Albertson and Wetterau 1970).

SCHEME 1



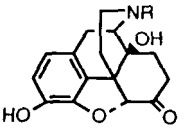
10, R = $CH_2CH{=}CH_2$, SKF 10047
11, R = $CH_2CH{=}C(CH_3)_2$, PENTAZOCINE
12, R = $CH_2C_3H_5$(cyc), CYCLAZOCINE

3,4-Dimethylpyridine,4, was benzylated to give the quatemary salt,5. Treatment of the latter with p-methoxybenzylmagnesium chloride furnished 6 which in turn was catalytically reduced to 7. Acid-promoted cyclization gave 8 as the major product. Debenzylation to 9 followed by alkylation gave the following: 10, SKF-10047 (Gordon et al., 1961),11, pentazocine (Archer et al., 1962, 1964, Keats and Telford 1964))and 12, cyclazocine. It should be recalled at the time that this work was done no methods were available for determining the agonist activity of these compounds. The antagonist activity was determined in rats (Harris and

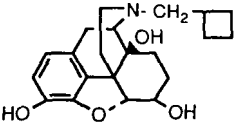
Pierson 1964) and the analgesic activity was determined clinically by Keats and Lasagna (Keats and Telford 1964a). SKF-10047 had an AD_{50} = 0.047mg/kg vs meperidine and the analgesic dose in man was >15mg/70kg. At these doses dysphoric effects were observed. Cyclazocine showed an AD_{50} = 0.018 mg/kg and in man 0.25 mg/70kg was equivalent in analgesic potency to 10 mg of morphine. However dysphoric effects were observed near the clinically effective dose. Pentazocine was a weak antagonist (AD_{50} = 3.9mg/kg) in rats. Doses.of 20-30-mg/70kg were found to be equivalent in analgesic potency to 10mg of morphine. At these doses no psychotomimetic effects were noted. Subsequent studies showed that at higher doses dysphoric effects did occur. Similarly, early direct addiction studies indicated that pentazocine produced littlle or no physical dependence but when tested under more severe conditions abrupt withdrawal of the drug resulted in a withdrawal syndrome judged to be milder than that produced by morphine but was accompanied by drug-seeking behavior.

Cyclorphan,3, a drug related to levallorphan,2, prepared by Gates (Gates and Montzka, 1964), is even more potent that cyclazocine as antagonist in rats and as an analgesic in man. However psychotomimetic effects were noted at analgesic doses. Studies on the dextro and levo isomers of pentazocine showed that the analgesic and other effects of the drug resided almost entirely in the levo isomer. (Forrest, Jr. et al., 1969). The absolute configuration of cyclazocine was shown to be the same as that of morphine by means of single crystal X-ray analysis (Karle et al., 1969). Since the biologically active benzomorphans are all derived from the nor-base,9, they are all configurationally related to natural morphine.

The studies discussed above led to the synthesis and development of a number of other narcotic antagonists. Naloxone, 13, and naltrexone,l4, are pure antagonists devoid of any analgesic activity.(Jasinski et al., 1967, Gritz et al., 1970) Nalbuphine,l5, a closely related analog of naltrexone is a mixed agonist-antagonist producing analgesia in man with less dysphoric effects than pentazocine (Jaffe and Martin, 1990). These three compounds were synthesized from thebaine.

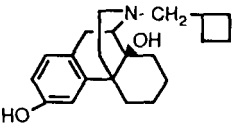


13, R = $CH_2CH{=}CH_2$,NALOXONE
14, R = $CH_2C_3H_5$(cyc), NALTREXONE

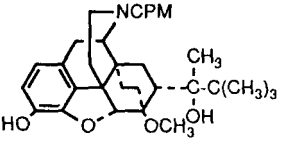


15, NALBUPHINE

Butorphanol,l6, is a totally synthetic agonist-antagonist whose clinical properties closely resemble those of nalbuphine (Jaffe and Martin 1990). Buprenorphine,l7, prepared from the adduct of thebaine and methyl vinyl ketone (Bentley et al., 1967) is a mixed agonist-antagonist with low abuse potential and is devoid of psychotomimetic effects. (Jasinski et al., 1978). It is being considered as a modality for treating heroin and cocaine addicts.



16, BUTORPHANOL



17, BUPRENORPHINE

As a result of his investigations on agonist-antagonists Martin (Martin et al., 1976) introduced the concept of multiple opioid receptors by proposing three such entities: mu for the morphine receptor, kappa for the cyclazocine receptor and sigma for the SKF-10047 receptor. The latter was originally thought to mediate the psychotomimetic effects of the agonist-antagonists but Herz (Pfeiffer et al., 1986) suggested that the kappa receptor was responsible for the dysphoric side effects of this class of opioids.

The synthetic efforts of Everette May and his group at the NIH led directly to the synthesis of the first clinically acceptable agonist-antagonist. Nathan B. Eddy, Chief of the Laboratory on Analgesics NIH,who in his capacity as the Secretary of the Committee on Narcotic and Drug Addiction, had a profound influence during the early stages of the work on mixed agonist-antagonists. The contributions of these two men to this field cannot be overestimated.

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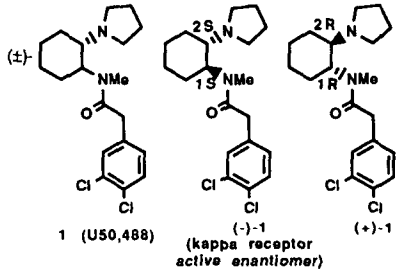
Novel Kappa Opioid Receptor and Sigma Ligands

B. de Costa, R.B. Rothman, W.D. Bowen, L. Radesca, L. Band, A. Reid, L.D. Paolo, J.M. Walker, A.E. Jacobson and K.C. Rice

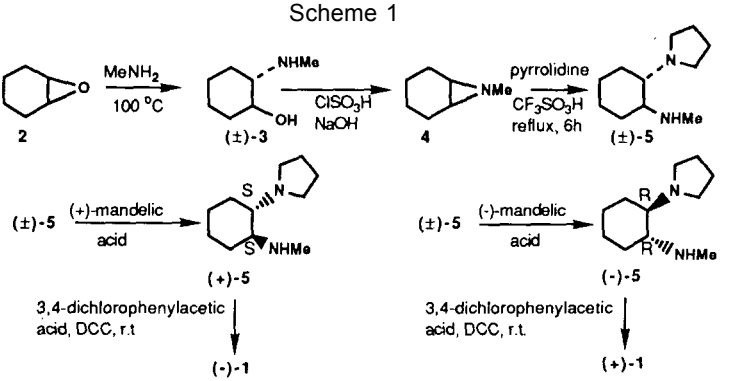
Specific site directed electrophilic affinity ligands have been employed with a great deal of success in identification of the structure and function of receptors in the central nervous system (CNS) (Newman, 1991). In our hands, the isothiocyanate (-NCS) functionality has served admirably as a suitable electrophilic group for the development of electrophilic affinity ligands. We have successfully employed the NCS group in electrophilic affinity ligands for a number of unrelated CNS receptors which include phencyclidine, opiate receptor subtypes, peripheral and central benzodiazepine receptors (for a detailed review, see Newman, 1991) and cannabinoid receptors (Richardson et al., 1990). However, until recently (de Costa, Rothman et al., 1989 and de Costa, Band et al., 1989), no specific irreversible ligands were available for the kappa opioid receptor.

We wished to approach this problem using the kappa selective opioid agonist, U50,488 as our template. However, U50,488 is a racemic drug and enantiomeric components of a racemic mixture can have different effects at receptors. Thus, we developed a synthesis of enantiomeric forms [(+)- and (-)-**1**] of U50,488 [(+)-**1**] (de Costa et al., 1987).



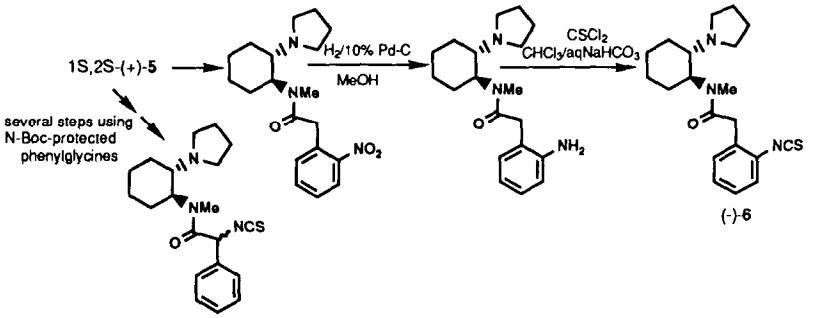
We successfully synthesized optically pure cyclohexanediamines **5** (Scheme 1) which served as precursors to the enantiomers of U50,488. The absolute configuration of these precursors was determined by single crystal X-ray analysis of the R-(-)-mandelate salt of (+)-**5** (Scheme 1). Coupling of (+)-and (-)-**5** with 3,4-dichlorophenyiacelic acid in the presence of DCC afforded (-)-and (+)-U50,488 of defined absolute configuration and optical purity (>99%).

Scheme 1



The 1S,2S-(-)-enantiomer of U50,488 proved to be the kappa receptor active enantiomer of U50,488, exhibiting a K_i value of 124 nM for displacement of [^{3}H]bremazocine while the (+)-enantiomer was essentially inactive (K_i=90,300 nM) (de Costa et al., 1987). With this knowledge, we then embarked upon the synthesis of 5 possible isothiocyanate derivatives of the S,S enantiomer of deschloro U50,488 (see Scheme 2). We decided to synthesize the isothiocyanate derivatives of deschloroU50,488 because it is known that compounds in this class lacking the chlorine atoms (eg U69,593) (Lahti et al., 1985) retain kappa receptor activity. We also synthesized the "inactive" (R,R) isothiocyanate enantiomers for the purposes of comparison. Evaluation of the ability of all compounds (total=10) in the series to cause wash resistant inhibition of [^{3}H]bremazocine binding indicated that all were inactive (de Costa, Rothman et al., 1990). However, evaluation of the capacity of these compounds to produce wash resistant inhibition of [^{3}H]U69,593 binding revealed that the ortho compound S,S-(-)-**6** was the only isothiocyanate able to irreversibly inhibit [^{3}H]U69,593 binding (de Costa, Rothman et al., 1990). Pretreatment of guinea pig brain membranes with a 1μM concentration of (-)-**6** followed by extensive washing indicated a 90% reduction in binding. In contrast, (+)-**6** was inactive.

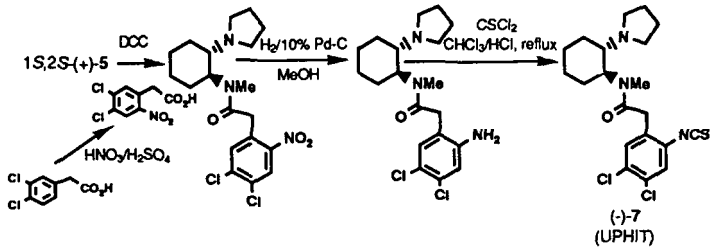
Scheme 2



In vivo (ICV) administration of (-)-**6** to guinea pigs indicated that it failed to affect either [^{3}H]bremazocine or [^{3}H]U69,593 binding 24h after the administration. Thus, we decided to synthesize the 3,4-dichloro analog (-)-**7** (UPHIT) of (-)-**6** (Scheme 3) (de Costa, Band et al., 1989). (±)-**7** was initially

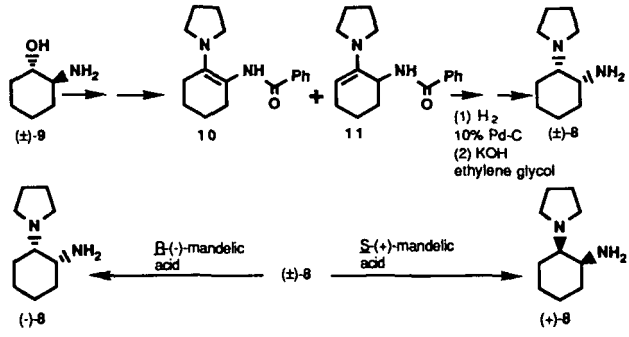
tested and proved to be a potent and selective in vivo affinity ligand for kappa receptors; ICV administration of a 100 µg dose of (±)-**7** to guinea pigs followed by removal of the brain 24 h later indicated that [^{3}H]U69.593 binding was reduced by 98% and bremazocine binding was reduced by 40%. The (-)-enantiomer was similarly active. UPHIT is proving to be of value in functional studies of kappa receptors (Horan et al., 1991).

Scheme 3



At this stage we decided to examine the kappa receptor affinity of the cis diastereomers of U50,488 with the hope of finding still further efficacious kappa receptor affinity ligands. We developed a practical synthesis of enantiomeric cis diamine precursors (+)- and (-)-**8** starting with the readily available aminoalcohol **9** (de Costa, Radesca et al., 1990). The cis geometry (Scheme 5) was obtained by stereoselective reduction of enamine mixture **10/11.**

Scheme 4



The N-methyl homologs of (+)- and (-)-**8** (de Costa, Bowen et al., 1989) served as intermediates for the synthesis of the (-)- and (+)-cis diastereomers of U50,488 while (+)- and (-)-**8** served to examine the effect of N-alkyl

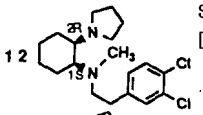
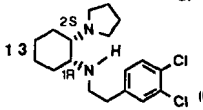
Table 1. Binding Affinities cis and trans Isomers of U50,488 at Sigma Receptors

COMPOUND	K_1(nM)[$_3$H](+)-3-PPP
cis-(1R,2S)-(+)-U50,488	221±36
cis-(1S,2R)-(-)-U50,488	81±13
trans-(1R,2R)-(+)-U50,488	1270±168
trans-(1S,2S)-(-)-U50,488	594±3

substitution (Radesca et al., 1991). To our surprise, the cis isomers of U50,488 exhibited no significant affinity for kappa receptors and showed high affinity and selectivity for sigma receptors (see Table 1) (de Costa, Bowen et al., 1989).

The SAR of these compounds was extended to find more potent and selective compounds for the sigma receptor (Radesca et al., 1991). This was especially of interest to us since sigma receptor research has been impeded by the lack of ligands showing both high affinity and selectivity for this site. The systematic SAR study resulted in novel cyclohexanediamines (de Costa, Rice et al., 1990 and Radesca et al., 1991) showing extremely high potency and specificity as sigma receptor ligands, two of which are illustrated in Table 2.

Table 2: Binding Selectivity of Cyclohexanediamine-type Sigma Receptor Ligands

COMPOUND	BINDING AFFINITY (K,nM)				
	Sigma	Kappa$_1$	Kappa$_2$	PCP	Dop-D$_2$
	[^{3}H](+)-3-PPP	[^{3}H]U69593	[^{3}H]BREM	[^{3}H]TCP	[^{3}H]SUL
12 		No-inhib	No-inhib	No-inhib	No-inhib
13 	0.49	N D	No-inhib	6880	8514

In general, the 1S,2R configuration and smaller N-substituents (eg R=H) resulted in more potent sigma ligands (Radesca et al., 1991). These novel diamine-type sigma ligands exhibited exceptional potency for sigma receptors (guinea pig brain vs [^{3}H](+)-3-PPP) and showed no significant cross-reactivity with dopamine-D_2, PCP or kappa receptors (those which commonly cross-react with most sigma receptor ligands). The high affinity of the (-)-benzomorphans for kappa receptors and the high affinity of their (+)-enantiomers for sigma receptors coupled with the results which we have observed with the cis and trans diastereomers of U50,488 may suggest a structural or evolutionary link between sigma and kappa receptors (Walker et al., 1990). Compounds **12** and **13** (Table 2) and related diamine-type sigma receptor ligands in this series have demonstrated antipsychotic and neuroprotective properties (Contreras et al., 1991) that may be linked to their sigma receptor affinity. These effects may represent a novel functional role for the sigma receptor.

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Three Generations of Electrophilic Affinity Ligands for the Phencyclidine Binding Sites

A.E. Jacobson, J.T.M. Linders, K.C. Rice, J.A. Monn, M.V. Mattson and C. George

Phencyclidine (PCP)-like ligands are known to bind within excitatory amino acid ion channels regulated by the NMDA/glutamate receptor. These ligands act as non-competitive antagonists and block excessive influx of endogeneous ligands and ions through the excitatory amino acid ion channel induced by traumatic events such as head injury or stroke. By this blockade, PCP-like ligands such as MK-801 (Dizocilpine) are said to exert their neuroprotective effects. PCP-like ligands also interact with the dopamine transporter complex to block dopamine reuptake (Schweri *et al.,* 1987, 1989, Rothman *et al.,* 1990, Zimanyi *et al.,* 1989, Sershen et al., 1988, Berger et *al.,* 1986, Reith et al., 1991).

PCP itself was originally introduced to clinical medicine as an intravenous anesthetic. It did not produce circulatory or respiratory depression (Marshall and Wollman 1985). PCP's unfortunate side-effects (Olney *et al.,* 1989, 1990), perhaps induced through interaction with the dopamine transporter complex, militated against its further use in man. Its spectrum of side-effects has been noted to mimic schizophrenia better than amphetamine. Ketamine, a structurally- and biologically-related molecule, however, is still used as an anesthetic for specific needs in children and in veterinary medicine.

PCP is a known drug of abuse. Although its use has decreased, coincident with the acceleration in the misuse of cocaine, PCP abuse reached epidemic proportions in several U.S. cities a few years ago. Nevertheless, the potential value of PCP-like drugs as anesthetics, anticonvulsants, and neuroprotective agents, and the recent finding that the tolerance and dependence caused by repeated morphine administration to rodents can be blocked by Dizocilpine (Trujillo and Akil 1991), has produced a resurgence of interest in this class of drugs.

Our exploration of new potential electrophilic affinity ligands for PCP-binding sites was guided by the utility of metaphit as a pharmacological tool. Metaphit, our first electrophilic affinity ligand, was based on the achiral PCP (Rafferty et al., 1985). Its use was instrumental in the discernment of the dopamine transport complex as one of the sites at which PCP-like ligands interact. Affinity ligands have become essential

tools for the discernment and exploration of receptors and binding sites. They have been used for the isolation and purification of receptors. Affinity ligands are also used for the study of the physiological function of receptors and to selectively deplete receptor subtypes, allowing investigation of the remaining receptor population. Furthermore, affinity ligands can be utilized to produce antibodies for the development of antiidiotypic antibodies, which are useful for the purification of receptors and identification of gene products (Newman 1990).

Metaphit is not a very potent affinity ligand and although it is reasonably selective in its receptor affinity, in that it does not appreciably interact with opioid, benzodiazepine or muscarinic receptors, it shows afffinity for at least two of the sites of action of PCP-like drugs. It irreversibly interacts with a binding site in the excitatory amino acid ion channel regulated by the NMDA/glutamate receptor and with the dopamine transport complex. In order to obtain a more potent and selective electrophilic affinity ligand we explored different types of molecules that were known to exhibit PCP-like activity, the dioxolanes and the MK-801-like compounds.

From our former studies we knew that the dioxolanes, such as dexoxadrol and etoxadrol, were at least as potent as PCP in binding affinity and *in vivo* (Jacobson *et* al., 1987). Dexoxadrol has two asymmetric carbon atoms, and etoxadrol has three such centers of asymmetry and therefore four sets of enantiomers. The synthesis of affinity ligands from the pure stereoisomers could provide potential irreversible agents which might be able to stereoselectively interact with their binding sites. We established, using single-crystal x-ray crystallography, that dexoxadrol had *S,S* stereochemistry at its two chiral centers, and etoxadrol displayed *S,S,S* stereochemistry at its three chiral carbon atoms. With these data, and similar data on another molecule from a different diastereomeric mixture in the etoxadrol series, we were able to assign the stereochemistry of each of the molecules which we synthesized.

We succeeded in isolating all of the parent diastereomeric etoxadrol-like compounds, and synthesized the *S,S,S*-etoxadrol-meta-isothiocyanate, and its *R,R,R*-enantiomer (Thurkauf *et al.,* 1988a). The isothiocyanate in the dexoxadrol series proved to be unsuitable because of its instability. The *S,S,S*-etoxadrol-meta-isothiocyanate was found to be very stereoselective and was considerably more potent than metaphit as an acylating agent (table 1). The *R,R,R*-enantiomer and the *S,S,R*-epimer were both more than 20-fold less potent *in vitro* and were inactive *in vivo.*

In an attempt to obtain an even more potent affinity ligand, our third irreversible ligand was based on MK-801, one of the most potent PCP-like drugs. We have now synthesized two MK-801 isothiocyanates, one of which has proven to be more potent (table 1) and more selective in its interaction with the PCP binding sites than metaphit or etoxadrol-meta-isothiocyanate. That is, it irreversibly blocks half of the binding sites labeled by [^{3}H]MK801 at a 100 nM concentration (table 1) and it appears to interact minimally with the dopaminergic reuptake system.

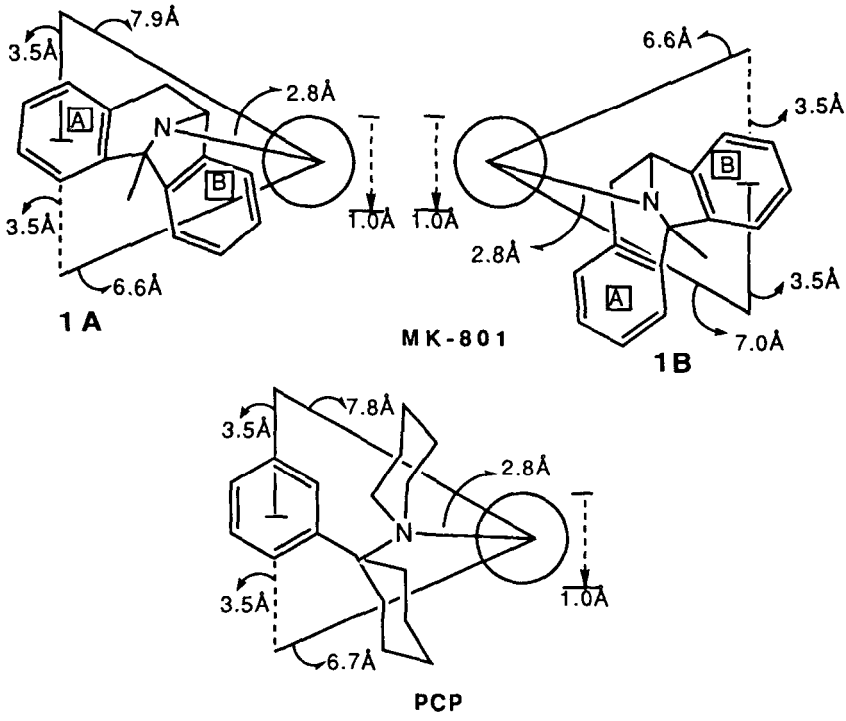
TABLE 1. Three generations of electrophilic affinity ligands for PCP binding sites

Ligand	50% Acylation (Irreversible) (μM)	~Ki (μM) (Reversible - Displacement of [^{3}H]MK-801)
Metaphit	10	0.5
Etoxadrol-meta-NCS	0.26	0.1
MK-801 NCS	0.1	0.04

Since PCP, etoxadrol, and MK-801 exert similar *in vitro* and *in vivo* effects, it is important to denote a pharmacophore, the minimal common structural features in 3-dimensional space which could cause those pharmacological effects. This has been determined for PCP-like compounds by F. I. Carroll and S. W. Mascarella, using computer-assisted molecular modeling, with Tripos Sybyl software (Thurkauf *et al.,* 1988b). A pictorial representation of that pharmacophore is shown for PCP in table 2, as we redetermined it with a Silicon Graphics 4D70GT minicomputer and the Polygen Quanta and CHARMm software. An area of 3-dimensional space required for interaction of the receptor with a PCP-like ligand is delineated by the 1Å, sphere. This is determined by drawing a spatial vector 2.8Å in the direction of the lone-pair electrons on the nitrogen atom. The distance from the aromatic ring and the 3-dimensional space occupied by the aromatic ring is obtained by a vector drawn 3.5Å above and below the centroid of the aromatic ring and subsequent triangulation to the point in the 1Å, sphere. Thus, the area of space occupied by the aromatic ring of PCP is marked by a distance of 6.7Å and 7.8Å to the sphere and the angular relationship which can be obtained by the triangulation.

Subsequently, Leeson *et al.* (1990) published their pharmacophore for MK-801 and related derivatives. We have recalculated the pharmacophore for MK-801 and PCP using Polygen Quanta and CHARMm software (table 2). Leeson and his colleagues noted that MK-801's ring B should be utilized for overlap with PCP's aromatic ring. As noted in table 2, considerably better overlap is obtainable using ring A of MK-801 (RMS displacement = 0.069 using the overlap of ring A in MK-801 with PCP and 0.281 for overlap of ring B in MK-801 with PCP). However, the data presented by Leeson *et al.* (1990) indicate that only the fit to ring B is possible, which would lead to the presumption that a more precise fit is not necessarily the most important parameter which must be considered with these molecules. The RMS displacement or fit of the two molecules was determined using three points, the centroid to the aromatic ring, a dummy atom 3.5Å above or below the centroid to the aromatic ring, and a 1.0Å sphere determined by a spatial vector extended 2.8Å from the nitrogen atom in the direction of the lone-pair electrons on the nitrogen atom. The pharmacophore shown for PCP,

TABLE 2. Comparison of minimum energy conformers of MK-801 and PCP using computer-assisted molecular modeling



	Overlap[a] Using A-Ring	Overlap[a] Using B-Ring
Sum Displacement^2	0.014	0.237
RMS Displacement	0.069	0.281
Average Displacement	0.062	0.272
Maximum Displacement	0.087	0.344
Distance between N atoms (Å)	1.17	0.42

[a]3-Point overlap between corresponding areas in energy-minimized conformer of MK-801 and PCP (in Å)including: 1) centroid in aromatic ring A (1A) or B (1B) in MK-801; 2) dummy atom representing 1.0Å sphere as determined by spatial vector extended 2.8Å from N atom in direction of lone-pair electrons; 3) dummy atom 3.5Å above or below centroid of aromatic ring.

when turned 180°, can be overlapped with 1B in table 2. The nitrogen atoms are very close in space when 1B and PCP are overlapped (table 2), and are considerably further apart in the overlap of 1A and PCP (0.42Å vs. 1.17Å respectively). Similarly, etoxadrol has been compared with PCP (Thurkauf *et al.,* 1988b). Indeed, all three molecules have common features in 3-dimensional space. The triangulated distances between

the aromatic ring and the 1.0Å sphere, which represents the receptor interaction site are similar but the aromatic ring is discernably closer to the l.0Å sphere in MK-801 (table 2, 1B) than in PCP or etoxadrol. That closer distance was noted by Leeson and his colleagues to be critically important for its increased potency.

In conclusion, we have prepared three generations of electrophilic affinity ligands with increasing potency and selectivity for the PCP binding sites, and we have utilized the PCP pharmacophore to indicate pertinent relationships which determine the interaction of their parent molecules with a binding site. We have found that this pharmacophore allows an area in 3-dimensional space for interaction with a part of the NMDA/glutamate receptor complex and, following the work of Leeson *et al.,* an area in a 180° direction to the 1Å sphere which is disallowed. It is conceivable that the space forbidden for the NMDA/glutamate receptor complex could pertain to an area for interaction with the binding site in the dopamine transporter complex. We have observed that most known PCP-like ligands can theoretically interact with either area. The two areas are depicted by the 1Å spheres in 1Å and 1B in table 2 which are 180° apart from each other. MK-801 appears to interact much less potently with the dopaminergic site than with the binding site in ion channels regulated by the NMDA/glutamate receptor, and presumably must interact as shown in 1B of table 2. The possibility that some PCP-like ligands could interact in two different modes with binding sites renders hopeful the eventual separation of anticonvulsant or neuroprotective actions from the side-effects shown by PCP-like drugs, if these side-effects are mediated by a binding site other than the NMDA/glutamate receptor complex.

REFERENCES - Available on request.

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The Cannabinoid Receptor-Pharmacologic Identification, Anatomical Localization and Cloning

M.R. Johnson, K.C. Rice, A. Howlett, L.S. Melvin and M. Herkenham

INTRODUCTION

The medicinal chemists search for the ideal analgesic has occupied thousands of scientists over the past century. Much of this research has focused on synthetic and endogenous opiates (Johnson and Milne 1981). We sought a structurally and mechanistically distinct approach to the problem when we entered the field in the 1970's. One of the areas we followed closely at the time involved the cannabinoids. Various preparations of Cannabis sativia have been used for a variety of social and medicinal purposes including the relief of pain (Lemberger 1980; Segal 1986). The availability of the pure psychoactive component, Δ¦9-THC, allowed for more definitive anaigetic studies (Mechoulam 1986). It wasn't until 1974, however, that evidence of structurally dissociable analgesia was presented by Wilson and May.

MAY - WILSON HYPOTHESIS

Wilson and May proposed that metabolic activation of Δ 9-THC yields the analgetically more active 11hydroxy- 9-THC. While others had proposed this before, May and Wilson reasoned that the synthesis of Δ 9-nor- Δ 9-THC, which cannot be converted to an 11-hydroxy metabolite, provided a means of examining the role of II-hydroxylation in the pharmacological make up of Δ 9-THC (Wilson and May 1974). They found that the O-nor derivatives were analgetically inactive suggesting that the active analgetic forms may be the II-hydroxy intermediates (Wilson and May 1975). More importantly, however, they discovered that HHC, a synthetic intermediate, possessed analgetic activity nearly equal to morphine in the hot plate test (Wilson and May 1976).

MEDICINAL CHEMICAL CONCEPTUALIZATION OF THE CANNABINOID RECEPTOR

Based on the clue from Wilson and May that analgetic activity is a structurally dissociable feature of the cannabinoid molecule and relying on structural insights from the prostaglandin overlap hypothesis (Johnson and Milne 1980A; Milne and Johnson 1981) we proposed a three point receptor interaction for producing analgesia. In the course of our work to optimize analgesia based on this hypothesis, we examined modifications of the side chain, the phenolic moiety, and, most significantly structures that lack the benzopyran ring present in THC and

HHC (Johnson and Melvin 1966). In our initial studies, we found that a new grouping, 5-phenyl-2 pentyloxy side chain, elaborates a unique lipophilic region. This more hydrophobic compound possessed 10-50 times the analgetic activity of HHC (Johnson et al., 1981). Introduction of a weakly basic nitrogen at C-5 and deletion of the axial methyl group in the B ring, two structural changes forbidden by traditional cannabinoid SAR, resulted in a unique family of benzoguinolines with potent analgetic activity. The prototype of this series, levonantradol exhibits potent and enantiospecific analgetic and antiemetic activity (Johnson and Milne 1980).

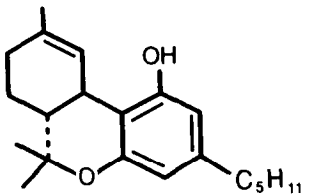
Synthesis of the first AC-bicyclic cannabinoid followed from our observation that the pyran ring of HHC was not a requirement of this structural class for expression of biological activity (Johnson et al., 1982). Together, this observation and speculation about the necessity of the lipophilic side chain, phenol and alcohol for biological activity was confirmed with the synthesis of a simple phenyl cyclohexanol. This compound was shown to possess a biological profile and potency similar to HHC (Melvin et al., 1964). Such activity and potency was highly dependent on the side chain but was not as influenced by substitution in the cyclohexanol ring. Further structural elaboration and development of SAR led to a more potent bicyclic derivative CP-55,940 (Melvin et al., 1983A, Johnson and Melvin 1986). Structural optimization of this derivative was achieved by incorporation of the hydroxypropyl chain as a new fused ring resulting in the synthesis of the first ACD-tricyclic cannabinoid, CP-55,244 (Melvin et al., 1983B). This rigid molecule again exhibits an increase in potency and significantly shows total enantiospecificity in favor of the levorotatory enantiomer. These findings of exceptional potency in the microgram/kilogram range, retained spectra of activity despite extremes of structural elaboration of simplification, regio-, stereo- and enantiospecificity of action and indirect effects of multiple biochemical systems mandated a novel site of action involving a distinct neurotransmitter system and lead us to intensify our search for a cannabinoid receptor site.

BIOCHEMISTRY OF THE CANNABINOID RECEPTOR

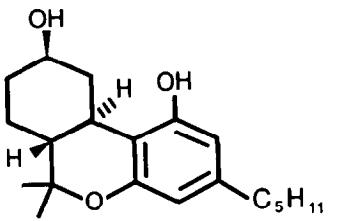
In vitro studies using cultured neuroblastoma cells, neuroblastoma x glioma hybrid cells and brain slice preparations demonstrated that one cellular action of cannabinoid drugs is the reversible inhibition of cAMP production (Howlett 1985; Devane et al., 1966). Further studies demonstrated that psychoactive cannabinoid compounds inhibit adenylate cyclase activity via the G protein, Gi, suggesting a receptor-coupled mechanism (Howlett 1985: Howlett et al., 1966). The inhibition of adenylate cyclase by natural and synthetic cannabinoids is enantioselective, and the pharmacological profile for regulation of adenylate cyclase correlates well with that observed for several animal models of cannabinoid activity (Howlett et al., 1988). These findings suggest that the receptor characterized in cell lines is the same as the receptor responsible for certain cannabinoid actions in the CNS.

LOCALIZATION AND FUNCTIONAL SIGNIFICANCE OF CANNABINOID RECEPTORS IN THE BRAIN

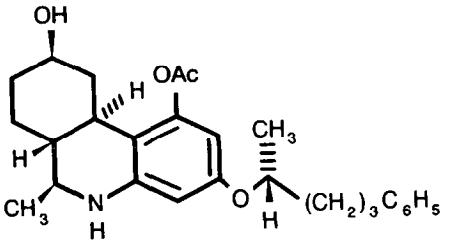
Synthesis of a potent radiolabeled ligand, [^{3}H]CP-55940, led to the development of membrane homogenate and tissue section binding assays for the characterization and localization of the cannabinoid receptor in brain (Devane et al., 1966; Herkenham et al., 1990 and 1991). The [^{3}H]CP-55940 binding site is saturable, has high affinity and enantioselectivity for agonist ligands, and exhibits characteristics expected for a neuromodulator receptor associated with a G protein.



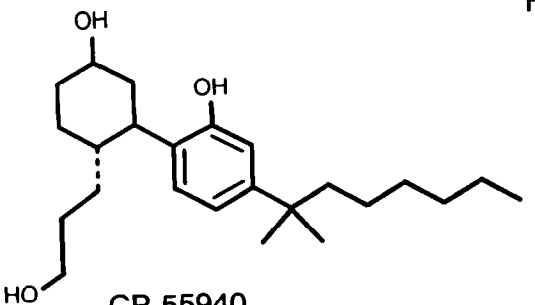
Δ^9 - THC



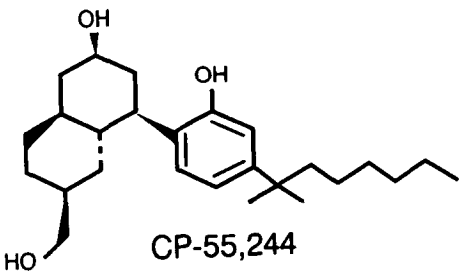
HHC



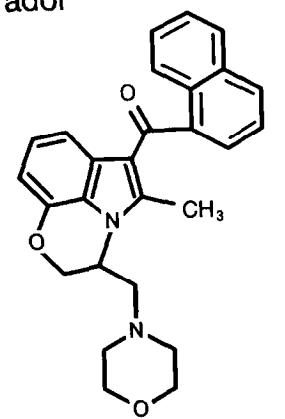
Levonantradol



CP-55940



CP-55,244



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The relative potencies with which cannabinoid compounds inhibit [^{3}H]CP-55940 binding parallels the abilities of these compounds to produce analgetic effects in animals. A similar structure activity profiles exists for receptor binding and the regulation of adenylate cyclase in vitro (Devane et al., 1988; Herkenham et al., 1990 and 1991). High affinity ligands for a variety of neurotransmitter. neuromodulator and hormonal classes, including adrenergic, cholinergic; dopaminergic, serotonergic, opioid, GABAergic, glutamatergic, steroid, and prostanoid agonists and antagonists, fail to displace [3H]CP-55940 binding at this receptor. [^{3}H]CP-55940 binding is also found in the nervous systems of lower vertebrate species. The cannabinoid receptor appears to be conserved across species in that the K_d, values are similar, and the non-hydrolysable GTP analog guanylyl-ß-γ-imidodiphosphate reduces the binding.

NEUROANATOMY OF THE CANNABINOID RECEPTOR

Autoradiography using [^{3}H]CP-55940 reveals a heterogeneous distribution of cannabinoid receptors throughout the brain (Herkenham et al., 1990 and 1991). A unique pattern of binding is conserved across several mammalian species, including humans, with the greatest abundance of [^{3}H]CP-55940 binding sites in the basal ganglia, hippocampus and cerebellum (Herkenham et al., 1990 and 1991). In rats, monkeys and humans, the greatest density of cannabinoid receptors is observed in the globus pallidus, the substantia nigra pars reticulate, the molecular layer of the dentate gyrus of the hippocampus, and the cerebellar molecular layer. Receptors are also dense in the cerebral cortex, the neostriatum and in the remainder of the hippocampal formation. Comparatively little binding is observed in the brain stem and spinal cord.

Current work is addressing the roles of cannabinoid receptors in receptor-rich regions such as the basal ganglia. There is evidence implicating the basal ganglia in the cataleptic response to cannabinoids seen in rodents and in the potentiation by Δ9-THC and levonantradol of reserpine-induced hypokinesia in a model of Parkinson's disease in rats and primates. Cannabinoid drugs attenuate the D_1-dopaminergic stimulation of cAMP production as do D_2-dopaminergic agonists and opioid agonists in striatal slices. This suggests that opioid and cannabinoid receptors may be co-localized on the same population of cells that respond to dopamine. Anatomical evidence for this comes from the observation that cannabinoid, D_1 and D_2-dopaminergic receptors in the striatum, globus pallidus and substantia nigra pars reticulata are lost following ibotinic acid lesions of the striatum.

Desacetyllevonantradol regulates cAMP production in brain regions exhibiting high receptor binding density. Populations of cells in the cortex, hippocampus and cerebellum. in which the ß-adrenergic agonist isoproterenol or vasoactive intestinal peptide stimulate cAMP production, are differentially regulated by cannabinoid drugs.

AFFINITY LIGANDS

Studies have been initiated to describe the biological activity of the isothiocyanate derivatives of CP-55,244 (Richardson et al., 1990). Positioning the isothiocyanate moiety as an extension of the hydroxymethyl group in ring D resulted in a loss of binding and functional activity compared with the parent compound. Placement of the isothiocyanate at the alkyl chain extending from the aromatic A ring resulted in a compound having the same affinity for the receptor and ability to

inhibit adenylate cyclase. Further studies will determine the abilities of these ligands to covalently attach to the receptor. Subsequent studies can then be performed to define the amino acids on the receptor which are targets for these potential affinity ligands.

CLONING OF THE GENE FOR THE CANNABINOID RECEPTOR

The recent report of the cloning of the cannabinoid receptor gene by Lisa Matsuda working in Tom Sonner's laboratory at the NIMH (Matsuda et al., 1990) has added the cannabinoid receptor to the increasing number of G-protein-coupled receptors for which the amino acid sequence is now known. These investigators isolated a DNA clone, SKR6, from a rat cerebral cortex library, using an oligonucleotide probe derived from the sequence of a receptor from this superfamily of homologous receptor types. This strategy to obtain novel receptor subtypes through molecular biological techniques has led to the concept of "reverse pharmacology", i.e., the cloning and expression of genes whose products remain to be matched with receptors for physiological responses. Thus, the approach of "reverse pharmacology" ultimately led to the successful identification of the SKR6 gene product as the cannabinoid receptor which had previously been well characterized by classical pharmacological approaches..

PROSPECTIVE CONCLUSIONS

Much work is left to be done to unravel and utilize our knowledge of cannabinoids and how they work. The tools that allowed us to discover the receptor and pinpoint neuro-anatomical distributions coupled with the design of new tools (e.g., affinity ligands) will help us answer many other questions.

There are at least four major areas where we look for progress in the coming decades. The discovery of physiologically relevant receptor subtypes will aid the ultimate goal of separating the traditional activities of cannabinoids (specific agonists) in search of therapeutically useful drugs. The discovery of an antagonist will be a key event both as a research tool and to combat cannabis overdose. Recent findings of agonists and an antagonist in the aminoalkylindole (AAI) series gives us a hope that this goal will be attained soon. The third area ripe for new developments is the discovery of the endogenous ligand. Three separate groups (Howlett, Childress, and Mechoulam) are currently working on identifying the endogenous cannabinoid(s). Finally, new areas will evolve from this research, e.g., the discovery of peripheral cannabinoid receptors and new pain mechanisms.

REFERENCES: Available from author upon request

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Opiate Total Synthesis and Contemporary NIDDK Analgesic Research-Natural and Unnatural Ligands for Computed Tomography Imaging of Receptors in Conscious Humans, Implications for Future Advances in the Neurosciences

K.C. Rice

INTRODUCTION

The contemporary analgesic program in the Laboratory of Medicinal Chemistry (LMC), NIDDK, has been strongly influenced by events which occurred in the mid 1970's including (a) the discovery of saturable, high affinity, enantioselective receptors in the CNS which were shown to mediate the effects of a wide spectrum of analgesics and their antagonists, (b) the identification of the endorphins as endogenous opioid peptide ligands which subserve these receptors, and (c) the subsequent discovery of opioid receptor subtypes. In order to determine the structure and function of the opioid receptor-endorphin system in normal, drug altered and pathological states, new drugs were needed as research tools. Our design and development of opioid receptor subtype specific affinity labels, together with the design and synthesis of ligands and other drugs for computed tomography imaging of opioid receptors in living animals and conscious humans, were initiated for these purposes. Another major research area which became part of the current LMC program was the development of a practical opiate total synthesis. This program partly emanated from the discovery of the opioid receptor endorphin system and the need for the unnatural opioid enantiomers as new research tools but was also prompted by the severe opium shortage on the world market during 1973-1975. This shortage required release of about half of the U.S. strategic materials reserves of opium for the domestic production of medical narcotics and their antagonists (Schwartz, 1980). The possible consequences of complete reliance on foreign sources of an essential plant product and the need for methodology which could be utilized for independent production of these drugs in time of national emergency or otherwise strongly influenced our research program. As briefly described below, major advances have been made in several research areas, a practical synthetic route to natural and unnatural opium derived narcotics and antagonists now known as the NIH Opiate Total Synthesis has been obtained, and quite fruitful results have resulted from studies in the affinity label (de Costa et al., 1990) and imaging agent areas. As will be seen, these areas are largely complementary and overlapping.

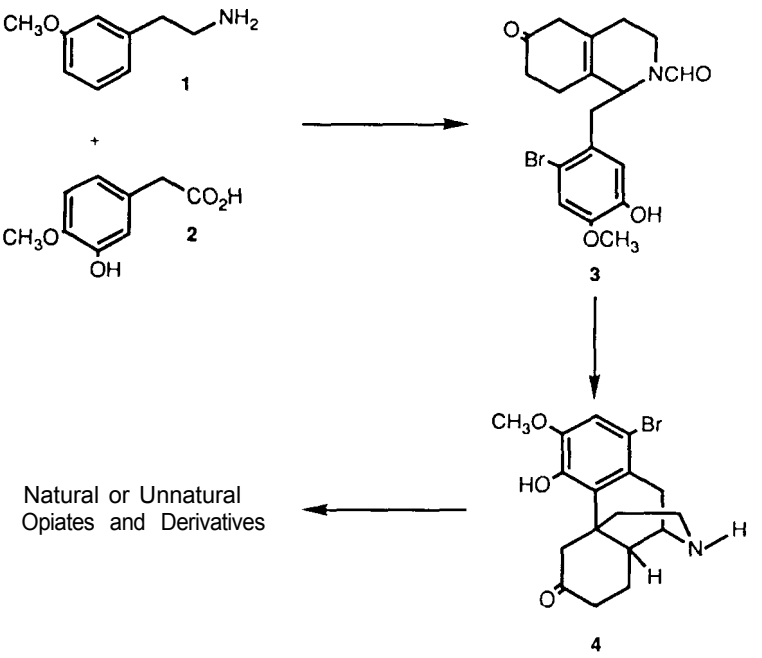
DEVELOPMENT OF THE NIH OPIATE TOTAL SYNTHESIS

The program for synthesis of the unnatural (+)-enantiomer of morphine as a receptor probe at NIH was conceptualized in 1975 and its origin has been described (Rice, 1985). This goal was extended at NIH to include unnatural (+)-naloxone and other drugs in this class. At that time, a number of synthetic routes

to appropriate intermediates had been described, including the brilliant first synthesis of morphine (Gates and Tschudi, 1952, 1956), however none seemed suitable for efficient laboratory synthesis of these drugs or their commercial production. Thus, the rare alkaloid sinomenine which had been previously converted to (+)-morphine (Goto and Yamamoto, 1954) was chosen as starting material to be certain of providing the target compounds in a timely manner, which was not guaranteed by attempts to develop a practical total synthesis. Work was first begun at NIH on March 2, 1976, in the Section on Medicinal Chemistry then under the direction of Dr. E. L. May, toward synthesis of the unnatural (+)-enantiomers of codeine, morphine, and naloxone (the latter via unnatural thebaine and oxymorphone). This program provided gram amounts of these compounds by improvements in the original routes (Iijima et al., 1977, 1978a, 1978b) and the introduction of efficient O-demethylation for conversion of codeine to morphine and oxycodone to oxymorphone (Rice, 1977; Iijima et al., 1978a). Pharmacological and biochemical studies soon revealed these drugs to be extremely versatile and effective tools for detecting receptor mediated effects of the active enantiomers as the unnatural enantiomers were found to have 10^3-10^4 fold lower affinity for opioid receptors (Rice, 1985).

By the late 1970's, however, sinomenine had become unavailable as starting material for further synthesis of unnatural opiates and derivatives, and efforts to develop a practical phenolic oxidation approach to the opium alkaloids had proven unsuccessful at NIH and elsewhere. As most of our stocks of the unnatural isomers had been depleted, it was clear that a practical opiate total synthesis was needed to continue research in the area, and to make available previously synthesized and numerous new unnatural isomers in multigram amounts. Requirements established by the writer were that such a synthetic route should provide 100+ gram quantities of correctly oxygenated, chiral morphinan intermediates per batch beginning on a 1 mole scale in the laboratory. The route should also be as short and simple as possible, utilize economical starting materials, be clearly amenable to scaleup for commercial production of any required quantity of medical narcotics and their antagonists, and provide optically pure opiates and derivatives. The latter consideration was critically important in the synthesis of unnatural enantiomers as research tools and potential drugs. Although substantial advances had been made by the Delft group (Lie et al., 1979) which were later extended (Crabbendam et al., 1983), it was thought that alternate methodology would be required to meet the above requirements.

Such a route was successfully developed at NIH by the writer and his associates and utilized a modified Grewe approach for direct formation of the morphinan carbon-nitrogen skeleton with appropriate functionalization for conversion to the morphine alkaloids and derivatives (Rice, 1980, 1981, 1985). The key step is the triflic acid catalyzed cyclization of the octahydroisoquinoline 3 to the correctly oxygenated 1-bromonordihydrothebainone ring system as in 4. It is now possible to synthesize either enantiomer of morphine, codeine and thebaine from amine 1 and acid 2 in about 25% overall yield with only 6-8 isolated (filtered and washed) intermediates. Since the entire spectrum of medically valuable opium derived narcotics and their antagonists consists of the (-)-enantiomers of these three alkaloids and their transformation products, the NIH Opiate Total Synthesis provides practical access to this entire group of drugs. Acid 2 is now easily prepared (Rice, 1991) from the inexpensive 4-methoxy derivative and has been synthesized in 84% yield on a 6.0 mole scale in the laboratory. At this time, hectogram quantities of both enantiomers of all medically useful opium derived analgesics can be easily and relatively inexpensively prepared in the laboratory.

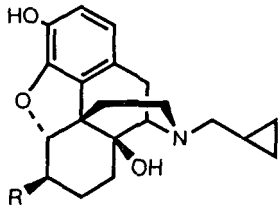


DEVELOPMENT OF AGENTS FOR IMAGING OPIOID RECEPTORS IN THE LIVING BRAIN BY COMPUTED TOMOGRAPHY: (-)-CYCLOFOXY AND (-)-IOXY

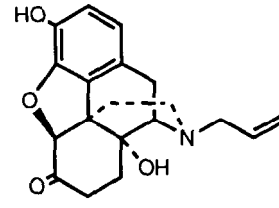
Positron emission tomography (PET) and single photon emission computed tomography (SPECT) scanning are at present the only techniques available for real time measurement of drug receptor occupancy in the living brain. These techniques are related and rely on incorporation of a suitable radioisotope into an appropriate ligand, administration to the subject, signal detection and processing using the principles of computed tomography, and measurement of total and nonspecific binding in order to estimate specific binding (Bmax) and receptor affinity (Kd). PET and SPECT can thus provide information on biochemical function of opioid and other receptors in conscious humans in contrast to magnetic resonance imaging (MRI) and computerized axial tomography (CAT) scanning which provide anatomical details of the tissue under study. However, major cost and technical constraints are imposed on PET scanning by the short half life (< 2hr) of all isotopes suitable for drug labeling as such isotopes must be produced in a cyclotron immediately prior to use. We planned to develop a ligand which could be used to quantitate opioid receptors in the normal conscious human and then to study patient populations with disorders possibly linked to dysfunction of the opioid receptor system. With this approach, we hoped to develop clinical correlates of receptor dysfunction with disease states.

Our studies have now resulted in the identification of [^{18}F]cyclofoxy, [^{18}F)(-)-3,14-dihydroxy-4,5-epoxy-6 β·-fluoro-l7-cyclopropylmethylmorphinan, as a near ideal PET ligand. Cyclofoxy was initally synthesized by reduction of naltrexone to 6α-naltrexol, selective acetylation of the phenolic hydroxyl, conversion to the 6α-triflate, introduction of a 6β-fluoro substituent by displacement of the triflate group and cleavage of the acetate function (Burke et al., 1985). Cyclofoxy is a pure narcotic antagonist and thus exhibits no

pharmacological effect (Aceto et al., 1987). Autoradiographic studies with (^{3}H]cyclofoxy in the rat revealed that it reversibly labeled opiate receptors in opioid receptor rich brain regions previously identified in independent studies and that this binding could be prevented and displaced by unlabeled (-)-naloxone (Ostrowski et al., 1987). Cyclofoxy readily enters the brain, is free of interfering metabolites, and shows affinities (Rothman et al., 1988) for mu and kappa receptors in the 1 nM range. Analysis of transport, metabolism and binding (Kawai et al., 1990a, 1990b; Rothman et al., 1987, 1988; Sawada et al., 1991) showed that cyclofoxy Bmax and Kd values obtained from in vitro vs. in vivo studies were in good agreement. In 1984, we reported the first definitive images of opioid receptor occupancy in the living primate brain (Pert et al., 1984). These studies with [^{18}F]cyclofoxy (administered as the 3-acetate prodrug) in the living baboon showed a rapid, reversible accumulation in the caudate nucleus and thalamus which could be displaced by a low, pharmacologically relevant dose of (-)-naloxone. The same dose of the unnatural (+)-isomer available by the NIH Opiate Total Synthesis had no effect, strongly emphasizing the value of the unnatural isomers in identifying opioid receptor binding. Studies are now in progress to quantitate opioid receptor Kd and Bmax in discrete human brain regions as a possible step toward gaining insight into various little-understood psychiatric disorders which may be linked to the opioid receptor endorphin system. Such quantitation may also be useful in following the effects of drug therapy in these patients. Ex vivo autoradiographic studies with [^{3}H]cyclofoxy in the golden hamster have indicated that mating produces elevation of endogenous opioids strongly suggesting that [^{18}F]cyclofoxy may find utility as a diagnostic agent for disorders involving the opioid receptor endorphin system (Ostrowski et al., 1986). Recently, studies in LMC have identified IOXY, the iodo analog of cyclofoxy, as a ligand potentially suitable for imaging of opioid receptors by SPECT scanning (de Costa et al., 1991).



(-)-Cyclofoxy, R = F
(-)-Ioxy, R = I



(+)-Naloxone

The binding profile of [^{125}1]10XY was shown to resemble that of cyclofoxy from in vitro and autoradiographic studies. SPECT provides data similar to that obtained by PET and does not require an on-site cyclotron. This technique may ultimately be much more widely used than PET. Studies are now in progress to assess the utility of IOXY as a tool for the SPECT study of the opioid receptor endorphin system.

OPPORTUNITIES FOR FUTURE ADVANCES IN THE NEUROSCIENCES

Progress in our group has been largely due to a potent synergism between chemical and biological investigators. Advances in neuroscience related to drug abuse research can be expected to accelerate due to intensification of such collaborative research in the future. Such advances will include much a more detailed characterization of the structure and function of opioid receptor subtypes

and the development of subtype specific agonists and antagonists required to activate/deactivate only the particular subsite in question. The cloning and elucidation of the exact molecular structure of each subtype and its combining site together with modern computer assisted molecular modeling will no doubt facilitate the development of such agents. The identification of the precise mechanism of opioid tolerance and dependence will be a major advance which should lead to dramatic new treatments for, and strategies in, the prevention of narcotic abuse. Additional study will provide further insight into the role of opioid receptors in the regulation of immune function and will lead to the discovery of new agents for manipulation of the immune system. The development and utilization of new receptor subtype selective PET and SPECT ligands will be required to aid in understanding various disorders which involve the opioid receptor system and in the development of clinical correlates between opiate receptor dysfunction and disease states. Finally, it has recently been recognized (Corda, 1990) that numerous proteins and enzymes involved in the signal transduction occurring after drug-receptor binding have a number of different forms (isoforms). Since not all cells produce all isoforms, it should be possible to design new agents which stimulate or suppress signal transduction by acting on one or more isoforms in some populations of cells but not others thereby altering the biological response resulting from drug-receptor binding. Identification of such agents and administration in combination with agonists/antagonists of exquisite specificity for opioid receptor subtypes will provide dramatic advances in the pharmacotherapy of CNS disorders. Thus, advances in our understanding of opioid receptor signal transduction mechanisms will present many opportunities for rationally designed pharmacological intervention in these mechanisms and at the drug-receptor complex. These are only some of the challenges which now await investigators in the Decade of the Brain and beyond. Most assuredly, each of these issues will ultimately be resolved and their mechanisms explained in the language of organic chemistry with astounding ramifications in the practice of medicine.

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Overview of Opioid Medicinal Chemistry

J.W. Lewis

When Dr. Harris and I talked about how we should handle these concluding remarks we realised that to try to divide our remarks into chemistry and pharmacology would be both difficult and undesirable so we decided we would make the division between the opioids and the rest. I am going to deal with the opioids and Dr. Harris will cover those other areas of neuroscience which we've heard about in the latter part of this symposium.

Amongst today's contributors I must recognise three medicinal chemists who are my seniors in the opioid field in that they were all well established when I arrived at Reckitt and Colman in 1965 to work with Ken Bentley. The first is Dr. May and of course it is remarkable that his involvement spans the whole of the fifty years we are celebrating. Not only that but his major achievements in the field have continued throughout the period.

The second is Dr. Archer, who, together with the third member of this illustrious trio, Dr. Harris, gave us pentazocine and cyclazocine. He has continued to make substantial contributions to the field whilst keeping his scientific wings well spread since his return to academia.

As for my co-summariser, some may not recognise him as a medicinal chemist, preferring to regard him as a pharmacologist. But since he has high affinity for both fields I think he well fits the category of mixed pharmacologist-medicinal chemist. I am sure that is a more appropriate classification than partial pharmacologist or partial medicinal chemist!

What we have heard today is more than the history of fields in which a pre-eminent group of medicinal chemists have had a major influence. We have been able to follow the evolution of the role of medicinal chemists in neuroscience research starting in simple partnership with pharmacologists to what we have seen now is a genuine multidisciplinary relationship involving chemists, both medicinal and theoretical, with biochemists, pharmacologists, geneticists, neurologists,

psychiatrists and physicians and others where each has an important part to play. Dr. Rice has given us not only a story of achievements over the last fifteen years but a vision for the future of even greater promise.

But it all started with Lyndon Small in the era when medicinal chemistry in the opioid field was still predominantly concerned with modification of the epoxymorphinan structure using the natural opiates as sources. As you will appreciate organic synthesis in those days was a discipline very different from what it is today. Not only were there no sophisticated reagents for effecting specific synthetic transformations but determination of structure was a painful, labour-intensive process based to a large extent on chemical degradation and identification of fragments, often involving unequivocal synthesis.

The structure of metopon, one of the notable achievements of Lyndon Small's work, was uncertain between 1936 when it was synthesised and 1953 when Gilbert Stork published an unequivocal synthesis of 7-methyldihydrocodeinone and showed that it was not the methyldihydrocodeinone prepared by 0-methylation of metopon. During that period Small published several papers of well-reasoned chemical investigation which nevertheless still left open the question of whether metopon had the 5-methyl or 7-methyl structure.

Nowadays this would have been regarded as a routine problem and an NMR spectrum would have given the solution in a relatively few minutes. But those were the days when technique in organic chemistry was based on the traditional empirical skills of purification both of starting materials to ensure clean reactions and of products. I'm told that samples from Small's work over fifty years ago even now pass today's criteria of purity which says a lot for the technique of the chemists but also something about the stability of the compounds.

At this stage I will show my only transparency. This shows in simple form the course of the fifty years of opioid medicinal chemistry showing the introduction of the various chemical classes and the target pharmacological profiles which they were aiming for.

The epoxymorphinans not only had been studied for over a century before 1940 but they have continued to be a major, perhaps the major, structural class for contemporary investigation. Until the mid fifties medicinal chemists had no biological rationale on which to base the design of new opioids of greater safety and lower abuse potential than morphine.

The major new chemical classes of opioids discovered during this period were the phenylpiperidines, notably mepiridine, and the phenylpropylamines represented by methadone. Everette May made one of his earliest significant contributions to the field in his detailed study of the reduction products of methadone. Out of this work came the development of LAAM, l-α--acetylmethadol as perhaps a superior alternative to methadone for the treatment of opiate dependence.
The major event of the mid fifties was the discovery by that nalorphine was an anlgesic in man and had very low abuse potential. Since it had earlier been shown to be an antagonist of morphine's effects we had the birth of the concept of the mixed agonist-antagonist or antagonist analgesic.

On the chemistry side the field was opened up by Everette's pioneering work on the synthesis of the benzomorphans which became a most rewarding area in which to search for mixed agonist-antagonists.

The concept of the mixed agonist antagonist was expressed by Bill Martin as receptor dualism recognising the existence of two receptors which he later designated μ and K. The simplistic idea of a mixed agonist-antagonist was, in today's terminology, a μ-antagonist,K-agonist. We now know that what we were actually dealing with were mixed u/K-partial agonists. At that time the term partial agonist was used only to describe μ partial agonists with activity at K-receptors limited by either low affinity or very low intrinsic activity.

The trouble with the so called partial agonists was that when evaluated in prisoner addicts at Lexington, they were clearly identified as opiates by post addicts and substituted for morphine in withdrawn, morphine-dependent addicts. The successful mixed agonist antagonists showed opiate effects only at low doses. At high doses they were dysphoric which we now associate with K-agonism.

One of the problems for the medicinal chemist involved in these programs was the lack of reliable animal models to identify analgesics with desirable profiles, the problem being that they had to have relatively low intrinsic activity which meant that they were active only in tests where the nociceptive stimulus was of low intensity.

The search for mixed agonist-antagonists was a preoccupation of the drug companies in the sixties and early seventies. As you know several were developed and four, or is it five, are on the market today. I'm sorry to have to say that their impact has been less than sensational. In fact if we judge our research efforts over the last fifty years in terms of therapeutic practice, you'd have to say that we haven't been all that

successful. For the truth is that in 1991 the most prescribed opioid is still codeine and the fastest growing is morphine in the form of the sustained-release oral tablet.

Of course the seventies was a momentous decade in opioid research when evidence for the existence of opioid receptors was obtained and isolation of the natural peptide ligands was achieved. For the medicinal chemist the challenge became the synthesis of specific ligands for the receptor types.

In terms of analgesics, the target was a specific K-agonist in the hope that the unwanted effects associated with non-specific opioids having some K-activity would be missing in the specific compounds particularly if they had no affinity for σ receptors which is a characteristic of the non-opioid (+)-enantiomers of the benzomorphans.

Following the discovery of selective K-agonism in trans cyclohexane-1,2-diamine derivatives of which U50488 was the first example, a whole range of more specific and more potent analogs have been announced by several pharmaceutical companies. But we are still waiting for the first clinical analgesic to reward all this effort.

Medicinal chemists have also provided their colleagues in neuroscience with specific μ and δ-agonists. We have both peptide and non-peptide μ agonists but only peptide δ agonists. As far as I know no-one has yet come up with a specific non-peptide δ–agonist. In addition there are now available the whole range of non-peptide antagonists-μ,K and δ. The name of Phil Portoghese must be mentioned here as the designer and discoverer of naltrindole, the δ-antagonist, and norbinaltorphimine the K-antagonist.

The standard competitive selective μ antagonist is now cyprodime which was synthesised by Helmut Schmidhammer in a program of investigation of 14-hydroxymorphinan derivatives which he started at NIH during Arnold Brossi's time as Chief of the Medicinal Chemistry Section.

Valuable as these specific competitive ligands have proved to be, non-equilibrium or irreversible ligands as you've heard several times today have even greater utility in a number of different fields of neuroscience. Providing such ligands has been a notable achievement of the Laboratory of Medicinal Chemistry under Dr. Rice's leadership. You've heard about FIT and SUPERFIT and their use in the purification of δ-receptors. They've also given us specific irreversible ligands for μ and K-receptors. Use of the isothiocynanato-analog of

U50488 provided a demonstration of the likely heterogeneity of K-opioid receptors.

Once they got into the synthesis of irreversible ligands, Kenner Rice's group quickly found themselves diversifying out of opioids and into other classes of psychoactive drugs including the benzodiazepines. But I suppose the principal route out of the more traditional opioid fields has been the σ receptor which started as opioid with Bill Martin's work on SKF 10,047. The relationship of the original σ receptor to PCP and the PCP receptor is a story that still has several unwritten chapters. Brian de Costa and Arthur Jacobson have shown us today how medicinal chemists are contributing to the story and that the structural requirements for K- and σ activity are, in some ways, tantalisingly similar.

I want to round up this very brief summary where we began with the epoxymorphinans because they have continued to provide us medicinal chemists with structures to meet most of our targets. Several of us must be grateful that the biosynthesis of morphine goes through thebaine which provides us with so versatile a starting material.

It is comforting to know that we do not have to be totally reliant on natural sources for our morphine, codeine and thebaine, because Kenner's total synthesis could, I'm sure, be made commerical. To have worked out a practical total synthesis of the natural opiates, and perhaps of equal importance the non-natural enantiomers, is a great achievement of Kenner and his group which stands alongside the other important achievements of the laboratory during the past fifty years.

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DSM-IV Substance Use Disorders: The View from DSM-III-R

B.J. Rounsaville

NEW MANAGEMENT/NEW PROCESS

My perspective on the DSM-IV revision process has been one of an advisor to the Substance Use Disorders Committee, while my whole role in DSM-III-R had been as the Chair of the comparable committee. As an advisor I have been kept informed with the minutes of meetings and have been able to review position papers regarding proposed changes. The most striking difference between the DSM-III-R and DSM-IV effort is the scope of the work. DSM-III-R was seen as a relatively minor revision of DSM-III which was to take place over a compressed time schedule and without the conduct of formal field trials. In contrast, DSM-IV is seen as a major revision and it is guided by several principles that were present but not systematically practiced in earlier manuals: 1. because of the inherent problems attendant with frequent changes in criteria, the process is to be conservative with revisions made on the basis of empirical data rather than the consensus of experts, 2. to marshal the appropriate data, formal literature reviews have been prepared to inform decisions made about suggested changes, and 3. formal field trials and secondary analysis of relevant data sets are taking place to provide data which is not already available to guide the decision making process. What these three guidelines have translated into is a project of enormous size and complexity and the sheer amount of work and meetings has been breathtaking.

BIG ISSUES: DEJA VU ALL OVER AGAIN

What questions have engaged this group of energetic and eminent experts? In reading over the memos which review the key issues for the DSM-IV revisions of Psychoactive Substance Use Disorders, I have had the same sort of experience that I get when I attend class re-unions attended by old friends and enemies alike. When working on DSM-III-R, I had the sense of creating a reference work of biblical endurance and authority in which issues were at last settled. Now it appears that the appropriate medium for the criteria should have been newsprint (with the attendant remark that "yesterday's newspaper is tomorrow's toilet paper"). A partial list of key issues shared in the two revisions includes: 1. Dependence - What are the essential elements? How should indicators of neuroadaptation (tolerance/withdrawal) be rated in relation to psychological/behavioral symptoms? What role should social consequences of pathological drug use play in the diagnostic criteria? 2. Abuse/ Dependence relationship - If dependence is broadened to include psychological/behavioral symptoms and consequences, is abuse a (a) separate diagnosis, (b)

early phase of dependence, (c) a symptom of dependence and indistinguishable from it? 3. Common Criteria vs. Drug-Specific Criteria - Are the patterns of pathological use of different psychoactive substances sufficiently similar to justify a common set of criteria (addiction syndrome) across different substances or are the differences so great as to require substance-specific diagnoses in a manner analogous to the substance-specific organic mental disorders? 4. Severity - What is the most powerful, valid and efficient method to denote problem severity: global clinician rating, adding symptoms, creating weighted "symptom total, psychometric scoring? 5. Time Frame of Symptoms/Syndrome? Many symptoms represent -behaviors that occur-in discrete episodes (attempts to cut down or stop) while others represent behaviors that occur more pervasively over time(continued use despite problems). How long should any given symptom be required to last and how much temporal overlap in symptoms should be required? 6. In Remission - While many individuals appear to be able to put patterns of pathological drug use behind them with ease, others maintain a lifelong vulnerability to relapse. How long should a diagnosis be applied when substance use or symptoms ate no longer displayed? 7. Drug-induced Organic Disorders -How broad or narrow should-the listed-group of drug-induced organic disorders be made? 8. Dual Diagnosis - When symptoms of substance dependence and psychopathology co-occur what guidelines should be used to denote the relationship between the two types of disorders: a. as independent disorders, b. with a primary/secondary denotation, c. with psychopathology seen as drug induced?

LIMITATIONS OF A SCIENTIFIC NOSOLOGY

As we think about the DSM-IV revision process, I believe that it is important to attend to a wise report by Kendler (1990) in which he points out the limitations on the type of help that empirical findings can provide in choosing among options for structuring our nosology. While it is a desirable aim to base nosological revisions on empirical findings, data do not substitute for clinical wisdom and expert opinion because data are only interpretable on the basis of underlying hypothesis about the clinical phenomena under study. Kendler lists four key shortcomings of a strictly empirical approach to nosological decision making: a. Constructs Seldom Allow Critical Tests, b. Different Validators May Disagree. c. No Standards Exist for Determining When Diagnostic Subtypes Are Needed. and d. No Standards Exist For Determining the Relative Importance of Reliability as Contrasted With Validity. I will briefly describe how these four problems relate to issues currently under consideration in the DSM-IV revisions of the Substance Use Disorders section.

CONSTRUCTS SELDOM ALLOW CRITICAL TESTS. This problem refers to the fact that much disagreement about labeling and subtyping of mental disorders arises because different experts adhere to divergent theories about the processes underlying the observed symptoms and behaviors. In psychosocial sciences, observations seldom provide a critical test which can disprove propositions related to competing theories since the determinants of human behavior can not be completely characterized or controlled. An example from current DSM-IV thinking is provided by work on the Dependence Syndrome, a paradigm on which the DSM-III-R Substance Dependence criteria are based. According to the thinking underlying the Dependence Syndrome, three propositions are asserted: 1. Dependence Syndrome Elements are highly intercorrelated, 2. Dependence is not all or none but arrayed along a continuum of severity, and 3. Dependence severity is not directly related to

severity of substance related consequences (social, psychological, legal, occupational). Consistent with this third proposition, social consequences are not included as criteria for substance abuse or dependence in DSM-III-R. Rather, continued substance USE Despite Consequences are included in the criteria. The empirical test of the validity of proposition#3 has typically taken the form of evaluating the relative strength of intercorrelations between dependence syndrome elements and the relationship between those elements and measures of social consequences. However, what does it prove if the dependence syndrome elements are highly correlated with social consequences? Does this mean that social consequences should be made part of the DSM-IV criteria because they are correlated with Dependence? I believe not. The reason is that the separation of consequences from the diagnostic criteria for DSM-III-R has more to do with CONCEPTUAL rather than empirical issues. Social consequences of an illness can be an important indicator of the severity of the illness, as in the case of an individual receiving a social security pension because of heart disease. However, the pathogenesis and pathophysiology of heart disease (and the diagnostic criteria for the disorder) are not based on social consequences. Likewise, if the process of developing substance dependence involves a gradated loss of control over drug use, as captured by the elements of the dependence syndrome, then the diagnostic criteria should be based on this process and not on the social consequences of the development of dependence. An alternative view would submit that substance abuse is an inherently socio/cultural disorder in which the only important way to determine whether the disorder exists is to evaluate whether or not consequences have occurred or are highly likely. The decision of which view is more accurate is not one that can be made on the basis of empirical findings.

DIFFERENT VALIDATORS MAY DISAGREE. In attempting to validate a diagnostic system, numerous criteria have been suggested including prognostic validity, concurrent validity, construct validity, relationship to modes of familial transmission, and responsiveness to available treatments. Differing indicators may support the adoption of competing alternative diagnostic models. In the substance abuse area, an example of this pertains to the issues of different types of validators of the dependence syndrome. One of the underlying constructs for the dependence syndrome is that the hypothesized elements are highly intercorrelated. If the dependence syndrome is valid across different drugs of abuse, these high intercorrelations should be seen for different drug categories. Generally. data available so far suggests that this is the case. Another important validator of the dependence syndrome is it's prognostic significance. For some drugs, such as alcohol, greater dependence severity has been repeatedly shown to be associated with poorer prognosis (earlier relapse, need for more intensive treatments). However, for other drugs, such as heroin, prognosis for treatment seeking individuals is not well characterized by severity of dependence but is better determined by severity of associated psychopathology. What does one do in such a case? Should severity of associated psychopathology become a method of subtyping opioid addicts instead of denoting severity of dependence? The answer is not straightforward and is related to an important general issue of the desirability of maintaining consistent criteria across different drugs of abuse.

NO STANDARD FOR DETERMINING NEED FOR SUBTYPES. When are two conditions considered to be variants of a single disorder and when are they different enough to be considered distinct? In biological taxonomy, the operational rule of ability to mate and bear young like the parents is a general rule for denoting a species. For

psychiatric disorders. no such clean operational distinction exists to note the boundaries of different disorders. In the Substance Use Disorders, this problem is evident in the debate over the proper name to give to mild cases of substance use disorders. Should mild disorders be considered to be nonsevere instances of dependence or should they be defined according to social consequences and denoted as "abuse?' The central feature of dependence is diminished control over substance use. One instance of diminished control over substance use might be repeated instances in which use is dysfunctional. Hence, dysfunctional use (i.e. use with consequences) might be seen as mild dependence even if no other dependence symptoms exist. Alternatively, socially dsyfunctional use in the absence of other dependence symptoms could be considered to constitute a different category, "abuse ".What might be indicators of the validity of the Distinction? Perhaps course of illness would be helpful. If abuse is a separate disorder, one might expect abusers to recover more rapidly or not to progress to dependence. However, this does not invalidate the concept of mild dependence because the dependence syndrome is seen as arrayed along a continuum and those-who are mildly dependent are not hypothesized to always progress to more severe dependence. Another validating feature might be prognostic significance, with abusers having a better response to treatment than those with dependence. Again, mildly dependent individuals are hypothesized to have a better prognosis than than those with more severe dependence, so that this does not help in validating the abuse/dependence distinction. A third type of validator might be patterns of familial transmission. Abusers might be hypothesized as having less heavy family history of drug abuse while those with dependence might have heavier familial loading for drug abuse. This still does not provide a critical test because-the dependence syndrome is seen as having multiple determinants that are social, psychological and biological. Perhaps those with more severe dependence need determinants from multiple areas while those with mild dependence might be influenced by nonfamilial factors.

RELIABILITY VS. VALIDITY. One of the key assets of the DSM-III approach is it's greater reliability. Those features which enhance reliability include distinctiveness and observability of the criteria. However, for complex psychopathological symptoms, the essential features may not lend themselves readily to events which can be objectively observed. Hence, emphasis on operationalization might result in a highly reliable system but one which fails to capture the important pathological phenomena. In the substance abuse arena, an example of this distinction is that between indices of quantity and frequency (e.g. number of days drinking, number of ounces/drinking, day) as contrasted with symptoms that relate to more complex aspects of diminished control over drug use such as "inability to cut down or stop drug use". In the DSM systems, no reliance is made on actual quantities of substances consumed despite the fact that such measures are readily obtained and generally reliable. The reason is that there is not a direct relationship between quantity consumed and clinically important dysfunctional behaviors, with some heavy users manifesting few difficulties and some comparatively light users having severe consequences.

CONCLUSION

Are Experts Obsolete?

My principal conclusion in this brief review is to assert that the major decisions to be made in the DSMIV process will ultimately

boil down to reliance on expert opinion on a small number of key controversial issues. I do not intend to devalue the importance of field trials which evaluate reliability. construct validity and prognostic validity of the competing sets of diagnostic criteria for Substance Use Disorders. Rather, I present this point of view as an invitation to experts in this field to voice their opinions to the review committee based on their reasoned, best judgment about the relative merits of different approaches. I identify three of these underlying questions that will not be resolved by the data in order to elicit the opinions of experts at this meeting and elsewhere.

First, is Dependence best conceived of as a narrowly physiological phenomenon of neuroadaptation or should the concept be broadened to consider behavioral and psychological elements? The DSM R took the stand that other aspects of drugs, such as their ability to result in rapid euphoria, are at least as important as the factors as tolerance and withdrawal. For that reason. behavioral indicators of compulsive drug seeking were put on an equal footing with tolerance and withdrawal. This issue has been re-opened for DSM-IV.

Second, are social/psychological/occupational/legal consequences of substance abuse best seen as correlates or criteria for diagnosis? For DSM-III-R, these features were seen as correlates. However, suggestions have been made to include consequences as part of the syndrome in DSM-IV.

Third, is Abuse a distinctive category in its own right or is is best seen as mild dependence residual category for otherwise undiagnosed cases? As noted above, this decision may have less to do with empirical findings than with expert preferences regarding the clinical, legal and social impact of using different labels. According to this view a client with mild dependence may be likely to reject such a label, which implies a relatively severe, physical condition.

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Effects of Drugs of Abuse and Treatment Agents in Women

M.J. Kreek

Most drug addiction treatment programs are aimed primarily at the treatment of male drug abusers. This is especially true of the treatment programs for "hard-core" heroin addicts and for cocaine dependent persons. However, the percentages of female patients in these various treatment programs range from 10 to 40 percent. Therefore, it is increasingly important that the special needs of women be met in treatment programs, as well as in prevention efforts. It is also especially important to perform further basic laboratory and clinical research to characterize and separately study the biological basis of the major addictive diseases in females as contrasted to males, and to characterize the effects of drugs of abuse as well as treatment drugs on those systems which may be important either in the biology of addictive diseases or in the natural history of concomitant diseases which may complicate addiction.

Studies of the physiological status of female drug abusers before entering treatment, with emphasis on neuroendocrine status and immune status, have been carried out to some extent by our group and others, but further studies are needed. Similarly, the special clinical needs of women entering treatment with various common infectious diseases related to parenteral drug abuse, including hepatitis B, hepatitis delta and AIDS need to be considered and addressed in treatment programs. When pharmacological treatment is to play a major role, such as long term methadone maintenance treatment of heroin addiction, then it is important to determine whether or not there are any special differences with respect to drug disposition and metabolism in females contrasted with males.

At this time it is estimated that 2 million persons in the United States have used heroin and that approximately 1 million have used heroin with some degree of regularity. It is also estimated that there are at least 500,000 "hard core" regular long-term heroin addicts, defined as multiple daily users of heroin for one year or more with the development of tolerance, physical dependence and drug seeking behavior. The most recent surveys continue to show that approximately 22 million persons in the United States have used cocaine at one time and that

approximately 2.9 million have used cocaine recently. It is now estimated that 862,000 persons have used cocaine regularly. The most common drug of abuse remains alcohol abuse; 164 million in the United States have used alcohol at some time but of these, 11.5 million are problem drinkers. It is currently estimated that there are 5.8 million alcoholics. In each of these groups 15 to 50 percent of the regular, frequent or addicted users are females.

In the National Institute on Drug Abuse Warning Network system (DAWN), which addresses the question of the prevalence of various types of drug abuse and its complications, two major indicators are used: emergency room visits primarily for drug abuse related problems and medical examiners' reports of deaths due to drug abuse. In the data released for 1989, the top three drugs mentioned both in emergency room episodes and in medical examiners cases were in order: cocaine, alcohol in combination with other drugs, and heroin. In the 1988 DAWN survey, emergency visits by females totaled 43% of the total mentions, almost equal to those made by men, although episodes related to cocaine and heroin were 33% and 31% respectively by females, less than those by males. Drug abuse in the setting of suicide attempts are far more common in women 'than in men; 43% of the emergency room visits of females were drug abuse related suicide attempts or gestures. Although the 20 to 39 year old age groups were the most common age groups seen in emergency room episodes for both males and females, 14% of the emergency room visits were by females between the ages of 10 and 18, over twice the number of visits by males in that age group. In contrast, 73% of the medical examiner cases were males, with only 17% of the drug abuse related deaths occurring in females. Cocaine abuse in 1988 was the cause of death in 54% of the male cases seen by the medical examiner and 36% of the female cases. In 1989 data released by the National Institute on Justice, reported from major urban centers, between 70 and 90 percent of all females arrested gave a history and also had objective evidence (urine monitoring) of drug abuse of one or more types. In many cities, including New York and Washington, cocaine was the major drug of abuse seen in female arrestees, with cocaine found in around 70 to 80 percent of all females; opiates were seen commonly also, present in 20 to 40 percent of all arrested females. In essentially all cities reporting, a very high percentage of females requested access to treatment; from 15 up to 55 percent of women in different locations requested treatment for cocaine, heroin, and alcohol abuse, treatment was the major need identified by the arrestees themselves.

The problems of the AIDS epidemic, first recognized as a disease in 1981 and now very widely studied, has increased the concerns of the general public about drug abuse and has in turn increased both support for increasing treatment modalities and recognition of the urgent needs for research related to drug addiction. From the beginning of the AIDS epidemic through June 1989, both in New York State and in the Nation as a whole, from 25 to 35 percent of the cases of AIDS occurred in intravenous drug abusers. In some regions, notably New Jersey and New York, since 1988-1989, the new cases of AIDS in parenteral drug abusers exceeds that of any other risk group. In 1989, 43% of the new cases of AIDS in New York City were intravenous drug abusers; 40% were bisexual and

homosexual men. Since the beginning of the epidemic in New York, women have comprised approximately 13 to 17 percent of the new cases of AIDS. In New York, 66% of the women with AIDS have been themselves intravenous drug abusers and 25% had sexual partners who were drug abusers. Therefore drug abuse is the major risk factor in over 90% of women with AIDS. Women are the major vectors of the spread of AIDS to infants. Over 80% of the infants born with AIDS disease have had mothers who were either intravenous drug abusers or who have had intravenous drug abusers as sexual partners. Female drug abusers are also probably the main source of heterosexual spread of AIDS. In a recent survey in the United States, it was found that 0.14% of U.S. military recruits were anti-HIV-1 positive, 0.02 to 0.20 women of child-bearing potential in various regions were anti-HIV-1 positive, and most strikingly, in a study of random anonymous blood samples from over 16,000 university students on 19 campuses, 0.2% were HIV positive. From the very beginning of the AIDS epidemic, based on retrospective analyses of banked blood specimens in our laboratory, it was found that approximately 14% of intravenous drug abusing women were anti-HIV-1 positive. It was also found that for both women and men, combined use of heroin and cocaine, with implied use of shared unsterile needles for intravenous administration of both of these drugs, along with possibly the increased high-risk sexual behavior of women and men taking cocaine, led to a highly significant increase in prevalence of HIV positivity in combined drug abusers, as compared to heroin addicts. In a period of 1978 to 1983, 83% of parenteral drug abusers found to be anti-HIV-1 positive were intravenous users of both cocaine and heroin, whereas 17% were users of heroin alone.

In 1964, in collaboration with Dr. Vincent Dole and Dr. Marie Nyswander, we developed a new pharmacologic approach for the maintenance treatment of heroin addiction using the long-acting orally effective opioid methadone. Early studies prior to the development of analytical technologies suggested that methadone had a 24 hour duration of action, and when appropriate doses were used, would result in no euphoric effects in tolerant, dependent addicts coming into treatment. It was also shown that methadone would prevent withdrawal symptoms for 24 dosing intervals and would prevent drug hunger. Based on clinical research studies, it was also shown that, through the mechanism of cross-tolerance, methadone would prevent any euphoric effects of other superimposed illicit narcotics such as heroin. Later when analytical techniques, using gas chromatography and mass spectometry were developed by our group, as well as later by others, it was shown that the pharmacokinetic profile of methadone in humans are essentially unique, with a very long plasma half life in humans of 24 hours for the racemic compound and over 48 hours for the active l enantiomer. This is to be contrasted with a one to two hour half life for heroin and the maximum of 4 to 6 hour half life for its major metabolite morphine in humans. Many studies in this country and throughout the world have now shown that methadone can be a very effective treatment for narcotic addiction, especially when combined with counseling, rehabilitation efforts and access to primary medical care and psychiatric care as needed. In such combined programs, voluntary retention in treatment for over two years has been shown to be as high as 80%, with even more limited programs having a retention of 45 to 50 percent. In good programs, after

stabilization less than 10% of the patients continue to use illicit narcotics. In addition, the perfusion of opiate receptors by the long-acting opiate methadone seems to result in normalization of many physiological functions including neuroendocrine, reproductive and immune function which have been made abnormal during cycles of heroin addiction. However when methadone maintenance has been withdrawn voluntarily from subjects, including both those who have been well rehabilitated as well as those who have not been so successful in treatment, over 80% will relapse to illicit heroin use within two years. In 1984-85 at a time when over 50% of the street addicts in New York City who were intravenous drug abusers were found to be anti-HIV-1 positive, we found that less than 10% of those patients who entered effective methadone maintenance treatment prior to the AIDS epidemic arriving in New York City in 1978 and who had remained in treatment were anti-HIV-1 positive.

Since methadone has been proven to be such a highly effective treatment, special studies have been carried out in females to determine whether disposition of methadone is any different from that in males. No differences have been seen in females except during pregnancy. In a highly controlled study of patients stabilized in methadone for minimally two months prior to entry into study, methadone pharmacokinetics were determined using a repeated measure design. Pharmacokinetics as well as symptom questionnaires were carried out between 20 to 34 weeks of gestation, again between 33 and 40 weeks of gestation, 1 to 4 weeks postpartum and between 8 and 9 weeks postpartum. All pregnancies resulted in the birth of normal healthy infants; approximately half of the infants required treatment with paregoric for withdrawal symptoms for up to 5 days; the other half required no treatment. No abnormalities beyond that period were noted in any of the infants. None of the mothers were poly-drug abusers or alcohol abusers. It was shown in these studies that the area under the plasma concentration time curve, as well as the plasma levels of methadone at every time point studied were significantly lowered during the third trimester of pregnancy as contrasted to the postpartum period. These studies extended earlier findings from our group in which it had been shown that in the third trimester, but not in the second trimester, the biotransformation of methadone is accelerated, presumably because of the effect of the progestin hormones of late pregnancy on P450 drug metabolism. Plasma protein binding of methadone changed modestly but not significantly during the third trimester of pregnancy. This change in protein binding was not adequate to account for the highly significant differences in either total clearance or unbound methadone clearance in the third trimester of pregnancy. These studies also showed that there was increased urinary excretion of the two major metabolites of methadone during late pregnancy. These studies also confirmed earlier findings of very low concentrations of methadone in breast milk.

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Cardiovascular Effects of Cocaine in Pregnancy and on the Fetus

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Cocaine use in pregnancy has been associated with an increased incidence of miscarriage, rupture of maternal intracranial aneurysm, abruptio placenta and delivery of small for gestational age babies.(Chasnoff et al., 1985; Henderson and Torbey 1988; MacGregor et al. 1987; Oro and Dixon 1987; Acker et al., 1983) These pregnancy-related complications are believed to be mediated primarily by cocaine's ability to block uptake and degradation of catecholamines at adrenergic nerve endings, thereby potentiating the vasoconstrictive actions of norepinephrine in regional vascular beds and increasing its concentrations in circulating blood. Much of the impact of cocaine upon the fetus results from the basic cardiovascular changes in pregnancy normally designed to support the developing fetal-placental unit.

During the past 4 years we have utilized the pregnant ewe to document the effects of maternal cocaine administration upon maternal-fetal cardiovascular function. High doses of intravenous cocaine when given to pregnant ewes produced significant cardiac and neurologic sequelae. At 3.0 mg to 5.0 mg/kg cocaine produced dose-dependent cardiac arrhythmias, abruptio placenta, respiratory distress, seizures or death.(Woods et al., 1989) Seizures were characterized by rigidity of the extremities, opisthotonos and tetany of the respiratory muscles. Death in all cases was preceded by significant cardiac arrhythmias. Although these cocaine-induced sequelae were also observed in nonpregnant ewes, they were only seen at much higher (10.0-15.0 mg/kg) doses. Of note, the lethal dose of cocaine in the nonpregnant human has been reported at approximately 20 mg/kg for a 70 kg person.(Smart and Anglin 1987) These data suggest that the risks of significant cardiovascular or neurologic complications from intravenous cocaine are greater during pregnancy than in the nonpregnant state.

At lower doses, IV cocaine produces significant maternal systemic cardiovascular responses in the pregnant ewe. When cocaine, in doses of 1.0 and 2.0 mg/kg, was given to pregnant ewes instrumented for continuous cardiac output recordings (via a

pulmonary artery blood flow probe), dose-dependent increases in HR, BP, cardiac output and systemic vascular resistance occurred. More significant, these changes were much greater than occurred in nonpregnant ewes administered similar doses/kg of cocaine.(Woods et al., 1988; Woods and Plessinger 1990) Moreover, when the nonpregnant ewes were then administered the pregnancy hormone, progesterone, and then retested with cocaine, an exaggerated cardiovascular response similar to that seen in pregnant ewes was observed.(Plessinger and Woods 1990) In contrast, the hypertensive response to intravenous norepinephrine was unaffected by progesterone treatment. These results indicate that the enhanced cardiovascular toxicity to cocaine seen in pregnancy or during progesterone treatment did not result from altered sensitivity of the peripheral vascular system. Instead, progesterone mediated cocaine toxicity was most likely occurring at the level of the maternal heart.

Cocaine is thought to act on uterine vessels by blocking reuptake of norepinephrine in the synaptic cleft, thereby potentiating the vasoconstricting actions of norepinephrine on alpha-adrenergic receptors in the uterine vasculature. To test this theory, four pregnant ewes were administered IV cocaine 2.0 mg/kg before and following alpha adrenergic receptor blockade with phenoxybenzamine (PBZ) 5.0 mg/kg.(Dolkart et al., 1990) Norepinephrine (NE) injection, 30 ug, produced significant increases in BP, decreases in uterine blood flow and increases in uterine vascular resistance before PBZ administration; after PBZ, NE injection resulted in no change in BP, uterine blood flow or uterine vascular, resistance. This lack of response to NE confirmed complete alpha-adrenergic blockade with PBZ. However, despite PBZ blockade, maternal cocaine injection produced decreases in uterine blood flow, increases in uterine vascular resistance and decreases in fetal oxygen levels which were less in magnitude but similar in time sequence to cocaine's actions prior to PBZ. We conclude that although cocaine produces reductions in uterine blood flow primarily by NE-mediated vasoconstriction, its vascular effects may involve other vasoconstrictive agents.

Low doses of intravenous cocaine at 0.5 to 2.0 mg/kg also produce dose-dependent changes in uterine blood flow. uterine vascular resistance and oxygen delivery to the fetus.(Woods et al., 1987) At 0.5 mg/kg, maternal IV cocaine produces a small drop in uterine blood flow but no changes in fetal PO 2 However, fetal responses to maternal IV cocaine in doses of 1.0 and 2.0 mg/kg are characterized by hypoxemia, tachycardia and hypertension. When cocaine is injected directly into the fetus, no changes in fetal PO_2 occur. Nevertheless the fetus exhibits tachycardia and hypertension, even in the absence of hypoxemia. These results suggest that while maternal cardiovascular actions are a direct drug response to maternal IV cocaine, the fetal responses result, in part, from fetal hypoxemia secondary to cocaine-induced uterine artery vasoconstriction. Additionally, via maternal-placental transfer to the fetus, cocaine increases fetal blood pressure and heart rate as a direct drug action. Support for the

maternal-fetal transfer of cocaine is provided from a study of pregnant ewes in which maternal and fetal cocaine levels were measured prior to and at 5, 15, 30 and 60 minutes following maternal cocaine injection.(Woods et al., 1989) By 5 minutes following maternal injection of 1.0 and 2.0 mg/kg cocaine, fetal cocaine levels are 14 and 17% of maternal levels, respectively. Cocaine values rapidly decline thereafter and are undetectable in either maternal or fetal circulation by 60 minutes. When maternal and fetal blood samples are drawn every minute during the first 15 minutes, maternal cocaine values peak immediately following IV injection while fetal values peak by 2-3 minutes; both then rapidly decline. These data indicate that cocaine rapidly crosses the placenta, does not equilibrate, and then rapidly disappears by 60 minutes in the two vascular compartments.

In our most recent studies of cocaine's action during pregnancy, we have focused upon cocaine toxicity upon the maternal heart. To do so, the isolated rat heart papillary muscle preparation was selected. The results confirm that progesterone alters cocaine's actions upon the adult heart.(Sharma et al., 1990) In this study, papillary muscles from pregnant, nonpregnant and nonpregnant progesterone treated rats were electrically stimulated while exposed to incrementally increasing concentrations of cocaine from 10 M, a no effect dose, to 10 M, a dose that in all cases produced nonfunctioning muscles. The results indicate that cocaine in nonpregnant rats produces a biphasic cardiac contractile pattern. Low dose cocaine produced a positive inotropic response, while at higher doses, a negative inotropic response was recorded. When these results were compared with those obtained from the pregnant or nonpregnant progesterone-treated rat, differing patterns of cardiac contractility were seen. At no times during the study of these latter two groups did cocaine produce positive inotropy; instead only negative inotropic responses to increasing cocaine concentrations were noted. Moreover, the heart preparations from pregnant and nonpregnant progesterone treated rats became nonfunctional at cocaine concentrations 1-3 orders of magnitude lower than those of nonpregnant untreated rats.

These studies, carried out on the chronically instrumented sheep and isolated rat heart preparation allow the following conclusions to be drawn:

1. Cocaine, when given to pregnant ewes in IV doses of 3.0 to 5.0 mg/kg, produces cardiac arrhythmias, respiratory distress, seizures or death.(Woods et al., 1989)
2. The systemic cardiovascular response to maternal IV cocaine is characterized by dose-dependent increases in heart rate, blood pressure, cardiac output and systemic vascular resistance.(Woods et al., 1988; Woods et al., 1987) These cocaine-induced responses are significantly greater in pregnant than in nonpregnant ewes and may result from the effects of elevated progesterone levels in the sheep.(Woods and Plessinger 1990; Plessinger and Woods 1990)
3. Maternal IV cocaine administration produces dose-dependent

increases in maternal blood pressure, reductions in uterine blood flow and increases in uterine vascular resistance. Fetal responses to maternal cocaine injection are characterized by increases in HR and BP and decreases in PO_2 .(Woods et al., 1987)

4. The action maternal IV cocaine upon uterine blood flow is mediated primarily but not exclusively by norepinephrine stimulated alpha-adrenergic receptors.(Dolkart et al., 1990)
5. Following maternal IV injection, cocaine rapidly crosses the placenta from maternal to fetal circulations, does not equilibrate and rapidly declines in both circulations to undetectable levels by 60 minutes.(Woods et al., 1989)
6. Progesterone's role in enhancing cocaine-induced cardiotoxicity is demonstrated in the rat heart isolated papillary muscle preparation. Progesterone supplementation to nonpregnant rats produces an alteration in the muscle contractile pattern during drug exposure to increasing cocaine concentrations and development of a nonfunctional contractile state at lower cocaine doses than seen in the nonpregnant untreated rat. Since this same pattern of cocaine toxicity is seen in the pregnant rat heart preparation, progesterone is the likely mediator of cocaine-induced cardiotoxicity during pregnancy.(Sharma et al., 1990)

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Neurological Outcome in Infants Exposed to Stimulants in the Perinatal Period

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The central nervous system has been identified as a particular target for injury associated with stimulant drug use. Stroke, ruptured aneurysms, intraparenchymal hemorrhage, primary respiratory arrest, a cerebral vasculitis have been described in adult users of all forms of these substances. Chronic mental impairment and psychoses that outlast the acute and subacute ingestion period also suggest more generalized CNS alterations. These drugs with particular pharmacologic action producing vasoconstriction through an increase in noreplnephrine accounts for some of the injury patterns. The pregnant user is no less and may be more vulnerable to these morbidities. Through alternative metabolic pathways and, variably a reduction in cholinesterases, the pregnant woman's effective exposure to these agents may be even greater than that of a non-pregnant woman using the same amount of substance.

Stimulant drugs readily transplacentally are passed to the fetus and active metabolites excreted into the amniotic fluid for internal "recycling". The fetal brain then becomes the target of potential injury of the same type. There may be additional consequences for the developing brain. For example, in primate models the role of dopamine and other neurotransmittters in the development of lateralization of hemispheric function in late gestation has been shown. Neural cell proliferation in a primate model appears regulated by catecholamine systems between seven and 17 weeks. The applicability of these observations to the human is unknown. However, we must bear in mind possible dynamic effects of these drugs when assessing the long-term developmental impact of gestational drug exposure to stimulants as opposed to the relatively static impact on the adult brain. This means research in this area must be longitudinal and is necessarily complex as developmental processes unfold over time.

Neonates with gestional exposure to stimulants do show behavioral abnormalities of several types. Early and ongoing reports from several institutions of infants with amphetamine exposure suggested a constellation of hypotonia, irritability when disturbed, sleep alterations and difficulty feeding. These clinical impressions have been substantiated many structured observations using the Brazelton Neonatal Assessment Scale. Poor alertness, poor visual attention, some motor dysfunction and aberrant sleep/wake regulation has been consistently described in our work and others. For example, Chasnoff reports BNAS data on 162 cocaine-exposed neonates to that from 59 alcohol exposed and a drug free control group. The cocaine exposed showed more behavioral dysfunction than the other groups in the

cluster of BNAS items dealing with attention and alertness. In another study, even neonates with only early pregnancy exposure showed impairment similar to those with cocaine use throughout. That early exposure produced changes evident in the neonates' behavior suggesting brain alterations occurred early. Only half of the early exposure group came to an alert state during the exam as opposed to all of the control neonates; only one-quarter of the continuous exposure group come to alertness, suggesting a poor outcome as related to more severe and/or prolonged exposure. This consistent observation of poor alertness and attention in early infancy is important as it is these types of measures of infant attention that have been shown to be most closely related to long-term mental development in children. Beckwith and Howard have shown that poor attention, poor visual memory and decreased attention to novel stimuli are still evident at six months.

Chasnoff and Griffith report that the cocaine-exposed neonates show essentially no change in behavior and neurologic status over the first three months; they continue to show poor alertness, poor wake/sleep organization, only brief alert periods and are easily stimulus overloaded. Specific motor assessments done at four months of age show motor impairments in over 80% of cocaine-exposed neonates. Our own work substantiates these observations of a period of significant impairment over weeks to months post-birth, long after the infant leaves the hospital. This may take the form of a hyperirritable state with poor sleeping and large appetite. Erroneously labeled "withdrawal"; this period provides enormous challenges to caretakers. Conversely, some infants, many with methamphetamine exposure, show an extremely hypoalert state, poor feeding, and excessive sleep profiles continuing for months. Small pilot studies have suggested that notepinephrine levels in neonates with antenatal cocaine exposure are 1.8-5 times higher for weeks after birth. Whether these neurochemical changes account for the altered behavior in this period or whether they only coexist with permanent CNS changes in this population is unclear.

Many adverse conditions exist in the perinatal histories of these children that contribute to poorer outcome. A central issue is impaired intrauterine growth that many conditions produce. Several observers have found there is significant reduction in neonatal size in cases involving perinatal drug use. Zuckerman et al.; have shown a symmetrical IUGR, i.e., a reduction in relative length and OFC, as well as weight, with a reduction in lean body mass as well as fat. This suggests chronic insult as opposed to late pregnancy placental insufficiency. The worst developmental outcome has historically been found in this form of IUGR. The reduction in brain growth exceeds the severity of growth failure, occurring in 10-30% in most studies. Little and Snell have reported an incidence of microcephaly similar to that seen with prenatal alcohol use. Investigators at UCLA report one-half the population of drug-exposed children have an OFC of $\leq$ 10%. This is clearly of concern in reflecting specific reduction of brain growth either through reduced neuronal cell protein synthesis, a specific effect observed in a mouse model or from chronic hypoxia or both. These observations of poor head growth in other drug-exposed groups (e.g., heroin) and has been associated with poorer developmental outcome.

The comparative roles of IUGR and the direct toxic effects of cocaine itself on the neonatal nervous system were evaluated by Lester and colleagues using neonatal cry analysis. This window into the developing CNS has been useful in other populations to identify neurologically/developmentally impaired children. Drawing cohorts of 80 cocaine-exposed and 80 drug-free neonates from a large multicenter study of recorded neonatal cries, Lester was able to identify two clusters of cry

characteristics using structural equation modeling. One cluster was associated with low birth weight. This included longer latency to cry, fewer utterances, lower amplitude and increased dysphonic sounds. All these suggest a depressed, lethargic CNS. A hyperirritable CNS is suggested by those characteristics associated with cocaine exposure: longer duration of cries, higher fundamental frequency, higher first format and more variability in pitch and amplitude. Cocaine-exposed, IUGR neonates will show both patterns. This is one attempt to tease out the several factors that may influence the neonate's neurological status after *in utero* drug exposure and may account for the variable behavioral profile in these groups.

In order to assess whether structural CNS changes were seen in any frequency in the neonate as they are in adult users, we began a study using cranial ultrasound in neonatal infants to look for perinatal cerebral infarction, reasoning that structural changes might be the bases for some of the observed neurobehavioral abnormalities. We evaluated all term neonates with positive toxicologies for cocaine or amphetamines. There were 74 infants born during a single calendar year with no other known adverse perinatal conditions. During the same time period, 87 term drug-free neonates had cranial ultrasound for clinical reasons. In our center and others, about 25% of such children will have evidence of cerebral injury in the perinatal period and a similar proportion; 25-30% will have some neurodevelopmental compromise over the long-term. A cohort of entirely normal neonates had an incidence of abnormalities of 5.3%, comparable to other studies. In comparing the clinically ill children with the "well" drug exposed children, we found no significant difference in incidence of abnormal cranial ultrasounds. That is, the drug exposed children had a similar incidence of cerebral injury as those children who were known to have conditions that put them at developmental risk. The drug subgroups of infants showed no significant differences between them in the incidence and type of abnormalities. The types of abnormalities seen in the ultrasound studies showed no significant difference between the clinically ill group and those with drug exposure with the exception of white matter cavitary lesions. These well developed echolucent areas are evidence of injury prior to birth. These lesions are older versions of the acute hemorrhagic lesions suggesting that there are several injuries of varying ages.

The locations of these areas of hemorrhage are important to observe. The lesions appear to be concentrated in the basal ganglia and frontal lobes, and with a pattern of subarachnoid hemorrhage in the posterior fossa. These are areas of the brain that are least likely to be damaged in the more classic forms of perinatal ischemia and those injury patterns seen in premies. This difference in location suggested a differing etiology of injury, and possibly a different behavioral outcome. The vascular system of a human fetus shows a more mature ontogeny of cerebral vessels that supply the base of the brain. With a more well developed muscular layer, these vessels may be more vulnerable to the specific vasoconstrictive action of cocaine. In contrast, the watershed areas of the cerebral cortex may be more vulnerable to drops in placental perfusion pressure or drops in oxygenation associated with birth trauma or difficulties of vasoregulation faced by the preterm infant.

Other forms of neuroimaging show that in some the damage may be more widespread. The computerized tomography study shown here shows a pattern of diffuse "delays in myelination". On ultrasound, these areas now appear to be multiple small areas of lucency. Note the preponderance of this "darkening" in the frontal lobes. The midline structures such as the septum pellucidum and the optic tract and pituitary axis appear to be additionally vulnerable to these drugs. In the

MR study we can see a lucency contingent with the ventricles in a coronal view here; in the parasagittal view we see that this lesion is in the frontal lobes and there is an additional lesion that goes into the area of the basal ganglia. From these studies and others, we can conclude that the human fetus is vulnerable to the cerebral injury associated with stimulant drug usage. Anywhere from 20-35% of children may show patterns of cerebral injury.

Electrophysiologic measures show changes with this drug exposure. The visual system appeared particularly behaviorally impaired so we did flash VEP evaluations on now over 100 neonates. In the vast majority, 60-7.5% of children with circulating drug on board, have abnormal flash evoked visual potentials. Increased latency as well as failure to respond to the flash stimuli are characteristic of these studies. These appear in parallel to animal studies which suggest that cerebral ischemia can produce this kind of pattern. Other studies show this pattern in preterm infants with known hypoxic insult. Over 90% of these children show complete resolution of these VEP abnormalities in the first year of life. Specific long-term impairment of the visual system has not been demonstrated. The peripheral visual system, the retina, iris and cornea1 vessels show patterns of vascular constriction. Several investigators have looked at the auditory in this group and have demonstrated an increased latency on brainstem system auditometry at the midbrain level. There are no clinical correlates of this observation and these findings also appear to be transient. EEG changes seen when these drugs are circulating at birth, as well as in those with early exposure show sharp-wave activity, suggestive of central nervous system irritability and/or cerebral injury. Other reports suggest that over half of infants 3-12 months continue to show these abnormalities. Vertex abnormalities are prominent, suggesting deep central brain injury. Sleep disturbances are noted on EEG, as well as behaviorally. Neonates spend one-third more time awake with stimulant drug exposure. Sleep shows decreased quiet sleep and more transition sleep--an immature pattern in the neonate.

Neurobehavioral abnormalities do not clear away when the urine tox does. Many studies describe neurodevelopmental morbidity. In a clinical sample of our own 64 drug exposed term children from 0-26 months of age with no other adverse conditions, a neurologic impairment was seen in appropriately 40% of the group. Suspicious findings included those with increase motor tone, tremulousness and abnormal reflexes. Severe abnormalities included major asymmetries, hemiparesis and fixed dysfunctional postures. There is a confusing pattern of changing motor asymmetries in this group that appears to cluster not in children with long-term neuromotor compromise but those with language and learning difficulties. Strange dystonias and persistent tremor have been seen in some children.

Most show improvement in neurological status over the first year. Development, however, looks worse over time, as well as showing some delays. Our own work shows a significant decrement with time both in a cross-sectional sample of 64 and a longitudinal sample of 35. Language, in particular, shows substantial delays and a significant downward regression with time. Recent studies suggest that after 18 months, Bayley test scores show significant deviation from the norm. The role of the environment in this decline must be taken into account as many children are raised in a chaotic environment. To investigate this further, a carefully selected sample of 16 in stable middle-class homes, and other assessments carefully assessed using the MacArthur CDI in the labs of Drs. Thal and Bates. Significant deviation from the norm is clear at 28 months in expressive vocabulary. Less than half of the children made two-word combinations, a landmark achieved by virtually

all children in the normal sample. Means of receptive language also shows significant differences from a matched-normal sample. Clinical observations of poor behavioral organization, poor attention and social difficulties, as well as difficulties at the point of acquisition of language suggest the frontal lobe function in these children may be the central impairment in these diverse areas of dysfunction. This hypothesis is being pursued.

We suggest that many stimulant drug-exposed children have a biologic insult that occurs prior to birth that is severely confounded by other adverse perinatal circumstances. Furthermore, the chaotic, non-contingent environment and/or the current foster care system contributes significantly to this developmental morbidity. Removing the drug from their pre- and postnatal environment would clearly improve the outcome for these children, but it may not solve all the co-morbidities that exist in families with substance abuse. A comprehensive approach to intervention for all families cries out to be done.

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Effects of Cocaine and Other Drugs on the infant

C.D. Coles

Recently, drug abuse, particularly of cocaine by pregnant women, has been the focus of attention. There are various estimates on how many pregnant women use illegal drugs. Some early estimates were as high as 20% (Zuckerman, et al., 1989) but Hollingshead et al., (1990) and Chasnoff et al.,1990) reported 8 to 14% of women had positive urine screens. With this increase has been a heightened concern about offspring, which arose initially due to observation of negative infant outcomes as well as troubling maternal behaviors (e.g., inconsistent caretaking, neglect). Effects were attributed to cocaine with other factors overlooked. However, women who abuse substances, particularly poor women with little access to drug treatment or prenatal care, often have pregnancy outcomes.

In addition, cocaine users are usually polydrug abusers and other drugs are associated with negative outcomes. Cigarette smoking causes growth retardation, while opiates are associated with Neonatal Withdrawal Syndrome which produces agitation, tremoring, autonomic arousal and may be life threatening (Finnegan, 1986). Maternal. alcoholism may lead to fetal alcohol syndrome (FAS) affecting growth, morphology, and the development of the CNS (Clarren and Smith, 1978).

The aim of the current study was to document the effects of cocaine and alcohol on newborn behavior while controlling other potentially confounding factors including perinatal complications, lifestyle variables, and polydrug exposure. The following hypotheses were tested:

1) Reported effects of cocaine may be related to confounding factors (e.g., gestational age, social class, maternal health). When these are controlled, many effects associated with this drug (i.e. growth deficits, behavior) may not be observed.
2) Effects of cocaine and other drugs may be related to the length of exposure or active drug effects.

Thus, duration of use is expected to be related to presence of growth and behavioral effects in neonates.

METHOD: This is a metaanalysis using 3 cohorts of data (collected from 1981 to 1991 on the effects of maternal substance abuse in pregnancy on neonatal outcomes, with a focus on the effects of alcohol, cocaine, cigarettes, and marijuana. The three cohorts were: 1) 1981- 1983 (N = 161); 2) 1984 - 1985 (N = 103); and 3) 1988 - 1990 (N = 120). In the first cohort, focus was on the effects of heavy alcohol use (mean AA/wk = 14 oz.); and in the second moderate use (mean AA/wk = 6 oz.); and in the third, on the interaction effects of cocaine and alcohol.

All studies were done in the same clinic, serving the same (low SES, 90% black) population by the same investigators and with all infants tested by the same team of professionals.

Subjects. Subjects were women (<19 years) receiving obstetrical services at Grady Memorial Hospital, Atlanta, Georgia. All were screened for alcohol/drug use and women reporting alcohol (1 oz. AA/wk) or any cocaine use were recruited. A contrast group of non-users was also recruited. The drug-use groups were categorized: (1) women who stopped using during pregnancy and (2) women who continued to use.

Measures. Prenatal drinking and cocaine use were assessed by maternal reports (Addiction Severity Index). Cocaine use was also assessed by urine analyses (EMIT).

Outcomes focused on growth (birth weight, head circumference and length) and neurobehavioral status as measured by the Brazelton Neonatal Assessment Scale (BNBAS) (Brazelton, 1984). Neurobehavioral measures were performed by certified examiners blind to the mother's drug/alcohol use.

Data Analysis. Mancovas and Ancovas were used to compare groups non-drug, stopped users, continued-to-use). Multiple regression procedures were used with various confounding factors (i.e., reproductive optimality, examiner effects) controlled. To isolate the relationship between specific drug parameters and particular outcomes, partial correlation procedures were used to find the association between cocaine and alcohol use and infant outcomes when other drugs were controlled. Substance use variables were: All Drugs: (1) duration of use in weeks: Alcohol: (2) oz. AA/wk, Cocaine: (2) frequency of use; Marijuana: (2) joints/wk; Cigarettes: (2) cigarettes/wk.

RESULTS AND DISCUSSION: Growth

In all three cohorts, significant differences were observed on growth variables with less exposure associated with greater growth. Since there is a relationship among the use of various drugs, regression procedures were used to identify the amount of variance associated with drug variables. Maternal reproductive

optimality, measured using the Obstetrical Complication Scale (Littman & Parmalee, 1973) (Table I), was also included in the analysis.

In these analyses, all drugs affected growth significantly with cocaine duration (the length of time during pregnancy during which the fetus was exposed) accounting for more variance (12-15%) than alcohol duration (4-5%). Partial correlations, controlling the effect of other drugs show the relationships between infant outcomes, and alcohol and cocaine use (Table 2).

Neonatal Behavior. BNBAS variables were clustered for this analysis (Lester et al., 1982)) producing 7 variables: Habituation, Orientation, Motor, State Regulation, Range of State, Autonomic, and Abnormal Reflexes. These outcomes were used in regression procedures with Examiner and Time of Testing as covariates. No drug effects were found for variables related to infant state control (i.e., State Regulation and Range of State). Among the 5 other clusters, the drug effects varied suggesting that multiple factors are affecting infant behavior. Alcohol had the strongest effect on Habituation, Autonomic Regulation, and Abnormal Reflexes. Cigarettes, particularly cigarette duration, were related to many behavioral outcomes, particularly Motor and Orientation. When partials were done (Table 2), alcohol per week and alcohol duration were related to infant behavioral outcomes, but there was no association of cocaine exposure with any of the infant behavioral measures.

Thus, effects seen in infant outcome are multiplily determined with both polydrug abuse and lifestyle issues (i.e., OCS) affecting outcome. Because the confounding effects of these other factors have often not been considered when studies of cocaine effects are done, many effects have been attributed to that drug which may not be really related.

A second conclusion is that cocaine does indeed have a strong effect on growth beyond that seen for alcohol and cigarettes. It is not sufficient to simply note that a child has been exposed prenatally. Rather, it is important to examine the extent and type of exposure since these factors appear to be related to outcome.

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Table 1
Neonatal Growth:
Effects of Substance Use in Pregnancy

ALCOHOL SAMPLE (N - 161)

GROWTH VARIABLE	FACTOR % Variance	R2	F	P
Birth Weight	Alcohol/wk Cigarette Duration OCS	5 3 2	8.78 5.90 3.93	.004 .002 .005
Head Circumference	OCS Alcohol/wk	5 2	7.78 3.99	.006 .05
Length	Alcohol/wk OCS		8.31 4.62	.005 .03

COCAINE SAMPLE (N = 120)

GROWTH VARIABLE	FACTOR % Variance	R2	F	P
Birth Weight	Cocaine Duration OCS	15 3	21.25 4.40	.0000 .04
Head Circumference	Cocaine Duration Alcohol Duration	13 4	17.68 4.97	.0001 .03
Length	Cocaine Duration Alcohol Duration OCS	12 5 3	16.60 5.92 4.35	.0001 .017 .04

Table 2

Correlations (1st Order Partials*) of Drug Variables with Outcomes

COCAINE (N = 161)

	Alcohol AA/wk	Alcohol Duration	Cocaine Duration
GROWTH			
Birthweight	-.15 (p < -.0l)	-.15 (p < .0l)	-.26 (p < .009)
Head Circumference	-.12 (p < .05)	-.13 (p < .03)	-.30 (p < .003)
Length	-.17 (p < .008)	-.11 (p < .05)	-.16
BEHAVIOR			
Habituation	-.15 (p < .03)	-.12 (p < .04)	.14
Orientation	-.02	-.10 (p < .05)	-.04
Motor	-.05	-.07	-.07
Range of State	-.07	-.05	-.05
State Regulation	-.07	-.10 (p < .05)	-.06
Abnormal Reflexes	.05	.06	.17
Autonomic	-.16 (p < .0l)	-.26 (p < .0000)	-.06

*Controlling for cigarettes and marijuana, plus alcohol and cocaine, when relevant

Fetal Alcohol Syndrome: Early and Long-Term Consequences

A.P. Streissguth

Perhaps one child in 600 to 700 is born with the Fetal Alcohol Syndrome (FAS). The exact number will vary with the drinking habits of women in the child-bearing years. Although FAS is a birth defect caused by maternal alcohol abuse during pregnancy, most children with FAS will be unrecognized at birth (Little et al., 1990). Probably many will go through life undetected because the signs of this disorder are subtle and involve a pattern of characteristics rather than a single major malformation like cleft lip and palate. Furthermore, although the most severely affected children can be diagnosed at birth, the time at which the characteristics are most distinguishable is between 8 months and 8 years of age.

Fetal Alcohol Syndrome is diagnosed when patients have a positive history of maternal alcohol abuse during pregnancy and (1) growth deficiency of prenatal origin height and/or weight); (2) a pattern of specific minor anomalies that include a characteristic facies generally defined by short palpebral fissures, midface hypoplasia, smooth and/or long philtrum and thin upper lip); and (3) central nervous system manifestations (including microcephaly or history of delayed development, hyperactivity, attention deficits, learning disabilities, intellectual deficits or seizures). Patients exposed to alcohol in utero with some partial FAS phenotype and/or central nervous system dysfunction, but without sufficient features for a firm diagnosis of FAS or strong consideration of any alternative diagnosis, are identified as "possible FAE", Fetal Alcohol Effect (Clarren and Smith 1978).

Fetal Alcohol Syndrome is a specific medical diagnosis usually given by a dysmorphologist, a geneticist, or a pediatrician with special training in birth defects or dysmorphology. FAS is not appropriately diagnosed by checklists or without a full clinical examination by a specially trained person. Unfortunately, most physicians have not received special training in syndrome identification and as dysmorphology is a rather new field, many persons with FAS go unrecognized. This is

a particular problem with regard to persons who have not been identified prior to puberty.

The developmental course of babies with FAS may include developmental delays, but this is not always the case. They are, however, at higher than normal risk for sucking and feeding problems and for sleep disorders. About 70% of the young children with FAS are hyperactive and many evidence language and perceptual motor problems during the preschool period. With the onset of school, attention deficits and behavior problems often emerge and academic problems are noted. The average IQ in children with FAS is around 68, (in the mildly retarded range) although the range of intellectual abilities is broad, from severely retarded to normal IQ.

Most of the patients with FAS described in the medical literature have been young children. However, in 1985, a 10-year follow up of the first 11 children diagnosed with FAS was published (Streissguth et al., 1985). Two out of the original 11 children were dead on follow up and 1 could not be located. Half of the remaining 8 were clearly mentally retarded and seemed to be appropriately cared for at home and at school. They were regarded as disabled and known to have FAS. The other 4, all of whom had IQ scores in the "borderline" range (IQ between 70 and 85) were not recognized as having FAS, were not identified as disabled and were all having difficulty in school where they were expected to be learning normally. One dropped out of school in the 5th grade; one left school in middle school and had a baby. The major psychosocial problems observed in these children with FAS prompted a larger and more comprehensive study of older patients with this disorder.

The first major study of the long-term consequences of FAS was recently published (Streissguth et al., 1991). In this study, 61 patients are described who ranged in age from 12 to 40 years. Their average age was around 17 years. Seventy-four percent of the sample were American Indian because the study involved a follow up of many patients originally examined for a FAS prevalence study carried out on several Indian reservations of the southwest. It also involved patients referred to dysmorphologists for diagnostic evaluations.

One important finding was that the physical features of FAS are less distinctive after puberty. The faces of the patients were not as characteristic as they had been in childhood. Growth deficiency for weight was

not as remarkable as in infancy and childhood, although the majority remained short and had microcephaly (small heads). These findings help explain why the initial identification of persons with this disability is difficult as they mature. It also points up the importance of early identification.

Intellectual development was extremely varied, with some patients being very mentally retarded and others having normal intelligence. The average intellectual level for the patients with FAS was in the mildly retarded range. Almost half of them, however, had an IQ of 70 or above, so they would not be technically classified as mentally retarded. This has important implications for obtaining community services, as many persons with FAS are not automatically eligible for programs designed for the mentally retarded. Although the average academic functioning of these patients was at the 2nd to 4th grade level, some did read and spell at a 5th grade level or beyond. In general, arithmetic skills were the most difficult, probably representing difficulty with abstract thought.

Unlike previous studies of younger children with FAS which have dealt only with IQ and achievement scores, this study carried out systematic evaluations of the patients' level of adaptive functioning. Although this subgroup had an average chronologic age of 17 years, their average age of adaptive function was at a 7-year level. Of the three domains making up this adaptive behavior score, they performed best on daily living skills (at an average 9-year level) and most poorly on socialization skills (at approximately the 6-year level). Although one or two patients had age-appropriate daily living skills, none was age-appropriate in terms of socialization or communication skills. Specific types of adaptive behaviors characteristic of adolescents that these patients with FAS/FAE, who were not technically retarded, had failed to accomplish were: failure to consider consequences of action, lack of appropriate initiative, unresponsiveness to subtle social cues and lack of reciprocal friendships.

These findings underscore the critical importance of keeping adolescents with FAS/FAE in the school setting, as they certainly do not have the adaptive living skills to survive well outside of a structured environment, even when they are not technically retarded. These findings also point up the necessity of schools taking a broad functional approach to education and the importance of job skills training and work experience. It is of interest to note that of those patients on whom information was available,

only 6% were in vocational programs, 2% were working and none was entirely independent.

Family environments of these patients with FAS/FAE had been remarkably unstable. On average, they had lived in five different principal homes in their lifetimes. Only 9% were with both biologic parents; 3% with their biologic mothers. Of those for whom accurate data could be obtained, 69% of their biologic mothers were known to be dead. This statistic demonstrates the severe impact of alcoholism in women (they died not only of cirrhosis, but of many other types of alcohol-related accidents and violent deaths). This information leads to the conclusion that an early diagnosis of FAS in a child is important from the standpoint of both mother and child. Mothers giving birth to children with FAS are clearly at risk for alcohol-related disability and premature death. Diagnosis of FAS in the child can not only help the child receive proper services early in life, but can help the mother come to grips with her own alcoholism.

It is now recognized that FAS is not just a childhood disorder. There is a predictable long-term progression of the disorder into adulthood, in which maladaptive behaviors present the greatest challenge to management. The outcomes documented represent the interactive influences of biology and environment. Most of these patients were born before mothers were generally aware that drinking during pregnancy was harmful. Most of these patients were undiagnosed as infants and young children, or if they were, this diagnostic information was not carried along with them through life. Thus, most were raised by caretakers who were unaware of their diagnosis and taught by teachers who had no knowledge that they had a lifelong disability.

Hopefully, with more widespread diagnosis of FAS and with clearer understanding of the long-term consequences, more reasonable and appropriate environmental interventions can be developed at home, in the school and in the broader community. Out of this realization can come the help for each child to develop to his own best potential in an environment that is ultimately the most enhancing.

The wide variation in intellectual levels in this group of patients confirms what we have known since the beginning, namely that the diagnosis of FAS does not carry with it any particular guarantees or inevitabilities about IQ or about academic achievement levels. Diagnosis of FAS does not mean that a person cannot graduate from high school or even attend

college. It does mean that some degree of brain damage has been sustained and that the manifestations of this will be apparent in the persons' adaptive behaviors. Furthermore, the more serious manifestations of FAS may well be experienced at that time in life when the expectations for independent functioning are the greatest. It is our hope that this knowledge can lead to better programming for children with FAS, more widespread help and support for parents and teachers, and more realistic expectations for the patients themselves. Unrealistic expectations can led to frustration, despair and hopelessness. Knowledge about disability should garner support for the disabled person and hope for a happier, more fulfilling future.

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Hallucinogens and Serotonergic Mechanisms

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Using a drug discrimination paradigm, we have shown that classical hallucinogens [indolealkylamines such as 5-OMe DMT and (+)lysergic acid diethylamide (LSD), and phenylalkylamines (PAAs) such as mescaline and 1-(2,5-dimethoxy-4-R-phenyl)-2-aminopropane where R = Me, Br, I (DOM, DOB, and DOI, respectively)] produce similar stimulus effects in rats (Glennon et al., 1982). Because the stimulus effects are antagonized by serotonin (5-HT) antagonists, we proposed that stimulus effects of these agents involve a 5-HT agonist mechanism. It was later demonstrated that there exists in the brain more than a single type of 5-HT receptor and several major populations are now recognized: 5-HTl, 5-HT2 and 5-HT3. Shortly after the discovery of the 5-HT2 antagonists ketanserin and pirenperone, we found that both act as potent antagonists of the DOM stimulus and of DOM-stimulus generalization to other hallucinogens such as LSD, mescaline and 5-OMe DMT; we proposed that classical hallucinogens act via a 5-HT2 agonist mechanism (Glennon et al., 1983). If hallucinogens act as direct 5-HT2 agonists, it should be possible to demonstrate binding at 5-HT2 receptors using radioligand binding techniques. In 1984, we showed that classical hallucinogens bind at 5-HT2 receptors (rat cortex), and that there is a significant correlation between 5-HT2 receptor affinity and the discrimination-derived ED50 values; a significant correlation was also demonstrated between receptor affinity and human hallucinogenic potency (Glennon et al., 1984). Follow-up studies showed that the correlation between hallucinogenic potency and binding still applies when human cortex is used as the source of 5-HT2 receptors (Sadzot et al., 1989).

The 5-HT2 *hypothesis of hallucinogenic activity* was proposed nearly 10 years ago (Glennon et al., 1983). Since that time, there have been challenges to this hypothesis; some have been resolved and some are still in question. For example, Pierce and Peroutka (1988) suggested that hallucinogens, LSD in particular, behave not as 5-HT2 agonists but as 5-HT2 antagonists. There is some evidence that under the appropriate conditions hallucinogens may indeed behave as antagonists in certain pharmacologic assays; however, this is most likely reflective of the partial agonist nature of some of these agents (Glennon 1990). There is no support for the concept that hallucinogens consistently produce the types of 5-HT2 antagonist effects typically associated with ketanserin and pirenperone, nor is there any evidence that the 5-HT2 antagonist ketanserin (which is currently undergoing clinical trials) produces hallucinogenic activity in humans.

The 5-HT2 hypothesis was proposed prior to the discovery of 5-HT1C receptors. We have now shown that DOM-related agents bind at 5-HT1C receptors, and that there is a significant correlation between the 5-HT1C affinity of these agents and, for example their ability to reduce hyperthermia in rabbits and their hallucinogenic activity in humans (Titeler et al., 1988; Glennon 1990) Thus, at this time, involvement of 5-HT1C receptors in hallucinogenic activity can not be

discounted. Nevertheless, because. there is a correlation between 5-HT2 affinity ([$_3$H]ketanserin) and 5-HTlC affinity ([^{3}H]mesulergine) for a series of 16 such agents (r = 0.95; agents included in the correlation are: (+)LSD, (-)DOB, (+)DOB, DOB, DOI , DOM, (-)DOM, DOET, DOPR, DOBU, 2,4,5-TMA, 2,5-DMA, 3,4,5-TMA, MDA, (-)MDA, and MEM, with Ki values ranging from 3.8 to 16,500 nM), it is not surprising that the SAFIR (structure-affinity relationships) for binding of DOM analogs at the two receptors are nearly identical.

SAFIR AND QSAR STUDIES

Using radioligand and binding data, we have conducted structure-affinity relationship (SAFIR) studies in order to determine what molecular features are important for binding (Seggel et al., 1990). An important structural feature is the 2,5-dimethoxy substitution pattern; substituents at the 4-position (C4) modulate affinity over a wide range. Taking this approach one step further, we examined quantitative structure-affinity relationships (QSAR). For a series of 23 DOM analogs varying only with regard to the substituent at C4,5-HT2 affinity (which ranges from < 20 to > 50,000 nM) can be modeled mathematically as Log : Ki = 6.01 + 0.75 π (r = 0.90) (Glennon and Seggel 1989). These studies indicate that the lipophilicity of the C4 substituent of 1-(2,5-dimethoxy-4-R-phenyl)-2-aminopropanes plays a significant role in determining 5-HT2 affinity and that derivatives with highly lipophilic C4 (i.e., R) substituents bind with high affinity. Novel lipophilic derivatives, predicted to bind with Ki values of between 1 and 15 nM, were subsequently prepared and evaluated. For example, the 4-hexyl and 4-octyl derivatives DOHX and DOCT (Ki = 2.5 and 3 nM, respectively) bind with greater affinity than DOM (Ki = 100 nM) and represent some of the highest affinity PAAs reported to date. Discordant with these findings, however, is that neither DOHX nor DOCT (nor several other related agents) reduce DOM-like stimulus effects in DOM-trained animals. [Figure 1 shows the relationship between the chain length of several 4-alkyl-substituted derivatives and both their 5-HT2 affinity and relative potency in the drug discrimination assay using 1 mg/kg of DOM as the training drug. Note that derivatives where the chain length is 5,6 (DOHX), and 8 (DOCT) carbon atoms lack agonist activity.] Because these highly lipophilic high-affinity agents lack agonist activity, the possibility existed that they constitute a novel class of 5-HT2 antagonists; several have now been shown to antagonize the contractile effects of 5-HT in the rat aorta preparation (Seggel et al., 1990).

DEVELOPMENT OF A NEW MODEL

With the realization that some of these agents constitute agonists and others antagonists, the QSAR study was repeated including only those agents with known agonist properties. With this subset (n = 10), the lipophilicity of the C4 substituent seemed slightly less important and an electronic term emerged suggesting that electron withdrawing substituents might, in addition to lipophilicity, contribute to binding (Glennon and Seggel 1989).

Teitler and co-workers (e.g. Titeler et al., 1987) have proposed that 5-BT2 receptors exist in low-affinity (5-HT2L) and high-affinity (5-HT2H) states. [^{3}H]-Ketanserin appears to label both states of the 5-HT2 receptors. We dveloped radiolabeled derivatives of the 5-HT2 agonists DOB and DOI (i.e., [^{3}H]DOB and [^{125}I]DOI) and demostrated that they label what appears to be the high-affinity state of 5-HT2 receptors. 5-HT2 antagonists bind with comparable affinit at the 5-HT2L and 5-HT2H states whereas 5-HT2 agonists bind with considerably higher affinity at the latter. McKenna and Peroutka (1989) have suggested that radiolabeled analogs of DOB/DOI and ketanserin label two distinct receptors (i.e., 5-HT$_{2A}$ and 5-HT$_{2B}$, respectively) and not two states of the same receptor. Results of recent cloning studies using rat and human 5-HT2 receptor genes suggest that this is unlikely (Teitler et al., 1991; Branchek et al., 1991 and provide evidence for the expression of a single two-state receptor type.

TABLE 1. Affinity and agonist activity of several serotonergic agents.

Agent	Ki (nM)[a] (KET)	Ki (nM)[b] (DOB)	Ratio[c] (nM)	EC50[d] (nW	Intrinsic Activity
5-HT	521	5.2 (±0.6)	100	125	1.0
DON	300	4.5 (±0.8)	75	156	0.6
R(-)DOB	24	0.4 (±O.1)	60	6	0.6
DOBZ	7	0.3 (±O.1)	23	24	0.4
DOTB	19	1.7 (±0.4)	12	ND	0.3
DOCT	3	2.5 (±0.7)	1	ND	–
DOPP	10	17 (±7)	1	- e	0

[a]Affinity at [3H]ketanserin-labeled 5-HT2 receptors; data previously reported (Seggel et al., 1990).[b]Affinity at [3H]DOB-labeled 5-HT2 receptors, followed by SEM. [C]Ratio: Ki(KET)/Ki(DOB). [d]Agonist potency at 5-HT2 receptors linked to phosphoinositide hydrolysis. [e]Antagonist IC50 value = 44 nM.

Nevertheless, it is clear that agonists, but not antagonists, tend to bind with different affinity at 5-HT2 receptors depending on the identity (i.e., agonist versus antagonist nature) of the radioligand employed. SAFIR studies conducted using [^{3}H]ketanserin could underestimate the affinity of agonists and partial agonists (e.g. the value for (±)DOB is 41 and 0.8 nM, respectively, depending upon whether [^{3}H]ketanserin or [^{3}H]DOB is the radioligand) (Glennon and Seggel 1989). Thus, a more definitive SAFIR investigation would be one based on binding data obtained using a tritiated agonist radioligand. We have recently begun such an investigation and it is already evident that a linear relationship between lipophilicity and 5-HT2 affinity is significantly less predictive of affinity than previously thought. These results confirm and extend the interpretation of our SAFIR studies using the agonist subset described above. A new trend is a parabolic relationship between binding and lipophilicity, with a decline in affinity once an optimal lipophilicity is achieved (Fig 2). Furthermore, although too few agents have been examined at this point to allow examination of multiple dependent variables in a statistically significant manner, the electron-withdrawing nature of the C4 substituent remains important.

Given that agonists bind with greater difference in affinity than antogonists for 5-HT2 sites labeled by [^{3}H]ketanserin and [^{3}H]DOB, and because some of the DOB analogs may be partial agonists, there may be a relationship between the Extent of this difference and agonists versus antagonists or partial agonists activity. A small series of agents was examined both at [^{3}H]ketanserin- and [^{3}H]DOB-labeled receptors and the results were compared with something agonists activity in a published (Barker et al., 1991) functional assay (i.e.. phosphoinositide hydrolysis in fibroblasts cultured from rabbit choroid plexus). 5-HT binds with a 100-fold higher affinity at [^{3}H]DOB- versus [^{3}H]ketanserin-labeled 5-HT2 receptors (5-HT2(DOB) vs 5-HT2(KET) receptors); 5-HT is a full agonist (Table 1). The 4-nitro analog DON and (-)DOB bind with 75- and 60-fold higher affinity at 5-HT2(DOB) receptors and are partial agonists, whereas the 4-octyl and 4-(3-phenlpropyl) derivative (DOCT and DOPP) bind with no selectivity and lack significant agonist activity. DOPP even acts as a potent 5-HT2 antagonist (Seggel et al., 1990 and Table 1). The results of this study signify the importance of using [^{3}H]DOB as a radioligand when examining agents of unknown functional or intrinsic activity and suggest that SAFIR formulated for agonists using [^{3}H]ketanserin as the radioligand are in need of reevaluation. They also suggest that the use of both radioligands may provide a useful indicator of whether PAA analogs are agonists or antagonists and may have a bearing on hallucinogenic activity.

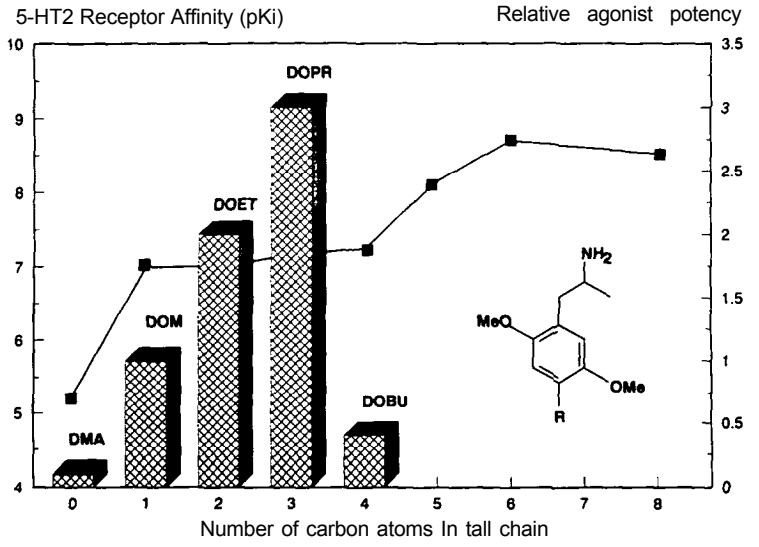

Figure 1. *Relationship between 5-HT2(KET) affinity (left), drug discrimination potency relative to DOM (right; bars), and the length of the C4 (R) alkyl chain*

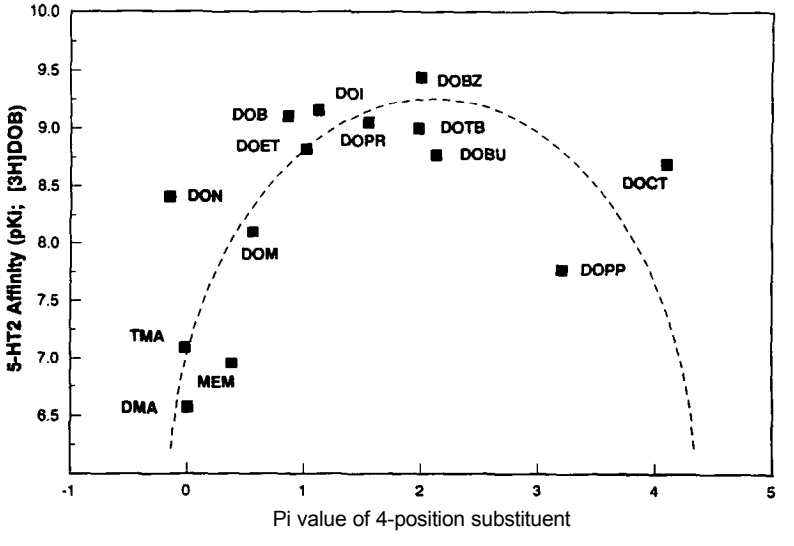

Figure 2. *Relationship between 5-HT2(DOB) affinity and lipophilicity (π value) of the C4 (R) substituent of 1-(2,5-dimethoxy-4-R-phenyl)-2-aminopropanes.*

SUMMARY
Classical hallucinogens possess 5-HT2 agonist activity; certain structurally related agents are antagonists, but there is no evidence that the antagonists are hallucinogenic. Both the discriminative stimulus and human hallucinogenic potencies of the agonists are significantly correlated with 5-HT2 receptor affinity when [^{3}H]-ketanserin is used as radioligand and Glennon et al., 1984). These agents appear to bind with higher affinity when [^{3}H]DOB, an agent that apparently labels the agonist high-affinity state of 5-HT2 receptors, is used as radioligand (Sadzot et al., 1989). For a series of DOB-related agents, 5-HT2(KET) affinity can be modeled by the lipophilicity of the 4-position substituent; lipophilicity alone does not completely account for the affinity of 5-HT2 agonists. Using [^{3}H]DOB as radioligand and to minimize differences observed between agonists and antagonists, 5-HT2 affinity is related both to the lipophilicity and electron withdrawing nature of the 4-position substituents. The difference between 5-HT2(KET) and 5-HT2(DOB) binding may be related to the intrinsic activity of DOB-related agents. Serotonin, with an intrinsic activity of 1.0, is not hallucinogenic whereas hallucinogens may be hallucinogenic because they are high-affinity 5-HT2 partial agonists.

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Self-Administration of Stimulants and Serotonergic Systems

D.S. Roberts

The principle mode of action of psychomotor stimulant drugs, such as amphetamine and cocaine, appears to be at noradrenaline (NA), dopamine (DA) and serotonin (5-HT) terminals. Cocaine blocks the re-uptake of monoamines, whereas amphetamine has the additional property of releasing newly synthesized monoamine stores. Such actions result in increased concentrations of transmitter substance at their respective synapses.

NA, DA and 5-HT possess characteristic distributions within the central nervous system. Each of these transmitters is generally localized to cell clusters in the pons/medulla and mesencephalon which send projections in varying densities throughout the neuro-axis. If every monoamine synapse is affected by cocaine or amphetamine, and if every region of the CNS receives a significant innervation from one or more of the monoamine transmitters, then it follows that all areas of the brain are probably influenced. If this is the case, the research question is not to identify the brain regions where these drugs have a pharmacological effect, but rather to identify those systems or areas that are essential for the rewarding actions of cocaine.

While a substantial number of drug self-administration studies has demonstrated a critical role for dopamine in the reinforcing effects of amphetamine and cocaine, an increasing number of papers are now focussing on the role of serotonin in cocaine and amphetamine self-administration behavior. Data will be reviewed here which suggests that serotonin has an important modulatory influence on psychostimulant reinforcement.

Lyness and coworkers were the first to examine the role of serotonin in amphetamine reinforcement. In their initial study, intraventricular injections of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) were used to deplete forebrain 5-HT. Lesioned animals consistently self-administered more amphetamine than control animals (Lyness et al.,1980). Leccese and Lyness (1984) subsequently demonstrated that 5,7-DHT lesions of the median forebrain bundle (MFB) also produced substantial increases

in amphetamine intake, indicating that an ascending system was involved, although curiously, 5,7-DHT infusions into the nucleus accumbens had no effect (Lyness et al., 1980). Consistent with these data are the results of Simon et al., (1980) who showed that radio-frequency lesions of the dorsal or median raphe, 5-HT nuclei which project to the forebrain, accelerate the acquisition of amphetamine self-administration. The findings of both groups support the idea that the effect of amphetamine on 5-HT systems is aversive, and by removing 5-HT, amphetamine becomes more reinforcing.

If an action at 5-HT terminals were in fact aversive, then it might be predicted that augmentation of 5-HT function would decrease amphetamine reinforcement. Some support for this idea comes from studies using pharmacological pretreatments. Leccese and Lyness (1984) presented some intriguing data showing that amphetamine self-administration was decreased following either l-tryptophan administration, which increases brain 5-HT content or fluoxetine administration, which acts as an indirect 5-HT agonist. Interestingly, dietary administration of l-tryptophan has since been shown to reduce intake of both amphetamine (Smith et al., 1986) and cocaine self-administration (Carrol et al, 1990). Compatible with the idea that these manipulations require intact 5-HT systems, Leccese and Lyness (1984) went on to show that l-tryptophan or fluoxetine did not alter amphetamine self-administration in 5,7-DHT lesioned animals.

In general, alterations in drug intake are difficult to interpret. While a reduced number of infusions may be suggestive of an attenuated reinforcing efficacy other interpretations are available. Often imaginative control measures are included or several reinforcing drugs are compared so that the specificity of the treatment may be assessed. An alternative strategy that has been used recently is the use of a progressive ratio (PR) schedule of reinforcement. Since primate studies (eg Griffiths et al.,1975; Beford et al., 1978) have shown that breaking points derived from the PR schedule yield an alternate measure of reinforced responding *our* lab has become interested in its application in the self-administration studies with rats (Roberts et al., 3989).

Consistent with the idea that the removal of serotonin systems would increase the reinforcing efficacy of psychomotor stimulants, we found that, following intraventricular administration of the neurotoxin 5,7-DHT, animals would respond to very high ratios in order to obtain a single injection of cocaine (unpublished observations). Intracerebral infusions of 5,7-DHT into the MFB or the amygdala also produced significant, although less spectacular increases in break point (Loh and Roberts, 1990). On average, the MFB lesioned group responded to breakpoints that were a third higher on the escalating scale than their previous baseline. These data provide clear evidence that 5-HT systems are critically involved in cocaine self-administration, and support the hypothesis that 5-HT systems are antagonistic to the reinforcing effects of psychomotor stimulants.

It is important to note that, even in rats that showed dramatic 5,7-DHT induced increases in breaking point, the rate of cocaine intake remained unchanged. These data appear contradictory since any change in the reinforcing efficacy would be expected to produce some change in rate of self-administration; however it now appears that rate of cocaine self-administration (at least at high unit dosages) is extremely resistant to change. For example, we have shown that breaking points established on a PR schedule for cocaine reinforcement are affected by the estrous cycle even though the rate of cocaine intake remains unchanged. We interpret these results to indicate that rate of drug intake reflects only the preferred drug concentration in the blood which might be unchanged by some treatment, even though the motivation to obtain drug might be altered.

Therefore, the fact that an experimental manipulation does not affect the rate of cocaine self-administration cannot be taken as evidence that the reinforcing efficacy is not altered. For example, it has been reported that fluoxetine decreases amphetamine self-administration (Leccese and Lyness, 1984; Yu et al., 1986) but not cocaine self-administration (Porrino et al., 1989) leading these authors to suggest that amphetamine and cocaine might be differentially sensitive to pharmacological manipulation. The importance of dose has been emphasized by Carroll et al., (1990) who have found that fluoxetine does in fact influence the rate of cocaine self-administration at lower unit cocaine dosages (0.1 and 0.2 mg/kg) but not at a higher dose (0.4 mg/kg). Recently we have re-evaluated the effect of fluoxetine on cocaine self-administration using the PR schedule and we found that even at relatively high doses (1.5 mg/kg) the breaking points are reduced following fluoxetine pretreatment.

While it is difficult to compare all the manipulations from various labs, the data seem to be consistent with the idea that both cocaine and amphetamine are similarly affected by aerotonergic manipulations. The reinforcing effects of both cocaine and amphetamine are enhanced by injection of 5,7-DHT into the ventricle or the MFB, and neither are affected by injections into the nucleus accumbens (Loh and Roberts, 1990; Lyness et al., 1980). Augmentation of serotonergic function through l-tryptophan diet or fluoxetine pretreatment appear to reduce the reinforcing efficacy of both cocaine and amphetamine.

The idea that there is an aversive or toxic reaction from psychostimulant drugs is consistent with the in vitro data of Ritz and colleagues. These authors studied a variety of cocaine and amphetamine analogues for their ability to compete for binding sites with agents specific for DA, WE and 5-HT transporters. They found that there was a positive correlation between the potencies of the various cocaine analogues for the DA transporter and their reinforcing potencies as measured in self-administration studies (Ritz et al., 1987). Of particular interest is the finding of a negative correlation between the reinforcing effects of

amphetamine-like compounds and their potencies at 5-HT transporter sites (Ritz and Kuhar, 1989). Both the in vitro data and the behavioral data are consistent with the idea that an interaction with the dopamine system is essential for the reinforcing effects and that a low intrinsic activity at 5-HT terminals (or removal of the terminal altogether) will result in an enhanced reinforcing effect.

Finally, the data involving 5-HT antagonists must be incorporated into the developing picture. If stimulation of serotonin receptors causes an aversive reaction then it might be predicted that serotonin antagonists would augment psychostimulant self-administration. The data, however, do not support this claim. It has been reported that methysergide, cyproheptadine or cinancerin produce a decrease in amphetamine self-administration (Leccese and Lyness, 1984; Porrino et al., 1989). Recalling that fluoxetine and 1-tryptophan also produced a decrease it would appear that augmentation and blockade of 5-HT function produce similar not opposite results.

Recently we have examined the effect of a number of specific 5-HT receptor antagonists on breaking points established on a PR schedule for cocaine reinforcement. To date we have found no significant effect of pretreatment with methysergide, MDL 72222 (a 5-HT3 antagonist) nor ketanserin (a 5-HT2 antagonist). While further testing is necessary with both amphetamine and cocaine, it would appear that 5-HT antagonists either reduce or have no effect on psychostimulant reinforcement. Are these data fatal for the hypothesis that serotonin has a negative impact on psychostimulant reinforcement? Probably not. It may be unreasonable to expect that any pretreatment would enhance the reinforcing effects of cocaine or amphetamine. Cocaine and amphetamine drugs already have diverse effects on the nervous system. Adding another drug on board that may itself have an aversive component is unlikely to amplify their positive effects.

In summary, there seems abundant evidence that dopaminergic mechanisms are essential for the reinforcing effects of amphetamine and cocaine. A small but growing literature seems to support a modulatory role for serotonin. While it remains unclear whether 5-HT antagonist drugs affect psychostimulant reinforcement, there is the growing impression that agents which augment 5-HT function may have clinical relevance in treating stimulant addiction.

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Serotonin and Alcohol Drinking

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INTRODUCTION

PRE-CLINICAL STUDIES

Over the last 20 years a considerable amount of evidence has accumulated to suggest that treatments which elevate central 5HT availability reduce ethanol consumption in rats. Thus, the 5HT precursors tryptophan and 5-hydroxytryptophan (Zabik et al, 1985), 5-HT agonists such as quipazine (Zabik et al, 1985), MK212 (Lawrin et al 1986), TFMPP (McBride et al, 1990), intracerebral 5-HT (Hill, 1974),and selective 5-HT uptake blockers, such as fluoxetine (Murphy et al, 1985, 1988; Haraguchi et al, 1990), zimeldine (Amit et al., 1984) and fluvoxamine (Murphy et al, 1985; Lawrin et al, 1986) all reduce alcohol intake in free choice paradigms. Also, genetically derived alcohol preferring rat strains, such as the P-line and the Fawn-hooded rat, show biochemical markers indicative of low endogenous 5-HT levels within the CNS by comparison to other strains (Murphy et al, 1987; McBride et al, 1990; Rezvani et al, 1990; Wong et al, 1990). As yet the receptor subtypes underlying these effects are unknown even though in theory, their identification could prompt the development of selective or relatively selective agonists as therapies for the treatment of alcohol abuse.

Perhaps paradoxical to a previous suggestion that enhancement of central serotonergic tone may diminish certain rewarding stimuli, 5-HT_3 receptor antagonists (e.g. ICS205-930, ondansetron) would appear to induce similar effects. For instance, ICS205-930 has been reported to reduce the elevations in dopamine mesolimbic activity produced by a number of abused substances, including ethanol (Carboni et al, 1988). Also ondansetron may, under certain conditions, reduce free-choice ethanol consumption in rats (Sellers et al, 1988) and marmosets (Oakley et al, 1988). Interestingly, 5-HT_3 antagonists also block the ethanol discriminative stimulus in pigeons (Grant, 1990) suggesting that some of the subjective effects of ethanol are mediated via this subtype.

Both pharmacological and biochemical manipulations designed to decrease 5-HT tone have been hypothesized to increase impulsive

conduct in rodents, leading Soubrie and co-workers to suggest that central 5-HT systems may not regulate specific behaviours as such but the way in which the animal responds to salient environmental stimuli (Soubrie, 1986).

CLINICAL STUDIES

There is clinical evidence to suggest that the cerebrospinal fluid of many alcoholics is deficient in the neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) and its metabolites, especially 5-hydroxyindolacetic acid (5-HIAA) (Eriksson and Humble, 1990; Linnoila, 1990). Interestingly, a similar biochemical deficiency has been proposed for other disorders, which like alcoholism, may be characterized by a loss of behavioural control. These include bulemia, obsessive compulsive disorders (OCD), seasonal affective disorders (SAD), pre-menstrual syndrome, aggression and suicide (Murphy et al, 1988; Peroutka, 1988). This hypothesis is largely based on the fact that a number of pharmacological agents that enhance serotonergic function, notably selective re-uptake inhibitors, are effective therapies for these conditions (Peroutka, 1988). Taken together, this has lead some workers to envisage that central serotonin systems may exert a modulatory role in controlling behaviours and impairments to this system may result in their impulsive or compulsive appearance.

The most widely studied group of serotonergic drugs in alcohol related disorders are the serotonin uptake inhibitors. Thus a number of short-term clinical trials (2-4 weeks duration) have demonstrated the effectiveness of fluoxetine, zimeldine, citalopram and viqualine in early stage problem drinkers (Naranjo and Sellers, 1989). This effect seems independent of their antidepressant action and appears to vary in magnitude across the patient population (Naranjo and Sellers, 1989). On average, the reduction in average alcohol consumption attainable by these drugs is rather low, being approximately 9-17%. (Naranjo and Sellers, 1989).

In other studies, the 5-HT_{1A} receptor partial agonist buspirone (Bruno, 1989) the 5-HT_2 antagonist ritanserin (Monti and Alterwain, 1991) and the 5-HT_3 antagonist ondansetron (Sellers et al, 1991) have also been reported to decrease alcohol consumption in abusers showing varying degrees of dependence. Since there is a frequent co-existence of mental disorder (particularly anxiety) and alcohol abuse (Reigier et al, 1990) it is prudent to question whether the effect on alcohol is secondary to the purported anxiolytic effects of this diverse range of drugs (Wilkinson and Dourish, 1991).

Perhaps related to this association between anxiety and alcohol abuse are the recent reports describing mCPP, a drug which may precipitate anxiety both in normals and in patients with anxiety disorders (Charney et al, 1987), to induce subjective effects of alcohol craving in hospitalized alcoholics (George et al, 1990). An alternative theory to account for this interesting finding may relate to the observation that TFMPP, a drug with similar pharmacological properties to mCPP, will generalize to an ethanol cue in a rodent drug discrimination paradigm (Signs and Schechter, 1988). Thus the craving elicited by mCPP may

be a response to the perception of an ethanol-like stimulus in the absence of the expected pharmacological effect.

PRE-CLINICAL STUDIES

Methods

Using a computerized drinkometer system, the effects of the following treatments on free choice ethanol and water consumption in ethanol preferring rats allowed continual access to either solution were examined:

a) the serotonin releaser dexfenfluramine and the serotonin uptake blocker sertraline;
b) the 5-$HT_{1/ic}$ antagonist ritanserin; the non-selective 5-HT antagonist metergoline; the 5-HT, antagonist ondansetron given one hour prior to dexfenfluramine (1.0 mg/kg);
c) a peripheral 5-HT receptor antagonist xylamidine given one hour prior to dexfenfluramine (1 .0 mg/kg);
d) the 5-HT, antagonist ondansetron.

In order to obtain a stable pattern of ethanol drinking, the rats selected from the screening phase were presented with a free choice of 5% ethanol solution (v/v) and water for a further 7 day period. On each of these days, all rats received i.p. injections of vehicle at 1800 h (one hour prior to lights off). Fluid intake was continuously monitored during the dark cycle and body weight and food consumption were measured daily. Rats then entered the first two day drug phase followed by two days of washout. In the sertraline experiment, rats received one of the following treatments: sertraline 1.0, 3.0 or 10.0 mg/kg or vehicle control. In the dexfenfluramine study, the treatments were: dexfenfluramine 0.5, 1.0 or 2.5 mg/kg or vehicle control. All drugs were administered i.p. at 1800 h on each test day. In a repeated measures, Latin square design, each rat received each treatment. In the case of the antagonist experiments there were three dose levels for the antagonist alone and combined with the dexfenfluramine, i.e. seven treatment groups.

Results: Dexfenfluramine and Sertraline

Under baseline conditions the rats drank 50-70% of their total fluid intake in the form of a 5% ethanol solution, predominantly during the dark phase. Intraperitoneal injection of sertraline (1, 3 or 10 mg/kg) or dexfenfluramine (0.5, 1 or 2.5 mg/kg) one hour before the onset of the dark period reduced subsequent ethanol and food intake; water consumption tended to increase. Dexfenfluramine, especially at the 1 mg/kg dose produced a marked attenuation in ethanol (-28%, $p < 0.01$) in relation to food (-4%, $p < 0.01$) intake. Sertraline, in contrast, appeared to affect both ethanol and food intake to a similar degree. Both drugs appeared to reduce ethanol intake by affecting the number of drinks; actual drink size was unaffected. Sertraline also delayed the initiation of ethanol drinking. The reduction in ethanol intake was due to an increase in the latency ($p < 0.01$) and a reduction in the number of drinks ($p < 0.01$); drink size was unaffected (Sellers et al, 1991; Tomkins et al 1991).

Identification of Receptor Subtypes Modulating Alcohol Consumption

Dexfenfluramine (1.0 mg/kg) depressed ethanol consumption and preference was reversed by pre-treatment with either metergoline (1 and 5 mg/kg i.p., $p < 0.01$) or ritanserin (1 and 3 mg/kg, $p < 0.01$) which when given alone had no significant effect on ethanol intake. Ondansetron (0.01, 0.1 and 1 mg/kg) and xylamidine (3 mg/kg) had no significant effect on dexfenfluramine induced reduction in ethanol intake. However, 0.1 mg/kg ondansetron alone significantly reduced ethanol intake by 17% ($p < 0.05$).

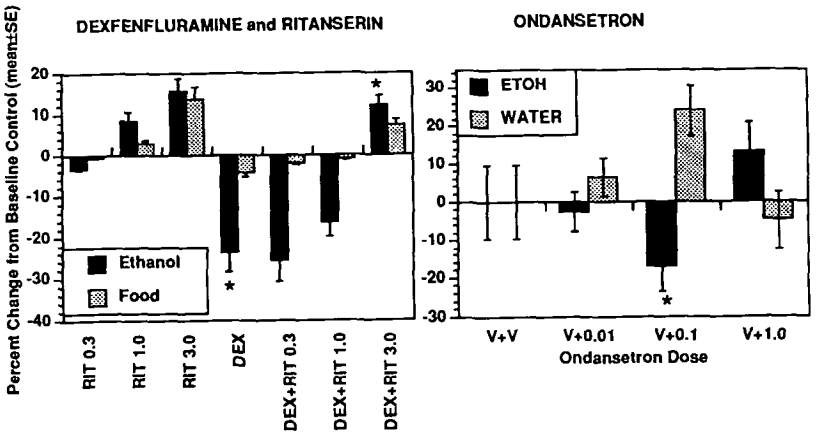

Figure 1: Left panel: Effects of dexfenfluramine alone and with ritanserin in 12 h ethanol and food intake in male Wistar rats expressed as a percent change from control. RIT = ritanserin; DEX = dexfenfluramine. Right panel: Ondansetron effects on ethanol and water consumption. V = vehicle control. *$p < 0.05$.

CLINICAL STUDY

Efficacy of Ondansetron in Alcohol Dependence

In a double-blind clinical trial, 71 male alcohol abusers, without anxiety disorders, were randomly assigned to received daily doses of ondansetron 0.25 or 2 mg or placebo p.o. b.i.d. for 6 weeks after a two week pre-treatment baseline period. All patients received concurrently a low intensity structured non-pharmacologic program which consisted of goal setting, daily recording of drinking, two homework assignments and weekly review of progress and problems by study staff. Alcohol consumption was determined by daily monitoring logs and daily urine alcohol determinations.

Comparison of pre-treatment vs. treatment 1-3 vs. treatment 4-6 vs. post-treatment week 1 showed an increasing reduction in ethanol consumption in the 0.25 mg group e.g. pre-treatment minus post = -2.6 standard drinks/day ($p < 0.05$) which was more pronounced if the

heaviest drinkers consuming > 10.5 drinks were excluded. When the analysis was restricted to this group a highly significant and clinically important effect (up to a 37% reduction in drinking compared to baseline and 20% compared to placebo) was found at the lowest dose studied (0.25 mg b.i.d.) for weeks 4,5,6 of the drug treatment period.

Table 1. Ondansetron Effect on Drinks on Drinking Days (mean ± SD)

	Placebo	Ondansetron 0.25 mg b.i.d.	Ondansetron 2.0 mg b.i.d.
Weeks 1-3	-1.1 ± 1.0	-1.3 ± 1.9	-0.2 ± 1.3
Weeks 4-6	-1.5 ± 1.5	-2.3 ± 2.0*	-0.8 ± 1.4
Post-Treatment	-1.2 ± 1.9	-2.8 ± 2.0*	-0.7 ± 2.0

*$p < 0.05$

CONCLUSIONS

1. The present findings suggest that dexfenfluramine, and to a lesser extent sertraline, may show some selectivity at certain doses in decreasing ethanol consumption relative to other consummatory behaviour in rats. The data suggest that the serotonin releasers may have greater utility in treatment of certain clinical impulse control problems than do uptake inhibitors.
2. The reversal of the dexfenfluramine induced reduction of ethanol intake by the non-selective 5-HT antagonist metergoline and the 5-$HT_{1C/2}$ antagonist ritanserin, but not by either ondansetron (5-HT_3 antagonist) or xylamidine (peripheral 5-HT_2 antagonist) suggest a role for central 5-$HT_{1c/2}$ receptors in the mediation of this effect.
3. Our data provide the first evidence that a 5-HT_3 antagonist may decrease ethanol consumption in the rat and that this animal model may have predictive validity since in a clinical trial ondansetron 0.25 mg b.i.d. decreased ethanol use by in ethanol dependent patients. The effect seems to be delayed in onset and is most prominent in moderately heavy drinkers.
4. Further studies are warranted with 5-HT_3 antagonists in various psychoactive substance use disorders. Pre-clinical data suggest that clinical application to opiate, nicotine, benzodiazepine, cocaine and alcohol dependence should be considered. In addition studies in patients with the dual disorders of psychoactive substance use disorder and anxiety would be appropriate in light of recent preliminary evidence for the potential clinical efficacy of ondansetron in generalized anxiety (Lader, 1991).

References available on request from the first author.

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Pharmacotherapy of Substance Abuse with Serotonergic Drugs

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Serotonergic neurons are substantially effected by cocaine's actions as a re-uptake blocker. This re-uptake blockade results in much larger amounts of serotonin remaining in the synaptic cleft leading to receptor dysregulation with chronic cocaine use. While dopaminergic pathways have been primarily implicated in the reinforcing properties of cocaine, the serotonergic actions of cocaine may be important in the chronic abuse of this drug. Therefore, treatment agents targeted at the serotonergic systems may be clinically helpful in the treatment of cocaine dependence.

Several of the serotonergic agents have been used to treat cocaine dependence, including fluoxetine, sertraline, gepirone, odansetron, mazindol, lithium, imipramine and carbamazepine. These agents act through a variety of mechanisms on the serotonergic systems, but all initially increase serotonergic activity. These increases in the serotonergic activity may be relatively generalized with a serotonin uptake inhibitor, such as fluoxetine, or more specific to serotonergic receptor subtypes with partial agonists, such as gepirone. Since cocaine is a re-uptake inhibitor, it nonspecifically effects serotonergic systems. Thus, potential substitution agents for cocaine might target this serotonergic re-uptake blockade.

Mazindol is a non-specific re-uptake inhibitor that has been tried in treatment of cocaine abuse. Although mazindol shares many characteristics of cocaine, including binding to the dopamine re-uptake carrier, it has little abuse potential. Thus, it had been considered a potential substitution or even blocking agent for cocaine abusers. Preliminary studies of mazindol by Berger et al. (1989) in methadone maintained cocaine abusers suggested that mazindol might indeed reduce cocaine craving and cocaine use. Seven patients were treated for one month at 2 mg once daily in an open study. They had a substantial reduction in cocaine craving within the first week of treatment, and cocaine abuse as documented by twice weekly urines also showed a substantial decline. As a

follow-up to this initial study, 19 methadone maintained patients were enrolled in a placebo controlled cross-over study examining cocaine use and craving (Diakogiannis et al., 1991). While there was a reduction from baseline cocaine craving and use, the placebo and mazindol period did not differ. The two week duration of study, however, was relatively short, and the washout between phases appeared to be too short, since there were carry-over effects from the mazindol into the placebo cross-over. Thus, a parallel groups design with a longer period of treatment is needed to adequately evaluate mazindol.

Three serotonin uptake inhibitors are available: sertraline, fluoxetine and fluvoxamine. The Yale group has examined sertraline for cocaine abuse in a four week open trial of 11 patients. They were compared to the placebo response in our desipramine trial and showed a significantly greater reduction in craving than the 24 patients on placebo (Gawin et al., 1989). For placebo craving scores dropped from 10.5 to 8.5, while for sertraline scores dropped from 10.5 to 6.5. Although on sertraline, cocaine use dropped from a baseline of 9.5 grams per week to 3.5 grams per week at week two, cocaine use did not significantly differ between the treated and placebo groups. Pilot studies have been more promising with fluoxetine. Pollack and Rosenbaum (1991) used fluoxetine in 11 methadone maintained cocaine abusers. Three patients dropped out of the study, and five patients were successfully treated for cocaine abuse. Because only eight patients took the medication for at least a week, the overall success was felt to be 63%, as supported by urine toxicology. Similarly, 16 methadone maintained cocaine abusers were treated with fluoxetine by Batki et al.,(1991), with doses ranging from 20 to 60 mg daily. They had a major decrease in reported cocaine use by week nine from an average of 14.4 to 1.2 times per week, and quantitative urine benzoylecgonine levels also fell significantly from approximately 6000 mg/ml to 3500 mg/ml at week nine. Cocaine craving significantly decreased from 14.2 to 4.1. Few adverse effects were noted, and no subjects had to discontinue fluoxetine.

Although these open studies have suggested that fluoxetine would be a useful treatment for cocaine addiction, a placebo controlled study by Covi et

al., using dosages of 20, 40 and 60 mg. daily has not been encouraging. This 12 week double-blind study, which included blood level monitoring of medication, showed little effect of fluoxetine on cocaine abuse in 50 pure cocaine abusers. No differences were found based on either dosage of fluoxetine or blood levels of the medication. Because this was a relatively small sample size and used pure cocaine abusers rather than methadone maintained patients, it will need replication.

Two other agents with relatively specific effects on serotonergic systems are also being examined for their efficacy in cocaine abuse treatment - gepirone and odansetron. Gepirone is a partial agonist at 5HT1A and 5HT2 receptors. In a multi-site, randomized clinical trial examining a 15 mg daily dosage of gepirone, an interim analysis bu Jenkins et al., was disappointing. Among 20 patients on placebo and 21 on gepirone, gepirone was not better than placebo in reducing cocaine positive urines. Work by Jasinski with odansetron, a putative 5HT3 agent, indicates that it appears to be medically safe for the treatment of cocaine abusers, although no efficacy data are available.

Other agents with potential serotonergic effects include imipramine, lithium and carbamazepine. Quitkin and Nunes have suggested that imipramine may be effective with the depressed cocaine abuser. While earlier reports suggested that lithium might be an effective blocking agent for treating cocaine dependence, the only double-blind study comparing lithium to placebo and desipramine found lithium was no better than placebo and significantly worse than desipramine in the attainment of cocaine abstinence (Gawin et al., 1989). Carbamazepine has been suggested by Hallkas et al. (1989) in open studies as a potential treatment for cocaine dependence, and its efficacy related to medication.

These studies of serotonergic agents for cocaine abuse generally lack adequate controls. Although pilot studies support the efficacy of most serotonergic agents, placebo controlled studies have generally shown no efficacy for cocaine abuse. Because better controlled trials are underway for fluoxetine, mazindol and carbamazepine, more definitive efficacy data should soon be available.

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Behavioral Strategies for the Evaluation of New Pharmacotherapies for Drug Abuse Treatment

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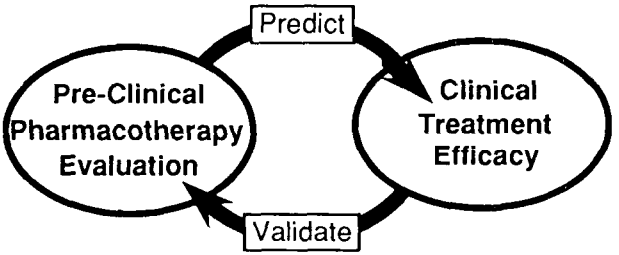
The goal of this symposium was to review established methods in behavioral pharmacology and to discuss how these procedures could be used to evaluate new pharmacotherapies for drug abuse treatment. Treatment evaluation has traditionally been the province of extensive clinical trials, but pre-clinical studies may also be useful for this purpose. This symposium focused on drug self-administration and drug discrimination procedures which have traditionally been used to address other questions in behavioral pharmacology. The potential advantages and limitations of drug self-administration and drug discrimination procedures for the evaluation of new pharmacotherapies for drug abuse treatment were examined.

One basic assumption is that if a new pharmacotherapy reduces drug self-administration by monkeys, it is more likely to be effective in humans than a pharmacotherapy that has no effect or increases drug self-administration. Monkeys will self-administer most drugs that are self-administered by humans (Griffiths et al., 1980; Mello 1985; Schuster and Johanson 1974), and the primate drug self-administration model has been used to predict the abuse liability of new compounds for several decades (Brady and Lukas 1984; Griffiths and Balster 1979; Thompson and Unna 1977). Since drug self-administration is the target behavior that is reduced by an effective pharmacotherapy, this model also should be useful for the pre-clinical evaluation of new medications. Yet, there have been surprisingly few studies directed towards this issue. It is critically important to concurrently examine responding maintained by another reinforcer such as food. If a pharmacotherapy reduces drug self-administration with only transient effects on behavior maintained by another reinforcer, this would suggest that the treatment drug's effects on drug self-administration are selective. However, if a new pharmacotherapy reduces both drug and food self-administration in a parallel and dose-dependent manner, this might indicate that there was a general suppression of behavior by the treatment drug or that the animal was sick or asleep.

Among the several advantages of the primate model for pre-clinical evaluation of new medications are: 1) compliance with the drug treatment regimen is ensured; 2) the treatment drug effects cannot be influenced by unreported polydrug abuse; 3) the treatment drug effects cannot be modulated by expectancy (i.e.. placebo responding); 4) accurate baseline measures of the daily dose and pattern of drug self-administration are available for comparison before, during and after treatment;

and 5) targeted pre-clinical trials are more cost-effective than extensive clinical trials.

As shown schematically below, validation of animal models for treatment drug evaluation will require measuring the degree of concordance between pre-clinical studies and outpatient studies of new medications. If the primate drug self-administration model shows good concordance with outpatient clinical studies, then it could be used to *predict* the potential effectiveness of new pharmacotherapies. If medications that effectively reduce drug self-administration in monkey are also effective in human drug abusers, this could significantly reduce the time required to introduce new medications into clinical treatment programs. But it is important to recognize that no pharmacotherapy can be expected to completely eliminate drug abuse since it is a complex and multiply-determined behavioral disorder. However, pharmacotherapy can be a very useful adjunct to a multi-modality treatment program.



Development of Pre-Clinical Models

In addition to the evaluation and prediction of therapeutic efficacy, a valid pre-clinical model could also be useful for the identification of new pharmacotherapies and could suggest novel approaches to treatment. One illustration of this application is our discovery that an opioid mixed agonist-antagonist, buprenorphine, significantly reduced cocaine self-administration by rhesus monkeys (Mello et al., 1989, 1990a). This finding has now led to confirmatory clinical trials in men with dual cocaine and heroin dependence (Gastfriend et al., 1991; Mello and Mendelson 1991; Mendelson et al, 1991).

Buprenorphine, an opioid mixed agonist-antagonist, and naltrexone, an opioid antagonist, each significantly reduced heroin self-administration by heroin-dependent men in in-patient studies (Mello and Mendelson 1980; Mello et al., 1981, 1982) and opiate self-administration by macaque monkeys (Mello et al., 1983). However, before 1989, the effects of buprenorphine on cocaine self-administration had not been studied, probably because the reinforcing properties of cocaine appear to be controlled by dopaminergic rather than by endogenous opioid systems (Johanson and Fischman 1989; Gawin and Ellinwood 1988). We observed that cocaine significantly stimulated pituitary release of luteinizing hormone, a hormone that is under endogenous opioid inhibitory control (Mello et al., 1990b and c). We speculated that since neuroendocrine systems are co-modulated by dopamine and endogenous opioids (Yen 1986; Mello et al, 1990b and c), perhaps an opioid would reduce the reinforcing properties of a dopamine agonist, such as cocaine (Mello and Mendelson 1991). There is accumulating evidence that dopamine and opioid systems interact to mediate behavioral and neurobiological effects of drugs.

We compared the effects of daily treatment with saline, buprenorphine (0.237, 0.40 and 0.70 mg/kg/day) and naltrexone (0.32 and 3.20 mg/kg/day) on self-administration of cocaine (0.05 or 0.10 mg/kg/inj) and food (1 gm banana pellets). Cocaine and food were available on an FR4 (VR 16:S) schedule of reinforcement. Buprenorphine, or an equal volume of saline control solution, were infused slowly over 1 hr through 1 lumen of a double lumen catheter at the same time each day. Saline and each dose of buprenorphine or naltrexone were studied for 60 sessions over 15 consecutive days. Buprenorphine significantly reduced cocaine self-administration in comparison to saline treatment in all subjects ($P < .001$ to .0001). Cocaine self-administration usually decreased on the first day of buprenorphine treatment and remained 72 to 93 percent below baseline (Mello et al., 1989, 1990a). Food self-administration was initially suppressed in some monkeys ($P < .01$) but tolerance developed to buprenorphine's reduction of food-maintained responding. Since food-maintained responding recovered while cocaine self-administration remained significantly suppressed, we concluded that buprenorphine had a selective effect on cocaine self-administration which did not reflect a generalized suppression of behavior (Mello et al., 1989, 1990a).

Naltrexone treatment also significantly reduced cocaine self-administration by an average of 28 to 25 percent, but the effect was not dose-dependent. Moreover, cocaine self-administration tended to increase over the 15 days of naltrexone treatment (Mello et al., 1990a). Food self-administration initially decreased, then exceeded base-line levels at higher doses of naltrexone. We concluded that naltrexone selectively reduced cocaine self-administration but less effectively than buprenorphine (Mello et al., 1990a).

Concordance of Pre-clinical and Clinical Evaluations

Since both buprenorphine and naltrexone reduce heroin abuse by heroin-dependent persons (Mello and Mendelson 1980; Mello et al., 1981, 1982; Mendelson and Mello 1991), our pre-clinical data suggested that these drugs might be useful for the treatment of polydrug abuse involving cocaine and heroin. Our ongoing clinical evaluation of buprenorphine treatment is very promising (Mendelson et al., 1990; Gastfriend et al., 1991). Our preliminary findings from an open clinical indicate that buprenorphine reduces both opiate and cocaine abuse in patients who meet DSM III-R criteria for dual dependence on cocaine and opiates. Clinical reports from the Yale group also showed that buprenorphine treatment of heroin abusers who were also polydrug abusers reduced the number of cocaine-positive urines more effectively than methadone treatment (Kosten et al., 1989a and b). Moreover, naltrexone treatment of opioid dependent polydrug abusers reduced cocaine-positive urines significantly in comparison to methadone (Kosten et al., 1989a).

The concordance between these clinical and pre-clinical data on buprenorphine's effects on cocaine abuse illustrate the feasibility of using the primate drug self-administration model to evaluate and predict the clinical efficacy of new pharmacotherapies. These data suggest that the primate drug self-administration model should be as effective for the evaluation of new medications as it has been for the prediction of new drug abuse liability (Mello 1991).

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Assessment of New Medications for Stimulant Abuse Treatment

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On the face of it, developing behavioral methodologies for preclinical evaluation of new pharmacotherapies for stimulant abuse should be a simple matter. Consider, for example, the as yet unavailable cocaine (COC) antagonist. We have known for many years that animals will self-administer COC. One could establish COC self-administration in an animal and give the antagonist before a self-administration session. If the drug antagonizes the reinforcing effect of COC, self-administration should stop. The purpose of the present discussion is to elaborate upon some of the complexities in such an apparently simple undertaking. We will limit our discussion to COC since this is the stimulant for which we have the most information. What is known about pharmacological mechanisms of psychomotor stimulant self-administration suggests that our discussion will have considerable generality. In addition, our discussion will largely be limited to issues related to potential antagonists. Clearly, agonist development is a viable option but may have its own set of considerations. We will rely to some extent on the opioid literature, where agonists and antagonists are well characterized, to illustrate some points. In addition, our discussion will be limited to data collected using non-human primates. Obviously, non-human primates are not the only species that can or are being used (see e.g., Carroll et al., 1990; Lyness et al., 1983). However, more examples of the points we wish to make are available in the non-human primate literature.

To the pharmacologist, the most straight forward way to study antagonism of a reinforcing effect would be to have a preparation in which the reinforcing effect varied between 0 and 100% as a direct function of drug dose. An antagonist of the reinforcing effect would be expected to shift that dose-response function to the right. Whether that shift was parallel or non-parallel would have the usual implications for the competitive or non-competitive nature of the antagonism. It is well established that the reinforcing effect of COC increases as a direct function of dose, at least over the range of intermediate doses that are reliably self-administered. In fact, however, the dose-response function relating rate of responding for injections to dose of COC is typically an "inverted U" or V shape. (This is true for other drugs as well). That is, there are doses that are too low to maintain self-administration, generally an abrupt transition to a dose that maintains high rates (the peak of the inverted V) and rate decreases as dose is increased further. The primary reason for the decrease in rate is that rate of responding under these conditions is determined by a combination of drug effects. Cocaine has reinforcing effects that tend to increase the rate of subsequent responding. However, shortly after the injection COC has other effects which may also alter rate of lever pressing. The ambiguous nature of those effects is captured by the multiplicity of names for them: direct effects, indirect effects, non-specific effects or satiating effects. Whatever the effect is called (we will use direct effect), the important point is that when a putative antagonist alters the rate of responding maintained by COC it is difficult or impossible to know whether the reinforcing

effect, the direct effect or some combination of these effects has been altered by the pretreatment.

This point was well illustrated in the first experiment to examine the effects of a potential COC antagonist. Wilson and Schuster (1972) allowed monkeys to self-administer one of two doses of COC (0.1 or 0.2 mg/kg/inj, IV) under FR schedules of reinforcement in daily experimental sessions. When responding was stable, chlorpromazine (CPZ) was administered IM 5 minutes before a session. CPZ was chosen because of evidence that it could antagonize other effects of psychomotor stimulants. Intermediate doses of CPZ increased COC self-administration. Self-administration was decreased only at the highest dose of CPZ, probably because of the severe motor incapacitation that was observed rather than blockade of the reinforcing effect of COC. Since a decrease in the dose of COC available for self-administration resulted in an increase in rate of responding, it is possible that the increase in rate of COC self-administration at intermediate doses of CPZ was the result of partial blockade of the reinforcing effects of COC. However, it is equally plausible that the direct effects, or some combination of effects of COC were altered by CPZ treatment. Although this ambiguity was well noted by Wilson and Schuster (1972), this wheel has been reinvented by any number of investigators with similarly ambiguous results. In any case, it requires significant legeredemain to argue that a treatment that increases COC self-administration is an obvious clinical goal.

With these considerations in mind, the first question is whether there are conditions under which rate of responding for COC injections is a direct function of dose. The basic task becomes one of eliminating the direct effects of the drug from the preparation Brady and Lukas, 1984). To do this, it is useful to divide time in reference to the drug injection. Reinforcing effects, although they are obviously a consequence of an injection, primarily influence behavior occurring before the next injection by increasing its probability of occurrence. The influence of a reinforcing effect on behavior is exerted well beyond the duration of the pharmacological actions of the drug. However, the direct effects of the injection begin Immediately after the injection and dissipate according to the pharmacokinetics of the drug (see Yokel and Pickens, 1974). Therefore, it should be possible to eliminate the influence of the direct effects of the drug on behavior by allowing sufficient time for them to dissipate before the next injection is available. This has been done by instituting a time-out (TO) after injections during which responding has no consequence. That rate is an increasing function of a range of doses of COC when a TO is programmed after injections has been well documented (Griftiths et al., 1979; Winger and Woods, 1985; Woolverton and Virus, 1989). For example, Griffiths et al., (1979) allowed baboons to self-administer COC under a FR 160 schedule of reinforcement and programmed a TO of either 3 or 12 hrs after each injection. With a 3-hr TO, response rate increased as a function of COC dose at intermediate doses (0.032-0.32 mg/kg/inj) and decreased as dose was increased above 1.0 mg/kg/inj. When the TO was increased to 12 hrs, response rate increased to asymptotic levels and did not decrease as dose was increased to 3.0-4.0 mg/kg./inj. This is persuasive evidence that under FR schedules with a TO programmed after an injection, response rate can be a measure of reinforcing effect uncontaminated by direct effects of the drug Moreover, the portion of the dose-response function over which rate increases includes more than two doses. Programming a TO after injections also puts a practical limit on the number of injections that an animal can take during a session. Although this may seem an artificial constraint, it means that there is a maximum possible effect in the preparation. As a practical consideration, these advantages must be balanced with the disadvantage that a long TO after injections slows down data collection.

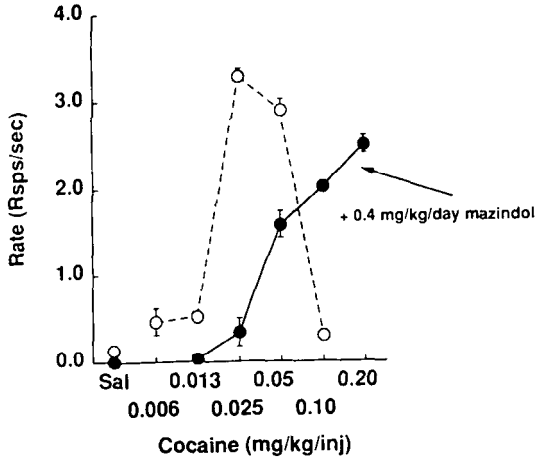
Perhaps the best validation of the utility of this sort of model for studying antagonists comes from a paper by Bertalmio and Woods (1989). Rhesus monkeys were allowed to self-administer codeine in 2/day experimental sessions under a FR 30 schedule with a 10 minute TO after each injection. Periodically, the mu agonist alfentanil was made available, with or without pretreatment with the mu antagonist quadazocine. Over the range of doses tested, rate of responding maintained by alfentanil was a direct function of dose. Quadazocine pretreatment shifted the alfentanil dose-response function parallel to the right, consistent with competitive antagonism of the reinforcing effect of alfentanil. In fact, these investigators were able to conduct an apparent pA_2 analysis that strongly supported the notion that mu opioid receptors mediate the reinforcing effect of alfentanil. Data of this type with COC would be persuasive evidence for blockade of its reinforcing effect.

A second issue relevant to the use of this model for medication development is behavioral. All other things being equal, one would prefer an antagonist that decreased behavior maintained by the drug while leaving behavior maintained by other positive reinforcers intact. To address this issue, behavior maintained by reinforcers m addition to the drug of interest must be examined. Although the theoretical and practical considerations for alternative reinforcers are myriad, in fact only two have been studied in non-human primates: another drug and food. Harrigan and Downs (1978) allowed rhesus monkeys to self-administer a drug IV for 15 minutes every 4 hrs, 24 hrs per day, under a FR 1 schedule of reinforcement. Drugs were available in a repeating sequence of morphine for three days, methamphetamine for two days and saline for two days. Naltrexone was then continuously infused intravenously for four consecutive weeks. Responding maintained by morphine decreased as naltrexone dose was increased while responding maintained by methamphetamine was unaffected by naltrexone. Thus, naltrexone selectively decreased morphine-maintained behavior. Most work with COC, including our own, has used behavior maintained by food to control for selectivity. Food has the advantages of being a non-drug positive reinforcer and of being easy to quantify and deliver. In addition, it is relatively easy to maintain comparable baselines of responding for COC and food.

These considerations have guided our method development. In our studies monkeys are trained to lever press under a three-ply multiple schedule of food and drug reinforcement. In the first and third components food pellets are available under a FR 30 schedule. Pellet delivery is followed by a brief TO. COC is available in the second component under the same schedule of reinforcement but with a longer TO after each injection. Although response rate is usually slightly lower for COC, patterns of responding are similar for the two reinforcers. This equivalence helps to control for effects of drugs that may depend upon rate or pattern of responding. To maintain a preparation that is at equilibrium, at least with respect to the test drug, test drugs are infused via a syringe pump, 24 hrs/day.

Because of the evidence that DA is involved in the reinforcing effect of COC, our initial efforts have concentrated on DA mechanisms. In one study, the D2 antagonist pimozide decreased COC-maintained behavior but only at doses that also decreased food-maintained behavior (Woolverton and Virus 1989). In another study, when the the D1 antagonist SCH 23390 was administered by continuous IV infusion, COC-maintained responding was decreased at doses that did not affect food-maintained behavior in half of the monkeys we tested (Kleven and Woolverton, 1990). However, the magnitude of the decrease in COC-maintained behavior diminished over the two-week infusion period as if tolerance were developing. When we redetermined the COC dose-response function after SCH 23390 exposure, it was shifted to the left in all monkeys, suggesting sensitization to the reinforcing effects of COC. These data strongly suggest that D1 receptors

play an important role in the reinforcing effect of COC (see also Bergman 1990; Koob 1987). Further, they suggest that D1 antagonists may block the reinforcing effects of COC in humans, although they may have substantial limitations when chronically administered. Third, they demonstrate that the method can detect compounds with selectivity for behavior maintained by COC.



A few additional points need to be made. Although we have discussed considerations for antagonist development, the development of agonists or, perhaps, mixed agonist-antagonists for pharmacotherapy is clearly a possibility. Clinical experience with opioids suggests that agonist have advantages in terms of patient compliance. Conceptually, our initial thought in considering agonists was that we could Just reverse our assumptions made for antagonists. That is, if an antagonist shifts the COC dose-response function to the right, then an agonist should shift it to the left. However, one study with opioids (no TO after injections) suggests that this may not be the case (Harrigan and Downs 1981). In that study, continuous infusion of methadone shifted the dose-response function for heroin down and to the right in much the same way as naltrexone did. Recently, we evaluated the dose-response function of COC in the presence or absence of the indirect catecholamine agonist mazindol in one monkey. In the presence of 0.4 mg/kg/day mazindol, the COC dose-response function was shifted to the right, just as would be expected with an antagonist (Figure 1).

Clearly, these results need to be verified in other animals. Nevertheless, they make the point that further basic studies with agonists need to be done to establish what effects of cocaine self-administration to expect. In addition, there are other behavioral approaches that we have not discussed. We have limited ourselves to circumstances in which multiple reinforcers are sequentially available to the organism and rate is the dependent variable. Procedures are available that instead allow simultaneous access to reinforcers, i.e., a choice, and measure frequency of choice rather than rate. These procedures have been used with both opioids and COC (Griffiths et al., 1976; Woolverton and Balster, 1981) and clearly merit further develpment. They have the advantage of offering the organism an alternative to drug self-administration and may, therefore, more closely mimic the human situation. Clearly, we find ourselves simultaneously involved in basic research into drug mechanisms, preclinical drug development and in method development. The stage is set for fascinating research that will provide basic insights into the mechanisms, both pharmacological and behavioral, controlling psychomotor stimulant self-administration.

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Preclinical Methods for the Development of Pharmacotherapies for Cocaine Abuse

R.L. Balster, R.S. Mansbach, L. Gold and L.S. Harris

With the increased effort to develop new pharmacotherapies for substance abuse, it is important to keep in mind that there are a number of different indications for which such medications could be used. Table 1 lists a number of intervention strategies that should be kept in mind when developing new treatments.

Table 1

Pharmacotherapeutic Strategies for Substance Abuse Treatment

I. Substitution for substance of abuse
   - examples: methadone and benzodiazepines

II. Antagonize substance of abuse
   - example: naltrexone

III. Treat immediate and/or protracted abstinence effects
   - example: clonidine

IV. Enhance the aversive effects of abused substance
   - example: disulfiram

V. Modify the biodisposition of abused substance

VI. Reduce the toxicity associated with substance abuse

VII. Treat the underlying psychopathology contributing to substance abuse

It should be apparent that quite different pharmacological profiles are needed for medications to be used in each of the intervention strategies shown in Table 1. It is also likely that different medications would be needed for treatment of opioid abuse, stimulant abuse, alcohol abuse, etc. Thus, there are many distinct indications for substance abuse medications; each of which probably requires a medication development program targeted for that indication. It follows that different types of research

methodologies will be needed to evaluate proposed medications for each of these indications.

In developing a program of animal research for the development of medications for substance abuse, it should be clear that no single model will be useful for all the types of interventions described in Table 1. Indeed, animal models of substance abuse may not even be particularly useful for developing medications that target underlying psychopathology, for instance, since it is not clear that comorbidity plays a role in animal models.

Medication Development Program at MCV

With the above considerations in mind, we are utilizing a series of animal test procedures in the Department of Pharmacology and Toxicology at the Medical College of Virginia of Virginia Commonwealth University to evaluate the possible efficacy of proposed treatments for cocaine and other stimulant abuse. These procedures are part of a larger contract supported by the National Institute of Drug Abuse (NIDA) for Pharmacological and Toxicological Evaluation of Treatment of Drugs (Louis S. Harris, Principal Investigator).

Table 2 lists the animal test procedures we have established.

Table 2

Procedures for Preclinical Efficacy Evaluation of Proposed Medications for Cocaine Abuse

I. Cocaine discrimination in rats
   a. Tests for substitution
   b. Tests for antagonism
   c. Tests for enhancement of cocaine's effects

II. Cocaine discrimination in squirrel monkeys
   a. Tests for substitution
   b. Tests for antagonism

III. Cocaine self-administration in rhesus monkeys
   a. Acute effects
   b. Repeated dosing

These models were selected to evaluate potential medications proposed to interact directly with the neural substrates of cocaine abuse and thereby alter the effects of cocaine that contribute to its abuse. We are primarily focusing on drugs which could substitute for cocaine or antagonize cocaine. It is also possible to evaluate drugs which might enhance the aversiveness of cocaine thereby leading to a decrease in its reinforcing effects. Medications which are found to substitute for cocaine may help satisfy drug craving, reduce abstinence-produced distress, and/or produce cross-tolerance. Cocaine substitutes might also increase treatment compliance and could be used interactively,

e.g. contingently, with behavioral interventions. It would be important to develop substitutes that differ sufficiently from cocaine to have lesser abuse liability and abstinence-producing properties of their own. A cocaine antagonist might be useful to treat overdosage, to block cocaine reinforcement, and to prevent relapse. In developing a potential antagonist, it will be important to find selective agents which would not alter other behaviors and that would be acceptable to patients. Although many antipsychotic medications have some ability to antagonize cocaine, they have not been found to be selective. They may also produce dysphoria that could be reversed by cocaine, thus providing increased motivation for cocaine abuse.

Drug Effects on IV Cocaine Self-Administration

We have completed the evaluation of a number of proposed medications for cocaine abuse treatment in the above-listed animal models. Because of the nature of our contract with NIDA, only data on some of these compounds have been approved for release. The purpose of this confidentiality arrangement is to allow NIDA to enter into agreements with pharmaceutical companies and other sponsors of novel drug entities which allow for their commercial development. Sponsors interested in having their compounds evaluated in these, or other tests, should contact the Medications Development Division at NIDA.

We have completed enough testing of drugs for their effects on rates of i.v. cocaine self-administration for a pattern of results to emerge. Briefly, the procedure involves the evaluation of treatments given intravenously just prior to daily 1-hour sessions of cocaine availability. The treatment drugs are also given i.v. to eliminate concerns about their bioavailability at the time their effects on cocaine self-administration are being assessed. We presume the drugs with qualitatively positive results would be evaluated more systematically using clinically relevant doses and routes of administration. In addition to assessing effects on rates of cocaine self-administration, concurrent measures of food intake and/or food-maintained operant behavior are obtained to assess the selectivity of drug pretreatment effects on cocaine-maintained behavior.

The results with a number of test drugs are shown in Table 3. Under these test conditions, we are finding that drugs which could be thought of as cocaine substitutes, e.g., cocaine itself, d-amphetamine and mazindol, all produce decreases in rates of cocaine self-administration. Dopamine receptor blockers such as haloperidol and a-flupenthixol, as well as some others we have tested, increase rates of cocaine self-administration. There are a number of explanations for the ability of antagonists to increase cocaine self-administration. It may be that they effectively lower the dose of cocaine shifting the dose-

Table 3

Drug Effects on i.v. Cocaine Self-Administration in Rhesus Monkeys

Drug	Effect
Cocaine	decrease
d-Amphetamine	decrease
Mazindol	decrease
Haloperidol	increase
α-Flupenthixol	increase
Naltrexone	no effect
Buspirone-acute	increase
Buspirone-repeated	no effect
Gepirone-acute	no effect
Gepirone-repeated	no effect

-effect curve to the right, resulting in higher rates of cocaine self-administration to produce the same total dose effect. It is also possible, and some of our data support this, that anti-psychotics may actually increase the maximal rates of cocaine self-administration suggestive of an increased efficacy of cocaine reinforcement. We have speculated that this may be due to the ability of cocaine to antagonize aversive effects of dopamine receptor blockers.

Naltrexone was without effects in this model, even at doses that decreased food-maintained responding. These results with naltrexone, as well as results with some other drugs we have tested, convince us that not all drugs we test would have positive results in this model. Finally, we found the results with buspirone and gepirone interesting. Buspirone, when given acutely, increased rates of cocaine self-administration. This may have been due to the well-characterized interactions of buspirone with dopaminergic neurotransmission. The failure to obtain effects with gepirone, which shares buspirone's actions on serotonergic system but lacks dopaminergic antagonist effects, provides further support for this interpretation. Neither buspirone nor gepirone consistently altered cocaine self-administration when given in single daily treatments for each of 10 consecutive days.

CONCLUSIONS

We have developed a series of animal tests for evaluating the efficacy of proposed pharmacotherapies for cocaine or other stimulant abuse. These models are being employed to evaluate drugs which would interact pharmacologically with cocaine and thus mimic or alter its discriminative and/or reinforcing effects. It is important to also understand the limitations of these models which are not specifically designed to study cocaine withdrawal effects, factors contributing to relapse, or psychopathology that may contribute to cocaine abuse. We are able to identify potential medications that would substitute for cocaine (among

these would be mazindol) and possible antagonists (e.g. α-flupenthixol and buspirone).

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A Laboratory Model for Evaluating Potential Treatment Medications in Humans

M.W. Fischman and R.W. Foltin

The model to be described combines the self-administration procedures developed for use with non-humans (see Mello, Woolverton and Kleven, and Balster, this symposium) with the traditional laboratory approach to studying drug-taking and its consequences (i.e., drug abuse) in humans. The validity of the non-human laboratory procedures is obvious; if we want to know something about a drug's potential to maintain self-administration, we can obtain the most focussed data by directly studying that behavior. Research with humans, however, has been less direct, often focusing on the physiological and subjective effects engendered by an experimenter-administered drug dose. The assumption in this latter case has been that "positive," "euphoria-like" effects are an indirect measure of a drug's reinforcing effects (Fischman, 1989). Because the behavior of interest when we discuss the treatment of drug abuse is drug-taking behavior, we adopted a strategy for evaluating potential treatment medications in the laboratory, using human research volunteers, which incorporates drug-taking as well as other effects of the drug.

Combining the direct measurement of the reinforcing effects of a drug with its other effects (e.g., physiological, self-reported) under conditions in which users have free-access to drug, has the added advantage of providing information about the effects of the drug when users can take it as they would outside of the laboratory. If we want to know the effects of a drug which is generally used (or abused) in repeated dosing patterns, studying it that way in the laboratory will provide maximal information about its effects. For example, the drug being used to illustrate this model, cocaine, is commonly abused in multiple-dose cycles (i.e., binges), with users taking the drug repeatedly until their drug supply is exhausted. In this model, cocaine is available for self-administration over several hours each day, and measures are made of both physiological and self-reported effects. Thus, a model which allows for multiple doses within each drug-taking occasion provides relevant information about natural patterns of drug use, which can then be used as a baseline to determine whether potential treatment medications impact drug-taking behavior.

THE MODEL

The proposed model compares behavior before and during maintenance on a potential treatment medication. Subjects are tested in a cocaine choice/self-administration paradigm using a range of cocaine doses (for 2-3 weeks), and can choose up to 7 injections in a 90 min session each day (Fischman et al, 1989). A 3-4 week outpatient medication maintenance period is inserted between two cocaine dose choice determinations, and the potential treatment medication is evaluated by comparing drug-taking behavior before and during maintenance. This model has the advantage of mimicking the clinical treatment methodology, allowing medication blood levels to stabilize before measurements are made. In addition, because actual drug-taking is measured, generalizations with non-human research studies are possible. Finally, since separate measures can be made of drug-taking, cardiovascular effects, and self-reported effects, it should be possible to isolate the specific effects of a treatment medication.

Subjects reside on a hospital Clinical Research Unit during the 2-3 week dose-choice determinations, and are tested daily in experimental sessions lasting 3-4 hours during which they receive either drug or placebo. All volunteers have histories of cocaine use as well as a wide range of other drugs, including marijuana, alcohol and nicotine cigarettes (see Cornell et al., this volume, for details).

Volunteers for these studies are given the opportunity to take repeated doses of cocaine, and the resulting patterns often approximate those reported in the natural ecology. Subjects, fitted with i.v. catheters in each arm, as well as physiological monitoring equipment, are told that they will be able to self-administer repeated doses of one of two solutions. One solution is associated with the left position and one is associated with the right position. This association remains constant within a day, but can change from day to day. Subjects determine which solutions are available each day by sampling from each, and are free to choose between the available solutions for the duration of each session (see Fischman et al., 1989, for details). Self-reported effects are measured repeatedly each day using the Profile of Mood States, a short form of the Addiction Research Center Inventory, and a series of Visual Analog Scales (see Fischman and Foltin, in press, for details).

EVALUATING THE EFFECTS OF DESIPRAMINE

Dose-choice determinations were completed before and during desipramine maintenance in a recently completed study (Fischman et al., 1989). The desipramine maintenance period was outpatient, with subjects reporting to the laboratory daily, and blood samples taken to monitor desipramine blood levels twice weekly. Desipramine blood levels were maintained at approximately 125 ng/ml during the final cocaine dose choice determination. This protocol allowed evaluation of desipramine's effects on drug-taking, dose preference, self-reported drug effects and cardiovascular effects under the conditions in which

subjects had the opportunity to administer cocaine as they would in a naturalistic setting (i.e., repeated dosing).

Desipramine had no effect on cocaine-taking behavior, with a mean of approximately 6 injections requested during sessions in which cocaine was available before and during desipramine maintenance.

Decreases in cocaine "craving" have been anecdotally reported for cocaine abusers being treated with desipramine. A Visual Analog Scale labeled, "I want cocaine", was administered as part of the self-report questionnaires in an effort to operationalize "craving." Before desipramine maintenance, subject's scores on this scale were close to the maximum of 100, while during desipramine maintenance, scores on this scale were substantially and significantly lower. Despite such shifts in reports of "wanting" cocaine, however, subjects' drug-taking behavior remained unchanged.

Desipramine maintenance also resulted in a change in cocaine's profile of subjective effects. Desipramine had the effect of attenuating scores on many of the scales that are sensitive to stimulant drugs, including Arousal and Positive Mood on the Profile of Mood States and the Benzedrine Group Scale on the Addiction Research Center Inventory.

A second pattern of desipramine-cocaine interaction effects was observed on other self-report scales. Desipramine maintenance resulted in lower placebo scores and significantly higher scores in response to cocaine on the Confusion and Anger scales of the Profile of Mood States and the Addiction Research Center Inventory LSD scale, a measure of dysphoric drug effects.

Desipramine maintenance was not always associated with changed cocaine effects. In some cases, desipramine had no effect on report of cocaine's effects. Most notable and consistent in this regard were the series of questions answered at the termination of each choice session, approximately 30 minutes after the last cocaine injection. Despite differential effects recorded during each session immediately after the drug was administered, and at approximately its time of peak effect, ratings made at the end of the session were related only to cocaine dose level, regardless of the presence or absence of desipramine maintenance. Thus, some properties of cocaine do not appear to change when it is taken in conjunction with desipramine maintenance.

Although desipramine maintenance, under these laboratory conditions, did not appear to affect cocaine self-administration, it did modify some of cocaine's effects, as reported by the subjects. This dissociation suggests that desipramine by itself is not an adequate pharmacological intervention for treating cocaine abusers: cocaine remains a potent reinforcer. It may, however, sufficiently alter cocaine's profile of effects so that users participating in a behavioral treatment intervention will learn to use other reinforcers in their environment rather than continuing to take cocaine. This procedure did not address the issue of what might

happen if there were non-drug alternatives to cocaine-taking during each test session.

INCLUSION OF A NON-DRUG OPTION

Such a procedure is currently under development, and preliminary data have been collected. During dose-choice determinations subjects are given the opportunity to select among three options. One option may be 16 mg cocaine, the second option, 8 mg of cocaine and the third option, 2 tokens. Subjects are required to sample each of the three options available that day once, after which they have the opportunity to make four additional choices during the session. They trade in their tokens at the end of each daily session for food, candy, cigarettes, videotapes, etc., and can use the items they have purchased any time until the next daily session.

We have hypothesized that if a treatment medication works selectively to make cocaine a less efficacious reinforcer, we might see this as a switch from drug-taking to token-taking, under some conditions. Tokens can serve as reinforcers, and under some conditions subjects will shift their choices from drug to tokens. For example, increasing the number of tokens/choice from 1 to 2 to 4 is frequently sufficient to engender changes in choice behavior from drug to tokens. Increasing the number of tokens or changing the dose has the desired effect for some subjects, while for others it does not. This procedure is currently being used with fluoxetine as the maintenance drug. Preliminary evidence suggests that neither cocaine-taking nor the self-reported effects of cocaine are affected by fluoxetine maintenance.

CONCLUSION

This research demonstrates the way in which procedures developed in the laboratory for use with non-humans can be adapted for use with humans and combined with additional measures of drug effect. Such designs provide data with cross-species generality, thus increasing their utility. Self-reports do not substitute for measuring actual drug-taking behavior, and in the absence of a good measure of drug-taking behavior there is no way to be certain about whether a drug will maintain self-administration, or about conditions that will influence self-administration. For example, based on verbal reports alone, the prediction would have been that desipramine-maintained subjects, who showed minimal drug-induced “euphoria-like” responses, would not self-administer cocaine. In addition, based on the decreases in the magnitude of verbal reports of stimulant effects and increases in the magnitude of “dysphoric” drug effects during desipramine maintenance, the prediction would have indicated a decrease in cocaine self-administration during desipramine maintenance. Neither behavioral outcome was observed.

The major disadvantage of this model is that it is still in development. And some of the disadvantages are not unique to the model, but due to the drug, cocaine, being tested. With that in mind, however, a few of the

disadvantages are: the procedure is time consuming, labor intensive and expensive, and the attrition rate due to medical issues is substantial. In the past 4 years we have tested approximately 135 subjects in various protocols, 20% of whom were discharged early. Of that 135, 18 were admitted into these medication studies and 50% were discharged early, most for medically related issues.

Nevertheless, we believe that the potential sensitivity of this model justifies its continued development. The model has the potential to provide information about medication-induced changes in behavior and conditions under which such changes should occur. The profile of effects being measured is important. While it is frequently the case that drugs which readily serve as reinforcers in human and non-human research subjects also cause effects which lead to self-reports related to "euphoria," or "liking" or some combination of self-reported effects judged to be "positive," the correspondence is far from perfect. Verbal reports of "euphoria" or other "positive" drug effects, and drug self-administration dissociate from each other under a number of conditions. Models such as this one, that measure drug-taking directly, as well as other drug-induced effects (i.e., cardiovascular, self-reports, etc), should clarify those issues.

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Clinical Parallels of Chronic Drug Self-Administration Models for Treatment Evaluation

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INTRODUCTION

Evaluation of new medications for the treatment of drug abuse would be greatly facilitated by the availability of objective and quantifiable measures of drug-seeking behavior. The efficacy and limitations of a new pharmacotherapy could be assessed rapidly by observing its effects on the amount and frequency of drug self-administration, and the behavioral consequences of drug use, without reliance upon retrospective self-reports. This report describes the application of techniques derived from the experimental analysis of behavior (Skinner 1938; Skinner 1953) to the study of human heroin self-administration. One goal of this study was to acquire basic information about patterns of heroin self-administration and its consequences, under controlled conditions, in which other factors such as polydrug use could not influence data obtained. A second goal was to compare the effects of naltrexone, buprenorphine and placebo on heroin self-administration on a clinical research ward.

NALTREXONE EFFECTS ON HUMAN HEROIN SELF-ADMINISTRATION

METHODS

Table 1 presents the sequence of conditions for the study carried out on a clinical research ward.

Table 1
Sequence of drug conditions

Nahrexone Group (N = 3)	Naltrcxone Placebo Group (N = 9)	Condition Duration
		days
Drug-free control period	Drug-free control period	9
Heroin (40 mg/day) + naltrexone (50 mg/day)	Heroin (40 mg/day) + naltrexone placebo	10
Naltrexone (50 mg/day)	Methadone detoxification (25-5 mg/day)	5
Naltrexone (50 mg/day)	Drug-free control period	7
Naluexone (50 mg/day)	Naltrexone (50 mg/day)	3
		Total days = 34

Six adult male volunteers with a history of heroin dependence for six to eight years provided informed consent for participation in these studies. All subjects had failed at least twice in conventional treatment programs. All subjects were in good health and showed no evidence of psychiatric or medical abnormalities as determined by clinical and laboratory examinations. Subjects age ranged between 22 and 29 years, and they had an average of 11 years of formal education.

Operant techniques were used to provide an objective quantitative measure of performance for two alternative reinforcers, heroin and money. The effects of naltrexone on heroin self-administration were measured in terms of duration, rate and pattern of operant performance for heroin rather than inferred from verbal behavior. The effects of heroin intoxication on operant performance for money was examined in comparison to performance during drug-free conditions. Subjects worked for money and for heroin on a second order schedule of reinforcement, FR 300 (FI 1 set: S). The response requirements and operant manipulanda used in this study have been described in the Journal of Pharmacology and Experimental Therapeutics (Mello et al., 1981).

RESULTS

Subjects maintained on placebo administered between 87.5 and 100 percent of heroin available. In contrast, subjects maintained on naltrexone administered only 2.5 to 7.5 percent of the heroin available ($P < 0.001$).

BUPRENORPHINE EFFECTS ON HUMAN HEROIN SELF-ADMINISTRATION

Methods

Male volunteers with a history of heroin abuse for an average of 10.4 years provided informed consent for participation in these studies. Subjects were selected from volunteers who had failed in conventional treatment programs. All subjects were in good health as determined by appropriate clinical and laboratory examinations. The average age of these subjects was 28.6 years; the average duration of formal education was 12.4 years. Six subjects participated in a single study in which three subjects were assigned to buprenorphine and three to its placebo. Four subjects participated in two separate studies and received both buprenorphine and placebo in a counter-balanced order. Table 2 presents sequence of drug conditions for this study. The operant procedures employed in

Table 2
Sequence of drug conditions

Buprenorphine Group (N = 7)	Buprenorphine Placebo Group (N = 7)	Condition Duration
		days
Drug-free baseline	Drug-free baseline	5
Buprenorphine (0.5-8 mg/day sc.)	Buprenorphine placebo (=volume s.c.)	14
Heroin (21 or 40.5 mg/day i.v.) + buprenorphine (8 mg/day s.c.)	Heroin (21 or 40.5 mg/day) + buprenorphine placebo	10
Buprenorphine detoxification (7 to 1 mg/day	Methadone detoxification (25 to 5 mg/day)	5
Drug-free baseline	Drug-free baseline	3
Naltrexone (10-50 mg/day)	Naltrexone (10-50 mg/day)	3
		Total days = 40

the evaluation of buprenorphine effects on heroin self-administration were similar to those described above for the naltrexone-related studies (Mello et al., 1982b).

RESULTS

Buprenorphine-maintained subjects took significantly less heroin than subjects maintained on placebo ($P < 0.001$). Buprenorphine-maintained subjects took only between 2 and 31 percent whereas the placebo maintained subjects took between 93 and 100 percent.

DISCUSSION

NALTREXONE EFFECTS ON HEROIN SELF-ADMINISTRATION

The opiate antagonist, naltrexone, significantly reduced heroin self-administration by heroindependent men. Naltrexone-maintained subjects took only between 2.5 and 7.5 percent of all the heroin available. Subjects stopped operant work for heroin after the first or second heroin injection and said they felt no effect from the heroin. Despite this compelling evidence that naltrexone reduces heroin use by dependent men, employing direct behavioral measures of heroin self-administration, outpatient naltrexone trials have been disappointing. Although a few motivated patients respond well to naltrexone maintenance, the majority discontinue naltrexone within days or weeks (Julius and Renault 1976; Meyer and Mirin 1979; O'Brien et al., 1975; Resnick and Washton 1978; Schecter 1980 for review). It is generally agreed that patients with the most social and employment resources and the most conventional life styles do best on naltrexone (Meyer and Mirin 1979; Resnick and Washton 1978; Suffett et al., 1978). Patients who refuse to take naltrexone or who quickly discontinue naltrexone are more likely to be those with marginal social and economic resources for whom heroin dependence is a central part of their lives.

Although naltrexone effectively antagonizes opiate effects, it is virtually devoid of agonistic properties and produces minimal side effects over a wide range of doses (Brahen et al., 1978; Martin et al., 1973). Despite the chemical dissimilarity and the different rationale for use, naltrexone appears to have many of the limitations of disulfiram (Antabuse) insofar as it lacks mood altering properties, and it can be abruptly discontinued without discomfort if the patient wishes to resume drug use. The absence of opiate-like agonist effects, which are perceived as positive by the dependent person, may be an important factor in the lack of outpatient acceptance of naltrexone.

BUPRENORPHINE EFFECTS ON HEROIN SELF-ADMINISTRATION

Buprenorphine significantly suppressed heroin self-administration ($P < .001$) over 10 days of heroin availability in comparison to placebo control. Placebo control subjects took between 93 and 100 percent of all the heroin available. Because these data are based on a direct behavioral measure of heroin self-administration, rather than on retrospective recall or an anticipatory self-report, we conclude that buprenorphine maintenance effectively suppresses heroin use by heroin addicts. The degree to which buprenorphine suppressed heroin self-administration appeared to be related to the maintenance dose of buprenorphine, as 8 mg/day produced a 69 to 98 percent suppression whereas in one subject, 4 mg/day produced a 45 percent suppression. Buprenorphine also produces a dose-related

suppression of opiate self-administration in a primate drug self-administration model (Mello et al., 1982a) Buprenorphine's advantages over the opiate agonist, methadone, are that it does not induce significant physical dependence in man, and the possibility of overdose is remote due to the opiate antagonist properties of buprenorphine. Its possible advantages over the narcotic antagonist, naltrexone, are in the realm of patient acceptance which in turn reflects its opiate agonist properties. Drugs which do not have an agonistic component, and consequently some abuse potential, may not be widely effective in the treatment of heroin dependence. The safety and potential therapeutic benefits of buprenorphine probably outweigh the possible risks associated with its abuse potential. Subjects liked buprenorphine and asked to be contacted when buprenorphine became available for outpatient treatment. Buprenorphine can be administered sublingually and rapidly absorbed into the venous plexus (Robbie 1979).

CONCLUSIONS

A useful model for assessing safety and effectiveness of new medications for the treatment of drug abuse has been based upon controlled human drug self-administration paradigms (Mello et al., 1982b; Mello et al., 1981). These paradigms have also been employed for assessing behavioral and biological concomitants of polydrug abuse. For example, human drug self-administration paradigms have enhanced our understanding of concurrent use of alcohol and marijuana (Mello et al., 1978). An increasing prevalence of polydrug abuse and the need for better pharmacologic interventions for this complex disorder prompts further development and application of controlled research ward studies which utilize human polydrug self-administration procedures.

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Use of Cocaine-Discrimination Techniques for Preclinical Evaluation of Candidate Therapeutics for Cocaine Dependence

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In recent years there has been a dramatic increase in both clinical and preclinical research aimed at understanding and treating the problem of cocaine addiction. Some of the most enlightening findings in this important area have come from drug-discrimination studies in which monkeys or other species are trained to distinguish between injections of cocaine and vehicle. These procedures have begun to provide fundamental information about neuropharmacologic mechanisms that presumably underlie the subjective effects of cocaine in people. They also are being used increasingly as preclinical screening devices for prediction of cocaine-like abuse liability and for evaluation of candidate therapeutics for the management of cocaine dependence. This brief review will consider these latter two uses of cocaine-discrimination procedures.

RELATIONSHIP BETWEEN COCAINE-LIKE STIMULUS AND REINFORCING EFFECTS

To provide a framework for evaluating the utility of drug-discrimination procedures as predictive screens for abuse liability it is instructive to examine the empirical relationship between the discriminative-stimulus and reinforcing effects of drugs with purported cocaine-like activity. There is now a substantial body of literature showing that many drugs capable of substituting for cocaine in drug-discrimination experiments also maintain robust self-administration behavior in monkeys. Among these are a number of cocaine derivatives (e.g., norcocaine, WIN 35,428), dopamine (DA) uptake inhibitors and releasers (e.g., GBR 12909, amphetamine), DA receptor agonists (e.g., quinpirole, PHNO), and a few drugs classified traditionally as local anesthetics (e.g., procaine) or histamine antagonists (e.g, diphenhydramine), which may also modulate DA uptake. As shown in Figure 1, there is a close overall correspondence between the potencies of several such drugs for producing cocaine-like stimulus effects and for maintaining i.v. self-administration in monkeys.

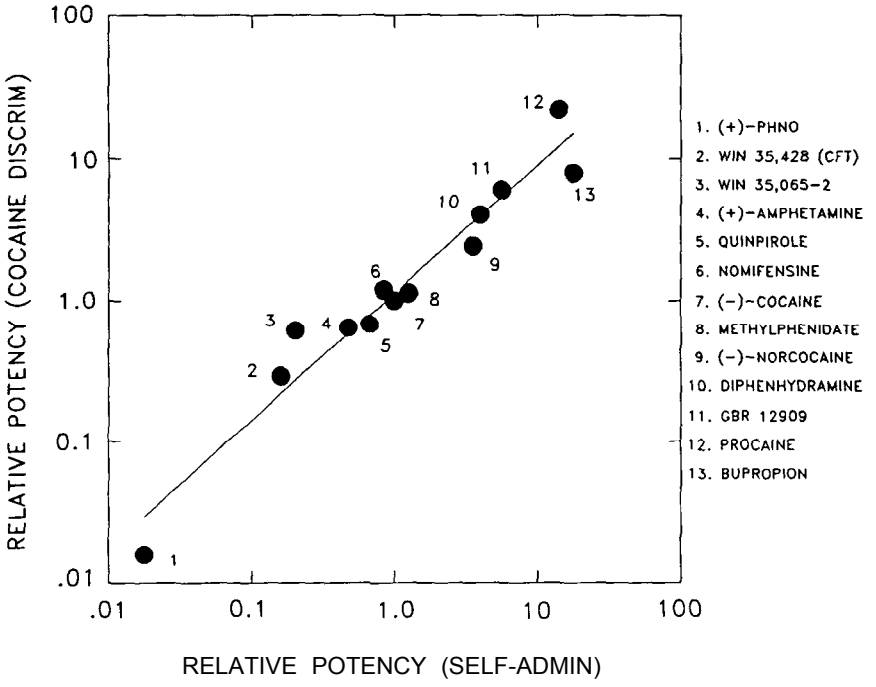

FIGURE 1. Relationship between the potencies of drugs (relative to cocaine) for producing cocaine-like stimulus effects and for maintaining i.v. self-administration in monkeys. Data are from Spealman and Kelleher (1981), Bergman and Spealman (1986), Ritz *et al.* (1987), Bergman *et al.* (1989), Melia *et al.* (1989), Spealman *et al.* (1991a,b) and unpublished observations.

It is important to recognize, however, that there are a number of notable exceptions to this general correspondence. Mazindol, amantadine and bromocriptine, for example, have been found to substitute either fully or partially for cocaine in drug-discrimination experiments (Melia *et al.,* 1989; Kleven *et al.,* 1990; unpublished observations), yet do not consistently maintain iv. self-administration in nonhuman primates (Woolverton *et al.,* 1984; Sannerud and Griffiths, 1988; Bergman *et al.,* 1989) and are considered to have low abuse liability in people. The dopamine D, receptor agonist SKF 81297 also has been found to partially reproduce the discriminative-stimulus effects of cocaine (Spealman *et al.,* 1991b), but preliminary studies suggest that it is not self-administered by squirrel monkeys (Bergman and Rosenzweig-Lipson, this volume). The lack of concordance between the stimulus and reinforcing effects of these drugs emphasizes the potential hazards in attempting to infer cocaine-like abuse

liability solely from results of drug-discrimination experiments. Viewed the perspective of drug discovery, however, the dissociation between stimulus and reinforcing effects may provide a rational basis for identifying candidate therapeutics for the management of cocaine dependence.

PARTIAL COCAINE MIMETICS AS CANDIDATE THERAPEUTICS

Drugs that are found to mimic the stimulus, but not reinforcing effects of cocaine in monkeys might have a role as pharmacological adjuncts for the treatment of cocaine withdrawal in people. By partially substituting for cocaine, such drugs could help alleviate the subjective symptoms of cocaine abstinence (e.g, craving) without themselves promoting abuse. Amantadine, bromocriptine and mazindol are examples of such partial cocaine mimetics currently under clinical evaluation. Other candidates include bupropion and the experimental compounds SKF 81297, Lu 19005, and quinelorane (Table l), all of which substitute at least partially for cocaine in drug-discrimination experiments with monkeys (Melia *et al.,* 1989; Spealman et al., 1991b, unpublished observations). Bupropion has been found to maintain i.v. self-administration in monkeys (Bergman *et al.,* 1989) and thus may have reinforcing effects by this route in people. Orally, however, bupropion is thought to have minimal abuse liability at recommended therapeutic doses. Lu 19005 and quinelorane have not been studied for their reinforcing effects in either monkeys or humans. Both, however, have relatively long onsets of action, which might be expected to limit self-administration as a result of delayed reinforcement. As noted above, SKF 81297 does not appear to have reinforcing effects in monkeys.

TABLE 1. Effects of selected partial cocaine mimetics.

	Cocaine-Like Activity		
Drug	Drug Discrimination (Monkeys)	Self-Administration (Monkeys)	Abuse Liability (Humans)
Amantadine	Partial	No	Low
Bromocriptine	Partial	Inconsistent	Low
Mazindol	Full	Inconsistent	Low
Bupropion	Full	Yes (i.v.)	Low (oral)
SKF 81297	Partial	No	?
Lu 19005	Full	?	?
Quinelorane	Partial	?	?

COCAINE ANTAGONISTS AS CANDIDATE THERAPEUTICS

A number of drugs classified pharmacologically as DA receptor blocker

have been shown to antagonize both the discriminative-stimulus and reinforcing effects of cocaine in monkeys. Included among these drugs are nonselective D_1/D_2 blockers such as chlorpromazine and cis-flupenthixol (Herling and Woods, 1980; Spealman *et al.,* 1991b), selective D_2 blockers such as eticlopride and YM 09151-2 (Bergman et al., 1990; Spealman *et al.,* 1991b), and selective D_1 blockers such as SCH 39166 (Bergman *et al.,* 1990; Spealman *et al.,* 1991b; Vanover *et al.,* 1991). Low-efficacy D_1 agonists such as SKF 75670 also are effective in attenuating the discriminative-stimulus and reinforcing effects of cocaine in squirrel monkeys (Bergman and Rosenzweig-Lipson, this volume). Conceivably, DA blockers and low-efficacy DA agonists could play a useful role in the management of acute cocaine intoxication (e.g., by ameliorating disruptive subjective symptoms) or as adjuncts in relapse prevention programs (analogous to the use of naltrexone in former opiate addicts). The utility of drugs with prominent D_2 blocking actions probably will be limited, however, by their extrapyramidal side-effects. On the other hand, reports that D_1 blockers are less likely to induce severe extrapyramidal symptoms in monkeys (Coffin *et al.,* 1989) suggest that some of these drugs may be suitable candidates for clinical evaluation.

TABLE 2. Effects of candidate cocaine antagonists.

	Cocaine Antagonist Activity		Extrapyramidal
Drug	Drug Discrimination	Self-Administration	Side-Effects
Flupenthixol	Yes	?	Yes
Chlorpromazine	?	Yes	Yes
Eticlopride	Yes	Yes	Yes
YM 09151-2	Yes	?	Yes
SCH 39166	Yes	Yes	?
SKF 75670	Yes	Yes	?

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The Use of Human Drug Discrimination Studies in Medication Development

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Drug discrimination studies with nonhuman species are numerous. A basic assumption underlying the evaluation of the discriminative stimulus effects of drugs in nonhumans is that these effects are related to the subjective effects of drugs in humans. Drug discrimination studies have been used for classifying drugs, elucidating their mechanisms of action, and predicting dependence potential. As other presentations in this symposium have illustrated, there may also be a role for this methodology in the development of medications for treating drug abuse disorders. If there is a role for nonhuman drug discrimination studies in this regard, it is likely that studies with humans can also help determine whether a particular medication may alter the subjective effects of a drug. Thus, to the extent that subjective effects influence the propensity for individuals to abuse drugs, this approach will be useful for evaluating ways of altering an abused drug's subjective effects. In this chapter, methods for evaluating the discriminative stimulus effects in humans will be described along with examples of obtained results. Finally, a discussion will be presented on the advantages and disadvantages of incorporating human drug discrimination studies into a battery of tests for developing medications.

METHOD AND SELECTED RESULTS

Several studies with psychomotor stimulants and benzodiazepines have been conducted using a procedure developed by Johanson and her colleagues (Chait et al., 1984, Johanson 1991a, 1991c). In this procedure, subjects are trained to discriminate an active drug from placebo. Subjects report to the laboratory in the morning several times a week for a total of 23 sessions. They fill out subjective effects questionnaires, ingest a capsule, and then are free to leave. They also fill out questionnaires 1, 3 and 6 hours after leaving. During the first four sessions (phase 1), they receive each of the two drugs (active vs placebo) on two of these four sessions. The drugs are identified to the subject prior to ingestion by letter code (drug A or drug B). During the next seven sessions (phase 2), the procedure is the same except the capsules are not identified to the subject. They receive drug A or drug B three or four times each, in a mixed order. Six hours after receiving the capsule, subjects telephone the experimenter to report their identification. When correct, they receive a monetary bonus. If the identification is correct on five of the seven sessions, subjects enter the third phase. This phase has six additional training sessions as previously described. They receive drug A on half of these sessions and drug B on the other half. During the other six sessions that are intermixed, subjects receive capsules that contain test drugs. Regardless of their

identification, they receive a monetary bonus, i.e., there is no correct identification. These additional drugs/doses are given in order to assess the specificity and selectivity of the discrimination.

Four studies have been conducted using 10 mg *d*-amphetamine and placebo as the two drugs (Chait et al., 1985, 1986a, 1986b, Chait and Johanson 1988). The procedure was identical for all four studies and a total of 100 subjects participated. Of these 100, 53 learned the discrimination. During test sessions, those subjects that learned the discrimination identified phenmetrazine, phenylpropanolamine, and mazindol as amphetamine. Half of these subjects identified fenfluramine, caffeine, benzphetamine and 5 mg d-amphetamine as amphetamine but only a few subjects identified diazepam and 2 mg d-amphetamine as the active drug, i.e., they were identified as placebo.

Two studies have been conducted using 10 mg diazepam and placebo as the two drugs (Johanson 1991a, 1991b). A total of 28 subjects participated and 22 of these learned the discrimination. During test sessions, most subjects identified 2 mg lorazepam and 0.25 mg triazolam as active drug, over half identified 50 mg pentobarbital, 10 mg buspirone, and 5 mg diazepam as active drug, but few identified amphetamine, tripellennamine, or lower doses of diazepam, lorazepam, buspirone, and triazolam as active drug.

These studies have indicated that drug discrimination procedures are feasible to use with human subjects, that the discrimination learned is sensitive to changes in dose (dose-response) and that drugs with pharmacological properties similar to the training drug are identified similarity. In contrast, to the extent that drugs have a different profile of effects, they are not identified as active drug. Furthermore, the results are generally comparable to those found with nonhumans trained to discriminate amphetamine and diazepam. The only exception to this comparability across species is the finding that buspirone substituted for diazepam as a discriminative stimulus. However, in an ongoing study with humans trained to discriminate 15 mg buspirone from placebo, both 10 mg diazepam and 0.25 mg triazolam substitute in over 75% of the subjects (unpublished observations).

RELATIONSHIP BETWEEN DISCRIMINATIVE STIMULUS AND SUBJECTIVE EFFECTS

As previously noted, the discriminative stimulus effects of drugs are considered a model of human subjective effects. To the extent that subjective effects are related to drug abuse and to the extent that blockade or mimicking of these subjective effects decreases the probability of drug abuse, drug discrimination procedures might be useful in medication development. It is imperative, therefore, to evaluate the relationship or concordance between discriminative stimulus and subjective effects, which can only be done in studies that use human subjects.

There are several strategies that can be used to evaluate the extent of concordance between subjective and discriminative stimulus effects. One strategy is to compare the profiles of subjective effects produced by the the two training drugs (active and placebo). In both the amphetamine and diazepam studies, the active drug was shown to produce a distinct profile of subjective effects that was significantly different than the profile of effects that was reported when placebo was administered (Chait et al., 1985, Johanson 1991a). Thus, while it is only presumptive evidence, it is clear that differences in subjective effects could form the basis of the discrimination. A second strategy which is related to the first is to

compare the subjective effects of the active drug in subjects that learn the discrimination to its subjective effects in those individuals that do not learn the discrimination. In the amphetamine series of studies, approximately half of the subjects learned the discrimination (N=53) whereas the other half did not (N=47). When these two groups were compared, it was found that only the subjects that learned the discrimination experienced subjective effects that differed from placebo (Chait et al., 1989). This analysis also indicates that subjective effects could be the basis of the discrimination. That is, in those subjects that report no change in subjective state, amphetamine did not function as a discriminative stimulus. A third strategy for evaluating the concordance between subjective and discriminative stimulus effects is to compare the subjective effects of drugs that are identified as active drug and those that are not. For instance, in Chait et al., (1986b), phenmetrazine was identified as amphetamine and in addition, its profile of subjective effects was similar to that of amphetamine. Likewise, in Johanson (1991a). lorazepam produced an almost identical profile of subjective effects as diazepam. Further, drugs that did not substitute for the training drugs did not produce subjective effects that were drug-like. A fourth strategy is to compare the subjective effects of a test drug that is identified as active drug in some subjects to its subjective effects in the group of subjects that identifies this drug as placebo. For instance, in Johanson (1991a), half of the subjects called 50 mg pentobarbital active drug (10 mg diazepam) and half identified this drug as placebo. Likewise, the subjective effects produced by pentobarbital in the former group were diazepam-like whereas in the other group, they were placebo-like. Taken together, these analyses indicate that there is a strong relationship between discriminative stimulus and subjective effects. While the relationship may not be perfect, there is enough correspondence to justify the use of drug discrimination methods as ways of evaluating the subjective effects of drugs.

MEDICATION DEVELOPMENT

There are several experimental outcomes in drug discrimination studies that might indicate that a drug would be useful clinically. First, drug discrimination studies might identify a drug that antagonizes the discriminative stimulus effects of the drug of abuse. It would be necessary to demonstrate the specificity of this effect by showing that this disruption occurred at doses that did not produce behavioral toxicity. Second, drugs might be identified that substitute for the drug being abused but do so at doses that are not severely disrupting of ongoing behavior. If this same drug were not self-administered, it might be useful as a replacement medication. That is, the replacement drug might produce enough agonist effect to deter patients from seeking these same effects, albeit at greater intensity, from the drug of abuse. For example, mazindol has been shown to substitute for amphetamine as a discriminative stimulus in humans although it is not self-administered by humans (Chait et al., 1985, 1987). Third, studies might identify a drug that had no discriminative stimulus effects even at doses that disrupted responding. While such a drug undoubtedly would never be abused itself, proving the absence of subjective effects would require an extensive testing with a wide range of active drugs.

ADVANTAGES AND DISADVANTAGES

There are several disadvantages of using drug discrimination methods in humans as a means of determining the subjective effects of drugs and conversely, the ability of medications to alter or mimic these subjective effects. First, unlike questionnaires methods used to assess subjective effects in experienced drug users, the procedure

requires training within the laboratory. Further, exposure to drugs is relatively extensive but is also limited to a narrow dose range. Thus, it is possible that different results would be obtained at doses more like those used by experienced drug users. Third, drug discrimination studies in both humans and nonhumans have not focused on methodological issues. The entire field of behavioral pharmacology has taught us that the stimulus effects of a drug are not invariant. Thus, it would be useful to extend the range of conditions used in the evaluation of the discriminative stimulus effects of a drug. This lack of understanding of the determinants of discrimination can only be ameliorated by additional studies. Fourth, while not unique to drug discrimination methods, the results it generates cannot indicate the potential usefulness of a medication in the absence of additional studies. Discriminative stimulus effects are not direct measures of reinforcing effects and unless a potential medication is also evaluated in a self-administration paradigm and found to decrease the reinforcing properties of the drug of abuse, it would be foolish to develop it any further. In addition, if a drug substitutes, it must itself be tested in the self-administration paradigm to determine whether it has abuse liability itself. Finally, the development of methods for assessing discriminative stimulus effects of drugs in humans has really only begun. While the results generally indicate that the discrimination is sensitive and specific, a great deal of additional research is needed before this method is adopted by those interested in medication development.

Despite all of these disadvantages, the procedure also has several characteristics that are advantageous. Although it is generally agreed that subjective effects are important determinants of a drug's abuse potential, these interoceptive events are difficult to measure objectively. Drug discrimination has the possibility to be an objective measure of these internal states. Second, unlike questionnaire approaches to the measurement of subjective effects, the drug discrimination procedure does not rely on the subject's ability to recollect past experiences and may minimize individual differences in response due to previous differential drug exposure. Third, drug discrimination studies are faster and cheaper to conduct compared to self-administration studies and thus should at least be useful as a first step to obviate the need for further testing of many drugs in self-administration paradigms. Finally, drug discrimination procedures might lend themselves to laboratory settings more than self-administration studies. In the latter type of study, there may be little reason for subjects not to self-administer an offered drug even if its subjective effects are largely antagonized by a medication. There is little to loose and "you never know. it might have an effect this time." There may also be reasons for human subjects to not self-administer a drug even if its effects are unaffected by a medication. Within a therapeutic context, for instance, (studies conducted prior to the initiation of treatment), subjects may wish to appear highly motivated to their therapists and show-off their "will power." In summary, there may be "demand" characteristics of the experimental setting that could alter self-administration behavior regardless of the ability of a medication to modify the subjective effects of the abused drug. These possibilities seem less likely in drug discrimination procedures where drug administration is not under the control of the subject. However, as animal studies have shown, experimenter- and subject-controlled drug administrations can produce profoundly different effects even at the biochemical level.

In summary, drug discrimination methods in humans may have an important role to play in the development of medications for the treatment of drug abuse disorders. While a great deal of additional research is necessary before they are unquestionably adopted, there are so few totally adequate procedures for the development of

medications for this indication that it would be foolish not to continue the development of drug discrimination methods in humans for this purpose.

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Cocaine-Antagonist Effects of Limited-Efficacy D_1 Agonists

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The prominent role played by cocaine's indirect actions as a dopamine agonist in its behavioral effects has suggested that directly-acting dopaminergic drugs may be useful adjuncts in the treatment of cocaine dependence. In this regard, dopaminergic D_2 receptor blockers can surmountably antagonize behavioral effects of cocaine and selective D_2 agonists already have been assessed in clinical studies. However, D_2 receptor blockers are known to produce clinically unacceptable side effects, and results with D_2 agonists have been mixed (e.g., Teller and Devenyi, 1988). Thus, the therapeutic benefit of drugs with selective actions at D_2 receptors remains unclear.

Recent evidence indicates that dopamine D_1 mechanisms also are involved in cocaine's effects. For example, the selective D_1 receptor blocker, SCH 39166, has been shown to surmountably antagonize rate-stimulant, discriminative-stimulus, and reinforcing actions of cocaine in monkeys (Bergman, et al., 1990a; Spealman, 1990; Vanover et al., 1991). Also, cocaine and high-efficacy D_1 agonists such as SKF 81297 may have comparable discriminative-stimulus and other behavioral effects in monkeys (Bergman et al., 1990b). These preclinical findings are especially encouraging, as D_1 receptor blockers may not produce the extrapyramidal symptoms associated with D_2 receptor blockade (Coffin et al., 1989) and dopamine D_1 agonists may be free of side-effects that limit compliance in treatment programs involving D_2 agonists (Tennant and Sagherian, 1987). The present studies were conducted to further examine the potential of dopaminergic drugs for treatment of cocaine dependence by evaluating cocaine-antagonist effects of drugs selective for D_1 receptors but with limited agonist efficacy. In these experiments, the cocaine-antagonist effects of SKF 75670 (low efficacy; Arnt, 1988) and R-SKF 38393 (moderate efficacy; Arnt, 1988) were compared with those of SCH 39166.

PROCEDURES

The cocaine-antagonist effects of SKF 75670 and R-SKF 38393 were evaluated in separate experiments. In the first, squirrel monkeys sat in Plexiglas chairs (Spealman, et al., 1977) within ventilated, sound attenuating chambers. Chairs were equipped with response levers, stimulus lights, and small stocks fitted with brass electrodes for delivery of brief, low intensity stimulation (200 msec; 3 mA). Responding by one group of monkeys was maintained by termination of a visual stimulus associated with electric shock to the distal portion of the tail (Morse and Kelleher, 1966). Briefly, completion of a 30-response fixed ratio

(FR30) terminated the visual stimulus and started a 10 second timeout (TO). Daily sessions consisted of five components, each comprising a 10-min TO followed by a 3-min period during which the FR schedule was in effect. Modification of the effects of cocaine on responding was studied by giving SKF 75670 or R-SKF 38393 10 minutes prior to determining the effects of a full range of cumulative doses of cocaine during the experimental session.

A second group of monkeys was trained to respond differentially on one of two levers after injections of cocaine (0.3 or 1.0 mg/kg, i.m.) or saline. Monkeys responded under an FR10 schedule of stimulus-shock termination (see above). Daily sessions comprised no more than 5 components, each composed of a 10-min TO followed by 10 presentations of the FR schedule. During training sessions, either saline or cocaine was injected during TO periods, following which responding only on the injection-appropriate lever was reinforced. During test sessions, responding on either lever was reinforced and effects of cocaine or other drugs were determined by administering cumulative doses during successive TO periods. Modification of the effects of cocaine was studied by administering pretreatment drugs 10 minutes prior to the test session.

Responding by two monkeys was maintained under a second order FI-3 min schedule of drug self-administration. In the presence of a green light, completion of a 30-response FR unit during the 3-min FI produced a 1-set change in illumination from green to amber; completion of the first FR unit after the 3-min FI elapsed produced both the stimulus change and an I.V. injection of drug or saline. A 60-set TO followed each injection and daily sessions consisted of 8 cycles of the FI schedule. Each dose of cocaine, alone and after pretreatment with SKF 75670, was studied for five consecutive sessions or until no systematic trends in rates of responding were observed.

Cocaine HCl was obtained from the National Institute of Drug Abuse. SCH 39166 was kindly provided by Schering Plough Corp, and SKF 75670 was donated by SK&F Research Laboratories.

RESULTS

Effects of Cocaine. Under the FR schedule of stimulus-shock termination, 0.3 mg/kg cocaine modestly increased responding to an average of 125% of control values; higher doses decreased responding in a graded, dose-related manner. The highest dose, 5.6 mg/kg, decreased response rates to an average of approximately 25%. In drug discrimination experiments, cocaine produced dose-related increases in the percentage of responding on the cocaine-associated lever. On average, doses of cocaine greater than 0.1 mg/kg engendered criterion levels of responding (> 80%) on the cocaine-associated lever. Cocaine maintained self-administration behavior under the second order FI schedule in both S-235 and S-370. The relationship between injection dose and response rate was characterized by a biphasic function; maximal rates of responding were maintained by 0.1-0.3 mg/kg in the two monkeys.

Antagonism of Effects of Cocaine. Pretreatment with SCH 39166 (0.03 or 0.1 mg/kg) antagonized the effects of cocaine on FR responding (Fig. 1). Cumulative dose effect functions were shifted 3-fold or more to the right for individual monkeys, indicating that the antagonistic effects of SCH 39166 were surmountable by cocaine. Antagonism between the two drugs was mutual: decreases in response rate produced by antagonistic doses of SCH 39166 were reversed by doses of cocaine lower than those that decreased responding in the presence of the D_1 antagonist. Doses of SCH 39166 that antagonized the rate-decreasing effects of cocaine also blocked the discriminative-stimulus effects of cocaine (Fig. 1). In all monkeys, responding on the cocaine-associated lever in the presence of SCH 39166 was restored by increased cumulative doses of cocaine.

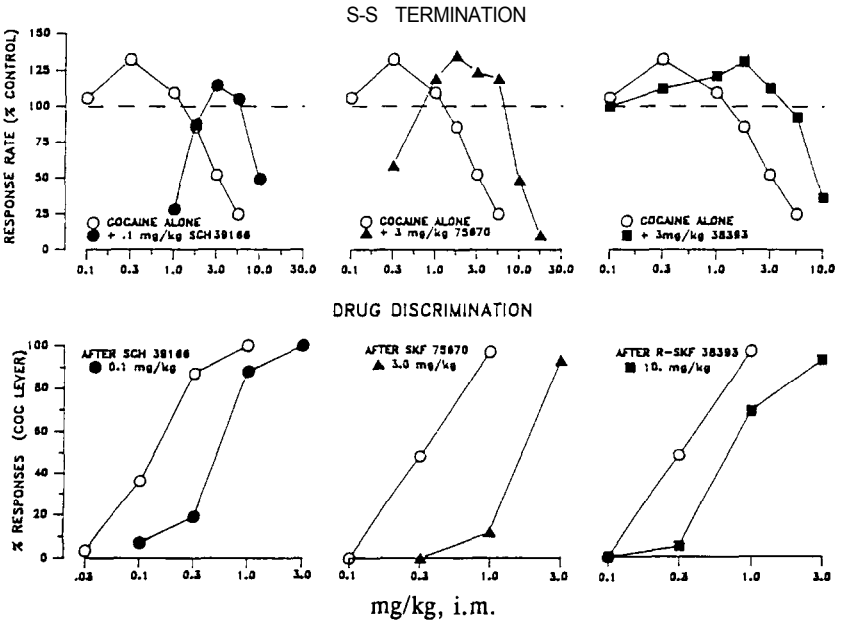

Figure 1. Cocaine-antagonist effects of SCH 39166 (left), SKF 75670 (center), and SKF 38393 (right). Abscissae: dose, log scale; ordinates: effects of cocaine expressed as percent of control rates of responding under the FR schedule of stimulus-shock termination (top) and as percentage of responses on the cocaine-associated lever in drug discrimination experiments (bottom).

The limited-efficacy D_1 agonists SKF 75670 (0.1-3.0 mg/kg) and R-SKF 38393 (0.1-10.0 mg/kg) did not have rate-stimulant, discriminative-stimulus, or reinforcing effects characteristic for cocaine (data not shown). However, presession administration of SKF 75670 or R-SKF 38393 modified the behavioral effects of cocaine in a manner comparable to that observed with SCH 39166. For example, 3.0 mg/kg SKF 75670 or R-SKF 38393 produced a 3-fold or greater rightward shift in the dose-effect function for cocaine under

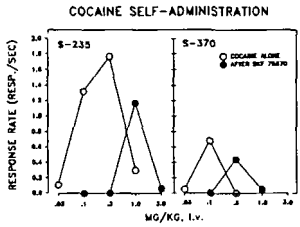

Figure 2. Antagonism of cocaine self-administration by SKF 75670 (0.3 mg/kg for S-235 and 1.0 mg/kg for S-370). Abscissae: injection dose, log scale; ordinates: rates of cocaine-maintained responding.

the FR schedule of stimulus-shock termination (Fig. 1). The effects of cocaine in drug discrimination studies also were shifted rightward by SKF 75670 and R-SKF 38393. For the group of monkeys, 1.0 mg/kg of SKF 75670 and 10.0 mg/kg of R-SKF 38393 attenuated the discriminative stimulus effects of cocaine, which were restored by increasing the dose of cocaine to 3.0 mg/kg (Fig. 1). Finally, the effects of cocaine under the second-order FI schedule of drug self-administration also were antagonized by SKF 75670 (Fig. 2). For both monkeys, the reinforcing effects of optimum injection doses of cocaine (0.1-0.3 mg/kg) were blocked by treatment with SKF 75670 (0.3-1.0 mg/kg), and generally were restored by a 3-fold increase in dose of cocaine per injection.

DISCUSSION

The present experiments provide further evidence that dopamine D_1 mechanisms play a prominent role in the behavioral effects of cocaine. As in previous studies in monkeys (Bergman et al., 1990; Spealman, 1990; Vanover et al., 1991), dose-effect functions for the behavioral effects of cocaine were shifted rightward by the dopamine D_1 receptor blocker, SCH 39166, in a manner indicative of surmountable antagonism. This view is further strengthened by the present findings that the limited-efficacy D_1 agonists SKF 75670 and R-SKF 38393 also can surmountably antagonize rate-altering, discriminative-stimulus, or reinforcing effects of cocaine in monkeys. These data represent the first demonstration that the effects of the indirectly-acting dopaminergic agonist, cocaine, can be antagonized by a limited-efficacy direct agonist, and might be predicted by classical receptor theory. These results also suggest that other mechanisms by which cocaine may act can be evaluated similarly. For example, a comparison of dopamine D_2 receptor blockers and low efficacy D_2 agonists might provide important information regarding the role of D_2 receptor mechanisms in the behavioral effects of cocaine.

The behavioral effects of limited-efficacy dopamine D_1 agonists may be of practical importance in the development of novel therapeutics for the treatment of cocaine abuse. For example, low-efficacy agonists such as SKF 75670 might be useful for the treatment of acute emergencies associated with cocaine overdose, as they may not produce troublesome side effects associated with conventional dopamine receptor blockers (Coffin et al., 1989).

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Potential Effects of Benzodiazepines on Cocaine Reinforcement in Rats

N.E. Goeders

Initial cocaine use is often reported by humans to produce profound subjective feelings of well-being and a decrease in anxiety. However, continued cocaine use or the administration of high doses of the drug can also induce severe anxiety. Cocaine has also been reported to precipitate episodes of panic attack in some individuals. Since panic disorder only became apparent after chronic cocaine use in many of these cases, the drug may have functioned as a precipitating as well as a causative factor in neurobiologically vulnerable individuals. In the emergency room, benzodiazepines (BZDs) are often used for some of the medical complications associated with cocaine intoxication. Convulsions are often apparent following an acute cocaine overdose, and these seizures can be treated with intravenous diazepam, but not dilantin. Furthermore, some of the major symptoms associated with cocaine withdrawal in humans also often include severe anxiety, restlessness and agitation. These data suggest that anxiety may be involved in the etiology of cocaine use and/or withdrawal in humans.

The binding site associated with cocaine reinforcement may be localized at the dopamine uptake site since the affinity of cocaine and related drugs for the dopamine transporter is highly correlated with the reinforcing efficacy of these drugs (Ritz et al., 1987). The mesocorticolimbic dopaminergic system may be involved in cocaine reinforcement since 6-hydroxydopamine lesions of the nucleus accumbens or ventral tegmental area attenuate intravenous cocaine self-administration in rats (Roberts et al., 1980; Roberts and Koob 1982). More recent investigations have also implicated the medial prefrontal cortex (MPC) in cocaine reinforcement (Schenk et al., 1991). Rats with a 70% depletion of dopamine in the MPC responded reliably for low doses of cocaine that were unable to consistently maintain responding in sham-treated rats during both the acquisition phase for self-administration or once the behavior was established, suggesting that dopaminergic activity within the MPC does indeed play a role in intravenous cocaine self-administration. Further support for the role of the mesocorticolimbic dopaminergic system

in cocaine reinforcement comes from intracranial self-administration data from our laboratory where we demonstrated that cocaine would maintain responding resulting in the discrete microinjection of the drug directly into the MPC (Goeders and Smith 1983).

The mesocorticolimbic dopaminergic system also appears to be activated by stress. It is well documented that dopaminergic neuronal activity in the prefrontal cortex is selectively activated following electric footshock in rodents (Thierry et al., 1976) and that these stress-induced increases in dopamine turnover can be inhibited or reversed by pretreating the animals with diazepam (Reinhard et al., 1982). More recent data suggest that both cocaine and footshock stress appear to selectively activate the mesocorticolimbic dopaminergic system and that these effects may be additive (Kalivas and Duffy 1989). Furthermore, the anxiogenic BZD inverse agonist, FG 7142, also selectively increases dopamine turnover in the prefrontal cortex, while BZD agonists decrease turnover, suggesting that BZD recognition sites may exert a selective and powerful modulatory influence on the mesocorticolimbic dopaminergic system (Tam and Roth 1989). The following experiments were therefore designed to investigate the effects of chronic cocaine administration on BZD receptor binding in the rat brain. We also investigated the effects of systemic and central BZD agonist pretreatment on intravenous cocaine self-administration.

In the first experiments, forty-two adult male Fisher 344 strain rats were injected once daily with saline (n=6) or cocaine (20 or 40 mg/kg, ip; n = 18 per group) for 15 days. The animals were sacrificed by cardiac perfusion under sodium pentobarbital anesthesia (50 mg/kg, ip) either 20 minutes, 2 days or 14 days following the final injection. The brains were rapidly removed and cut into 10 µm coronal sections, which were thaw-mounted onto subbed slides and stored at -20ºC. BZD receptors were visualized using [^{3}H]flumazenil under standard autoradiographic conditions. Briefly, slide-mounted tissue sections were incubated for 40 min at 4ºC with 2 nM [^{3}H]flumazenil (79.8 Ci/mmol) in 0.17 M Tris-HCl buffer (pH 7.4 at 4ºC). Non-specific binding was estimated by including 1 µM clonazepam in the incubation. Following incubation, the sections were washed for 2 min in ice-cold buffer to reduce non-specific binding. The slides were briefly dipped in ice-cold distilled water and immediately dried under a stream of cool, dry air. Slides were affixed to mounting board, placed in X-ray cassettes with radioactive standards and apposed to [^{3}H]Ultrofilm. After a ten week exposure, the film was developed and and the autoradiograms were quantified using computer-assisted microdensitometry. In general, cocaine decreased BZD receptor binding in terminal fields for the mesocorticolimbic dopaminergic system, while increasing labeling in terminal fields for the nigro-striatal system. BZD receptor labeling was significantly reduced in the MPC following 20 mg/kg cocaine when the animals were sacrificed 20 min or 2 days following the final injection, but returned towards saline control levels after 14 days. However, these effects persisted for up to 14 days with the 40 mg/kg dose. Similar decreases in binding were observed for

up to 2 days in the rostral (but not caudal) nucleus accumbens. Receptor labeling was significantly reduced in the sulcal prefrontal cortex only with the 40 mg/kg dose. In contrast, cocaine significantly increased BZD receptor binding in the caudate nucleus when the animals were sacrificed 20 min following the final injection. In the substantia nigra, BZD binding was decreased 20 min and 2 days following the final injection of 20 mg/kg cocaine, but these effects were not significant with the 40 mg/kg dose. In contrast, while no significant changes were observed in the ventral tegmental area 20 min after the final injection with either dose of cocaine, BZD receptor labeling was increased at 2 days and 14 days with either dose.

However, response-contingent and response-independent cocaine administration can result in different behavioral and neurobiological effects. We previously reported the involvement of BZD receptors in cocaine reinforcement processes (Goeders et al., 1991). In these experiments, 7 groups of 3 adult male Fisher strain 344 littermate rats were used. The self-administration (SA) littermate was trained to respond under a fixed-ratio 2 schedule of cocaine presentation (0.33 mg/200 µ1 infusion) during daily 6 hour sessions, while the yoked-cocaine (YC) and yoked-saline (YS) littermates received simultaneous infusions of cocaine or saline, respectively. After a 30 day exposure to the drug, the animals were sacrificed by cardiac perfusion, and the brains were rapidly removed and prepared for quantitative autoradiography of BZD receptors using [^{3}H]flumazenil as described above. Cocaine (SA and YC vs YS) delivery resulted in significant increases in BZD binding in the frontal cortex with decreases in the substantia nigra and ventral tegmental area. Response-contingent cocaine (SA vs YC) resulted in significant increases in BZD labeling in the MPC and nucleus accumbens. These data suggest that BZD receptors located in brain regions associated with the mesocorticolimbic dopamine system may be involved in cocaine reinforcement processes.

We previously described the effects of systemic pretreatment with the benzodiazepine agonist, chlordiazepoxide (CDP), on intravenous cocaine self-administration in rats (Goeders et al., 1989). In these experiments, 16 adult male Fisher 344 strain rats were trained to self-administer cocaine (0.5 or 1.0 mg/kg, iv) during daily 2.5 hour sessions conducted 5 days per week. The rats were trained to respond under a fixed-ratio 4 limited hold 300-set (FR4 LH300) schedule of reinforcement where cocaine delivery was contingent on the animal pressing the response lever 3 additional times within 5 min from the first response. Pretreatment (15 min) with low doses (0.3 to 1.0 mg/kg, ip) of CDP produced small increases in drug-intake with 0.5 mg/kg cocaine, while higher doses (10 mg/kg, ip) significantly decreased drug-intake in all rats tested. The effects of CDP on self-administration were attenuated when the concentration of cocaine was increased to 1.0 mg/kg, suggesting that CDP was opposing rather than augmenting the pharmacological effects of cocaine. However, since the decreases in drug-intake may have resulted from a non-specific effect on

the ability of the rats to respond with higher doses of CDP, the following experiments were initiated.

These experiments were designed to investigate the effectiveness and specificity of alprazolam on intravenous cocaine self-administration. Alprazolam was studied since this drug has been proven to be clinically effective in the treatment of anxiety and panic attacks and has been proposed to be useful in the treatment of some types of depression. Cocaine use and withdrawal have also been associated with anxiety, depression and even panic attacks in some cases. Alprazolam was tested in adult male Wistar rats under a multiple schedule of intravenous cocaine presentation and food reinforcement. Cocaine (0.25 or 0.5 mg/kg, iv) was available during the first hour of the session under a FR4 schedule of reinforcement. During the second hour, food presentation (45 mg pellets) was available under a discrete-trial FR10 schedule of reinforcement. The animals were pretreated with alprazolam (0, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/kg, ip) thirty minutes prior to the start of the behavioral session. Initial exposure to alprazolam resulted in non-specific decreases in both cocaine- and food-maintained responding. However, the animals quickly became tolerant to the effects of the drug on food reinforced responding upon subsequent testing. On the other hand, dose-related decreases in cocaine self-administration (0.5 mg/kg) were maintained throughout testing with alprazolam. Furthermore, significant increases in drug-intake were observed in some animals with 0.25 mg/kg cocaine following pretreatment with low doses of alprazolam (e.g., 0.25 or 0.5 mg/kg, ip), suggesting that the animals were attempting to overcome a drug-induced blockade of the reinforcing properties of cocaine. These data also suggest that pharmacological effects inherent to alprazolam (i.e., anxiolytic?) specifically altered cocaine reinforcement without affecting responding maintained by food.

The final series of experiments was designed to determine the specific involvement of BZD binding sites within the MPG in cocaine reinforcement processes. Experimentally naive adult male Wistar rats were implanted with chronic indwelling jugular catheters and bilateral guide cannulae into the MPC under sodium pentobarbital anesthesia during the same surgery. The animals were trained to self-administer cocaine during daily 2 hour sessions 5 days per week under a FR4 schedule of reinforcement where 4 depressions of the response lever resulted in a 200 µl intravenous infusion of 0.5 mg/kg cocaine delivered over 5.6 seconds. CDP HCl was injected into the MPC 5 minutes before the start of the session in a volume of 200 nl/side over 30 seconds using a 1 µl microsyringe. The injection cannula was left in place for an additional 30 seconds to prevent the diffusion of the drug up the guide cannulae. Doses of CDP tested ranged from 25 pmol (8.5 ng) to 500 pmol (168 ng) per side, and each dose was tested at least twice. CDP was dissolved in saline, which also served as the vehicle. All rats self-administered cocaine, with stable rates of responding obtained within 2 to 3 weeks. In general, intracranial CDP delivery did not affect the total number of cocaine infusions/session. Furthermore, no observable signs of

ataxia or sedation were seen in any animal following any dose of CDP tested. However, significant increased in intravenous cocaine self-administration were observed during the first 30 minutes of the session following the microinjection of extremely small doses of CDP (e.g., 8.5 to 34 ng) into the MPC. This increase in drug-intake following intracranial microinjections of CDP may represent an attempt by the animal to over-come a drug-induced blockade of cocaine reinforcement. This mechanism of action may involve a decrease in dopamine turnover in the MPC induced by the binding of this agonist to the BZD receptors localized in this brain region (Tam and Roth 1989). This purported decrease in dopamine turnover could theoretically decrease in dopamine uptake) that may be important for cocaine reinforcement. The short duration of the effects of CDP on drug-intake (i.e., less than 10 to 20 minutes in most cases) is consistent since the small amount of CDP injected would likely be rapidly metabolized or would quickly diffuse away from the injection site. The results of these experiments suggest that BZD receptors localized in the MPC are involved in intravenous cocaine reinforcement in rats.

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Effects of Magnesium on Cocaine-Reinforced Responding in Mice, Rats and Squirrel Monkeys

K.M. Kantak

INTRODUCTION

This paper reviews several experiments on the interactions between magnesium chloride ($MgCl_2$) and cocaine in mice, rats and squirrel monkeys. The experimental findings across species center on 3 reoccurring themes: 1) $MgCl_2$ has reinforcing stimulus properties, but low potential for abuse; 2) under some experimental conditions, $MgCl_2$ maintains or potentiates the effects of cocaine; and 3) under other experimental conditions, $MgCl_2$ attenuates or blocks the effects of cocaine.

MOUSE STUDIES

The conditioned place preference procedure was used to investigate the reinforcing stimulus properties of $MgCl_2$ in mice. In one experiment (Lawley and Kantak 1990a), 5 groups of mice were conditioned with 5 mg/kg cocaine to the non-preferred side of a three compartment chamber over an 8 day period. Prior to cocaine conditioning, mice spent between 20-30% of their total time on the non-preferred side. After cocaine conditioning these same mice spent between 70-80% of their total time on the previously non-preferred side. Following conditioning, mice were injected with a test drug to determine what effect that drug had on the conditioned cocaine effect. Compared to an injection of saline, 30 mg/kg $MgCl_2$ and 1 mg/kg *d*-amphetamine potentiated the conditioned cocaine effect. In contrast, 10 mg/kg pentobarbital and 0.25 mg/kg haloperidol blocked the conditioned cocaine effect. These data suggest that $MgCl_2$ shares stimulant-like stimulus properties with cocaine.

In another experiment in mice it was determined if $MgCl_2$ could induce a conditioned place preference (Lawley and Kantak 1990b). Prior to $MgCl_2$ conditioning, mice spent 25-40% of their total time on the non-preferred side. Following conditioning with $MgCl_2$ over an 8 day period, significant shifts in place preference were obtained with 15 and 30 mg/kg $MgCl_2$, but not with 125 mg/kg. Mice now spent between 5560% of their total time on the previously non-preferred side. The conditioning effects of $MgCl_2$ were not as robust as those of cocaine because only 50% of the mice showed a strong conditioning effect. With cocaine as the conditioning agent, 100% of the mice showed a strong conditioning effect. These data suggest that $MgCl_2$ has reinforcing stimulus properties because it induces a conditioned place preference and potentiates a cocaine-conditioned place preference. However,

$MgCl_2$ has low potential for abuse compared to cocaine because it is not as efficacious as cocaine in producing a conditioned place preference.

RAT STUDIES

The intravenous self-administration procedure was used to investigate the reinforcing stimulus properties of $MgCl_2$ in rats (Kantak et al., 1991). In groups of rats given access to the 0.75 mg/kg/infusion training dose of cocaine under a FR 1 schedule, $MgCl_2$ maintained responding in a dose-dependent manner when cocaine availability was discontinued. NaCl and 1.5 mg/kg/infusion $MgCl_2$ engendered typical extinction responding. Doses of 6 and 12 mg/kg/infusion $MgCl_2$ produced response rates and patterns of responding that were most similar to 0.75 mg/kg/infusion cocaine. These doses of $MgCl_2$ maintained responding for at least 10-20 days after cocaine was discontinued. Responding was maintained above NaCl rates by $MgCl_2$ under a FR 5 schedule for at least 5 days in cocaine-trained rats. As FR value incremented from 1 to 5, the rate of responding maintained by 0.75 mg/kg/infusion cocaine or 6 mg/kg/infusion $MgCl_2$ increased in a similar fashion in order to maintain the intake of drug. Intake of cocaine was 2 to 3 mg/hr, and intake of $MgCl_2$ was 16 to 25 mg/hr. $MgCl_2$ also maintained responding above NaCl rates under different progressive ratio (PR) schedules in cocaine-trained rats. As PR value incremented from 1 to 3, breakpoints for 0.75 mg/kg/infusion cocaine and 6 mg/kg/infusion $MgCl_2$ increased and were significantly above the NaCl breakpoints for PR 2 and PR 3 schedules. The breakpoints for cocaine were relatively high which indicates that cocaine has a high potential for abuse, while the breakpoints for $MgCl_2$ were relatively low which indicates that $MgCl_2$ has a low potential for abuse. A low potential for abuse for $MgCl_2$ was also suggested by data showing that responding was not maintained by $MgCl_2$ in cocaine-naive rats (Kantak et al., 1990a).

Although it appears that the availability of $MgCl_2$ substitutes for cocaine because it maintains responding, the availability of $MgCl_2$ for 4 weeks prior to access to cocaine blocks the reinforcing effects of cocaine when the rats are previously cocaine-naive, and reduces the potency of cocaine when the rats are previously cocaine-experienced. In cocaine-naive rats, preexposure to 3 mg/kg/infusion $MgCl_2$ blocked the reinforcing effects of 0.75 mg/kg/infusion cocaine until days 8-10 of access when responding was instated and maintained. Cocaine will typically maintain responding on day 1 of access. In cocaine-experienced rats, preexposure to 3 or 12 mg/kg/infusion $MgCl_2$ caused 50% and 80% increases from baseline cocaine-maintained response rates during the 5 reacquisition days, respectively. This suggests a reduction in the potency of cocaine under these conditions.

MONKEY STUDIES

To investigate interactions between $MgCl_2$ and cocaine in squirrel monkeys, FI 3-min food-maintained (Kantak 1991) and stimulus shock termination-maintained responding was used. Experiments demonstrate that $MgCl_2$ potentiates and blocks the effects of cocaine, depending upon dose of $MgCl_2$ and dose of cocaine. Responding maintained by food was not influenced by 30 or 100 mg/kg $MgCl_2$ when these doses were injected alone. Injections of cocaine alone produced typical inverted-U dose-effect curves where low doses were ineffective, intermediate doses increased response rates and high doses decreased response rates. When 100 mg/kg $MgCl_2$ was combined with cocaine, the cocaine dose-effect curve was flattened. When 30 mg/kg

$MgCl_2$ was combined with cocaine, three kinds of effects were produced. First, in 2 of 3 monkeys, $MgCl_2$ increased the rate of responding associated with an ineffective dose of cocaine. This was about a 150% increase in rate. Second, in all 3 monkeys, $MgCl_2$ attenuated the rate-increasing effects of cocaine. Third, in all 3 monkeys, $MgCl_2$ attenuated the rate-decreasing effects of cocaine.

Under conditions of stimulus shock termination, similar effects were measured. In this experiment, a cumulative dosing procedure was used to determine the effects of cocaine (0, 0.1, 0.3, 1 and 3 mg/kg i.m.) and $MgCl_2$ (0, 30 and 100 mg/kg s.c.) on stimulus shock termination responding maintained under a FI 3-min schedule with a 3-set limited hold in a single pilot monkey. Each of 5 components contained 5 FI sequences and a I0-min timeout preceded each 15 min component. Injections of cocaine were made midway through each timeout and the single injection of $MgCl_2$ was made midway through the initial timeout only. Data are expressed as responses per 15 min for all conditions in Figure 1. Baseline is the average of 10 days of responding where no drugs or injections were given. The results demonstrate that cocaine produced an inverted-U dose-effect curve with a low dose being ineffective, intermediate doses increasing the rate of responding and a high dose reducing the rate of responding. $MgCl_2$ produced increases in the rate of responding in the absence of cocaine. The significance of this finding is that $MgCl_2$ can act as a stimulant in a primate species. In addition, $MgCl_2$ potentiated the effects of a low dose of cocaine and attenuated the effects of a high dose of cocaine on responding maintained by stimulus shock termination.

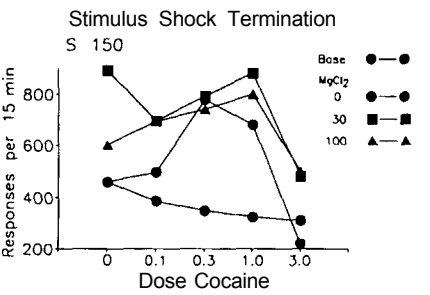

Figure 1

In another experiment, the pilot monkey that was exposed to a stimulus shock termination schedule had a 1/2 log unit shift to the right in the cocaine dose-effect curve after it was redetermined following 2 months of semiweekly $MgCl_2$ dosing which was discontinued during the cocaine determinations (Figure 2). A dose of 3 mg/kg cocaine which typically decreases FI 3-min responding in squirrel monkeys, produced an increase in the rate of responding. A dose of 0.3 mg/kg cocaine which typically increases the rate of responding under these conditions, was ineffective. These data indicate that the semiweekly injections of $MgCl_2$ for 2 months reduced the potency of cocaine. The cocaine dose-effect curve returned to normal 2 weeks after $MgCl_2$ was discontinued.

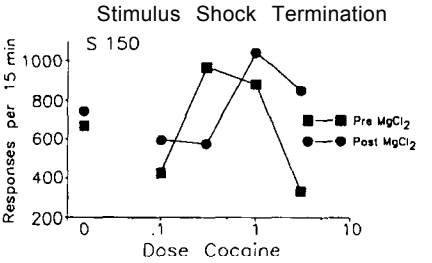

Figure 2

DISCUSSION

These data suggest that there is a species generality to the effects of $MgCl_2$ on cocaine-reinforced behavior. $MgCl_2$ can potentiate or maintain the effects of cocaine, and it can block or attenuate the effects of cocaine. These kinds of interactions have been demonstrated in mice (Lawley and Kantak 1990a, 1990b; Kantak 1989), rats (Kantak et al., 1990b, 1991), and squirrel monkeys (Kantak, 1991). The influence of $MgCl_2$ is complex; low doses have different effects than high doses, and acute administration has different effects than chronic administration. Dose of cocaine also has a bearing on the interaction with $MgCl_2$ in all 3 species.

Given the diverse actions of $MgCl_2$, it might be useful in one or more ways to treat cocaine dependence in humans. According to H.D. Kleber, cocaine withdrawal can be divided into three phases, with phases I and II containing stages where there is high craving for cocaine and phase III containing a stage where there is episodic craving for cocaine. These stages represent points where interventions which reduce craving for cocaine, substitute stimulus properties for cocaine, or attenuate or block the effects of cocaine might be efficacious in treating cocaine dependence. The experiments reviewed above suggest that $MgCl_2$ may be efficacious to either substitute for cocaine during the initial stages of withdrawal, or to block or attenuate the effects of cocaine during the latter stages of withdrawal and thus prevent relapse.

An interesting point of speculation is how does $MgCl_2$ produce its various effects on cocaine-reinforced behavior? On the dopamine post-synaptic nerve ending, there exists a high affinity Mg^{2+} binding site associated with the *a* catalytic subunit of the G-protein (Freissmuth et al., 1989). When Mg^{2+} binds to this site, the receptor is in its high affinity state and low concentrations (nM) of agonist are needed to activate the receptor. Without $Mg^{2'}$ bound to this site, the receptor is in its low affinity state and high concentrations (μM) of agonist are needed to activate this receptor. Mg^{2+} therefore can increase the ability of dopamine to bind to its receptor (Devries and Beart 1985). It may be through this Mg^{2+} binding site that $MgCl_2$ produces its stimulant-like behavioral effects and potentiates the indirect dopamine agonist effects of cocaine. A low affinity Mg^{2+} binding site is associated with the NMDA/PCP receptor complex (Reynolds and Miller 1988). When Mg^{2+} binds to this site, it acts as a noncompetitive antagonist to the NMDA receptor. Consequently, through this binding site, Mg^{2+} has been shown to inhibit the release of dopamine which is normally stimulated by excitatory amino acids (Werling et al., 1990). This mechanism could account for the ability of $MgCl_2$ to block or attenuate the effects of cocaine which rely on released dopamine for expression. A possible way in which the long-term administration of $MgCl_2$ blocks the effects of cocaine and reduces its potency may be related to an interaction at the cocaine receptor on the presynaptic dopamine transporter. High concentrations of Mg^{2+} have been shown to inhibit the binding of cocaine to its receptor (Kennedy and Hanbauer 1983). Further research on the physiological as well as the behavioral mechanisms by which $MgCl_2$ interacts with cocaine in various species might result in its use as novel strategy to treat cocaine dependence in humans.

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Access Schedules of Oral Cocaine and Ethanol in Rats

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INTRODUCTION

Typically if one wishes to demonstrate that a drug is a reinforcer, one usually confines the animal in an operant chamber for an hour or two a day and makes the delivery of some small quantity of the drug contingent on some arbitrary response of the animal, e.g., a lever press. If the rate of pressing maintained by the drug exceeds that of its vehicle, then the drug is said to be a reinforcer. This procedure has proved to be a very valuable research tool. It has allowed the identification of numerous substances that have abuse potential (Weeks, 1962; Pickens, 1968; Deneau et al. 1969) as well as the identification of a number of pharmacological therapeutics that block the reinforcing effects of a number of drugs (Weeks and Collins, 1964; Thompson and Schuster, 1964; and Goldberg et al. 1971).

However, the procedure may not be the best preparation for assessing the behavioral consequences of drug self-administration or for identifying behavioral strategies for the treatment of drug dependence because it does not accurately capture how the animal normally forages for commodities in the natural environment.

In everyday foraging several commodities are concurrently available. Animals eat and drink an discrete meals and bouts. For example, a rat may eat 10 to 20 or so meals and drink the same number of water bouts per day (Collier et al., 1972). Animals are not food deprived to some percentage of their free feeding weight but the animal is often required to emit a good deal of behavior to gain access to food or water, i.e., each commodity has an associated procurement cost (Collier et al. 1972). The animal also determines when a meal or bout is initiated. Once access is obtained the animal determines the amount consumed during a particular meal or bout. In the standard conditioning preparation, the emission of a number of responses produces a small portion of the food that the animal would consume during a meal or a bout, i.e., the experimenter manipulates what is known in the foraging literature as consumption cost. The procurement cost is a between meal or bout manipulation while the consumption cost is a within meal or bout manipulation.

In everyday foraging, the animal's economy is closed in that it gets all of its daily requirements through interacting with its environment, i.e., the animal receives all of its food and water within the experimental session. In the standard conditioning procedure the economy is open in that the animal is often fed a supplement after each session in order to maintain its body weight at some experimenter determined level. Both Collier et al., (1972) and Hursh (1980) have demonstrated that the relation between response rate and response cost (responses/reinforcer) is opposite in a closed versus an open economy. For example, in a closed economy, response rate increases as a fixed ratio schedule is increased while in an open economy response rate increases as the schedule is increased to fixed ratio (FR) 80 and decreases at higher fixed ratios.

The present experiments examined oral ethanol and cocaine self-administration in an experimental situation that contained these ethological and ecological features of everyday foraging. The experiments determined the quantity of behavior that the drugs could maintain when the animal controlled the amount of cocaine or ethanol consumed per bout and compared that to how much behavior food or water could maintain when all the substances were available concurrently in a closed economy. The experiments also determined how the availability of the drug in the animal's economy influenced the amount of behavior that food or water could support. Patterns of drug intake were also examined as a function of the access ratio of ethanol and of cocaine as well as the access ratio of the other concurrently available commodities.

METHOD

Sprague-Dawley rats lived in the experimental chambers with access to food, water and a 8 % w/v ethanol or 0.05 or 0.1% w/v cocaine solution. Cages were located in an approved animal facility located within the laboratory. On each of two sides of each cage, two graduated fluid reservoirs were mounted. Each reservoir was connected via tubing, first to a Gilmont capillary valve, then to a liquid solenoid valve, and then to a stainless steel drinking spout. Both spouts were attached to electric motors which allowed each tube to be projected into or retracted from the chamber.

The left tube contained water and the right tube, either water or a drug solution. Completion of a response ratio on either an adjacent water or drug lever resulted in the projection of the appropriate drinking tube into the chamber. Each tube lick operated the liquid delivery system calibrated so that an average of 0.006ml of fluid was delivered per lick. Following 10 min without a lick, the tube was retracted from the chamber.

Completion of a response ratio on the food lever, located on the third wall of the chamber, was followed by the illumination of a light located directly above the lever. Each subsequent lever press was followed by the delivery of a 45 mg Noyes food pellet. After 10 min had elapsed without a lever press, the light was turned off. Once the food light was turned off or the tubes retracted, the animal could regain access to any of the substances by completing the response ratio. The procedure was adapted from that of Collier (1983).
At 11:00 A.M. daily, the animals were removed from their chambers, weighed,

and then placed into a separate holding cage during a one hour maintenance period. All animals were exposed to a 12-h light-dark cycle.

ETHANOL

When an 8% w/v ethanol solution was concurrently available with food and water the following results were obtained. Ethanol maintained responding on ethanol access ratios as high as 300 responses even though rats had concurrent access to food and water on a fixed ratio 1 (FR 1) schedule throughout the experiment. As the ethanol access ratio was increased the amount consumed per bout increased and bout frequency decreased. Baseline performances were also recovered. The rats behaved toward ethanol as they did toward the other commodities in their environment (Collier et al, 1972). They decreased bout frequency and increased the amount consumed per bout as access to ethanol was constrained by increasing the access ratio. The results of a control experiment demonstrated that ethanol not some other feature of the liquid delivery system e.g., a tube preference, maintained responding. Several rats were given access to water and food according an FR 1 schedule with water available from both spouts. After the preferred spout for each rat was identified, the access ratio of that spout was increased. Access ratios above FR 20 were not maintained. Thus ethanol was clearly demonstrated to maintain substantial responding with food and water concurrently available, however, it was also clear that it maintained much less behavior than either food or water. During control experiments in which only food or water was available, both food and water maintained access ratios as large as 20,480 responses per meal or bout.

When the water and ethanol access ratio was held constant at FR 1 and the food access ratio was increased from 1 to the point where responding collapsed and 3 days elapsed without a meal - ratio between 5120 and 20,480 - ethanol intake increased. Most animals did not increase ethanol intake appreciably until very high food access ratios were reached. When the food access ratio was returned to FR 1 ethanol responding returned to its previous level.

Animals behaved quite differently with respect to increases in the water access ratio. In this experiment the food and ethanol access ratio was kept at FR 1 and the water access ratio was increased until the animals consumed no water for 5 days. All animals consumed substantial amounts of ethanol at much lower water access ratios than did the animals whose access to food was restricted by increasing the food access ratio. With ethanol available, the range of access ratios at which rats stopped responding for water ranged from 80 to 150 while for food they ranged from 2560 to 20,480. When the water access ratio was returned to FR 1 ethanol intake remained elevated. During control experiments when water was the only fluid available, water access ratios as high as 20,480 were maintained.

COCAINE

Prior to conducting a number of experiments with a .05% cocaine solution instead of ethanol available from the right tube rats were exposed to a home cage sucrose fading procedure (Samson, 1986). Animals were first given

unlimited access to 10% w/v sucrose, food, and water. Cocaine was introduced into the sucrose solution at .02% w/v. After 3 days it was increased to .05% then to 0.1% and then reduced to 0.05%. Thereafter, the sucrose concentration was gradually decreased to 0%. By the end of the fading procedure animals were drinking from 4 to 19 ml of the .05% solution per day. The animals were then placed into the chambers and shaped to lever press for food, water, and 0.05% cocaine. Once stable responding was obtained at the FR 1 access ratio, cocaine access ratio was increased. Responding of all animals was maintained by 0.05% sucrose at an access ratio of FR 1. Two animals stopped responding at FR 10, one at FR 20 and the fourth stopped at FR 80.

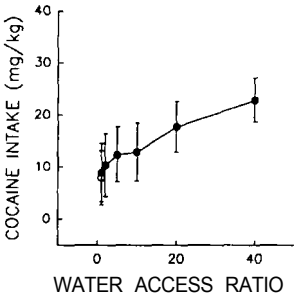

Figure 1

In another experiment with the cocaine and water access ratios held constant at FR 1, increases in the food access ratio decreased cocaine responding of two animals drinking cocaine at the FR 1 food access ratio. Cocaine consumption decreased to zero at very high food access ratios.

Increases in the water access schedule to FR 40 produced significant increases in consumption of the cocaine solution (Figure 1).

Attempts to establish self administration of a 0.1% w/v cocaine concentration by first presenting the cocaine in a 10% sucrose solution and then gradually lowering the sucrose concentration failed. Animals with food and water available on a FR 1 access schedule would self administer substantial amounts of the cocaine-sucrose solution until the sucrose was removed. Prior to the removal of the sucrose the cocaine concentration was varied from 0.02 to 0.3%. Maximum intake occurred at 0.1% (Figure 2). As with the 0.05% cocaine solution, slight increases in the water access ratio resulted in a substantial increase in cocaine intake. While the animals would not self administer a 0.1% cocaine solution when both cocaine and water were available on a FR 1 access schedule, all animals switched completely from water to cocaine consumption when the water access ratio was raised to 150.

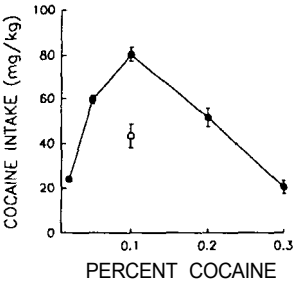

Figure 2

CONCLUSION

In conclusion, Collier's foraging procedure seems to provide a useful way to study the behavioral aspects of drug dependence. In the present experiments it was demonstrated that while rats responding could be maintained by a 0.05% cocaine or a 8% ethanol solution neither solution could support the quantity of behavior supported by food or water. Thus, while one could conclude that both substances were serving as reinforcers they did not generate the quantity of behavior that would have had an impact on the animals' other behavior. It remains to be seen whether drugs

delivered in a manner that shows them to be highly reinforcing in the standard conditioning preparation, e.g., intravenous cocaine or heroin will be able to support levels of responding in the foraging preparation, that will disrupt responding for the other available commodities.

With the growing interest in identifying behavioral strategies for the treatment of cocaine dependence experimental preparations that mimic the everyday environment of either the experimental animal or clinic patient will be important. Behavioral strategies that effectively compete with drug taking in the natural environment will be more likely identified in experimental environments that resemble the human drug taking environment.

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Effects of Cannabinoids on Synaptosomal Transmembrane Potential

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An important function of the neuronal membrane is to maintain a potential difference across it. In order to find a useful in vitro model for studying neuronal actions of the cannabinoids, we have examined effects on the functioning of neuronal membranes by measuring changes in the transmembrane potential difference in synaptosomes prepared from rat brain. Transmembrane potential was estimated by measuring the accumulation of tetraphenylphosphonium (TPP^+), a cationic probe that passively equilibrates across the membrane depending upon the membrane potential. TPP^+ accumulation is increased when the synaptosome becomes hyperpolarized and is decreased when the membrane potential becomes less negative. Δ^9-THC produces a concentration related decrease in TPP^+ accumulation. Effects were biphasic and seen at concentrations as low as 0.3 μM. The reduction in TPP^+ accumulation produced by THC administration suggests that it depolarizes synaptosomes. We have also examined the structural specificity of this action. 11-OH-Δ^9-THC, a behaviorally active THC metabolite, also produced biphasic decreases in TPP^+ uptake. Cannabinol and cannabidiol, non-psychoactive cannabinoids, produced little effect. However high concentrations (30μM) of all of the cannabinoids tested produced large increases in accumulation suggesting that these high concentrations disrupt the synaptosome. In order to further define the mechanism by which THC reduces synaptosomal uptake of TPP^+ we examined the effects of THC on the depolarizing action of increasing concentrations of potassium (5 mM-50 mM K^+) . K^+ was more effective in decreasing TPP uptake as the concentration of THC increased. The effect of potassium upon the THC dose-response curve was also examined. At a concentration of 10 mM K^+, THC produced its characteristic biphasic depolarization at lower THC concentrations compared to a normal physiologic potassium concentration of 5 mM. The possible role of calcium in the depolarizing effect of THC on synaptosomes was also explored by the removal of Ca^{2+} from the standard assay buffer and the addition of 1 mM EGTA to chelate any contaminating calcium. Removal of this ion had no effect upon the decrease in TPP uptake produced by THC, suggesting that calcium entry into the synaptosome does not play a role in the depolarizing effects of THC. This data indicates that this functional model of the neuron is responsive to the cannabinoids and selective alterations in the ionic environment. (Supported by NIDA grant DA-03725.)

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Comparison of Lipophilicity and Antinociceptive Effect of Intrathecally Administered Cannabinoids

P.B. Smith, A.H. Lichtman and B.R. Martin

Δ^9-tetrahydrocannabinol and other cannabinoids have been demonstrated to be potent antinociceptive agents. Although their specific neuroanatomical sites of action have not yet been determined, several lines of evidence indicate that they produce antinociception, in part, by spinal action. It has recently been proposed that the potency of spinally administered analgesics is inversely related to their lipophilicity. In order to determine the correlation between lipophilicity and pharmacologic effects in various cannabinoid compounds, antinociception (evaluated by the tail-flick assay) and hypothermia (evaluated by rectal temperature) were measured following intrathecal injection in male ICR mice. Sixteen cannabinoid compounds with a wide range of lipophilicities and pharmacological activities were administered. Dose response curves were generated following intrathecal administration and an ED_{50} was calculated for each compound. A correlation coefficient between lipophilicity and ED_{50} of active agents was demonstrated to be 0.76. Delta^8-THC-DMH had the highest lipophilicity of those examined [9.22 X 10^9(Po/w)] and was also the least potent (ED_{50}=95.7nmol). Levonantradol, a cannabinoid with a relatively low lipophilicity (5.04 X 10^5), displayed an ED_{50} of 2.1 nmol. Delta^9-THC, which has a higher lipophilicity (9.44 X 10^6), was also less potent in the tail-flick assay (ED_{50}=44.5 nmol). The hypothermia data were also consistent with this trend. These data suggest that the lipophilicity influences the potency of cannabinoids.

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Delta^9-THC-Induced Antinociception is Mediated by Spinal a_2 Noradrenergic Receptors

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It is well established that delta-9-tetrahydrocannabinol (Δ^9-THC) and other cannabinoid compounds produce antinociception in mice and rats as assessed by the tail-flick response to radiant heat. However, little is known about the underlying pharmacology of this effect. Because descending noradmnergic and serotonergic mechanisms have been implicated in antinociception, the present study examined whether these two monoaminergic spinal systems mediate cannabinoid-induced antinociception. In addition, the involvement of spinal noradrenergic and serotonergic systems on two other indices sensitive to cannabinoids, body temperature and immobility in the ring test, were examined. Adult male rats were administered vehicle or Δ^9-THC (10 mg/kg, i.v.) and subsequently given an intrathecal (i.t.) injection of either the α_2 noradrenergic antagonist, yohimbine (10, 30, or 100 µg), or the nonspecific serotonin (5-HT) antagonist, methysergide (5,20, or 50 µg), through chronically implanted spinal catheters. Δ^9-THC alone led to 76±8%MPE and yohimbine (30 and 100 µg) administered at the lumbar level of the spinal cord decreased this antinociception to 14±8 and 21±18 %MPE, respectively. However, yohimbine (30 µg) administered to the upper thoracic region failed to have an effect on Δ^9-THC-induced antinociception, thus implicating a_2 receptors at the lumbar level. In contrast, it. administered methysergide failed to have an impact on the antinociception at all doses tested. As previously reported, Δ^9-THC led to a 1.8±0.2° C decline in body temperature as well as 68±6% immobility, which were not blocked by it. administration of either monoamine antagonist. In fact, i.t. administered methysergide (20 and 50 µg) increased the hypothermic effect of Δ^9-THC by an additional 1.3-1.4° C. These findings indicate that cannabinoids activate descending noradrenergic neurons resulting in antinociception via the stimulation of spinal α_2-adrenoceptors.

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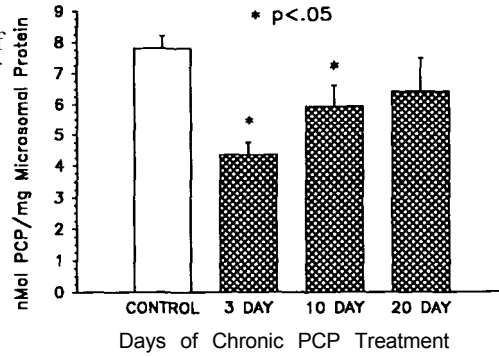
[3H]PCP Covalent Binding to Liver Microsomal Proteins is Significantly Affected by the Length of Chronic Dosing

S.M. Owens, M. Gunnell and M.J.J. Ronis



Adult male Sprague-Dawley rats were used to determine if the time period for chronic PCP (phencyclidine) dosing affects the ability of microsomal enzymes to irreversibly bind PCP metabolites. Rats were dosed for 3, 10 or 20 days (n=3 for each group) at 18 mg/day/kg via subcutaneous osmotic pumps. On the last day of dosing the pumps were removed and PCP was allowed to clear from the animal for 24 hrs before removing the livers. Analysis of the serum samples for PCP using a RIA showed no PCP was detectable. After preparing liver microsomal proteins by standard techniques, the concentration of metabolic-dependent covalent binding was determined using 100 μM of unlabeled PCP and [^{3}H]PCP as a tracer. The mean (±SD) concentration of PCP metabolite covalent binding in the 3 day, 10 day, 20 day and control group was 4.35 (0.397), 5.93 (0.684), 6.75 (0.851) and 7.80 (0.389) nM PCP equivalents/mg of microsomal protein, respectively. ANOVA followed by Dunnett's t test indicated the 3 and 10 day dosing groups were statistically different from the control group. These data suggest that there are time-dependent changes in in vivo cytochrome P-450-dependent PCP metabolism which could have important implications for PCP-induced toxicity. (Supported by NIDA rant DA 04136 and NIDA Research Scientist Development Award (SMO) K02 DA 00110).

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Dependence on Dizocilpine (MK-801) in Rats

W.D. Wessinger

Previous studies in our laboratories have demonstrated the dependence producing potential of phencyclidine (PCP) as evidenced by dose-related disruptions in rat operant behavior when chronic administration of PCP was stopped (Wessinger and Owens, 1991). The present study investigated the dependence producing liability of MK-801 in Sprague-Dawley rats. MK-801 shares many pharmacological actions with PCP, but has selective affinity at only one of the several sites with which PCP is known to interact, namely the PCP receptor which is associated with an NMDA-gated ion channel. Using S.C. implanted osmotic minipumps 0.1, 0.32, 0.56 mg/kg/day of MK-801, or vehicle, was infused to rats (n=5 per treatment group) trained to respond under a fixed-ratio 30 schedule for food reinforcement during four daily half-hour sessions occurring every 6 hours. During the infusions, the effects of MK-801 were modest. The highest dose of MK-801 decreased response rates to 72% to 75% of pretreatment levels during days 2 to 5 and response rates subsequently returned to control levels. When the osmotic pumps were removed after 10 days to stop chronic dosing, the two highest dose groups exhibited a marked and statistically significant ($P < .05$) suppression of response rates (to 41% and 27% of control, respectively). These effects were much larger than the effects seen during the infusions and lasted until the third or fourth day after cessation of dosing. The response rate suppression seen at the two highest infusion doses of MK-801 were similar in magnitude and duration to that seen after cessation of chronic PCP at 10.0 or 17.8 mg/kg/day for 10 days under comparable conditions. These results demonstrate that MK-801 produces dependence, as evidenced by the emergence of a behavioral abstinence syndrome after cessation of dosing.

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Dextrorphan (DO) but not Dextromethorphan (DM) Induces PCP-Like Behavior in Rats

J.I. Szekely, L.G. Sharpe and J.H. Jaffe

Recent media reports of the abuse of OTC cough suppressants containing DM prompted us to explore the possibility that the formation of DO, the immediate metabolite of DM, might contribute to the abuse of these medications. We previously showed (CPDD, 1990) that DO potentiated, and DM inhibited, phencyclidine (PCP)-induced increases in locomotor activity. In this report we monitored locomotor activity, stereotypy and ataxia after different doses of DO, DM and PCP. We also assessed the effects of DO and DM on PCP-induced locomotor activity using a drug-interaction paradigm in which various doses of DM and DO (15 to 120 mg/kg) were injected 15 min before 10 mg/kg PCP. DO and PCP (1.25 to 20 mg/kg) produce similar dose-dependent increases in locomotor activity, stereotype and ataxia. DM produced PCP-like effects only at 60 and 120 mg/kg. increasing locomotion 45 min after treatment. DO dose-dependently facilitated, whereas DM inhibited PCP-induced locomotion. We conclude that, if consumed in large amounts, antitussives containing DM may produce PCP-like effects by the metabolic conversion of DM and DO.

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The 5HT3 Antagonist MDL 72222 Selectively Blocks the Discriminative Stimulus Effects of Ethanol in Rats

K.A. Grant

Recent evidence indicates one possible target for the actions of ethanol in the CNS is the 5-HT_3 receptor/channel complex. Ethanol potentiates the actions of 5HT at this receptor (4), and a variety of procedures have shown that 5-HT_3 receptor antagonists attenuate the effects of ethanol (1,2). To further investigate (3) the role of $5HT_3$ receptor-mediated activity in the behavioral actions of ethanol, the ability of the selective 5-HT_3 antagonist MDL 72222 to block the discriminative stimulus effects of ethanol was studied. Rats were trained to discriminate ethanol (1 .0 g/kg (n=5), 1.5 g/kg (n=6), 2.0 G/KG (N=3); i.g.) from water. On test sessions, the 5-HT_3 receptor antagonist MDL 72222 (3.0-17.0 mg/kg; i.p.) was given in combination with the training dose of ethanol. MDL 72222 treatment resulted in a dose-dependent attenuation of ethanol-appropriate responding, to less than 15% of total responses emitted. The 5-HT_2 antagonist ketanserin (5.6 and 10.0 mg/kg; i.p.) given prior to the training dose of ethanol did not decrease ethanol-appropriate responding. MDL 72222 treatment did not block the discriminative stimulus properties of pentobarbital (PB) in a group of rats (n=5) trained to discriminate 10 mg/kg PB from saline. In addition, MDL 72222 (5.6 and 10.0 mg/kg) prior to PB did not block the substitution of pentobarbital for ethanol. The results demonstrate that the discriminative stimulus effects of ethanol can be selectively blocked by $5HT_3$ receptor complex antagonists.

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Self-injection, Discrimination and Tolerance with Zolipdem in Baboons

R.R. Griffiths, C.A. Sannerud, N.A. Ator and J.V. Brady

This study examined in baboons various behavioral effects of zolpidem, a short-acting imidazopyridine hypnotic which has selectivity for the central BZ_1(ω1) benzodiazepine receptor subtype. Zolpidem self-injection was studied in eight baboons under a fixed-ratio 80- or 160-response schedule with a 3 h time-out after each injection. Maximal rates of self-injection maintained by zolpidem (0.01-1 mg/kg) were consistently higher than those maintained by the benzodiazepine hypnotic triazolam. In a second experiment, eight baboons were trained to discriminate either lorazepam (1.8 mg/kg p.o.) or pentobarbital (10 mg/kg p.o.) from the no-drug condition. Zolpidem (0.32-10 mg/kg p.o.) occasioned drug appropriate responding (>80%) in a dose dependent manner. In a final experiment, administration of zolpidem (3.2 or 5.6 mg/kg i.m.) to four baboons produced ataxia and sedation which progressively decreased over 7 consecutive days of administration. The discriminative stimulus effects and tolerance shown with zolpidem are similar to those previously shown with benzodiazepines under similar conditions. The rates of self-injection of zolpidem are higher than those maintained by eleven different benzodiazepines previously studied under similar conditions. Further research on the reinforcing effects of zolpidem may provide useful insights into mechanisms underlying the maintenance of behavior by compounds acting through the benzodiazepine receptor.

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Chronic Administration of the Imidazopyridines Zolpidem and Alpidem, Does Not Produce Physical Dependence in Mice

D.J. Sanger, G. Perrault, E. Morel and B. Zivkovic

Zolpidem and alpidem are two novel imidazopyridine compounds with clinical hypnotic and anxiolytic activity, respectively. Both drugs have high affinity for the ω(BZ) sites associated with $GABA_A$ receptors with marked selectivity for the ω 1 subtype. As it is well known that withdrawal from repeated benzodiazepine administration produces in mice increased sensitivity to different convulsant challenges, an effect which could be taken as an index of physical dependence, the possibility that zolpidem and alpidem would give rise to similar proconvulsant effects was studied. After repeated administration of diazepam (2 x 5 mg/kg po daily for 10 days) there was increased sensitivity to isoniazid - and pentylenetetrazole-induced convulsions between 42 and 67 hours after the final injection. This proconvulsant action was also observed after flumazenil injection in diazepam-treated mice. In contrast, twice daily oral administration of zolpidem (30 mg/kg) or alpidem (100 mg/kg) for 10 days produced virtually no proconvulsant activity after spontaneous drug withdrawal and no signs of drug withdrawal were observed after flumazenil treatment. These results are consistent with clinical findings indicating that zolpidem and alpidem practically do not produce withdrawal signs.

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Effects of Benzodiazepines on Memory in Monkeys

J.M. Moerschbaecher

The technique of repeated acquisition and delayed performance was used to assess drug effects on memory in monkeys. Each session a subject was required to acquire a conditional discrimination (acquisition phase), retain the discrimination for a specified period of time (delay phase) and then was retested on the same discrimination (performance phase). The effects of various drugs were examined following both short (30 or 60 min) and long (24 hr.) delays. Retention of the acquired discrimination, as measured by percent savings in errors to criterion, was greater at the short than at the long delay. Alprazolam, triazolam and temazepam each were found to produce both dose and delay dependent disruptive effects on retention when administered prior to the performance phase. Triazolam was also administered immediately after acquisition. When evaluated 24 hr later triazolam was also found to exert a pronounced amnestic effect on performance. Interestingly, however, tolerance developed to this effect of triazolam. The nonbenzodiazepine anxiolytic, buspirone was also found to exert a pronounced disruptive effect on retention, decreasing percent savings at doses which had no effect on overall response rate. In contrast, the opioid agonists heroin and U50488H exerted no amnestic effects except at doses which produced substantial rate-decreasing effects. The data suggest that inherent differences exist among various drugs of abuse in terms of their effects on memory. (Supported by DA 04775 and DA 03573)

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Effects of Pentazocine, Nalbuphine and Buprenorphine in Humans Trained to Discriminatamong Saline, Hydromorphone and Butorphanol

G.E. Bigelow, K.L. Preston and LA. Liebson

To assess the stimulus properties of opioid mixed agonist-antagonists in humans, post-addict volunteers were trained in a 3-choice procedure to discriminate among IM saline (4 ml), hydromorphone (3 mg), and butorphanoi (6 mg). Discrimination and subjective effect measures were collected. Following training, generalization curves for hydromorphone (0.375 - 3 mg), butorphanol(0.75 - 6 mg), pentazocine (7.5 - 60 mg), nalbuphine (3 - 24 mg), and buprenorphine (0.075 - 0.6 mg) were determined. In generalization testing both hydromorphone and butorphanol produced dose-related increases in drug-appropriate discrimination responding and in subjective effects characteristic of mu-agonists and kappa-agonists, respectively. Nalbuphine produced dose-related increases in butorphanol-like discriminative and subjective effects. Buprenorphine produced dose-related increases in hydromorphone-like discriminative and subjective effects. Pentazocine produced partially butorphanol-like and partially hydromorphone-like effects. These results emphasize the differences among mixed agonist/antagonist opioids, may reflect differing opioid recector -activities (butorphanol and nalbuphine, kappa-agonist; pentazocine, both kappa-agonist and mu-agonist; buprenorphine, mu-agonist), and may reflect analogous differences in abuse liability. (Supported by USPHS grant DA 04089).

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Subjective and Behavioral Responses to Intravenous Fentanyl in Healthy Volunteers

J.P. Zacny, J.L. Lichtor, J.G. Zaragoza and H. De Wit

Fentanyl is a mu opiate agonist which is occasionally abused by medical personnel who have ready access to the drug. We examined in healthy volunteers (N=13) the subjective and psychomotor-impairing effects of intravenous fentanyl (0-100 µg/70 kg). A randomized, placebo-controlled, crossover design was used in which subjects were injected with 0, 25 (N=6), 50 and 100 µg/70 kg of fentanyl in a double-blind fashion. Subjects completed several questionnaires commonly used in abuse liability testing studies before drug injection and at periodic intervals for up to 3 h after drug injection. Subjects also completed several psychomotor tests at these times. Some aspects of psychomotor functioning (e.g., eye-hand coordination) were impaired by fentanyl. Fentanyl produced dose-related increases in ratings of “high” and “sedated,” but also tended to produce dysphoria and somatic symptomatology. Most subjects reported liking the effects of the two higher doses of fentanyl for at least a brief time after injection, but they varied widely in their liking ratings across the 3-h post-drug injection period. Despite the transient increases in liking ratings, fentanyl did not increase scores on a widely-used measure of drug-induced euphoria (Morphine-Benzedrine Group scale of the Addiction Research Center Inventory). Studies of the subjective effects of opiates in healthy volunteers are important to test the generality of findings obtained with addict populations and to document and attempt to understand the nature of individual differences in the subjective effects of psychoactive drugs in non-addicted individuals.

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Effects of Multiple Doses of Smoked Cocaine-Base

D. Hatsukami, P. Pentel, R. Nelson and J. Glass

Limited knowledge exists on the effects of multiple doses of crack or smoked-cocaine base in humans. Further, methodological issues pertaining to a safe dosing regimen and a placebo dose have not been addressed for smoked cocaine-base. The purposes of this study were to determine: (1) the safest interval between doses to deliver cocaine; (2) whether 5 mgs of cocaine-base can be considered an adequate placebo dose; and (3) responses to multiple doses of cocaine. Six black males were given 10 doses of either 5 or 35 mgs of cocaine-base in a semi double-blind manner at 15, 30 and 45 min intervals. The dependent measures included physiological (heart rate, blood pressure), subjective (high) and performance (reaction time) responses. Each of these measures were taken at baseline and at time intervals before and/or after each dose of cocaine. Doses were withheld for some of the subjects during the 15 min interval sessions due to elevated blood pressures and no subjects during the 30 min interval. The results showed: (1) a greater change in plasma cocaine levels with shorter intervals between doses; (2) significant differences between the 35 vs 5 mg dose on the physiological and subjective but not the reaction time measures; and (3) a sustained level of heart rate, blood pressure and subjective ratings in spite of increasing plasma cocaine levels. The results also preliminarily showed that a great deal of individual variability exists in the responses to cocaine and the pattern of physiological response does not necessarily associate with subjective responses. In conclusion, it appears that a dosing regimen of 35 mgs every 30 min is a safe and feasible method of smoked-cocaine delivery; and a delivery of 5 mgs results in minimal effects and can therefore be considered as a placebo dose. Finally, a great deal of individual variability exists in responses to smoked cocaine. This variability may be an important factor to consider in research examining the reinforcing effects from cocaine or treatment of cocaine abusers.

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Behavioral Effects of Ethanol and Marijuana, Alone and in Combination with Cocaine in Humans

R.W. Foltin, M.W. Fischman and T.H. Kelly

Intranasal cocaine (COC) and oral ethanol (ETOH) were administered to one group of seven research volunteers during daily experimental sessions. Following the determination of baseline subjective and performance indexes, an ETOH cocktail (0, 19.4, 38.7, or 58.1 gm of ETOH in lemonade) was consumed over a ten-minute period. COC hydrochloride (4, 48, 96 mg) was inhaled 25-min after the start of ETOH drinking. In a separate experiment, seven research volunteers received intravenous cocaine and smoked marijuana (MJ), alone and in combination, during daily experimental sessions. Following the determination of baseline indexes, a one gram MJ cigarette (0-2.7% Δ^9-THC, w/w) was smoked using a controlled puffing procedure. COC hydrochloride (0, 16, 32 mg) was given intravenously 13-min after the start of MJ smoking. These drug doses had minimal effects on performance. Intranasal COC increased ratings of "stimulated" and "high," which were unaffected by ETOH. In contrast, increased measures of "sedation" and Confusion following ETOH alone were attentuated by i.n. COC. Intravenous COC and smoked MJ alone increased ratings of "stimulated" and "high." Combinations of i.v. COC and MJ produced larger and more prolonged elevations in these measures. The results suggest that combinations of ETOH and COC block some of the undesired effects of ETOH alone, and combinations of COC and MJ produce some greater desired effects than observed with either drug alone.

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Clinical Effects of Daily Methamphetamine Administration

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The purpose of this study was to investigate the possible alteration of the disposition and pharmacodynamics of methamphetamine HCl after its daily administration. Six male, paid volunteers familiar with the use of amphetamines participated in the study. Each subject was administered 10 mg of methamphetamine HC1 as a slow release preparation (Desoxyn Gradumets) at 9 a.m. for 13 consecutive days (Days 2 through 14 of the study). This dosage regimen was designed to minimize unwanted effects (e.g., anorexia, insomnia and dependence) while affording the possibility for enzyme induction and tolerance to develop. On Days 1 and 15 the subjects were challenged with 10 mg of oral deuterated methamphetamine HC1. Deuterated drug was used to differentiate the plasma concentrations of challenge doses from those of daily doses. The heart rate effects, subjective perception of "high", and plasma concentrations of methamphetamine were examined on Days 1 and 15. Repeated measures ANOVAs indicate that a significant decrease in heart rate acceleration in response to methamphetamine challenge occurred on Day 15 [$F(1,5) = 8.26$, $p \leq 0.035$]. However, no significant change in either the subjective ratings of "high" or the plasma concentrations of deuterated methamphetamine occurred. These findings indicate that the disposition of methamphetamine and its subjective effects were not altered by this period of daily exposure to a low dose of the drug. In contrast, tolerance to the heart rate accelerating effect was observed.

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Effects of Acute Cocaine on Cerebral Glucose Utilization in Human Polydrug Abusers: Relations Between Regional Metabolism and Subjective Responses

E.D. London, J.M. Stapleton, B.C.K. Yung, R.L. Phillips, M.J. Morgen, N.G. Cascella, D.F. Wong and J.H. Jaffe

The purpose of this study was to identify brain regions which may contribute to the feeling states produced by cocaine in experienced drugs users. The regional cerebral metabolic rate for glucose (rCMRglc) is an index of local brain function, and it can be measured noninvasively in human volunteers by positron emission tomography (PET) scanning and radiotracer 18F-fluorodeoxyglucose (FDG). This method has been used to show that acute treatments with either cocaine or morphine produce diffuse reductions in rCMRglc (London et al., 1990a, 1990b).

Subjects for the study were 18 male volunteers between the ages of 23 and 43 years. A requirement for admission to the study protocol was a history of intravenous cocaine use. The group of subjects examined also generally used opioids, marijuana, ethanol, caffeine, and nicotine. None were currently dependent on illicit drugs or alcohol. In a double-blind, crossover study, subjects received placebo or cocaine (40 mg., i.v.) in randomized counterbalanced order. Data were collected using the FDG PET technique after the intravenous infusion of 5 mCi of the radiotracer. Subjective responses were measured, using various rating scales, including the Addiction Research Center Inventory, *the* Cocaine Sensitive Scale (CSS), Visual Analogue Scales (VAS), and the responses to "beep" prompts, as described previously (London et al., 1990b).

Cocaine produced positive feelings, as measured by the various subjective rating scales, and it reduced rCMRgic by 3 to 18% of placebo values in the brain regions assayed. The greatest decrease was measured in the left temporal pole, and the smallest decrease was found in the cerebellum.

Several measures of the strength of the drug effect, including responses to CSS questions, "How high do you feel?", How much do you feel the drug?", and "beep" prompt responses to the question, "How much do you feel the drug?", were negatively related to rCMRglc in the left temporal pole. When the difference scores for rCMRglc (cocaine minus placebo) were used in analyses, the change in rCMRglc in the right amygdala was negatively correlated with measures of strength of the drug effect on CSS and VAS.

Although the effect of cocaine on cerebral glucose utilization is widespread, the findings implicate only few regions as being involved in the subjective responses to this drug. The areas implicated are the temporal pole, which has an established involvement in various affective states, and the amygdala, a limbic region which has altered electrical activity in human subjects receiving cocaine. The findings demonstrate how noninvasive brain imaging techniques such as PET scanning, can be used to delineate the brain substrates for various feeling states induced by abused drugs, such as cocaine.

REFERENCES: Available upon request of senior author.

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Caffeine Drug Discrimination in Humans: Effects of Theophylline, Methylphenidate and Buspirone

A.H. Oliveto, W.K. Bickel, J.R. Hughes, S.Y. Terry and S.T. Higgins

Previously, we have shown that the discriminative stimulus effect of caffeine in humans differs from that of the benzodiazepine triazolam. This study further examined the pharmacological specificity of the caffeine discriminative stimulus. Four healthy male volunteers (ages 21-23 yrs) were trained to discriminate between 320 mg/kg of caffeine (e.g., drug A) and placebo (drug B). During the first 4 daily sessions (Training Phase), drug A and drug B were administered orally in capsules 90 min prior to the session on alternate days and subjects were informed of the drug label at the time of drug administration. Then during the Test of Acquisition Phase, drug A and drug B were administered in a randomized-block fashion and subjects were informed of the drug code post-session. Discrimination was assessed by measuring: 1) percentage of points accumulated using the drug-appropriate manipulandum under a concurrent fixed-interval 1-set schedule; 2) identification of the appropriate drug code under a discrete choice procedure; and 3) number of points out of 100 allocated to the appropriate drug code. Once the criterion for discrimination was met (i.e., correct drug code identification on 4 consecutive sessions), dose-effect curves for caffeine (56-320 mg/70 kg), theophylline (56-320 mg/70 kg), methylphenidate (10-56 mg/70kg), and buspirone (1-32 mg/70 kg) were determined during the Test of Novel Doses and Drugs Phase. Subjects met the criterion for discrimination within 4-9 sessions. Caffeine produced dose-related increases in caffeine-appropriate responding. Theophylline and methylphenidate showed partial generalization to the caffeine stimulus, as evidenced by the fact that they produced 50-75% caffeine-appropriate responding at at least one dose tested. In contrast, buspirone produced predominantly placebo-appropriate responding. These results agree with nonhuman drug discrimination data and indicate that the caffeine disriminative stimulus has pharmacological specificity in humans. (Supported by Grants DA-06205 and DA-04843.)

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Profile of Bhavioral Effects of the Benzodiazepine, Triazilam, as Compared to Ethanol

J.D. Roache, R.H. Bennett, D.R. Cherek, K.A. Cowan and R. Spiga

The effects of triazolam (0,2.0,4.0, and 8.0 ug/kg) were compared to those of ethanol (0, 0.25, 0.5 and 1.0 g/kg) using a double-blind, double-dummy, within-subject design in 8 normal male volunteers. Dose sequences were counter-balanced by an 8 x 8 balanced Latin Square. Subject ratings of mood and perceived drug effect and objective measures of psychomotor and memory task performance permitted relative efficacy and potency comparisons of the two drugs. Repeated measurements over a 6 hr post-drug period assessed the time-course of drug effects and allowed for an analysis of the peak drug effects achieved, regardless of measurement timepoint.

Both drugs produced dose and time related effects reaching a peak magnitude at 1-2 hrs post-drug. Valid relative potency determinations usually were not possible because ethanol had a steeper dose response function or the two drugs showed differential efficacies. Consequently, many between drug comparisons were based upon the relative effect sizes of the highest doses of each drug. At the highest dose, tiazolam and ethanol produced comparable degrees of psychomotor performance impairment (e.g., circular lights, below; darkened symbols are significantly different than placebo). In contrast, triazolam produced lesser subject ratings of perceived drug effect and greater degrees of short-term memory impairment (e.g., delayed number recall) than did ethanol. These same directional trends were also obtained with other measures of mood and psychomotor and memory performance.

Previous studies have shown that benzodiazepines produce relatively less subjective drug effect and greater memory/cognitive impairment than do other sedatives in sedative drug abusers. The present results systematically replicate these observations and extend them to normal populations and ethanol solutions.

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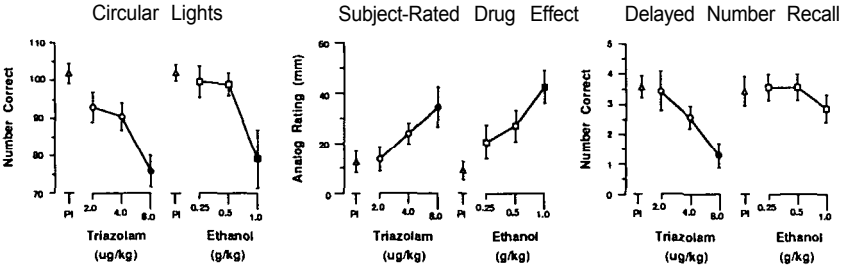

Ethanol Preload Increases Ethanol Preference in Normal Social Drinkers

H. de Wit and M.A. Chutuape

This study assessed the effects of a small preload ("priming") dose of ethanol on preference for ethanol in normal social drinkers. Twenty-eight moderate, non-problem drinkers (average 8-10 alcoholic drinks/wk) participated in a 6-session, double-blind choice procedure. On the first two sessions subjects sampled two color-coded beverages, containing ethanol (0.8 g/kg) and placebo (mix alone). On session 3 they were given a choice between the two beverages, and if they indicated a preference for ethanol, they were offered enough money to switch their choice to the placebo (placebo-plus-$). On the next three choice sessions, subjects were first given a preload dose of ethanol (0, 0.25 or 0.5 g/kg ethanol). One hour later they were allowed to choose between i) the ethanol beverage and ii) the placebo-plus-$ established in session 3. They could also regulate the dose of their preferred beverage on choice sessions. It was hypothesized that subjects would be more likely to choose the ethanol beverage (over the placebo-plus-$) after ethanol preloads. Subjective effects measures were obtained throughout the sessions. Eight subjects chose placebo without $ on session 3. For the 20 subjects who initially preferred ethanol on session 3, amounts of money ranging from $1 to $30 were needed to switch their choice. Of these 20 subjects, 4/20 chose the ethanol-containing beverage after the placebo preload, 7/20 chose ethanol after the low dose ethanol preload, and 1 1/20 chose ethanol after the higher ethanol preload (significant linear trend, Mantel-Haenszel test, $p<.03$). Subjective effects measures did not vary systematically with ethanol preload dose. These results suggest that ingestion of a low dose of a preferred substance (ethanol) increases an individual's tendency to consume more of the substance.

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Chronic Tolerance to Cardiovascular, Subjective, and Behavioral Effects of Nicotine in Humans

K.A. Perkins, J.E. Grobe, L.H. Epstein, R.L. Stiller, R. Solberg-Kassel and R.G. Jacob

Chronic tolerance to acute effects of nicotine may be important in understanding long-term physiological adaptation to nicotine intake and provide direction for the study of tobacco dependence. Using a measured-dose nasal spray dosing procedure developed in our lab, we have investigated differences in responses to nicotine as a function of smoking status. In this study, smokers and nonsmokers (n=7 each; 3 male, 4 female) participated in 3 morning sessions, each after overnight abstinence from smoking and food. During each session, subjects initially engaged in at least 4 practice and baseline trials with the assessment battery to obtain stable responding. The battery consisted of heart rate (HR) and blood pressure (BP) measurement followed by subjective measures (POMS; several visual analog scales [VAS]), sensitivity to a thermal (i.e. pain) stimulus, and psychomotor tasks of handsteadiness and finger-tapping speed. Subjects were then administered 0, 7.5, and 15 ug/kg nicotine (similar to yield of 1 typical cigarette) every 30 mins for 2 hrs, with each dose presented on a separate day. Order of doses across days was counter-balanced. In addition, smokers subsequently participated in a fourth, exploratory session involving administration of 30 ug/kg nicotine.

Nicotine produced dose-dependent changes in cardiovascular and subjective responses, handsteadiness, and most subjective measures. Finger-tapping and response to the thermal stimulus were less consistently related to nicotine dose. Contrary to some of our previous findings, smokers and nonsmokers generally had similar HR, BP, and psychomotor responses to nicotine. However, nonsmokers showed a decreased sensitivity to the thermal stimulus (i.e. antinociception) following nicotine which was not observed in smokers except at the very highest dose (30 ug/kg). Relative to nonsmokers, smokers also showed reduced subjective responses on POMS scales of Tension, Vigor, and Confusion, and the VAS of 'Head Rush' (related to euphoria). These results suggest the existence of chronic tolerance to nicotine in humans but clearly indicate that the magnitude of such tolerance is not uniform across response modes.

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Substance Dependence Diagnosis Using DSM-III-R and DSM-IV Criteria for Cocaine Users

K.J. Bryant and B. Rounsaville

A direct comparison of the performance of DSM-III-R and proposed DSM-IV substance dependence diagnoses was made for 399 cocaine users. The criteria of Tolerance, Withdrawal and Withdrawal Avoidance were examined in Community (n=lOl), Inpatient (n=149) and Outpatient (n=149) samples. Four types of analyses were carried out to contrast the two systems of diagnosis: 1) estimation of diagnostic agreement rates for dependence diagnoses for DSM-IV (requiring Tolerance or withdrawal criteria to be present) and DSM-III-R, 2) examination of diagnostic rates within Community, Inpatient and Outpatient samples; 3) estimation of the comparative test-retest reliability over a one week period (n=80) and, 4) examination of concurrent and predictive validity of the criteria for a one-year follow-up sample (n=94) using interviewer ratings.

Tolerance was one of the most frequently endorsed criteria (83%) while Withdrawal and Withdrawal Avoidance were the least frequently reported (52% and 36% respectively). When dependence diagnosis were assigned according to the two systems, 96.8% received dependence diagnoses under DSM-III-R and 90.5% under DSM-IV. However, further examination of rates of diagnosis for Community, Outpatient, and Inpatient samples showed the greatest diagnostic difference for Outpatient samples: DSM-III-R with 98.7% and DSM-IV with 89.3%, a 9.4% difference. No difference existed for Inpatient samples. Examination of the test-retest reliability for Tolerance and Withdrawal criteria showed that the Withdrawal criteria were less sensitive and equal or lower in reliability than other criteria.

Finally, the criteria of Tolerance and Withdrawal showed a low to moderate degree of association with concurrent ratings of need for Employment, Drug, Social and Psychiatric treatment as rated on the Addiction Severity Index. After one year criteria related only to need for psychiatric treatment not to need for drug treatment. It was concluded that Tolerance and Withdrawal criteria should not receive a special status in the diagnosis of drug dependence in cocaine users.

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DSM-III-R Dependence Without Tolerance or Withdrawal

J.E. Helzer, L.B. Cottler, D. Mager, E.L. Spitznagel and W.M. Compton, III.

With proposed criteria for DSM-IV substance dependence imminent an evaluation of the impact of changes from DSM-III to DSM-III-R would be informative. Recent admissions to St. Louis drug treatment centers were interviewed with the DIR-III-R, which covers criteria from both systems, thus allowing an evaluation of the systems to be made. Kappa values for systems agreement, diagnostic overlap and percent positive agreement reported by substance. The DSM-III-R system cast a wider net for dependence than DSM-III for alcohol, tobacco and and amphetamines Neither system predominated for cannabis, opioids and arbiturates/sedatives Hypnotics. Our data show that 34% of cannabis dependence, one-third of amphetamine dependence, 17% of alcohol dependence, and a significant proportion of hallucingen, PCP and inhalant dependence is not associated with tolerance or withdrawal. A closer look shows that physiological dependence (tolerance or withdrawal) is associated with the number of positive dependence criterion items. Additionally, our data show that the DSM-III-R "B" duration criterion is unnecessary since almost all who are labeled dependent meet the duration criterion. Reasons for differences and implications of findings for DSM-IV are discussed.

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Prevalence of Comorbidity Among Cocaine Users in Treatment

B.E. Havassy and D.A. Wasserman

Early comorbidity results are reported from a study of the efficacy of inpatient and outpatient treatments for cocaine dependence. Ss began treatment for cocaine abuse at one of four private, hospital-based, 12-step chemical dependency treatment sites in northern California. Ss were assigned to inpatient or outpatient treatment in a randomized clinical trial coupled with a regression discontinuity design. Assignment was based on an objective measure of addiction severity. Severely addicted Ss were assigned to inpatient treatment, low-severity Ss to outpatient. Moderately addicted Ss were randomly assigned. Ss were working to professional class and had insurance benefits for part or all of treatment or self-paid.

The aims of the comorbidity investigation were: (1) To gather data on the co-occurrence of cocaine dependence with psychiatric disorders, specifically major depression and dysthymia, post-traumatic stress disorder (PTSD), and antisocial personality disorder (ASPD). (2) To assess the co-occurrence of cocaine dependence with alcohol, marijuana, and heroin dependence. (3) To examine risk factors for and symptom patterns leading to these diagnoses and assess event exposure in relation to PTSD.

Data are from the NIMH Diagnostic Interview Schedule. Ss completed the DIS-III-R 14-21 days after treatment entry. Of the first 213 Ss who met DSM-III-R criteria for cocaine dependence in the prior six months, 78% were inpatients, 20% outpatients, and 2% in day-treatment. Most Ss were crack smokers. 75% were men; 54.5% were Caucasian and 38.5% African-American. 80% of the sample met lifetime criteria for a DSM-III-R disorder other than substance dependence and 77% for a substance-dependence disorder in addition to cocaine. ASPD prevalence was 41%. Lifetime rates of major depression and dysthymia were 16% and 13%; current rates (in the six months prior to treatment) were 13% and 8%. 28% of Ss met lifetime criteria for PTSD; 16% met current. Alcohol dependence was the most frequent other substance use disorder (66% lifetime, 51% current), followed by cannabis dependence (45%, 23%). Heroin dependence was rare (5%, 2%). Comorbidity rates obtained to date argue for thorough psychodiagnostic assessment of cocaine users in treatment and have important implications for conceptualizing and treating cocaine dependence.

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Psychiatric Histories Among Drug Abusers: IVDU vs. Non-IVDU

L.B. Cottler, W.M. Compton, III, D. Mager, G.A. Starch and J. Hudziak

Drug treatment programs which take into consideration the psychiatric histories of the abuser have been found to be more effective than those neglecting this relevant history. We have previously reported on methods for ascertaining psychiatric symptoms among drug users. Our NIDA-funded longitudinal study of HIV risk factors has interviewed 514 substance users recently admitted to treatment and their sexual partners (N=91). The interviews include the DIS-III-R, which elicits information necessary to make DSM-III-R diagnoses and a modified NIDA high risk behaviors assessment. The sample is 70% African American and 60% male; 50% reported lifetime IV drug use. Of these IV drug users, 60% have injected in the last 6 months. The findings indicate that the most prevalent disorders are tobacco dependence (67%), alcohol dependence (61%), phobias (37%) and ASP (33%). To determine the degree of comorbidity between drug use and psychiatric illness among IVDUs and non-IVDUs, odds ratios were calculated for present psychiatric illness, past illness, and no illness. After adjusting for the effects of age, gender and race, IVDUs are nearly 4 times as likely as non-IVDUs to meet criteria for a current ASP disorder; almost 2 times as likely to meet criteria for current phobic disorder and almost 3 times as likely to have current GAD. The findings indicate different patterns of illness, substance use histories, sociodemographic characteristics and ages of onset among IVDUs compared to non-IVDUs. Epidemiological impediments in studying dual diagnosed subjects are presented.

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Consistency in Measurement of Psychopathology

D.L. Hailer, J.S. Knisely and S.H. Schnoll

Recently, there has been increased interest in psychopathology among substance abusers and research has indicated that the presence of certain disorders impacts directly on treatment outcome. However, if psychiatric illness is to be considered, it must be adequately measured. This study compared the "hit" rates for psychiatric diagnosis in 30 addicted women who completed an extensive battery of standardized psychological tests prior to beginning treatment in a NIDA funded perinatal addictions program.

With regard to addictive disorders, the ASI, SCID, and program clinicians did equally well in detecting both alcohol and drug problems. However, the MCMI failed to identify half of the known cases in both instances while the MMPI (MacAndrews Alcoholism Scale) detected alcohol abusers but also failed to identify half the known drug abusers. The prevalence rates for alcohol and drug problems (based on agreement on at least 3 of 5 measures) was 36-42% and 90-93% respectively.

With regard to depression, base rates were totally test dependent and varied from a high of 58% on the Beck to a low of 10% on the SCID. However, of these who exhibited depressive symptomatology, about 80% were detected by multiple measures and a substantial minority (13%) were detected on 6 of 7 measures.

In comparison, reliable measurement of Axis II disorders was a problem. While every subject was given at least one personality disorder diagnosis by at least one instrument, diagnoses did not hold up from test to test. Overall, the bulk of diagnoses were in Cluster B, with ASP being the most frequently diagnosed personality disorder. The rate for detection of ASP varied from a high of 73% on the MMPI (Morey Scales) to a low of 7% by clinical diagnosis. Some Axis II disorders were relatively stable in their rate of detection including Schizoid, Borderline, and Passive/Aggressive while other fluctuated wildly (e.g., Paranoid, Histrionic, and Narcissistic). While the "experimental" personality disorder categories were not able to be measured by all instruments, the Self-Defeating diagnosis is worthy of further exploration.

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Psychiatry Comorbidity in Methadome Maintained Patients

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Severity of psychiatric illness has been associated with differential treatment outcome in methadone maintained patients (MMP's), with greater severity related to poorer outcome. Diagnosing the presence of secondary psychiatric disorders, and related areas of impairment, may serve to identify subtypes of MMP's with differing courses and indications for ancillary treatments. METHOD: Substance abuse was characterized with the Addiction Severity Index (ASI), and DSM III(R) diagnoses were made in a subset of MMP volunteers with the computerized Diagnostic Inierview Schedule (DISI. Mental status was assessed with the Mini Mental Status Exam (MMSE). The Family History - RDC (modified for this study) estimated the prevalence of substance abuse and psychiatric disorders in MMPs' family members. SUBJECTS were 53 males and 50 females with an average age of 28.3 years. MMP's were in treatment an average of 54.1 (sd 60.6) months, with an average methadone dose of 62.9 (sd 27.2) mgs. gd., at the time of their interviews. RESULTS: Overall severity of psychopathology correlated significantly ($p<.01$) with the number of days of polysubstance abuse in the 30 days prior to the interview, and the number of current DIS diagnoses correlated significantly with the ASI drug composite score ($p< .01$). MMP's met criteria for an average of 2.3 current DIS diagnoses, excluding drug abuse/dependence, with the following disorders most prevalent: depression (51.4%), phobic (45.3%), antisocial personality (with childhood conduct disorder at $\leq$ 15 years of age; 36.5%), anxiety (32.0%), alcoholism (24.0%), obsessive compulsive (20.0%;, and somatization (18.7%). No conclusions can be reached from these data regarding chronology of onset of psychiatric disorders relative to onset of substance abuse. However, interviews with a subsample revealed that MMP's who met criteria for. Childhood Conduct Disorder (x age of onset = 12.4 years, sd = 1.5) tended to do so prior to the onset of any drug abuse, including nicotine (x age of smoking onset = 12.6 years, sd =2.3). Alcohol (43.0%), cocaine (44.2%) and cannabis (36.0%) were the most commonly abused substances by MMP's. Nearly half (47.6%) of MMP's failed the delayed recall item of the MMSE. There was a high prevalence of drug abuse and other major psychiatric disorders in the families of MMP's. DISCUSSION: This study found an association between substance abuse and psychopathology in MMP's. Additionally, MMP's tended to come from families with a high prevalence of drug abuse and psychopathology. Perhaps as a function of their dysfunctional families, the majority of the MMP's (72%) tended to manifest serious disturbances in conduct that occurred prior to the onset of substance abuse. which has implications re: prevention.. Possibly as a function of treatment, only 36.5% of MMP's continued to meet criteria for ASP. The high failure rate on the delayed recall item of the MMSE has treatment implications, in that such MMP's may require special strategies to augment retention of treatment concepts. (DAP50-05130) Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, NY 10021

Comparison of Antisocial Personality and Psychopathy in Opiate Addicts

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Psychopathy and Antisocial Personality Disorder, APD, were asssessed in a sample of 70 male opiate addicts in outpatient methadone-treatment. The Psychopathy Checklist-Revised, PCL-R, is a measure of Cleckley's notion of psychopathy. It is suggested that a cutoff score of 30 be used to identify psychopathy. As only 7% of the sample had a score of 30 or above, a more liberal cutoff of 25 was employed identifying 19% of the sample as psychopathic. In contrast,*APD was the most prevalent personality disorder (33%) in this sample of opiate addicts. Although, some subjects were diagnosed with both disorders, there were subjects that met criteria for one diagnosis but not the other. Although, the correlation of PCL-R scores with an APD diagnosis was moderate it was not at a level which supports unifying the two diagnostic concepts. These results indicate that the diagnoses of APD and psychopathy are not interchangable, nor is psychopathy merely a severe form of APD.

As found in previous research with opiate addicts, the Axis I disorders: depression, alcohol and other non-opiate drug abuse/dependence disorders were the most frequently occurring in this sample. A diagnosis of APD or psychopathy did not yield differential rates of Axis I disorders. A high percentage of the sample met criteria for at least one personality disorder (67%). A diagnosis of any personality disorder was related to higher rates of alcohol dependence. The only personality disorder that APD compared to non-APD subjects were more likely to receive was that of sadistic personality disorder. A diagnosis of psychopathy was also related to an increased likelihood of a sadistic personality disorder. Additionally, APD, borderline, and Cluster B diagnoses, in general, were more likely to occur in psychopaths compared to non-psychopaths. Finally, those subjects with any personality disorder had poorer status with respect to alcohol,legal, and family/social problems than those subjects without a personality disorder.

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The Personality Dimensions of Male and Female Drug Abusers With and Without Antisocial Personality Disorder

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The five-factor model (FFM) of personality is an empirically-derived, comprehensive representation of personality structure that includes Neuroticism (N), Extroversion (E), Openness to Experience (0). Agreeableness (A), and Conscientiousness (C). The NEO-PI is a standardized self-report measure of this FFM (Costa and McCrae. 1989). N reflects the degree to which individuals are prone to experience chronic psychological distress. E refers primarily to the quantity of social stimulation preferred. O reflects the degree to which individuals are prone to seek out new experiences. A reflects the characteristic quality of interpersonal interaction ranging from self-centered antagonism to altruistic warmth and nurturance. C reflects the degree to which individuals are scrupulous, hard-working, and organized versus disorganized, hedonistic, and easily bored.

The present study examines NEO-PI computer generated profile scores in 203 male and female drug abusers with and without a diagnosis of Antisocial Personality Disorder (ASP). Their mean age was 34 years, 46% were male, 67% were white, 47% were single, and 69% were unemployed with a mean of 11 years education.

Male and female drug abusers obtained similar NEO-PI profiles with the exception that males had significantly higher scores on the O ($p=0.05$) and A factors ($p=0.01$), indicating that females were more closed to new experiences and more antagonistic than males. Male and female factor scores on both A and C were also close to one standard deviation below the normative sample, indicating that both groups were more antagonistic and less prone to manifest conscious control over their pleasure-seeking impulses.

Twenty-three percent of the sample met ASP criteria. The ASP group obtained a significantly higher score on the N factor (t-score of 60 vs. 55, respectively) and a significantly lower score on the A factor (t-scores of 31 vs 38, respectively) than the non-ASP group; they also obtained a lower score on the C factor (t-scores of 39 vs. 43, respectively); although this difference was not statistically significant. The ASP group obtained higher scores on the Hostility, Depression, and Vulnerability facets of N and lower scores on the Interpersonal Warmth and Gregariousness facets of E. Using post-hoc analyses, male and female ASP drug abusers obtained significantly higher scores than ASP-male and female drug abusers on the N and A factors, higher scores on the Hostility facet of N, and female ASP drug abusers also had significantly higher scores on the Impulsiveness facet and lower scores on the Gregariousness facet of E than non-ASP females.

In summary, the NEO-PI characterized the ASP group as significantly more vulnerable to stress, more prone to anger, more impulsive, more emotionally cold and distant, and as more disagreeable and vindictive than the non-ASP group. Thus, the behavioral framework utilized in the DSM-IIIR definition of ASP identified a subset of drug abusers that possess many of the personality traits thought to characterize the underlying dynamic structure of those with an antisocial personality.

REFERENCES: Available upon request of senior author.

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Validity of the Drug Abuse Screening Test (DAST-10) in Inpatient Substance Abusers

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There is a need for brief valid instruments for the identification of patients with drug use disorders. Gavin et al. (1989) reported that the DAST accurately classified DSM-III drug abuse/dependent patients. We assessed the diagnostic validity of the DAST in a sample of inpatients from a substance abuse treatment center. DSM-IIIR diagnoses were generated independently by structured lay interview alone (SCID) and by research clinicians using clinical observation, toxicologic and other laboratory data, and interview of patients and collateral informants. Ninety patients (39 males) aged 33 ± 8.5 years were evaluated. There was good concordance between the DSM-IIIR Current and Lifetime Substance Use Disorder diagnoses generated by non-clinicians (using the SCID) and those made by experienced clinicians using all available data. Eighty-four percent of patients interviewed had lifetime drug abuse or dependence, and 60% had lifetime alcohol abuse or dependence. Thirty percent of subjects had Current Major Depression, and 31% had Antisocial Personality Disorder. Other measures obtained revealed that DAST-10 scores were correlated with MacAndrew scores, suggesting that the DAST-10 reflects a personality factor that predisposes to drug abuse. The DAST showed good construct and discriminant validity: DAST-10 scores were not significantly correlated with measures of alcohol use or related problems, a measure of recent alcohol consumption, and MAST and CAGE scores. ANOVA revealed significantly ($p < .001$) different DAST-10 scores for patients with and without lifetime and current drug use disorders. Using Receiver Operating Characteristic analysis, the DAST-10 was found to discriminate well (AUC = 0.97 ± 0.03) between patients with lifetime drug use disorders and those without such diagnoses. Using a threshold score >3, the DAST-10 correctly classified > 93 % of patients, regardless of whether the criterion diagnosis was derived from the SCID or by research clinicians. Routine DAST screening of patients seeking substance abuse treatment is warranted, and the potential for use of the DAST for drug use disorder screening in settings such as medical clinics, hospital wards, and forensic facilities should be examined.

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[^{125}1]RTI-55: A Potent Ligand for the Cocaine Receptor

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3ß-(4-Iodophenyl)tropan-2-carboxylic acid (RTI-55) was prepared by treating the diazonium salt obtained from 3ß-(4-aminophenyl)tropan-2-carboxylic acid with diiodomethane. The [^{125}1]-analog of RTI-55 was prepared at a specific activity of 2200 Ci/mmol and selected for binding studies in male Sprague-Dawley rat brain. Saturation studies with 10 pM of [^{125}I]RTI-55 and 11 increasing concentrations of unlabeled RTI-55 were conducted in the striatum and cerebral cortex. Scatchard transformation of the data indicated a two site model was statistically preferred over a one site model in the striatum while a single site model was obtained in the cortex. Analysis of the striatal data revealed a high affinity binding site with a K_d value of 0.11 ± 0.01 nM and a B_{max} of 0.16 ± 0.02 pmol/mg tissue (original wet weight) and a low affinity site with a K_d value of 2.57 ± 0.30 nM and a B_{max} of 0.57 ± 0.03 pmol/mg tissue. Analysis of the data from the cortex revealed a single high affinity site was present with a K_d of 12.49 pM and a B_{max} of 0.45 pmol/mg tissue. Pharmacological characterization of the striatal binding indicated that the binding was consistent with the dopamine transporter. The rank order of potency was GBR 12909 > mazindol > (-) cocaine > (+) cocaine. Haloperidol, citalopram and desipramine where not potent inhibitors of striatal [^{125}I]RTI-55 binding. Binding of [^{125}I]RTI-55 in the cortex was consistent with that of the serotonin transporter. In the cortex, citalopram and clomipramine were more potent displacers of [^{125}I]RTI-55 binding than either desipramine or GBR 12909. Autoradiographic studies with [^{125}I]RTI-55 in the rat brain revealed a rank order of binding of striatum > lateral hypothalamus = substantia nigra > nucleus accumbens > hippocampus > cortex. The high affinity and specific activity of [^{125}I]RTI-55 makes this compound an ideal ligand to study the dopamine transporter. In vivo studies suggest RTI-55 has application in PET and SECT studies.

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Characterization and Localization of ^{3}H-WIN 35,428 Binding Sites in Rabbit Brain

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The binding of the potent cocaine analog, WIN 35,428 (WIN) (Canfield et al., 1990) was investigated using adult rabbits. Coronal brain sections incubated with ^{3}H-WIN in the presence or absence of 30 µM [-] cocaine revealed a high density of specific WIN binding sites in the caudate-putamen. Binding assays were performed using a crude membrane fraction prepared from caudate-putamen at 0° C in 20 mM phosphate buffer, pH 7.4, containing 0.32 M sucrose and ^{3}H-WIN. Nonspecific binding was defined as the binding remaining in the presence of 1 µM WIN. To validate the assay, we demonstrated that the binding was protein and time dependent. Time course data revealed that equilibrium was reached at about 20 minutes, and binding remained stable for 210 minutes; therefore, a 40 min. incubation time was used for all subsequent studies. Scatchard analysis revealed a single binding site with a K_d of 3 nM and a B_{max} of .27(±.05, SEM; n=5) pmoles/mg tissue. The dissociation curve also suggested the presence of a single site with an off time t1/2 of 4.2 minutes. [-]Cocaine completely displaced bound WIN and Hill plot analysis demonstrated a single site (n = 0.93 and an IC_{50} of 62nM). Dopamine also competed with WIN binding at a single site; Hill analysis (n = 0.92 and an IC_{50} of 3.3 µM). The presence of a single WIN binding site in rabbit brain, in contrast to the two sites in the monkey brain (Madras et al., 1989), will facilitate investigation of the properties of cocaine binding sites.

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Imaging Probes for Cocaine Receptors in Human and Non-human Primate Brain

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Probes which identify, map and monitor the molecular targets of cocaine are needed to clarify the mechanisms mediating the behavioral effects of cocaine. The cocaine congener CFT (also designated WIN 35,428, 2ß-carbomethoxy-3ß-4-(fluorophenyl)tropane) is a potent inhibitor of [^{3}H]cocaine binding in monkey caudate-putamen. [^{3}H]CFT labels sites in the caudate-putamen and nucleus accumbens of human or monkey brain that are associated with the dopamine transporter, are localized primarily presynaptically, and appear to be relevant to the behavioral effects of cocaine (Madras et al., 1989a,b, 1990, Kaufman and Madras, 1991). Thirty minutes after administration of [^{3}H]CFT (approximately 2 nmol/kg) to squirrel monkeys, the ex *vivo* distribution of [^{3}H]CFT in brain closely paralleled [^{3}H]CFT distribution in tissue sections of brain *in vitro* (Kaufman et al., 1991). The highest densities of [^{3}H]CFT binding sites were localized in dopamine-rich brain regions. In apparent contrast, the iodo analog of CFT, [^{125}I]RTI-55 (Baja et al., 1991) distributed to thalamus and brain stem nuclei in addition to dopamine-rich brain regions. In view of the promising results with [^{3}H]CFT, [^{11}C]CFT was evaluated as a PET (positron emission tomography) imaging ligand in cynomolgus and squirrel monkeys. Several minutes after administration, [^{I1}C]CFT binding became prominent in the caudate-putamen and remained stable for at least 90 minutes. The striatal:cerebellar ratio was 4 or higher and was reduced following administration of the dopamine uptake blocker mazindol. These results suggest that cocaine recognition sites can be imaged *in vivo* with positron emission tomography.

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Evidence for Multiple [^{3}H]GBR12935 Binding Sites Associated with the Dopamine Transporter in Rat Striatal Membranes

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A number of radiolabeled ligands (e.g. [^{3}H]GBR12935, [^{3}H]mazindol, [^{3}H]CFT and [^{3}H]BTCP) are used to label the dopamine (DA) transporter, which is implicated in mediating the rewarding effects of drugs. The main objective of the present study was to test the hypothesis that there exist multiple transporter binding sites by conducting quantitative binding studies with each of the [^{3}H]ligands using the same experimental conditions. We report here the results obtained with [^{3}H]GBR12935 and [^{3}H]mazindol. Rat striatal membranes were prepared by two methods: (i) using caudates dissected from completely thawed frozen rat brain and homogenized while thawed (membrane A), and (ii) using caudates homogenized while frozen (membrane B). Two concentrations of each [^{3}H]ligand was each displaced by eight concentrations of nonradioactive drugs: GBR12935, mazindol, CFT and BTCP. This was done for three separate preparations of membrane A and membrane B, generating 216 data points per [^{3}H]ligand and membrane type. Nonlinear least squares curve fitting demonstrated that both the [^{3}H]GBR12935 and [^{3}H]mazindol data sets were best described by a two site binding model. Although [^{3}H]mazindol site 2 corresponded to [^{3}H]GBR12935 site 1, [^{3}H]mazindol site 1 did not correspond to [^{3}H]GBR12935 site 2. Qualitatively similar results were obtained with membrane A and membrane B. Control studies demonstrated that [^{3}H]GBR12935 was not labeling either the piperazine acceptor site or the sigma binding site. Viewed collectively, these studies suggest that in addition to labeling a common binding site/state, [^{3}H]GBR12935 and [^{3}H]mazindol each label a distinct binding site/state. Additional independent data will be needed to confirm these findings.

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Comparison of the Molecular Weight of the Dopamine Transporter in Rat Nucleus Accumbens and Striatum

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The biochemical and molecular properties of the dopamine transporter from rat striatum has been extensively investigated. However, there are conflicting studies comparing the homogeneity of the dopamine transporter in rat striatum and nucleus accumbens. The present study examines the apparent molecular weight of the dopamine transporter in rat nucleus accumbens and striatum using ^{125}I-DEEP and SDS-PAGE electrophoresis. Membrane homogenates from rat nucleus accumbens and striatum were prepared and irreversibly photolabelled with ^{125}I-DEEP. Afterwards, photolabelled samples were treated with neuraminidase (2 U/ml) and processed for SDS-PAGE electrophoresis. ^{125}I-DEEP specifically bound to a binding site of higher apparent molecular weight in the nucleus accumbens (77 kDa) than that observed in striatum (72 kDa). Specific binding in the nucleus accumbens was blocked by mazindol, nomifensine, GBR 12909 and (-)-cocaine but not by (+)-cocaine, desipramine and citalopram (at 10 μM), thus indicating binding in the nucleus accumbens was to the dopamine transporter. Treatment of photolabelled samples from both brain regions, with neuraminidase, reduced the apparent molecular weight of the dopamine transporter in the nucieus accumbens and striatum to similar apparent molecular weights, thus, indicating the presence of sialic acids. In conclusion, it appears that the dopamine transporter in the nucleus accumbens is of greater molecular weight than that in the striatum and that the difference in molecular weight can be attributed to a difference in glycosylation. Further studies will be required to determine the functional role that glycosylation of the dopamine transporter has in the two brain regions.

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Structure Activity Relationship of Dopamine Transporter Ligands

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Cocaine binding to the dopamine/transport protein appears to be related to its observed reinforcing properties. We have therefore begun systematic theoretical studies aimed at the characterization of molecular properties that can be related to the mechanism of recognition of cocaine binding site on the dopamine transporter. All ligands that bind to cocaine's binding site on the dopamine transporter appear to require three elements for its recognition: a) a basic center; b) an aliphatic lipophilic region; and c) an aromatic moiety. In this work, we have begun elucidation of the mechanistic function of each of these elements. The first conclusion reached is that cocaine and its congeners bind in a nonprotonated form and that the function of the basic region is as a proton acceptor center. Cocaine cannot be recognized in a protonated state, because in this state, an internal hydrogen bond between the NH and the carbonyl of the ester substituent is formed, which makes this proton unavailable for intermolecular interaction. Moreover, the pKa of cocaine is significantly lower than the physiological pH. In a second aspect, we have explored the mechanistic function of the aromatic ring by studying a series of closely related analogs of WIN-35,428 that differ only in the substituents on the aromatic ring. For each of these analogs, their optimized structures and electronic properties were computed using the AM1 semiempirical technique. The results indicate a strong correlation with the computed partition coefficient of each analog, as well as to their ability to form a $\pi-\pi$ stacking complex with an aromatic counterpart in the receptor. Finally, we tested our basic pharmacophore by using a two-dimensional database searching strategy, that retrieved approximately 400 compounds that satisfied the stipulated criteria for recognition. A subset of these consisting of piperazine derivatives and tetrahydropyridine analogs were then tested for binding in rat striatum membranes using [3H] WIN-35,428 as radioligand. Analogs in these families display affinities similar to those of 4-phenylpiperidine, allowing a design of other novel families of high affinity analogs.
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Differential Effects of Cocaine and Cocaethylene on Extracellular Dopamine and Serotonin in the Rat Striatum

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Cocaethylene is a metabolite of cocaine that is formed in the presence of alcohol by the activity of liver enzymes. Toxicological analysis of cocaine-related deaths has demonstrated that cocaethylene is frequently detected in high concentrations in postmortem blood, liver and brain (Heam et al., J. Neurochem. 56: 698-701,1991). The present study was carried out to evaluate whether cocaethylene is pharmacologically active *in vivo*. We have used intracerebral microdialysis to measure the acute effects of cocaine and cocaethylene on the extracellular levels of dopamine and serotonin in the rat striatum. Dopamine and serotonin were quantified in the striatal perfusates by HPLC with electrochemical coulometric detection. Unilateral microdialysis sampling demonstrated that basal levels of endogenous dopamine and serotonin were stable before drug administration. Both cocaine and cocaethylene increased the extracellular levels of dopamine to the same degree, with a maximal response occurring within 10 mm of drug administration. Acute iv bolus injections of cocaine and cocaethylene (1.5 mg/kg) increased extracellular dopamine levels to 250% of baseline. Cocaine and cocaethylene had minimal effects on dopamine metabolite levels, while producing high extracellular levels of striatal dopamine. In contrast to these results, cocaine caused a 500% increase in striatal serotonin content, while cocaethylene increased serotonin by only 100% above baseline. In both treatment groups, dopamine and serotonin levels returned to baseline by 60 min after drug administration. In agreement with these results. cocaethylene was equipotent to cocaine in inhibiting [^{3}H]-mazindol binding to the dopamine transporter assayed in rat striatal membranes. Cocaethylene was 35-fold less potent than cocaine at blocking striatal [^{3}H]-paroxetine binding to the serotonin transporter. The inhibition of dopamine re-uptake by cocaine is considered to be the mechanism underlying cocaine's reinforcing effects. The results of the intracerebral microdialysis studies reported here demonstrate that cocaethylene directly affects the nerve terminal region, rapidly elevating extracellular dopamine levels in the striatum. The blockade of dopamine re-uptake in the synaptic cleft by cocaethylene may account for the enhanced euphoria reportedly associated with combined cocaine and alcohol use. Cocaethylene's targeted action on the dopaminergic synapse may explain the high abuse liability associated with this two way drug combination. Since serotonin is thought to attenuate the reinforcing effects of cocaine. its reduced action at the serotonergic synapse may make cocaethylene a 'purer' rewarding substance than cocaine itself.

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Repeated and Chronic Cocaine Treatments Have Differential Effects on Serotonin and Dopamine Uptake in Rate Striatum and Nucleus Accumbens

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Cocaine inhibited [^{3}H]dopamine uptake more potently than [^{3}H]serotonin uptake *in vitro* in a chopped tissue preparation from rat nucleus accumbens and striatum. The cocaine inhibition curve for [^{3}H]dopamine uptake was biphasic whereas the inhibition curve for [^{3}H]serotonin uptake was much steeper, suggesting a single component of uptake. In a synaptosomal preparation, both curves were apparently monophasic. Male rats were treated daily with cocaine hydrochloride (15 mg/kg ip x 3 days), a regimen which leads to sensitization to the locomotor stimulating effects of cocaine. Twenty-four hours after the last injection, there was a decrease in [^{3}H]dopamine uptake and an increase in [^{3}H]serotonin uptake in the nucleus accumbens with no changes in the uptake of either monoamine in striatum. Furthermore, in the nucleus accumbens from cocaine-treated animals, the *in vitro* uptake of [^{3}H]dopamine was more sensitive to inhibition by cocaine than in saline-treated animals, with no change in potency for inhibition of [^{3}H]serotonin uptake. When animals were treated chronically with 50mg/kg/day cocaine for 7 days via subcutaneously implanted osmotic minipumps, there were no changes in total uptake of [^{3}H]dopamine or [^{3}H]serotonin in either brain region. However, tolerance to the inhibition of [^{3}H]dopamine uptake was observed in both striatum and nucleus accumbens. No changes were seen in the potency for cocaine to inhibit [^{3}H]serotonin uptake. Thus, there are different effects of these cocaine treatments on [^{3}H]dopamine and [^{3}H]serotonin uptake. Furthermore, intermittent and chronic cocaine administration have different effects on monoamine uptake and the development of tolerance or sensitization may depend on the schedule of administration. (Supported by a grant from NIDA).

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Cocaine Sensitization is Associated with Modified Serotonin Function

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Repeated cocaine exposure can result in a gradual development of behavioral sensitization in animals and psychosis in humans, two phenomena that have been associated with a cocaine-induced inhibition of dopamine uptake processes. However, cocaine also inhibits serotonin (5-HT) reuptake and potently depresses the spontaneous activity of dorsal raphe (DR) 5-HT neurons.

Using standard single-unit in vivo recording techniques, we investigated whether cocaine sensitization altered the responsiveness of 5-HT DR neurons to an iv challenge of (-)cocaine or the specific 5-HT reuptake inhibitor fluoxetine. Changes in the density of 5-HT uptake sites labelled by [^{3}H]-imipramine or 5-HT$_{1A}$ receptors labelled by [^{3}H]-8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) were also assessed in cocaine-sensitized rats.

The inhibitory response of 5-HT DR neurons to iv injections of (-) cocaine or fluoxetine was significantly enhanced after chronic cocaine exposure. There was a 3-fold decrease in the mean ID$_{50}$ (dose at which spontaneous activity was reduced to 50% of baseline) and mean ID$_{100}$ ulative dose at which unit activity was completely suppressed) for an iv challenge with (-)cocaine, while there was a 5-fold decrease in the mean ID$_{50}$ and ID$_{100}$ for a similar challenge with fluoxetine. Chronic cocaine treatment also elevated [^{3}H]imipramine labelling of 5-HT reuptake sites in cortical (medial prefrontal, frontal, sulcal prefrontal) and DR (but not median) raphe nuclei; [^{3}H]8-OHDPAT labeling of 5-HT$_{1A}$ receptors was unchanged except for a decrease in the central medial amygdala.

In summary, chronic cocaine treatment enhances the inhibitory response of 5-HT DR neurons to (-)cocaine and fluoxetine. The increased density of [^{3}H]-imipramine binding suggests that this electrophysiological change is related to an increase in the number and/or affinity of 5-HT reuptake sites. Thus, behavioral sensitization to cocaine may be associated with modifications of 5-HT reuptake processes in specific somal and terminal regions.

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Neurotransmitter Receptor-Effector Alterations in Rhesus Monkey Brain Following Repeated Cocaine Injections

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Previously, we have shown that repeated administration of cocaine (COC) to rats and rhesus monkeys causes long-lasting decreases in dopaminergic Dl and transporter binding sites (Kleven, et al., 1990; Farfel, et al., 1990). In this study, we examined changes in binding site densities and activity of phosphoinositide-phospholipase C (PI-PLC) in rhesus monkey hippocampus and amygdala after repeated COC injections. Rhesus monkeys were administered either saline (SAL; n=4) or COC (n=5; 3-4 mg/kg) IM, qid for 14 days. One animal did not survive the drug regimen, and the remaining animals were sacrificed 2 wks after the last injection. [3H] SCH 23390, [3H] spiperone and [3H] DPAT were used in saturation studies to label D1, D2 and 5-HTlA binding sites, respectively. Densities are reported as fmole/mg protein (SAL v. COC, mean ± S.E.). To assay for PI-PLC activity, membranes were incubated with 3H-phosphoinositol 4,5-biphosphate, and accumulation of 3H-inositol phosphate reaction products was measured in cpm/mg protein (SAL v. COC, mean ± S.E.).

In hippocampus, repeated cocaine injections caused a significant decrease in the density of D1 binding sites (317 ± 52 v. 193 ± 37) and a significant increase in the density of 5-HT1A binding sites (683 ± 180 v. 317 ± 67) with no change in the density of D2 binding sites (356 ± 115 v. 294 ± 75). The Kd of [3H] DPAT binding was also increased in cocaine-treated animals (588 ± 165 pmole/L) relative to controls (1381 ± 339). Basal activity of PI-PLC in hippocampus was significantly lower in the COC-treated animals (9676 ± 1966 v. 4762 ± 562), although nonspecific PI-PLC activity stimulated by 1 mM deoxycholic acid did not differ significantly between the groups (28,563 ± 4591 v. 20,658 ± 4866). PI-PLC activity measured after incubation with the Dl agonist SKF 38393 (0.01 mM) did not differ significantly between groups (14,842 ± 2666 v. 9271 ± 2242), nor did 5-HT-mediated (0.1 mM) stimulation of PI-PLC activity (14,204 ± 2787 v.10,491 ± 2522). In amygdala, neither the density of Dl binding sites (511 ± 152 v. 345 ± 103) nor the density of 5-HTlA binding sites (4133 ± 1714 v. 2030 ± 343) was changed after repeated cocaine injections. However, there were no significant differences between groups in the activity of PI-PLC in the basal, deoxycholate-, SKF-38393- or 5-HT-stimulated conditions. In summary, repeated cocaine causes decreases in Dl and increases in 5-HT1A binding sites with an accompanying decrease in basal, but not receptor-mediated second messenger activity in rhesus monkey hippocampus. These results suggest repeated cocaine may cause an increase in receptor-effector efficiency, which in turn may be related to behavioral sensitization. (Supported by DA-00085.)

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Pharmacologic and Behavioral Effects of High Doses of Intravenous Buprenorphine

E. Cone, B. Holicky, W. Pickworth and R.E. Johnson

Intravenous abuse of burprenorphine has been reported in a number of countries. Little information is available on the effects or abuse liability of buprenorphine by this route. As part of a dose run-up and safety evaluation study we administered intravenous buprenorphine to healthy, non-dependent male heroin abusers. Doses were administered under blind conditions every 2 days in ascending order: placebo; 0.3 mg; 0.6 mg; 1.2 mg. Pharmacologic and behavioral measures were monitored prior to and periodically after drug administration for 48 hrs. The duration of effects was characterized as short-acting (4 hrs), intermediate (4-12 hrs) and long acting (>12 hrs). These effects included: short-acting increases in LSD (dysphoria) scores, pulse and systolic BP; intermediate increases in MBG (euphoria), self-reported "Liking", diastolic BP and decreases in respiration; and long-acting increases in "Liking" and "Feel Drug" Scales and decreases in pupil diameter and a psychomotor task. The cardiovascular effects were consistently modest and did not appear to be dose-related, whereas most other effects varied with dose. An apparent biphasic response was observed on the PCAG (sedation) scale. The data suggest that intravenous bup-renorphine has long-acting behavioral effects and abuse liability.

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The Acute Effects of High Dose Buprenorphine in Non-Dependent Humans

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Buprenorphine is a mixed opioid agonist-antagonist which is currently being investigated as an opiate addiction treatment agent. The purpose of this study was to characterize the subjective and physiological effects of buprenorphine over a wide range of doses and to evaluate its safety in non-dependent humans. Four nondependent male subjects with histories of opioid abuse were tested once weekly with ascending sublingual, double-blind doses of buprenorphine: 0, 1, 2, 4, 8, 16 and 32 mg. Subjects were monitored before and for up to 96 hours after drug administration, during which time a number of physiological and subjective variables were assessed. The dose-effect function for subject-rated magnitude of drug effects was characterized by dose-related increases up to 8 mg, with no further increases at 16 and 32 mg. The PCAG (sedation) scale of the Addiction Research Center Inventory was elevated at all doses compared to placebo. Score. elevations were equivalent in magnitude at doses ranging from 4 to 32 mg. There were no significant elevations of the MBG (euphoria) scores at any dose tested, though ratings of "liking," "good effects" and "high" did show increases which reached a ceiling at the 16 mg dose. Dose-dependent increases in LSD (dysphoria) scores were observed up to 8 mg, with 16 and 32 mg producing no further increase in dysphoria rating. Buprenorphine produced significant and long-lasting pupillary constriction, which peaked at approximately 2 hours post-drug and was present for 48 hours or longer. Buprenorphine produced minimal respiratory depression and had no effect on percent arterial oxygen saturation as measured by pulse oximetry. Buprenorphine produced small increases in heart rate across all doses, which coincided with drug onset. Buprenorphine did not produce any serious adverse effects, although several subjectively unpleasant side effects were noted, including nausea, vomiting, constipation, urinary retention and insomnia. These data suggest a ceiling on the magnitude of buprenorphine's subjective and physiological effects in humans. Thus high doses of buprenorphine do not appear to have increased abuse liability over lower doses, nor do they present an increased risk of overdose in non-dependent subjects. These findings are encouraging for the clinical use of buprenorphine as a treatment for drug abuse.

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Comparison of Agonist and Antagonist Sensitivity in Opiate-Naive and Opiate-Experienced Humans

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Several previous studies have reported naloxone-precipitated withdrawal after single injections of morphine in opiate-experienced individuals; however, few reports exist concerning this phenomenon in opiate-naive humans. The purpose of this study was to compare the responses to morphine and naloxone in opiate-naive and opiate-experienced human research volunteers.

Participants were 10 males reporting a history of i.v. opiate abuse and 10 males who had never used an opiate i.v., had not taken an opiate by prescription during the past year, and never for more than 7 days. All subjects resided on an inpatient unit and participated in one daily session in which they reveived i.m. morphine (10 mg/70 kg) at 0900 and i.m. naloxone at 1500. Half of each group received 10 mg/70 kg naloxone and the other half, 30 mg/70 kg. Subjects were closely monitored throughout the day by assessing physiological, subjective, and observer measures of agonist and antagonist effects.

After morphine, degree of miosis and mean ratings of drug high, liking, and strength did not differ between the two groups. However, opiate-experienced subjects reported greater increases in good drug effects, and observer agonist effects were greater than those for opiate-naive subjects, who reported increased bad drug effects and sickness after morphine. Naloxone equivalently reversed miosis in both groups and reversed lingering agonist effects in opiate-experienced subjects, whereas two opiate-naive subjects reported increased good effects. After naloxone, both groups also reported bad drug effects and antagonist symptoms, but ratings were greater for opiate-experienced subjects. Opiate-experienced, but not naive, subjects reported increased ratings of sickness and urge and need for an opiate, and number of yawns was greater in experienced subjects. In general, there was not orderly differentiation between the 10 and 30 mg naloxone doses.

Although there were large individual differences, on most subjective and observer measures demonstrating positive agonist effects and naloxone-precipitated withdrawal, the magnitude of response was less in opiate-naive compared to opiate-experienced subjects. However, precipitated withdrawal was documented in some opiate-naive subjects.

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Human Pharmacology of the Opioid Antagonist, Nalmefene

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Nalmefene is potentially a clinically useful μ-opioid antagonist. It is active after oral and parenteral administration and has a plasma half-life of 8-9 hours. Two residential studies were conducted with opiate users, who were not currently physically dependent, to investigate nalmefene as a potential treatment medication.

The first study was designed to assess the abuse potential of nalmefene by examining its ability to produce morphine-like effects. Six male research volunteers reporting histories of opiate abuse were randomly administered nalmefene (25, 50, and 100 mg, p.o.), morphine (15 and 30 mg, i.m.), and placebo every fifth or sixth day under double-blind, double-dummy conditions according to a 6 x 6 Latin square design. Physiological, subjective, and observer measures were repeatedly assessed over 3 days after each treatment condition. Morphine, but not nalmefene, produced miosis and increased MBG scale and drug liking scores. Nalmefene was associated with adverse effects, including agitation, irritability, and muscle tension. It was concluded that nalmefene has no apparent abuse potential.

The purpose of the second study was to assess the ability of nalmefene to antagonize the physiological, subjective, and observer effects of morphine. Using a double-blind, double-dummy, randomized Latin square design, seven male research volunteers participated in seven weekly conditions in which they received nalmefene (0, 50, or 100 mg, p.o.) on Monday and morphine (0, 10, or 20 mg, i.v.) on Tuesday through Friday. A battery of physiological, subjective, and observer measures was administered each day before drugs were given and 1, 2, 4, 6, 10, and 12 hours postdrug. Preliminary results indicate that nalmefene was effective in antagonizing the effects of morphine in a dose-dependent manner. There was minimal evidence of differential antagonism between the various measures. Nalmefene 50 mg blocked the miosis and increases in MBG scale and drug liking scores produced by morphine 10 mg for 72-96 hours and those of morphine 20 mg for 48 hours. Nalmefene 100 mg antagonized the same effects of morphine 10 mg for 96 hours and those of morphine 20 mg for 72-96 hours. Data from both studies suggest that nalmefene has clinical utility as a treatment medication for opioid dependence.

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Plasma Norharman (ß-Carboline) Levels are Significantly Higher in Alcoholics than in Nonalcoholics

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Based on the hypothesis that reaction products of pyruvate with neurotransmitters, e.g. ß-carbolines, are involved in the pathogenesis of alcoholism, norharman and harman were determined in the blood plasma of alcoholics, cannabis-dependent subjects and heroin addicts. Thirty-four alcoholics who had been admitted to a large community hospital for detoxication purposes spent venous blood at day 1, 4, 8 and 9-21. The concentration of norharman in blood plasma from alcoholics was 99.5 + 22.6 pg/ml and that of controls 26.9 + 10.7 pg/ml; $p < 0.001$. The concentration did not change significantly during a 3 week detoxication period. In the subgroup of alcoholics with delirium or hallucinosis, a slight increase of norharman could be detected while in alcoholics with vegetative withdrawal symptoms norharman levels dropped slightly over time ($p=0.07$). No difference was found with respect to harman. In a second study, 15 alcoholics of a psychiatric university hospital were examined day 1 and 8. Most but not all patients had increased concentrations of norharman and some of them dropped to control levels at day 8. Thus, it seems that the regulation of norharman levels is still able to adapt to ethanol intake in alcoholics who are little depraved.

All three cannabis-dependent patients had elevated concentrations of plasma norharman at the day of admission to the university hospital. During the observation period of 18 to 65 days the level dropped to control levels in one patient whereas in the other two the concentrations remained about three times higher than in control subjects. A preliminary analysis revealed that the duration of misuse and the dose of cannabis plays a role for the duration of the pathological changes.

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References available upon request.

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Pharmacokinetic Comparisons of Smoked and Intravenous Delta^9-Tetrahydrocannabinol (THC) in Humans Before and After Daily Smoking of Marijuana

C.E. Cook, M. Perez-Reyes, A.R. Jeffcoat, B. Jeffcoat, B.M. Sadler, D.R. Brine, R.D. Voyksner, W.R. White and S. McDonald

To examine the induction of pharmacokinetic tolerance to THC, we conducted a study involving the daily smoking of a 1% THC marijuana cigarette for 13 days.

Six healthy male paid volunteers participated. They had used marijuana an average of 4.0 ± 1.4 times per month over the past 6 months but agreed to abstain from marijuana and other illicit drugs for 2 weeks before and during the study except for laboratory use (checked by urinalysis). On day 1 they smoked a NIDA marijuana cigarette (1% THC) within a 15 min period. 5',5',5'-Trideutero-THC (2.6 mg) was then infused over a 50 min period. Blood samples were obtained for analysis. On days 9-21 each subject smoked 1 cigarette. On day 22 they were tested as before.

Plasma samples were treated with internal standard (20 ng/mL of [$^2H_9$]-Δ^9-THC), allowed to equilibrate, and then subjected to extraction (acetonitrile and solid phase extraction) followed by GC/MS analysis as the trimethysilyl ethers. After GC separation on a 30 m DB-1 capillary column, the three species (do, d3, and d_g) were determined by electron impact mass spectrometry. The limit of analysts was 0.2 ng/mL.

A tri-exponential equation was fit to the concentrations of THC in plasma (C_t) after smoking and intravenous administration. Model independent estimates of some parameters were also calculated.

None of the pharmacokinetic parameters for either the smoked or infused THC were statistically different between days. For example, mean maximum plasma concentrations were 38 and 47 ng/mL for smoking on days 1 and 22 and 34 and 39 ng/mL for infusion. Mean clearances of 0.74 and 0.69 L/min and 0.63 and 0.85 L/min were found, respectively.

In conclusion, although tolerance to the cardioacceleratory effects of THC was found in this study (Perez-Reyes et al., 1991), the pharmacokinetics of THC were not significantly changed in these subjects by 13 days of smoking one daily marijuana cigarette.

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Caffeine Intake as a Cause of Nicotine Withdrawal

J.R. Hughes, A.H. Oliveto, S.Y. Terry, S.T. Higgins and W.K. Bickel

Smoking cessation increases blood levels of caffeine and many symptoms of caffeine intoxication and nicotine withdrawal overlap; thus, caffeine intake during abstinence may be a significant cause of nicotine withdrawal. In two prospective studies of 105 and 620 self-quitters, we tested whether 1) coffee users had more nicotine withdrawal than non-coffee users; 2) heavy coffee users had more withdrawal than light users; and 3) increased coffee use post-cessation was related to the severity of withdrawal. There was little support for these hypotheses. An experimental study assigned 10 smokers to a within-subject 2x3 design with smoking status smoking vs abstaining for 4 days) and caffeine (0, 50 or 100 mg/svg x fixed number of servings) factors. Physiological, performance caloric intake, and observer- and subject-rated variables were recorded on an outpatient basis. Caffeine blood levels did not consistently increase post-cessation. Temporary smoking cessation produced the expected withdrawal symptoms. Caffeine did not increase withdrawal symptoms but did decrease post-cessation hunger. Our negative results may be due to 1) too short a duration of abstinence, 2) tolerance to caffeine effects, or 3) our restricted range of caffeine dosages.

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Termination of Normal Dietary Caffeine Produces Clinically Significant Withdrawal Symptoms

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This study assessed the clinical significance of abrupt termination of unconstrained dietary caffeine in 62 normal, daily caffeine consumers. Each subject participated in three seven-day experimental conditions. During the first condition, no constraints on diet were imposed (baseline condition). No constraints on diet were imposed on the first five days of the next two conditions. However, during the final two days of each of those conditions, subjects followed a caffeine-free diet and orally ingested identically-appearing capsules under supervised and double-blind conditions. During one of those conditions the capsules contained placebo (placebo condition); during the other, capsules contained caffeine (caffeine condition). The order of the caffeine and placebo conditions was counterbalanced across subjects. The caffeine doses were individualized to match each subject's daily caffeine intake estimated from the baseline condition food diaries. Caffeine intake averaged 235 mg per day. Although subjects were informed as to the study's procedures, the subject instructions and dietary restrictions did not reveal the fact that caffeine was the drug under study. Experimental measures were collected once during the baseline condition and then again at the end of the last day of both the placebo and caffeine conditions. Relative to the baseline and caffeine conditions, the placebo condition increased ratings of classic caffeine withdrawal symptoms, increased the percentage of subjects showing clinically significant scores on the Beck Depression Inventory (i.e., scores greater than 15), and disrupted motor performance. Significantly more subjects reported unauthorized use of OTC analgesic or cold medications during the placebo than the caffeine condition. These results suggest that abrupt termination of unconstrained daily dietary caffeine can produce clinically significant disruptions in normal daily caffeine consumers.

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Antisera Generated Against a Peptide Complementary to H-ß-Endorphin Inhibit B-Endorphin Binding to Human U937 Cells

N. Shahabi, K. Bost and B. Sharp

Antisera to the naloxone-insensitive receptor for ß-endorphin expressed on the U937 cell line were generated using the complementary peptide strategy. A peptide complementary to a fragment of h-ß-endorphin was synthesized using an amino acid sequence predicted from reading its antisense RNA 3' to 5'. Using ELISA, rabbit antisera specific for the ß-endorphin complementary peptide (C-ß-endorphin) were demonstrated. With the exception of C-ß-endorphin, preabsorption of the antisera with the following failed to reduce the ELISA titer: h-ß-endorphin$_{1.31}$ and 10 unrelated peptides of 5-21 amino acids. Sucrose gradient separation was also used to determine whether the antisera recognized ^{125}I-ß-endorphin. Incubation of ^{125}I-ß-endorphin with antisera to C-ß-endorphin, rabbit IgG, or irrelevant hyperimmune sera all failed to alter the expected sedimentation pattern, whereas antisera to h-ß-endorphin eliminated the expected peak. Antisera to C-ß-endorphin were purified to obtain IgG using NH_4SO_4 precipitation, ion exchange and KLH affinity chromatographies. IgG to C-ß-endorphin (2-800 ug/tube) progressively inhibited the binding of ^{125}I-ß-endorphin (1-2nM) to intact U937 cells, whereas normal IgG was ineffective. The approximate IC_{50} was 6.25±0.26 uM (mean±S.D.; N=3); maximum concentration of IgG completely eliminated specific binding. Following binding and crosslinking of ^{125}I-ß-endorphin to U937 cell membrane in the presence of either h-ß-endorphin (10^{-5} M), normal IgG or anti-C-ß-endorphin IgG, SDS-PAGE showed that anti-C-ß-endorphin IgG completely inhibited binding to a 44Kd and partially to a 59Kd site. The same pattern was found with h-ß-endorphin, but IgG was completely ineffective. In summary, using the complementary peptide strategy, antibodies selective for C-ß-endorphin were generated which displace h-ß-endorphin from the naloxone-insensitive receptor on U937 cells. The efficacy of the antisera with intact U937 cells suggests that the ß-endorphin receptor is on the cell surface.

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Comparison of μ-Opioid Binding in Intact Neuroblastoma Cells and in Broken Cell Preparations

L. Toll

Binding studies were conducted in intact SH-SY5Y neuroblastoma cells, and compared with studies conducted in broken cell membrane preparations. Binding studies used the μ-selective peptide antagonist [^{3}H]CTOP. For all compounds tested, inhibition curves were steep, suggesting binding to a single receptor site, and a single, low affinity, state of the μ receptor. Pseudo-Hill coefficients, derived using the program "Allfit", were greater than 1.0 for all of the agonists tested, but not significantly different than 1.0 for antagonists. This suggests the possibility of positive cooperativity of binding for agonists but not antagonists in intact cells. Binding results in SH-SY5Y cell membranes were significantly different. In Tris buffer, the affinities determined for agonists DAMGO and morphine, but not etorphine, were significantly higher in membrane preparations. In broken cell preparations, for agonists DAMGO and morphine, though not etorphine, Pseudo-Hill coefficients were significantly less than 1.0.

Binding studies were also conducted to intact cells, or cell membranes after treatment of the cells with pertussis toxin. Pertussis toxin caused no change in binding affinity of any compound to intact SH-SY5Y cells. However, when binding was conducted in cell membranes, pertussis toxin treament lowered the affinity for agonists DAMGO and morphine, though not etorphine or antagonist CTOP. Taken together, these results suggest high and low affinity states are present in membrane preparations, but not in intact cells, and that DAMGO and morphine, but not etorphine have different affinities for these two states. The fact that etorphine has high affinity for all receptor states, may explain its high analgesic potency.

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Opioid Receptor Subtype Selectivity of the Phenylmorphans and Analogs

M. Froimowitz, C. Pick and G.W. Pasternak

The morphine-like (+)-phenylmorphan, the atypical (-)-enantiomer, and analogs were tested in binding assays selective for opioid μ_1, μ_2, δ, κ_1 and κ_3 receptors. All compounds, except one, including the atypical (-)-phenylmorphan, had their greatest affinity for μ_1 and μ_2 receptors. The exception was the (+)-9-methyl analog which had slightly greater affinity for the κ_1 receptor. The receptor binding assays provide evidence that opioids in which the phenyl ring is constrained to be equatorial on the piperidine ring can also have considerable affinity for u-receptors. Dose-response curves were also determined for (+)- and (-)-phenylmorphan in the mouse tail flick assay with the (+)-enantiomer found to be about 10 times more potent. Pretreatment with the selective opioid antagonists ß-FNA (μ_1 and μ_2). naloxonazine (μ_1), nor-BNI (κ_1), and naltrindole (δ) suggest that the analgesic activity of both enantiomers is mediated through μ receptors. Intrathecal administration of (+)- and (-)-phenylmorphan showed that both are μ_2 agonists. Based on the relationship between their affinities for μ receptors and in vivo analgesic potencies, it appears that both phenylmorphan enantiomers are full agonists with respect to analgesia. Conformational energy calculations on the compounds were also performed using the MM2-87 program. Consistent with previous results for the phenylmorphans (Froimowitz 1984), the most potent analgesics preferred a particular orientation of the phenyl ring.

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Effect of ß-FNA Administration on In Situ μ Opioid Binding

T.J. Martin and J.E. Smith

ß-FNA has been shown to bind irreversibly to μ opioid receptors *in vitro* in brain homogenate and sections. However, the effect of i.c.v. ß-FNA administration on *in situ* binding to μ opioid receptors has not been documented. Male Fischer 344 rats (250-350 g) were implanted bilaterally with guide cannulae in the lateral ventricles and injected with saline or 40 nmol of ß-FNA in a volume of 16 μ1. Approximately 24 hr later, the animals were sacrificed and serial 20 μm sections were taken at -20 °C in a cryostat and dessicated overnight at 0-4 °C. Binding to μ opioid receptors was assessed by incubating sections in triplicate in various concentrations (0.1-20 nM) of [^{3}H]DAGO in 50 mM Tris (pH=7.4) at 25 °C for 2 hr. Following a 2 min wash in ice-cold buffer, sections were removed and placed in vials containing scintillation cocktail overnight. Nonspecific binding was assessed in the presence of 1 μM DAGO. [^{3}H]DAGO binding to sections of saline-treated animals was found to be to a single site with a Kd and Bmax of 0.22 nM and 22.8 fmol/section, respectively. These parameters were not significantly different from control animals. The Kd and Bmax of ß-FNA-treated animals were 0.32 nM and 14.0 fmol/section, respectively. [^{3}H]DAGO binding was also assessed in animals given two or three injections of 40 nmol of B-FNA approximately 24 hr apart. These animals were sacrificed approximately 24 hr after the last injection. In animals given two administrations of 40 nmol of ß-FNA, [^{3}H]DAGO binding was found to be to a single site with a Kd and Bmax of 0.36 nM and 12.4 fmol/section, respectively. However, [^{3}H]DAGO binding to sections from animals given three injections of ß-FNA was best fit by a two site model. The Kd and Bmax values of the high and low affinity sites were found to be 0.15 nM and 5.2 fmol/section, and 11 nM and 35.7 fmol/section, respectively. Therefore, ß-FNA binds irreversibly to a subset of μ opioid binding sites *in vivo.* Furthermore, repeated ß-FNA administration may be useful for the characterization of the pharmacology of subtypes of the μ opioid receptor. This research was supported in part by USPHS grants DA-01999 and DA-00114.

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Opioids Regulate hCG Release from Trophoblast Tissue of Term Human Placenta

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Components of an opioid system (receptors and peptides) are present in the human placenta. Kappa opioid receptors were purified from human placental villus tissue and opioid peptides B-endorphin, dynorphin 1-8, 1-13; methionine and leucine enkephalins are present in extracts of the same tissue. Trophoblast tissue synthesizes and releases human chorionic gondotropin (hCG) which is similar to pituitary luteinizing hormone (LH). In vitro opioids regulation of hCG release from trophoblast tissue is the subject of this report. Opioids investigated are a non-selective opioid receptor agonist morphine, a partially selective ethylketocyclazocine and the kappa selective agonists U-69,593 and U-50,488H. All agonists stimulated basal hCG release to different extents and their dose response curves were biphasic (bell shaped). Potency of agonists to stimulate hCG release correlated with their kappa receptor selectivity. Doses of agonists causing maximum stimulation of hCG release (control set at 100%) were: 10^{-6} M morphine, 122 ± 4%; 10^{-8} M EKC, 149 ± 14%, 10^{-9} M U-69,593, 144 ± 9%; 10^{-13} M U-50,488H, 142 ± 6%. Both the non-selective opioid antagonist naltrexone and the kappa selective nor-binaltrophimine (nor-BNI) caused an inhibition of hCG release. from trophoblast tissue. Antagonists dose response curves were diphasic and the potency of each corresponded to its kappa receptor selectivity. Maximum inhibition of hCG release was caused by naltrexone at a dose of 10^{-6} M (29 ± 4%) and nor-BNI at a dose of 10^{-9} M (30 ± 6%). A combination of either antagonist with any of the most stimulatory dose of an agonist resulted in hCG released levels comparable to control, i.e. total reversal of agonists stimulation by an antagonist. Data cited above indicate that opioid agonists and antagonists regulate the in vitro basal release of hCG from full term human placental trophoblast tissue and that this effect is mediated by the tissues kappa receptors.

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In Vivo Pharmacology of Potent, μ-Selective Opioids: Variations in Opioid Efficacy

C.P. France, G. Winger, M.R. Seggel, K.C. Rice and J.H. Woods

Several fentanyl-related 4-(heteroanilido)piperidines with interesting pharmacological effects were reported by Bagley and coworkers (Bagley *et al.*, 1989, 1990). One of these derivatives (compound 28, Bagley *et al.*, 1989) was studied under a variety of conditions in rhesus monkeys and compared to other μ opioids (e.g., alfentanil) as well as to a second fentanyl derivative (compound 32; mirfentanil; France *et al.*, 1990, in press). Compound 28 and mirfentanil maintained rates of self-administration responding comparable to rates maintained by alfentanil; these positive reinforcing effects of compound 28 and mirfentanil were attenuated by quadazocine. Compound 28 substituted completely and mirfentanil substituted only partially for alfentanil in monkeys discriminating between saline and alfentanil; neither compound 28 nor mirfentanil substituted for ethylketocyclazocine in monkeys discriminating between ethylketocyclazocine and saline. In morphine-treated monkeys discriminating between naltrexone and saline, mirfentanil but not compound 28 substituted for naltrexone. In morphine-abstinent monkeys compound 28 but not mirfentanil completely reversed naltrexone-lever responding. This withdrawal-reversing effect of compound 28 was attenuated in a dose-related manner by opioid antagonists; Schild analyses of these antagonisms indicated μ-receptor mediation of this effect of compound 28. Both compound 28 and mirfentanil had analgesic effects in a warm-water tail-withdrawal assay; this analgesic effect of compound 28 was an opioid effect as evidenced with antagonism by quadazocine. In contrast, the analgesic effect of mirfentanil under these conditions appears to be nonopioid (France *et al*, in press). Compound 28 but not mirfentanil markedly decreased respiratory function in monkeys breathing air or 5% CO_2 in air. The respiratory-depressant effects of compound 28 were antagonized by quadazocine. In contrast, mirfentanil antagonized the respiratory effects of other *μ* opioids (e.g.; alfentanil).

Compound 28 is structurally very similar to mirfentanil; however, the small difference in structure between compound 28 and mirfentanil appears to confer a significant increase in efficacy for compound 28 at opioid *μ*-receptors. Further studies on the structure-activity relations of these and other fentanyl derivatives might provide important information on the molecular requirements of opioid efficacy.
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REFERENCES: Available upon request from senior author.

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Displacement of μ and δ Opioid Receptor Ligands by Morphine in Mouse and Rat Brain

B.C. Yoburn and B.A. Billings

The density and affinity of DPDPE(δ) binding sites is greater in whole mouse brain than in rat brain; while the affinity and density of DAGO (μ) sites in whole brain are very similar in the 2 species (Yoburn et al., 1991). In the present study, displacement of [^{3}H]DPDPE and [^{3}H]DAGO by morphine in mouse and rat brain was examined to determine if the affinity of morphine for these 2 binding sites differed in the 2 species. In both species, [^{3}H]DAGO (0-7-2.0nM) and [^{3}H]DPDPE (1.0-6.5nM) were competitively displaced by morphine (0-1-1,000nM). The K_i (nM) values for morphine displacing [^{3}H]DPDPE were 82.8±2.5sem, 76.84±10.6 in rat and mouse, respectively. The K_i values for morphine displacing [^{3}H]DAGO were 1.4±0.2, 1.8±0.3 in the rat and mouse, respectively. In further studies, mice were implanted S.C. with 1, 75mg morphine pellet and rats were implanted S.C. with 2, 75mg morphine pellets. Placebo pellets were implanted in controls. In vivo studies indicated this treatment produced tolerance to morphine. After 72hrs. animals were sacrificed and morphine displacement binding studies conducted in whole brain. Morphine treatment did not significantly alter the morphine K_i in rat or mouse brain for either [^{3}H]ligand. Thus, morphine has a different affinity for [^{3}H]DPDPE and [^{3}H]DAGO binding sites, although the affinity for each site is similar in rat and mouse. Furthermore, chronic morphine treatment does not alter morphine affinity for the μ or δ binding site. (Supported by NIDA DA 04185).

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Lack of Antinociceptive Cross-Tolerance Between [D-Pen^2, D-PEN^5]Enkephalin and [D-ALA^2]Deltorphin II: Evidence for Delta Receptor Subtypes

A. Mattia, T. Vanderah and F. Porreca

This study has investigated the development of antinociceptive tolerance to, and cross-tolerance between, two highly selective δ agonists, [D-Pen^2, D-Pen^5]enkephalin (DPDPE) and [D-Ala^2]deltorphin II as well as to [D-Ala^2, NMePhe^4, Gly-ol^5]enkephalin (DAMGO), a highly selective μ agonist. Antinociception in male ICR mice was determined from the latency to tail-flick with warm water (55 °C) as the thermal nociceptive stimuli. Pretreatment with i.c.v. DPDPE twice daily for 3 days resulted in tolerance to DPDPE as shown by a 4.8-fold rightward shift in the dose-response curve. In DPDPE pretreated mice, the dose-response lines for [D-Ala^2]deltorphin II and DAMGO did not change versus naive mice. Following *i.c.v.* [D-Ala^2]deltorphin II, the [D-Ala^2]deltorphin II dose-response line was displaced to the right by more than 37-fold, but no changes in the dose-response lines for DPDPE and DAMGO compared to those obtained in naive animals were observed. Pretreatment with i.c.v. DAMGO produced a rightward displacement of the DAMGO dose-response line of 47-fold. In DAMGO pretreated mice, the dose-response lines for DPDPE and [D-Ala^2]deltorphin II were the same as those obtained in naive mice. Thus, the data indicate that antinociceptive tolerance develops to DPDPE, [D-Ala^2]deltorphin II and DAMGO but that there is no cross-tolerance between them. As both DPDPE and [D-Ala^2]deltorphin II are highly selective δ agonists, this unexpected lack of cross-tolerance may suggest that these compounds produce antinociception via interaction with subtypes of δ receptors.

AFFILIATION: University of Arizona Health Sciences Center, Tucson, AZ

Characterization of ^{3}H]DTG, [^{3}H](+)-3-PPP and [^{3}H] (+)-Pentazocine Binding Sities in NB41A3 Neuroblastoma, C6 Glioma, and NG108-15 Hybrid Cells: Further Evidence for Sigma Receptor Subtypes

B.J. Vilner and W.D. Bowen

We have recently proposed multiple forms of sigma receptors, and suggested the terms sigma-1 and sigma-2 to describe these sites (Brain Res 527:244-253, 1990). While these two sites bind [^{3}H]DTG with equal affinity, they can be differentiated by stereoselectivity for opiate benzomorphans. Sigma-l sites exhibit higher affinity for (+)-benzomorphans than (-)-benzomorphans and are enriched in guinea pig brain. Sigma-2 sites exhibit the reverse stereoselectivity, (-) > (+), and were first identified in PC12 cells.

We have now characterized the binding of sigma ligands to crude membrane homogenates from NB41A3 neuroblastoma, C6 glioma, and NG108-15 hybrid cells. [^{3}H]DTG bound with K_d (nM) and B_{max} (fmol/mg protein) of 62±6 and 7324±670 (NB41A3), 101±7 and 5507±537 (C6), and 75±1 and 3134±229 (NG108-15) respectively. The corresponding values for [^{3}H](+)-3-PPP were 137±11 and 2256±642 (NB41A3), 500±31 and 6268±682 (C6), and 183±25 and 1673±344 (NG108-15) respectively. Competition studies with various sigma ligands vs. 5 nM [^{3}H]DTG gave the following rank order of potency: DTG > haloperidol > (-)-pentazocine > (+)-3-PPP = fluphenazine > (+)-pentazocine > (-)-SKF 10,047 > (+)-SKF 10,047. Ligands for opiate (morphine, DAMGO, DSTLE), PCP (MK-801) dopamine (apomorphine), and amino acid (GABA, glutamate) receptors failed to displace [^{3}H]DTG binding at 10 uM. Low affinity for (+)-pentazocine (K_i= 1.7-3.3 uM) and (+)-SKF 10,047 (K_i= 19.3-91.8 uM), and higher affinity for (-)-pentazocine (K_i= 43-162 nM) and (-)-SKF 10,047 (k_i= 3.5-6.1 uM) suggest that these cells express sigma-2 receptors similar to those found in PC12 cells. Studies with [^{3}H](+)-pentazocine, a selective probe for sigma-l sites (FEBS Lett 251:53-58, 1989), suggest that sigma-1 sites are also present in these cells, but at lower densities than sigma-2. The rank order of sigma ligands at this site was: haloperidol > dextrallorphan > (+)-SKF 10,047 > (-)-SKF 10,047 >> MK-801. High affinity for haloperidol and 27-fold stereoselectivity for (+)- over (-)-SKF-10,047 confirms the presence of sigma-l.

Investigation of the subcellular distribution of [^{3}H]DTG and [^{3}H](+)-pentazocine binding in NGl08-15 cells showed that the highest level of binding was in the P2 fraction (enriched in plasma membrane), while only a low level of binding was detected in the P3 fraction (enriched in endoplasmic reticulum). This is in contrast to brain, where higher sigma binding is associated with the P3 fraction compared to P2. Also, preliminary studies show that these ligands bind to intact cells. Thus, sigma binding appears to be associated with the cell surface. These cells will be useful tools in studies of sigma receptor function.

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ß-Endorphin Stimulates Opioid Receptor-G Protein Activation at μ and δ Receptors

D.E. Selly and J.M. Bidlack

Opioid receptors are coupled to GTP-binding regulatory proteins of the G_i/G_o family, which transduce the binding of an agonist into an effector response, such as the inhibition of adenylate cyclase or the alteration of ion channel activity. The efficacy of receptor-G protein activation varies considerably, depending on the agonist. Since the opioid peptide ß-endorphin binds with approximately equal affinity to μ and δ opioid receptors, we investigated the ability of human ß-endorphin 1-31 (R-END) to stimulate opioid receptor-coupled G protein activity in membranes prepared from two opioid receptor-containing cell lines: the rodent neuroblastoma x glioma hybrid NG108-15, which contains only δ-type opioid receptors, and the human neuroblastoma SK-N-SH, which contains predominantly μ-type opioid receptors together with a small number of δ sites. As an indicator of receptor-mediated G protein activation, agonist-induced stimulation of the membrane-bound low Km GTPase was measured. S-END stimulated low Km GTPase activity in membranes from both NG108-15 and SK-N-SH cells in a concentration-dependent and saturable manner. Maximal stimulation of the enzyme was achieved with 100 and 300 nM B-END in the NG108-15 and SK-N-SH cell membranes, respectively, and was reversed by the inclusion of 100 μM naloxone. ß=END-stimulated GTPase activity was also blocked by treatment of either cell line with 40 ng/ml of *Borderella pertussis* toxin (islet activating protein) for 18 hr prior to the preparation of membranes. These results indicated that G proteins of the G_i/G_o-type were involved in the ß-END-stimulated reaction. Stimulation of low Km GTPase by ß-END had identical sodium requirements as DADLE-stimulated activity in NG108-15 cell membranes and DAGO-stimulated activity in SK-N-SH cell membranes, with maximal agonist-induced GTPase activation achieved at 100 mM NaCl. In NG108-15 cell membranes, ß-END-stimulated GTPase was inhibited in a concentration-dependent manner by the δ--selective antagonist ICI 174,864. However, ICI 174,864 was ineffective in reversing the activation of low Km GTPase by ß-END in SK-N-SH cell membranes, suggesting the involvement of μ opioid receptors in the ß-END-stimulated reaction in this cell line. These results are the first demonstration of the stimulation of low Km GTPase activity by ß-END at both μ and δ–opioid receptors.

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Opioid Enhancement of Enkephalin Release May Require Phosphatidylinositol-Derived Second Messengers

A.R. Gintzler and H. Xu

Previous work from this laboratory has demonstrated that the electrically evoked release of methionine-enkephalin (met-enkephalin) from the myenteric plexus can be modulated by mu-delta-and kappa-types of opiate receptor. This modulation is biomodal. Low concentrations (nanomolar) enhance whereas higher concentrations (10-100 nM) inhibit the magnitude of stimulated release. Both opioid effects are antagonized by the opiate receptor antagonist naloxone (0.1-1 uM). Stimulation-induced release of met-enkephalin is mediated by a cAMP-dependent process. Elevation of intracellular levels of this second messenger greatly enhances (approximately 2 fold) the effectiveness of electrical stimulation to induce release. The opioid enhancement or inhibition of release requires different second messengers. The enhancement requires a cholera toxin-sensitive G_s-like protein whereas opioid inhibitory effects require a pertussis toxin-sensitive G_i (or Go)-like protein. We now demonstrate that each opioid effect is differentially affected by blockade of enteric cholinergic receptors.

The muscarinic cholinergic receptor antagonist, atropine, blocks opioid enhancement but is without effect on the opioid inhibition of stimulated enkephalin release. Moreover, the previously demonstrated reversal of opioid inhibition to enhancement in enteric ganglia treated with forskolin is also abolished by cholinergic receptor blockade. Thus, opioid facilitation of release requires a cholinergic sensitive process that is not needed for opioid inhibitory action. Pretreatment with a calcium inophore, A23187, completely restores opioid excitatory responses despite the blockade of muscarinic receptors. A23187 does not alter opioid facilitation of release in the absence of atropine. This suggests that the deficit responsible for the abolishment of facilitory opioid effects when cholinergic receptors are blocked is the lack of stimulation-induced generation of elevated cytosolic calcium. The known coupling of muscarinic receptors to phospholipase C activation and the generation of IP3 (which raises cytosolic calcium levels) suggests that this second messenger is critical for the manifestation of opioid facilitation of enkephalin release.

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The Effects of Chronic Administration of Morphine on the Levels of Calcitonin Gene-Related Peptide (CGRP) in Brain Regions of the Rat

S.P. Welch, P.P. Bass, K.G. Olson, A.R. Schatz and W.L. Dewey

CGRP release has been reported to be decreased by the acute administration of opiates (Pohl et al., 1989), and has been shown to modulate the antinociception induced by acute administration of morphine (Welch et al., 1989). Mice were implanted with either a morphine pellet or a placebo pellet and received either chronic intrathecally (i.t.)-administered CGRP (2 pg/mouse) or vehicle two times per day for 3 days. Mice implanted with the morphine pellet and injected with CGRP chronically showed increased sensitivity to naloxone-precipitated withdrawal jumping versus mice treated with vehicle chronically. In morphine-tolerant mice receiving chronic i.t. vehicle, naloxone (0.03 mg/kg, s.c.) precipitated withdrawal jumping in 16% of the mice versus 83% jumping in the mice receiving chronic CGRP i.t. Increased lethalities were also observed in the group receiving chronic CGRP in combination with chronic morphine.

Acute administration of morphine (8 mg/kg) to rats did not significantly alter CGRP levels in the cerebellum, cortex, hippocampus, hypothalamus, midbrain, medulla, and the spinal cord However, a decrease in CGRP levels was observed in the corpus striatum (from 300 to 95 fmol/mg protein) following acute morphine. In rats, chronic administration of morphine via ALZET pumps for 7 days (2 mg/kg/hour) produced tolerance to the antinociceptive effects of morphine. Chronic administration of naltrexone (2mg/kg, s.c., twice per day) blocked the development of tolerance to morphine. Tolerance to morphine was determined by the use of the tail-flick test and a challenge injection of 15 mg/kg, S.C. morphine. Levels of CGRP in the vehicle, morphine and morphine + naltrexone groups did not differ in the cerebellum, cortex, medulla, midbrain, and spinal cord. However, rats tolerant to morphine had significant reductions in CGRP levels in the hippocampus (244 to 125 fmol/mg protein), and the hypothalamus (472 to 176 fmol/mg protein). These decreases were reversed upon the chronic administration of naltrexone S.C. Chronic naltrexone administration alone did not significantly alter CGRP levels. In rats that are tolerant to morphine, precipitation of withdrawal with naloxone did not alter CGRP levels in the cerebellum, cortex, hippocampus, hypothalamus, midbrain, medulla, and spinal cord. However, levels of CGRP rose from 257 fmol/mg protein in tolerant rats to 1117 fmol/mg protein in rats in withdrawal from morphine. Thus, the effects of chronic administration of opiates appears to alter CGRP levels in selective brain regions. We hypothesize that the changes in CGRP levels represent a compensatory homeostatic neuronal mechanism associated with opiate tolerance. The effects of withdrawal on CGRP levels is selective for the corpus striatum and may correlate with the enhancement of withdrawal jumping by CGW observed in mice. (Supported by grants DA-06031 and the Commonwealth of VA Center on Drug Abuse Research). AFFILIATION: Dept. of Pharmacology/Toxicology, MCV/VCU, Richmond, VA

Effects of Morphine Treatment on mRNA Levels of Pro-Opiomelanocortin and Proto-onocogene c-fos in a Neuroblastoma Cell

S.L. Chang, L. Spriggs and J. Zadina

Morphine has been previously shown to increase expression of the proto-oncogene c-fos in rat brain. The FOS nucleoprotein complex was proposed to couple morphine binding to alteration of expression of proenkephalin (PE) and pro-opiomelanocortin (POMC), whose promoter contains an AP-1 sequence (Chang et al., 1988). The SH-SY5Y human neuroblastoma cells express predominantly b-subtype opiate receptors (Yu amd Sadee, 1986) with receptor density increased by retinoic acid and decreased by TPA (Zadina et al., 1990). In this study, we examined the time course of change in c-fos and POMC mRNA concentrations upon two different methods of morphine treatment in SH-SY5Y cells. For treatment 1, the repeated treatment design, SH-SY5Y cells were grown in T75 flasks to achieve approximately 80% confluency. Seven days before harvesting, all the flasks of cells were changed to fresh medium and 10 μM or 1 μM of morphine sulfate (MS) was added to the flasks designated as 7 days morphine treatment. Five days before harvesting, this procedure was repeated with all flasks and the same amount of MS was added to the flasks designated as 7 days and 5 days morphine treatment. Three, one, and one-half days before harvesting, the procedure was repeated at each time point. Thus, in this design, cells exposed to morphine for 12 hrs or more were periodically given fresh morphine. During the final 6 hrs, morphine was added to the flasks without changing medium at zero, 15 min, 30 min, 1 hr, 2 hrs and 6 hrs. For the 2nd set of treatments, the single dose method, 10 μM MS was added to the flasks at the designated time from 6 days to zero time without changing the medium. At the end of each treatment, the cells were harvested, and RNA was extracted. Total cellular RNA from each sample was subjected to slot-blotting analysis using a ^{32}P-fos-CDNA probe. With the first set of experiments, MS caused both an early transient induction of c-fos and a later prolonged increase in c-fos. With the 2nd set of experiments, the single treatment of MS caused only a transient and rapid induction of c-fos. Slot-blotting hybridization with a ^{32}P-POMC cRNA probe revealed that POMC mRNA was significantly activated at 6 hrs and remained significantly elevated up to 7 days in the cells with repeated MS treatment. In the single-dose experiments, however, the POMC mRNA was not significantly elevated at 2 days or less. It was significantly activated at 6 days, but at a much lower level that that seen in the repeated dose design. These results indicate that repeated exposure to MS is required for sustained c-fos expression which may be optimal for the significant activation of POMC mRNA.

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REFERENCES: Available upon request from senior author.

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Excitatory Amino Acid Neurotransmitters and Morphine Withdrawal: Differential Effects of Central and Peripheral Kynurenic ACI Administration

J.H. Krystal, K. Rasmussen and G.K. Aghajanian

This study evaluated the capacity of the non-selective excitatory amino acid antagonist, kynurenic acid (KYN), to suppress withdrawal-induced activation of the locus coeruleus (LC) and the display of withdrawal behaviors. Central (i.c.v.) and peripheral (i.p.) effects of KYN were evaluated because this compound does not readily cross the blood-brain barrier. METHODS: STUDY 1. BEHAVIOR: KYN effects were assessed in rats implanted with 75 mg. morphine pellets daily for 2 days beginning 3 days prior to testing. CENTRAL KYN (0.1 µmole i.c.v. over 10 min. (n=6); 0.25 µmoles, i.c.v. (n=8); or saline (n=8)) was administered 15 minutes before naltrexone (10 mg/kg, s.c.) administration. PERIPHERAL KYN (saline, 10, 100, or 500 mg/kg (n=6/group)) was administered 15 minutes before naltrexone administration. Withdrawal behaviors were assessed using previously described methods. STUDY 2. LC ACTIVITY: KYN effects were evaluated using methods described above to produce dependence, elicit withdrawal, and administer KYN. CENTRAL KYN: Rats (n=3) were anesthetized with chloral hydrate. Rats were mounted in a stereotaxic apparatus and a burr hole was made 1.2 mm posterior to lambda and 1.1 mm lateral to the midline. Extracellular recording electrodes were single-barrel glass micropipettes, broken back to a tip diameter of 2-3 µm and filled with 2M NaCl solution containing 2% Fast Green. PERIPHERAL KYN: Comparison of KYN (500 mg/kg, i.p. vs. saline) was conducted using procedures described above. RESULTS: STUDY 1: Dose-dependent suppression of opiate withdrawal behaviors were observed in rats following both i.c.v (ANOVA, dose x time, $F=2.2$, $p=0.03$) and i.p. (ANOVA, dose x time, $F=2.8$, $p=0.00l$) KYN administration. Significant reductions were observed in the display of several individual withdrawal behaviors following i.c.v. and i.p. KYN. STUDY 2: Dose-dependent suppression of LC activation occurred following i.c.v, but not i.p. KYN administration. IMPLICATIONS: These data suggest that excitatory amino acid neurotransmitters contribute to LC activation and display of withdrawal behaviors associated with the naltrexone-precipitated abstinence states. Also, both central and peripheral excitatory amino acid receptors modulate opiate withdrawal. These data suggest that excitatory amino acid antagonists might be developed to suppress withdrawal in humans.
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C-FOS Expression is Induced by Opioid Withdrawal and Blocked by NMDA Antagonists

C.E. Inturrisi, M. Brodsky and K. Rasmussen

Naltrexone-precipitated withdrawal in opioid dependent rats results in the induction of the proto-oncogene, c-fos (Hayward et al., and NMDA antagonists (MK-801 and LY-274614) block the behavioral signs of withdrawal (Rasmussen et al.). Levels of c-fos mRNA were measured by a quantitative solution hybridization assay employing a ^{32}P-labeled riboprobe which was adapted from a procedure described by Franklin et al. for proenkephalin mRNA. In morphine dependent rats c-fos mRNA levels are increased 2-4 fold at 1 hour after naltrexone administration in locus coeruleus, amygdala, nucleus accumbens, frontal cortex and hippocampus (but not in striatum or spinal cord). Pretreatment of dependent rats with MK-801 (1 mg/kg SC) or LY-274614 (100 mg/kg SC) 15 or 60 min prior to naltrexone prevented or reduced the naltrexone withdrawal-induced increase in c-fos mRNA in amygdala, nucleus accumbens and hippocampus but not in locus coeruleus or frontal cortex. The NMDA antagonists given to morphine dependent rats (not withdrawn) did not alter c-fos mRNA compared to control (no pretreatment or withdrawal). These results demonstrate that NMDA antagonists can block both the behavioral manifestations of withdrawal and c-fos induction in those brain regions (amygdala, nucleus accumbens and hippocampus) associated with opioid effects and withdrawal. These effects are produced with either MK-801, a non-competitive or LY-274614, a competitive NMDA antagonist.

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Genetic Factors in Human Drug Abuse

R.W. Pickens, D.S. Svikis and M. McGue

Genetic risk for drug abuse was determined in a subsample of alcoholic twins where at least one member of each pair also met DSM-III criteria for drug abuse and/or dependence. The subsample included 41 male and 19 female pairs of monozygotic (MZ) and 32 male and 13 female pairs of dizygotic (DZ) twins.

For males, a significant difference in concordance for any type of drug abuse (excluding tobacco) was found in MZ (.63) and DZ (.44) twins ($p<.05$), suggesting genetic influences in the etiology of the disorder. For females, no significant difference in concordance for drug abuse was found in MZ (.22) and DZ (. 15) twins, although the M2/DZ ratios for males and females were similar (1.45 and 1.37, respectively). Using NIMH Epidemiological Catchment Area data as a measure of population baserate, estimated heritability of drug abuse in males was .34 while that in females was .22, which were similar to heritabilities found for alcoholism in the larger sample (Pickens et al., 1990).

In a more detailed analysis of these data, pairwise concordance rates were calculated for specific types of DSM-III drug abuse and/or dependence. While showing no significant MZ/DZ differences for drug abuse in general, females also showed no MZ/DZ differences for specific types of abused drug (including barbiturate, opioid, cocaine, amphetamine, hallucinogen, and cannabis). Males showed significant MZ/DZ differences for both amphetamine ($p<.03$) and cannabis ($p<.006$) abuse and/or dependence.

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AFFILIATIONS

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Risk for Alcoholism in Relatives of Drug Addicts

L. Handeisman, M. Branchey, J. Silverman and L. Buydens-Branchey

We studied the morbidity risks for alcoholism and addictions in the first degree relatives of male cocaine addicts with or without alcoholism. Of the 71 addicts, 40 (56.3%) had a history of alcoholism, and 37 (59.1%) had a history of opioid dependence. 22 patients (30.1%) also met criteria for a lifetime diagnosis of a major psychiatric disorder. Increased morbidity risks for alcoholism were found among male relatives of cocaine addicts with co-morbid alcohol dependence, compared with relatives of addicts without alcohol co-morbidity. Among fathers, risks were .69 vs. .32 ($z=2.98$, $p<.003$), while among brothers, risks were .38 vs. .15 ($z=2.35$, $p<.03$). Significantly increased risks were also observed when probands with a psychiatric diagnosis were excluded from the analyses. Co-morbid opioid dependence was not related to increased risk for alcohol or drug abuse in relatives. Whether this increased risk for alcoholism in relatives of alcoholic drug addicts is the expression of a specific vulnerability to alcoholism or a more general trait, could not be ascertained in this study.

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Prolactin and Cortisol bevels After Acute Ethanol challenge in Women with and Without a Family History of Alcoholism: A Pilot Study

B.W. Lex, J.E. Ellingboe, S.K. Teoh, J.H. Mendelson and E.M. Rhoades

In a pilot study, 5 matched pairs of family history positive (FHP) and family history negative (FHN) female social drinkers (mean age 23.6 yr) received 0.56 g/kg ethanol and isocaloric placebo in a double-blind cross-over design. FHP women had biological fathers who met DSM-III-R criteria for alcohol dependence, and FHN women had no relatives who met these criteria. FHP and FHN pairs were matched for age, education level, history of alcohol use, and quantity and frequency of current alcohol consumption. Repeated measures of blood alcohol levels (BALs), prolactin, and cortisol were obtained before and after beverage administration. FHP and FHN women had comparable peak BALs of about 70 mg/dl 60 min after ethanol. FHP subjects had significantly lower prolactin levels 40, 60, and 80 min after ethanol, but higher cortisol levels 130 and 150 min after ethanol. No significant differences in levels of either hormone occurred after placebo. Lower prolactin levels in FHP subjects confirm findings from young FHP men studied in another laboratory. Changes in prolactin, and possibly cortisol, levels observed in this pilot study are an indication that hormonal responses to alcohol might serve as predictors of risk for development of alcohol-related problems. Further study will increase the number of matched FHP and FHN women.

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Improving Treatment Outcomes in Pregnant Opiate Addicts

G. Chang, K. Carroll, H. Behr and T.R. Kosten

While the potential medical and social costs of opiate addiction during pregnancy are great, the impact of methadone treatment, either alone or in combination with other therapies, is equivocal. The purpose of this study is to compare outcomes for pregnant opiate addicts enrolled in enhanced methadone maintenance with those receiving treatment as usual. We hypothesized that women enrolled in the enhanced program would benefit by reducing illicit substance use, increasing prenatal care, and delivering healthier infants.

The enhanced outpatient methadone program offered weekly on-site prenatal care, weekly relapse prevention groups, thrice weekly urine toxicology screens with positive contingency awards for abstinence, and therapeutic child care during treatment visits. The enhanced program was delivered in addition to treatment as usual, which consisted of daily methadone medication, weekly group counseling, and random urine toxicology screens at the minimum rate of one per month.

Outcomes for six enhanced treatment subjects are compared for those of six in treatment as usual. Study patients differed from the comparison group by having three tines as many prenatal visits, 8.8 vs 2.7, and delivering heavier infants, 2943 vs. 2280 grams. Study patients had somewhat fewer positive cocaine screens, 39% vs. 49%. These results suggest that enhanced drug treatment can improve pregnancy outcomes, and in particular, reduce low birth weight for this high risk population.

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Can a Weekly Support Group for Pregnant Addicts Improve Meternal and Fetal Outcome?

D.S. Sivikis, M.E. McCaul, T. Feng, T.B.R. Johnson and E.J. Stokes

The sequelae of drug use during pregnancy to both mother and fetus have been well-established. Since many drug-abusing pregnant women are resistant to formal substance abuse treatment, less intensive, cost-effective interventions are needed to impact this high-risk population. The present study examined the usefulness of a weekly substance abuse support group offered on-site in an inner-city, hospital-based obstetrical clinic. Pregnant women identified as having an alcohol or drug problem via urinalysis screening and/or personal interviews with medical personnel, were referred to a substance abuse specialist who completed a thorough evaluation using the Addiction Severity Index (ASI). If the severit of the substance use disorder warranted intervention, the patient was referred to the OB support group. During each weekly 90-minute support group meeting, patients discussed target topics (e.g., consequences of drug use to the fetus, relapse prevention); established social support networks (e.g., exchanged phone numbers): and contracted to attend the following group session. To examine if group attendance was predicted by patient demographics, the present study compared women who attended two or more OB support groups (attenders, N=41) to women who attended 0 or 1 support groups (nonattenders, N=57). No significant differences were found between the attenders and nonattenders, with women in both groups being predominantly Black, sin le. in their mid-20s, and with less than a high school education. In addition, in patients for whom ASI data were available, no differences were found in psychosocial characteristics (including patterns of alcohol and drug use) and ASI interviewer severity ratings for all seven ASI domains. However when attenders and nonattenders were compared for maternal and fetal outcome several significant differences emerged. Group attenders showed greater maternal and fetal weight gains during pregnancy, improved 1-min infant APGAR scores, and significantly fewer low birthweight infants (<2500 gms) than group nonattenders. There was also a significant reduction in medical costs incurred for infants of attenders versus nonattenders. These findings suggest that a weekly substance abuse support group can provide a low-cost, effective therapeutic opportunity that is able to attract and retain many pregnant addicts who might not otherwise receive substance abuse treatment services.

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Fetal Stress Associated With Cocaine Withdrawal

J.T. Christmas, J.S. Knisely, S.H. Schnoll and S. Ruddy

Cocaine used by pregnant women is associated with numerous complications of pregnancy. Spontaneous abortion, preterm labor, intrauterine growth retardation, fetal death in-utero, and placental abruption, deceased birth weight and head circumference, certain congenital anomalies, neonatal emotional liability, and an increase incidence of SIDS have all been shown to occur with increased frequency in cocaine users, The etiology of most cocaine associated obstetic and neonatal complications remains obscure. The current recommendation for pregnant cocaine users is to abstain from use. The effects of abrupt withdrawal on the pregnant woman and her fetus have, however not been studied. There are anecdotal reports of changes in fetal movement.

The purpose of this ongoing clinical investigation therefore is to examine, using subjective and objective measures, the effects of acute cocaine withdrawal on the human fetus.

Pregnant. cocaine dependent women who seek treatment are offered inclusion in this study. Patients are admitted to the Clinical Research Center (CRC) and are abruptly withdrawn from cocaine. While in the CRC, the-patients undergo three times daily objective (Stimulant Withdrawal Scale) and subjective (Cocaine Craving Scale) evaluation of withdrawal. Daily maternal measures include vital signs, Profile of Moods States (PMOS), and urine and blood assays for cocaine and its metabolites. The fetus is evaluated with twice daily fetal movement counts (FMC) and daily Non-Stressed Fetal Heart Rate Testing (NST). Biophysical Profile (BPP), and uterine (UtA) and umbilical (UA) arterial doppler assessment of blood flow. Eight patients have been admitted and evaluated to date in this ongoing clinical investigation. Gestational age at the time of withdrawal has ranged from 24.0-33.7 weeks. Length of hospitalization has ranged from 5 to 10 days. Subjective rating of cocaine craving was high at the time of admission and gradually fell to how levels at the time of discharge. The POMS Depression score appeared to correlate well with cocaine cravine. With regard to fetal measures. there was a great deal variability in hourly FMC (range 14-398). Increased FMC did not clearly correlate with day of withdrawal or cocaine craving. Results of BPP and NST suggest that the fetus were at no time in danger of fetal death. Increased UA doppler S/D ratio noted early in withdrawal and returning to normal by the time of patient discharge suggests acute cocaine withdrawal may be associated with deceased fetoplacental blood flow. These preliminary results suggest that the maternal withdrawal state may be associated with stress that may help to explain some of the complications seen in pregnant cocaine users.

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A Comparison of the Antinociceptive Effects of Opioid in Neonatal and Adult Rats

C.R. McLaughlin and W.L. Dewey

It has been reported previously that recent changes in the attitudes about neonatal pain and pain management have resulted in increases in the administration of opioids to neonates. However, little was known about the relative efficacy of the various opioid agonists commonly employed, especially in comparison to adult responses. Therefore, in the present study we sought to compare the dose responsivity of neonates and adults to various opioids in the formalin test of tonic nociception, a clinically relevant model of pain in unrestrained animals. This test is especially appropriate for ontological comparison because the responses observed in neonates and adults are very similar, in both quantity and quality, thereby allowing direct comparison not possible in other nociceptive tests.

The subjects were 250 gm adult male Sprague-Dawley rats (Dominion Labs, Dublin, VA) and 3-6 day-old rat pups of both sexes bred in our colony. A small amount (5 ul in pups, 50 ul in adults) of dilute formalin (15%) was injected into the dorsal surface of a hindpaw. Behavior was then time-sampled and recorded at 30-second intervals 30-40 minutes following the formalin injection. This time period corresponded to peak nociceptive response to the formalin-induced inflammation. Behaviors were categorized as: pawlift, pawlick or other.

Our results indicate that buprenorphine (ED50 = 2.4 ug/kg), fentanyl (ED50 = 18.3 ug/kg), morphine (ED50 = 0.1 mg/kg) and meperidine (ED50 = 4.0 mg/kg) all produce antinociception in 3-day-old rat pups in the formalin test of tonic nociception. We observed a four-fold increase in the morphine ED50 in adults, however, indicating increased sensitivity to its analgesic efficacy in the pups (ED50 adult = 0.4 mg/kg).

These preliminary results suggest that opioids are effective antinociceptive agents in neonates in our adaptation of the formalin test, and that the relative efficacies of these agents appear to be similar to that observed in adults. Our morphine data, however, suggest an increased sensitivity to this opioid in the pups. Studies are currently underway to evaluate these trends with other opioids in the formalin and other nociceptive tests in both neonates and adults.

This research was supported by: PHS grants DA-01647, DA-07027 and the Commonwealth of Virginia Center for Drug Abuse Research.

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Effects of Early Postnatal Exposure to Morphine on Rat Pup Distress Vocalization and Analgesia

G.A. Barr and S. Wang

Opiates produce a myriad of behavioral and physiological effects in the infant animal. Whereas chronic treatment with opiates in the adult results in tolerance to many of these effects, there fewer reports on the biochemical and behavioral consequences of chronic opiates at different stages of development. The goal of these experiments was to assess the effects of chronic morphine treatment during the early postnatal period on subsequent opioid mediated behavior prior to weaning. Specifically we studied the effects of chronic morphine treatment on morphine induced analgesia and on the ability of morphine to quiet the ultrasonic vocalizations (USV) of a pup isolated from its mother and littermates.

Infant rats were untreated or injected b.i.d. with saline or morphine (0.6, 3.0, or 15.0 mg/kg, s.c) on postnatal days 1-7 (n=7-9 litters/treatment). On day 7, 5-7 hours after the last injection, pups were tested for 1) morpine-induced analgesia in a hot-water immersion test; 2) baseline levels of USV in response to isolation from the dam and litter-mates; and 3) the ability of morphine (0.03 - 0.30 mgkg) to suppress USV At 10 days of age baseline levels of vocalization and the ability of a littermate to reduce USV ("companion" effect) were measured. There was profound tolerance to the analgesia induced by morphine. Even the lowest chronic dose (0.6 mg/kg) produced tolerance. USV was significantly elevated at the two lower doses of chronic treatment (0.6 and 3.0 mg/kg) at 7 days of age, although chronic injections with saline had a similar if less robust effect. The chronically treated pups also showed less of a decline in USV when tested twice, a procedure that normally is quieting. There was, in contrast to the analgesia data, no evidence of tolerance to morphine's suppressant effects on USV; morphine decreased calling equally in all conditions. At 10 days of age pups, now abstinent for three days, vocalized more each minute of a six minute test, in contrast to controls who showed no change in USV over time. There was no alteration in the ability of a littermate to quiet the pup. The differences in tolerance suggest fundamental differences in opioid control of USV and analgesia. Alterations in USV, induced by chronic morphine, are characterized by the failure of pups to decrease vocalizations following repeated testing or during longer periods of separation from their mothers and littermates.
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Maternal and Fetal Plasma Disposition of Cocaine (C) in Near-term Macaque Monkeys

M.G. Paule, J.R. Bailey, C.M. Fogle, M.P. Gillam, H.M. Duhart and W. Slikker, Jr.

To address the hypothesis that cocaine (C) exposure in pregnant subjects also results in significant concomitant fetal exposure to C, plasma disposition profiles of C were obtained for both maternal and fetal monkeys on gestational days 147-154 (term=165 days). Anesthesia was induced with ketamine HCl and maintained with halothane/nitrous oxide/oxygen ventilation. Maternal plasma samples were collected from a uterine vein while fetal samples were collected from cannulated intraplacental or fetal femoral arteries [n=4 (1 cynomolgus, 3 rhesus)] for up to 8 hr after the maternal intramuscular injection of 1.0 mg/kg C and a tritiated C tracer. C and norcocaine were isolated via HPLC and quantitated using liquid scintillation spectrometry. Maternal plasma concentrations of C peaked within 10-20 min and ranged from 132-312 ng/ml while fetal levels peaked within 30 min to 2 hr at 18-329 ng/ml. Norcocaine levels were essentially undetectable in both maternal and fetal plasma. These data clearly demonstrate that maternal cocaine exposure results in substantial concomitant exposure to cocaine in the fetus. Since the fetus is clearly exposed in utero to C after maternal use, and since the consequences of such developmental exposure to C are currently unknown, studies evaluating the effects of cocaine exposure throughout gestation are currently underway.

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Period-Specific Effects of Developmental Cocaine Exposure: Neuroanatomical Findings

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The incidence of birth complicated by maternal cocaine abuse has been estimated at beeween 14-25% at inner-city hospital according to peripartum urine screens. While deficient infant growth and development occur primarily with long-term maternal cocaine abuse, neurobehavioral abnormalities are identified in offspring of women who use cocaine only during the first timestar. Animal studies afford us the possibility of examining the effects of cocaine exposure during selected developmental periods periods on the ontogeny of specific neuronal circuits.

Pregnant rats were assigned to one of three cocaine-exposure periods: gestation day (G) 8-22, postnatal day (PND) 1-10 or PND 11-20. Controls received sterile water. Gestational exposure consisted of daily gastric intubation of the dams with 6O mg/kg cocaine while PND) exposure consisted of daily sc injections of the pups with 5O mg/kg. When the rats were 60 days of age, the quantified deoxyglucose method of Sokoloff et al (1977) was used to determine cerebral glucose utilization. Alternate brain sections were collected for examination of the distribution of binding of ^{3}H SCH 23390, a ligand with high specificity for the D-1 receptor. Autoradiographs were evaluated using the MCID or Loats computerized imaging systems.

We found that prenatal exposure depressed glucose utilization in selected cortical and subcortical structures particularly the hypothalamus and nigrostriatal pathway. The substantia nigra also showed an increase in the concentration of SCH 23390 binding. PND 1-10 exposure produced significant increases in glucose utilization in several components of the limbic system in females. At the same time, there was little effect in males. The caudal portion of the caudate nucleus showed a significant decrease in SCH 23390 binding. PND 11-20 exposure produced significant increases in activity in several cortical regions as well as the extrapyramidal motor pathways in adult females and decreases in structures of the medial forebrain bundle in adult males. There was an increase in SCH 23390 binding in the caudal caudate nucleus in the females and an increase in the in the N. accumbens in the males.

These data indicate that cocaine has specific long-term effects on brain development which depend upon the period of drug exposure as well as the gender of the animal examined.

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These studies were supported by a grant from NIDA, DA04118.

Preclinical Strategies in the Development of Cocaine Abuse Pharmacotherapy

R.S. Mansbach and R.L. Balster

Behavioral models in animals are the latest tools being used in the investigation of novel medications for the treatment of cocaine abuse. Specifically, we are exploring three procedures: i.v. cocaine self-administration in rhesus monkeys, cocaine discrimination in squirrel monkeys, and cocaine discrimination in rats. In one series of experiments, a number of sympathomimetic agents are being studied for their efficacy in 1) altering cocaine- and food-maintained responding; 2) producing cocaine-like stimulus effects; and 3) modifying cocaine's stimulus effects. Mazindol and d-amphetamine suppressed cocaine-taking behavior in monkeys, although at higher doses food-maintained behavior was also affected, indicating incomplete behavioral specificity of these drugs in modifying cocaine-maintained responding. Both drugs also produced cocaine-like responding in rats trained to discriminate cocaine (10 mg/kg) from saline under a two-lever, fixed-ratio schedule of food presentation. Co-administration of mazindol did not augment the discriminative or response-rate effects of cocaine in rats. Mazindol also produced cocaine-appropriate responding in squirrel monkeys trained to discriminate cocaine from saline. On the other hand, phenylpropanolamine produced cocaine-appropriate discrimination behavior in rats yet suppressed cocaine-maintained responding in monkeys only at doses which also severely disrupted food-maintained lever-pressing. It is hoped that these strategies will lead to methods by which novel medications can be rapidly and accurately identified. (Supported by NIDA contract 271-89-8156)

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Effects of Central Chlordiazepoxide Infusions on Intravenous Cocaine Self-Administration in Rats

G.F. Guerin and N.E. Goeders

The following experiment was designed to investigate the role of benzodiazepine (BZD) receptors in the medial prefrontal cortex (MPC) in cocaine reinforcement in rats. Experimentally naive adult male Wistar rats were implanted with chronic indwelling jugular catheters and bilateral guide cannulae into the MPC. The rats were trained under a fixed-ratio 4 schedule of reinforcement where 4 depressions of the response lever resulted in a 200 µl intravenous infusion of 0.5 mg/kg cocaine delivered over 5.6 seconds. Chlordiazepoxide (CDP) was injected into the MPC 5 minutes before the start of the session in a volume of 200 nl/side over 30 seconds. Doses of CDP tested ranged from 25 pmol to 500 pmol per side, and each dose was tested at least twice. CDP was dissolved in saline, which also served as the vehicle. All rats self-administered cocaine, with stable rates of responding obtained within 2 to 3 weeks. In general, intracranial CDP delivery into the MPC did not affect the total number of cocaine infusions/session. However, there was a significant increase in drug-intake (i.e., 2-to 3-fold) during the first 15 to 20 minutes of the session following 25, 50 or 100 pmol of CDP. These data suggest that BZD receptors in the MPC are involved in cocaine reinforcement in rats. Increases in drug-intake following the microinjection of extremely small doses of CDP (e.g., 8.5 to 34 ng) may represent an attempt by the animals to overcome a drug-induced blockade of cocaine reinforcement resulting from a decrease in dopamine turnover in the MPC induced by the binding of CDP to BZD receptors localized in this brain region. The short duration of the effects of CDP on drug-intake is consistent since the small amount of CDP injected would likely be rapidly metabolized or would quickly diffuse away from the injection site. (Supported by NIDA grant DA06013)

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The Effects of Buspirone and Gepirone on Cocaine Self-Administration in Rhesus Monkeys

L.H. Gold and R.L. Balster

Buspirone and gepirone are members of a novel chemical class developed to treat anxiety and depression. Both drugs seem to act at the 5HTlA receptor, however,buspirone also binds to D2 receptors and has some dopamine antagonist effects. Buspirone and gepirone were evaluated as potential medications for cocaine abuse by studying the effects of acute and repeated treatment on iv cocaine self-administration in rhesus monkeys. Subjects had established stable rates of responding for cocaine (N=4, 0.02-0.05 mg/kg/infusion) under fixed-ratio 10 schedules during daily 60-min sessions. Doses of buspirone (0.01-1.0 mg/kg) or gepirone (0.03-1.0 mg/kg) were injected iv, 15 min prior to the start of selected sessions. Five min later, 50 gms of food was presented to the monkeys. Acute pretreatment with buspirone increased rates of cocaine self-administration without disrupting food intake. Rate increases often occurred without altering the within-session pattern of responding. The highest dose of buspirone severely disrupted self-administration behavior and food consumption in 3 of the monkeys. In contrast, acute doses of gepirone had little effect on rates of cocaine self-administration in 3 of 4 monkeys; modest increases were measured in 1 subject. Disruption of food intake and changes in the within-session pattern of cocaine self-administration were obtained only at the highest dose of gepirone. Repeated daily treatment with either buspirone or gepirone (0.1 mg/kg) produced no systematic effects on cocaine self-administration. These results suggest that while gepirone was ineffective in altering rates of cocaine self-administration, acute buspirone treatment increased rates of cocaine-maintained responding similar to increases produced by dopaminergic antagonists. The evidence that buspirone altered rates of cocaine self-administration at nontoxic doses suggests that further evaluation of this drug as a pharmacotherapy for cocaine abuse may be warranted, although the lack of effects with repeated dosing is not encouraging. On the other hand, no evidence is provided that gepirone modifies the behavioral effects of cocaine in this model. Supported by NIDA Contract 271-89-8156.

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Effects of Continous Administration of the Monoamine Uptake Inhibitors, Fluoxetine, Sertraline, and Mazindol on Behavior Maintained by Cocaine or Food in Rhesus Monkeys

W.L. Woolverton and M.S. Kleven

Several monoamine uptake inhibitors, including desmethylimipramine, maprotiline, and mazindol have been suggested as possible treatments for cocaine abuse. The purpose of the present study was to determine whether the serotonin uptake blockers sertraline and fluoxetine or the catecholamine uptake inhibitor mazindol could alter responding maintained by cocaine injections without altering behavior maintained by food. Six rhesus monkeys were trained to press a lever in daily experimental sessions under a 3 component multiple schedule of reinforcement. In the first and third components, food (1-g banana-flavored pellets) was available for 10 mins under a FR 30 schedule. In the second component the dose of cocaine that maintained maximum rates of responding (0.03 or 0.05 mg/kg/inj) was available for 30 mins under a FR 30 schedule. There was a brief time-out after each reinforcer. Two additional monkeys were tested under a different multiple schedule in which cocaine (0.05 or 0.10 mg/kg/trial) and food availability (4-7 pellets/trial) alternated every 6 mins for a total of 48 trials. When responding was stable, monkeys received continuous infusions of several doses of sertraline (0.5-8.0 mg/kg/day), fluoxetine (0.4-3.2 mg/kg/day), or mazindol (0.4-3.2 mg/kg/day) for either a minimum of 21 days (fluoxetine and sertraline) or the same number of sessions that were required for responding to decline to low levels when the monkeys were allowed to self-administer saline (mazindol). Each drug decreased cocaine-maintained responding in a dose-related manner. In most cases, food-maintained responding was disrupted at doses less that or equal to those that decreased cocaine-maintained responding. Additionally, the higher doses of each drug decreased food intake outside of the daily sessions. These results indicate that monoamine uptake blockers with prominent effects on either serotonin or dopamine neurotransmission can decrease cocaine self-administration but only at doses that also alter behavior maintained by food.

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Mediation of A10 Somatodendritic Dopamine Release by Opioids and GABA

M.A. Klitenick and P.W. Kalivas

Opioid administration into the A10 region (VTA) elicits an increase in dopamine (DA) metabolism and locomotor activity. Repeated exposure to opioids results in a progressively augmented behavioral response as well as a decrease in DA utilization within the VTA. Intra-VTA injection of $GABA_A$ agonists activate the mesolimbic DA system while $GABA_B$ agonists inhibit it, suggesting GABAergic regulation of DA transmission. In the present study, the modulatory role of both opioids and GABA on the mesolimbic system was further assessed using *in vivo* microdialysis. Adult male Sragues-Dawley rats (300±20g; N=S-10/group) were prepared with bilateral guide cannula 3mm above the VTA. After 8 days of recovery, a removable dialysis probe was unilaterally inserted into the guide cannula 24 hrs prior to the experiment. Samples of dialysate were collected every 20 min and the levels of DA, 5-HT and their metabolites were measured by HPLC/EC. Analysis of the data with repeated measures ANOVA revealed that administration of morphine through the dialysis probe elicited a dose-dependent increase in DA release which could be blocked by coadministration of baclofen or by peripheral injection of naloxone. Administration of amphetamine through the probe elicited a dramatic increase in DA release in the VTA that was not blocked by baclofen. The data provide further evidence for a modulatory role of opioids and GABA in the mesolimbic DA system. Other evidence indicates that μ-opioids indirectly activate A10 DA cells via a presynaptic inhibition of a tonic inhibitory input. Activation of $GABA_A$ receptors results in an indirect disinhibition and activation of $GABA_B$ receptors directly inhibits dopaminergic activity by hyperpolarizing the cell. Thus the morphine-induced increase in somatodendritic release of DA can be blocked, in an indirect manner with opioid antagonists or blocked directly with $GABA_B$ agonists, in contrast to amphetamine-induced release.

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A complete reference list may be obtained from M.A. Klitenick

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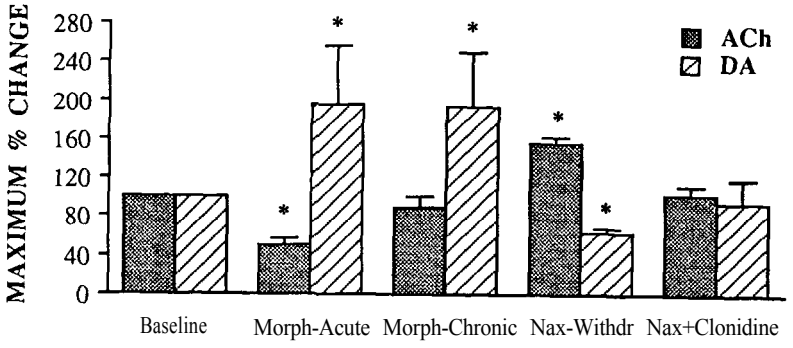
Extracellular Acetylcholine and Dopamine in the Nucleus Accumbens folling Morphine and Naloxone-Precipitated Withdrawal: Possible Role in Addiction

P. Rada, G.P. Mark, E. Pothos and B.G. Hoebel

Microdialysis was used to measure acetylcholine (ACh) and dopamine (DA) in the nucleus accumbens (NAC) of freely moving rats. Systemic injections of morphine (20 mg/kg, IP) in non-dependent subjects significantly decreased ACh (50%; p<.0l) and increased DA (95%, p<.05). ACh and DA were also measured during chronic morphine followed by naloxone-precipitated withdrawal (Nax-Withdr.) with and without clonidine pretreatment. Morphine in dependent rats again increased DA (95%; p<.05) but failed to reduce intracellular ACh. After morphine dependence had been established, naloxone caused withdrawal symptoms such as wet dog shakes and teeth chattering accompanied by an increase in ACh levels (55%; p<.05) and a decrease in DA (40%; pc.05). These withdrawal symptoms and the reciprocal changes in ACh and DA were eliminated by pretreatment with clonidine. The results suggest involvement of accumbens ACh and DA in the aversive aspects of opiate withdrawal which contribute to reinstatement of drug abuse and maintenance of the drug habit.



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Interactions of Ibogaine With Morphine in Pasts: Drug Self-Administration and In Vivo Microdialysis

S.D. Glick, I.M. Maisonneuve, J.N. Carlson and R.W. Keller

It has been claimed that ibogaine, a naturally occurring alkaloid, is effective in treating opiate addiction (patent No. 4,499,096). In an attempt to determine if there is a rational basis for this claim, behavioral and neurochemical studies of potential interactions between ibogaine and morphine were conducted in rats. In almost all rats, ibogaine (40 mg/kg, i.p.) decreased intravenous morphine self-administration (0.04 mg/kg/infusion) for at least a day afterwards, at a time when ibogaine should have been entirely eliminated from the body and when there was no obvious indication of ibogaine exposure; in some rats, there was a persistent decrease in morphine intake for several days or weeks after a single injection of ibogaine. Using in vivo microdialysis as well as assays of brain homogenates, ibogaine (40 mg/kg, i.p.) was found to produce both acute and persistent changes in brain dopamine release and metabolism. Acutely (up to 3 hours), ibogaine decreased extracellular dopamine levels in the striatum, increased them in the prefrontal cortex, and had no significant effects in the nucleus accumbens; however, when measured one hour after the same dose of ibogaine, homogenate tissue levels of dopamine were decreased in all three structures. When administered the day before a morphine challenge (5 mg/kg, i.p.), ibogaine prevented the rise in extracellular dopamine levels normally observed in the striatum, nucleus accumbens and medial prefrontal cortex after a morphine injection. A high dose of morphine (30 mg/kg, i.p.), administered alone, produced no increase in extracellular dopamine levels; it was therefore unclear whether ibogaine antagonized or potentiated the effects of the lower dose of morphine. Analysis of ibogaine pretreatment effects on locomotor activity after a range of morphine doses (0.5-30.0 mg/kg, i.p.) indicated that ibogaine either blocks a stimulant effect or enhances a depressant effect of morphine. Further investigation of mechanisms underlying a possible anti-addictive property of ibogaine is warranted.

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Interactions of Ibogaine and D-amphetamine in Rats: Motor Behavior and In Vivo Microdialysis

I.M. Maisonneuve and S.D. Glick

Ibogaine, an indolalkylamine, has been claimed to be effective in treating opiate (Lotsof, patent No. 4,499,096) and stimulant addiction (Lotsof, patent No. 4587,243). Consistent with the first of these claims, Glick et al. (this volume) have reported that, in rats, ibogaine decreases intravenous morphine self-administration and blocks the increase in limbic and striatal dopamine release induced by a low dose (5 mg/kg, i.p.) of morphine. The aim of the present study was to determine if ibogaine would have any effects on the neurochemical and motor changes induced by d-amphetamine that might substantiate the anti-addictive claim with regard to stimulants. Using in vivo microdialysis, we studied the time course of extracellular dopamine levels after d-amphetamine (1.25 mg/kg, i.p.) in rats pretreated with ibogaine (40 mg/kg, i.p.) or saline 19 hr beforehand. Ibogaine pretreatment potentiated the rise in dopamine levels induced by d-amphetamine in the nucleus accumbens and striatum. Using photocell activity cages, we found that a single dose of ibogaine (40 mg/kg, i.p.) injected 19 hr prior to a d-amphetamine challenge (0.625, 1.25, 2.5 or 5 mg/kg, i.p.) enhanced the stimulatory motor effects induced by all doses of d-amphetamine. It has been reported that high doses of d-amphetamine can be aversive, and that a high dose of d-amphetamine can cause the rejection of subsequent low doses. Ibogaine, by amplifying the d-amphetamine response, may make d-amphetamine aversive and thereby reduce its reinforcing efficacy.

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Ibogaine Alters Cocaine-Induced Biogenic Amine and Psychostimulant Dysfunction but not [^{3}H]GBR-12935 Binding to the Dopamine Transporter Protein

P.A. Broderick, F.T. Phelan and S.P. Berger

Ibogaine is a naturally occurring indole alkaloid made from the root bark of the African shrub, *Tubernanthe iboga.* It is claimed that ibogaine can reduce craving for cocaine (U.S. Patent #4,587,243 to H. Lotsof). Because dopaminergic (DA) mesolimbic neuronal circuitry is thought to closely correlate with reinforcement, we studied the effects of ibogaine treatment on (1) cocaine-induced DA dysfunction in nucleus accumbens of freely moving rats, simultaneously with its effect on cocaine-induced psychostimulant behavior, and (2) on [^{3}H]GBR-12935 binding to the dopamine transporter protein. Additionally, the effect of ibogaine on cocaine-induced serotonin (5-HT) dysfunction was studied. Neurotransmitter detection was accomplished by semiderivative *in vivo* voltammetry (Broderick, P.A., *Bruin Res.* 495:115-121, 1989). Ibogaine's interaction with the dopamine transporter protein was evaluated by the [^{3}H]GBR-l2935 binding assay (Berger, P.; Janowsky, A.; Vocci, F. et al., *Eur. J. Pharmacol.* 107 :289-290, 1985) and behavioral effects were measured by Activity Pattern Analysis (Geyer, M.A. In: *Testing and Evaluation of Drugs of Abuse.* (M.W. Adler and A. Cowan, eds), A.R. Liss, NY, 1990,81-99). Ibogaine HCI and cocaine HCI (Sigma, St. Louis, MO) were administered at doses (40 mg/kg ip x 4 days) and (20 mg/kg scx 1 day), respectively. The results show that ibogaine pretreatment significantly reduced the cocaine-induced rise in accumbens DA ($p<0.0001$), partially enhanced the cocaine-induced decrease in accumbens . 5-HT and significantly inhibited cocaine-induced hyperactivity ($p<0.001$). Studies, using the impulse flow inhibitor γ-butyrolactone, showed that the DA and 5-HT alterations induced by cocaine were dependent on presynaptic release mechanisms. Interestingly, in rats not treated with cocaie, ibogaine did not change DA but significantly increased 5-HT release ($p<0.0l$). Moreover, ibotaine, at concentrations as high as 100 μM did not significantly inhibit [^{3}H]GBR-12935 binding to the dopamme transporter protein. Likewise, 1 μM of ibogaine had no effect on *in vitro* striatal DA reuptake by a P_2 membrane fraction. Thus, ibogaine's effects are consistent with current views regarding rational strategies for cocaine treatment. Supp: NIDA R0l-04755 & PSC/CUNY Award 6-61188. [1]Dept. Pharmacol., CUNY Med. Sch., Convent Ave. & W. 138 St., NY 10031 & [2] Sec. Mol. Pharmacol., NIMH, Beth, MD 20892.

Nor-Binaltrophimine Interacts with Dynorphin-Induced Regulation of Morphine Effects on EEG and EEG Power Spectra

N.C. Paquette and G.A. Young

Previous evidence suggested that co-pretreatment with i.c.v. dynorphin A-(1-13) and morphine resulted in a qualitative change to kappa-like EEG power spectra and associated behavior when i.c.v. morphine was given to rats 24 hrs later. Correlated changes in sensitivity to antagonism of these EEG effects by naloxone were also found. A 10-fold increase in naloxone dose was needed to suppress EEG bursts in rats that received dynorphin/ morphine pretreatment. The present study further characterized this phenomenom using the selective kappa antagonist, nor-binaltorphimine (nor-BNI). Rats were implanted with cortical EEG electrodes and i.c.v. and intravenous (i.v.) cannulae. Injections of i.c.v. morphine in rats pretreated with i.c.v. dynorphin/morphine 24 h earlier produced qualitatively EEG power spectra with a predominant peak in the 4-6 Hz band, qualitatively similar to the EEG power spectra seen after acute administration of *kappa* opioids. After 20 min of morphine-induced EEG bursting, nor-BNI (20 nM) was administered which produced an enhancement of the '*kappa*-like' EEG bursts. In contrast, nor-BNI had no apparent effect on morphine-induced EEG power spectra in rats pretreated with either morphine alone or dynorphin alone. These data suggest that nor-BNI and dynorphin regulate morphine-induced effects on EEG power spectra in a similar manner. (Supported by NIDA Grant DA-01050 and NIDA Grant DA-05398-01).

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EEG Changes with Acquisition of Morphine Self-Administration

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We hypothesized that opioid effects on the EEG power spectra would differ between animals that self-administered morphine infusions through lever pressing (contingent subjects) and those that received an identical pattern of infusions independent of their behavior (noncontingent, or yoked subjects). Seven, male Sprague Dawley rats with chronic electrocortical electrodes were exposed to 30 microgram/kg-infusion of morphine sulfate contingent on lever-pressing, and three animals served as yoked controls that received a similar patterns of infusions, independent of their behavior.

A criterion that gave the usual upper range of self-administered infusions was established for each animal by taking the value that included 85 percent of the frequency histogram of infusion number per episode of self-administration. Times for the onset of periods of self-administration were identified by the combination of an increased number of infusions (greater than one half criterion) with relatively few infusions over the preceding six hours (less than criterion). The trend of EEG power with the onset of self-administration was then quantified by ensemble averaging. Off-line routines performed period-amplitude analysis on values for total EEG power averaged over five minute intervals. All days for which the number of self-administered infusions exceeded criterion were included in analysis.

The onset of morphine self-administration was associated with a desynchronization of the EEG in contingent subjects. EEG power was significantly lower than yoked control subjects when morphine was self-administered at intermediate or high levels. Both the ultradian period and amplitude were prolonged in animals self-administering morphine at intermediate or high levels, with similar changes in both contingent and yoked subjects.

In conclusion, the EEG differs in animals that are self-administering morphine compared to subjects that are passively receiving infusions. This may arise because of a relationship between the EEG and the skeletal activity required for lever pressing. Alternately, the tendency of morphine to desynchronize the EEG in contingent subjects may be related to its reinforcing effect on brain activity.

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Cocaine-Induced Seizures are Inhibited by Serotonergic $5HT_2$ and $5HT_3$ Receptor Antagonists

F.R. George and M.C. Ritz

We have shown that seizurgenic effects of cocaine and related compounds are primarily associated with drug binding to serotonin transporters, and that the $5HT_2$ antagonists cinanserin could completely block cocaine-induced seizures, without influencing the frequency of lethal responses. We have now assessed the effectiveness of other $5HT_2$ antagonists as well as $5HT_3$ antagonists in inhibiting cocaine seizurgenesis. The occurrence of and latency to seizures in male C57BL/6J mice were observed following pretreatments with test drugs and following subsequent administration of cocaine. Specifically, animals were injected i.p. with either drug or vehicle and observed for 15 minutes prior to contralateral i.p. injections of various doses of cocaine. Animals were subsequently observed for an additional 15 minutes for the occurrence of seizure activity or death. The results of our study confirm and extend our previous findings. All of the $5HT_2$ antagonists studied, including cinanserin, ketanserin and pirenperone significantly inhibited the occurrence of cocaine-induced seizures. Also, the $5HT_3$ antagonist zacopride, but not MDL72222, inhibited seizurgenesis. None of the compounds tested significantly shifted the dose response curve for cocaine-induced lethality. However, with the exception of cinanserin, all other compounds produced enhanced lethality at high doses of cocaine.

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Antidepressants Appear to Enhance Cocaine-Induced Seizures and Lethality

M.C. Ritz and F.R. George

Several antidepressant drug therapies for human cocaine abuse are currently being studied. However, we have recently reported that the seizurgenic and lethal effects of cocaine and related compounds are primarily associated with drug binding to serotonin and dopamine transporters, respectively. Since many clinically effective antidepressants have high affinities for monoamine transporters, we determined whether these compounds may enhance the toxic effects of cocaine. The occurrence of latency to seizures in male C57BL/6J mice were observed following pretreatments with antidepressant drugs and following subsequent administration of cocaine. Specifically, animals were injected i.p. with either drug or vehicle and observed for 15 'minutes prior to contralateral i.p. injections of various doses of cocaine. Animals were subsequently observed for an additional 15 minutes for the occurrence of seizure activity or death. In general, antidepressant drugs were not inherently seizurgenic, although at high doses, tremors were observed. Further, pretreatments with antidepressants generally increased the incidence and severity of cocaine-induced seizures, such that seizures were more frequently followed by lethality. Sertraline was found to be an exception in that it did not significantly increase seizures or lethality. Further, bupropion, at antidepressant doses, did not enhance cocaine-induced toxicity, although at higher doses it was highly lethal in combination with cocaine. These results have important implications for the clinical use of antidepressants in patients who have histories of cocaine use.

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NMDA Modulators Protect Against Cocaine Convulsions Resistant to Other Anticonvulsants

F.C. Tortella, R.J. Marley and J.M. Witkin

Convulsions associated with cocaine abuse can be life-threatening and resistant to standard anticonvulsant drugs. Diazepam and phenobarbital were devoid of anticonvulsant activity against 75 mg/kg cocaine (CD_{100}) in male Swiss Webster mice. Nonopioid antitussive anticonvulsansts (dextromethorphan, caramiphen, and carbetapentane) and serotonin-2 receptor antagonists (cinanserin and ICI 169,369) were also inactive. In contrast, the non-competitive NMDA antagonists (+)-MK801, (-)-MK801 and PCP provided dose-dependent protective effects against these diazepam-insensitive convulsions. The competitive NMDA-antagonists, CPP and NPC 12626, were also anticonvulsant but without producing the behavioral disturbances associated with non-competitive' antagonists. Compounds acting at putative modulator sites on the NMDA receptor complex, including the strychine-insensitive glycine site and the polyamine site of the NMDA receptor ionophore, were also protective. Namely, the glycine antagonists 7-chlorokynurenic acid and ACPC, as well as the polyamine antagonist ifenprodil, all produced dose-related protection against diazepam-insensitive cocaine seizures.

In studies using diazepam-sensitive cocaine (60 mg/kg) convulsions a heterogenous response profile has emerged for various putative sigma ligands. For example, pretreatment with doses of (+)-3-PPP (1-30 mg/kg) which alone caused no overt behavioral effects produced 100 % protection against the cocaine convulsion. In contrast, sublethal doses of DTG (<30 mg/kg) and subconvulsive doses of BMY14802 (<100 mg/kg) were only moderately protective (40-60%). For haloperidol only behaviorally effective doses associated with neuroleptic activity provided partial (50 %) seizure protection.

When used as an adjunct against the diazepam-insensitive cocaine convulsion, pretreatment with diazepam improved the anticonvulsant potency of various NMDA/sigma drugs. For example, doses of ACPC, NPC12626, (+)-PPP and caramiphen, which alone were ineffective against cocaine convulsions, possessed significant anticonvulsant activity when administered with 1 mg/kg diazepam.

Collectively, these results suggest a potential role for the NMDA receptor complex in the convulsant actions of cocaine, providing a novel molecular target for drug discovery in treating cocaine toxicity.

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Cocaine Has No Direct Vasoactive Effects

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Cocaine has been related to ventricular arrhythmias, myocardial infarction and sudden death. Coronary artery spasm is believed to be the underlying mechanism. The purpose of this study was to determine if cocaine, alone, has a direct vasoconstrictor effect on arteries. Our hypothesis was that the cardiovascular effects of cocaine are mediated by norepinephrine and that cocaine alone would not cause significant vasoconstriction of arteries. We examined the effects of cocaine on rabbit thoracic aorta using a standard isolated vascular ring preparation. Eighteen thoracic aortic rings obtained from 5 New Zealand White rabbits were studied. One half of the rings were denuded. Endothelial condition was verified by methacholine challenge. Methacholine is an endothelial dependent vasodilator, thus, if the arterial endothelium is intact, dilation of smooth muscle will be seen in response to methacholine. Dose response curves to norepinephrine and cocaine were conducted in random order for each ring. Norepinephrine doses ranged from .01 μM to 10 μM, while cocaine concentrations ranged from 1.3 to 13.5 ug/ml. In both denuded rings and non-denuded rings, norepinephrine caused a dose dependent increase in arterial tension. This is the anticipated result, since norepinephrine is known to be a powerful constrictor agonist with direct effects on the vascular smooth muscle cell. In our experiments, cocaine failed to elicit any change in arterial tension, either for the non-denuded or denuded rings. Our data is contrary to that of other laboratories demonstrating vasoconstriction to cocaine in isolated rat aorta and human umbilical vein. Differences between our study and that of others may be explained by the amount of arterial tension generated. These studies show less than 1 g of arterial tension in response to 1 μg/ml cocaine. Typically, arteries show up to 10 g of tension in response to norepinephrine, thus one must question the significance of studies reporting such small changes in arterial tension. Discrepancies between our work and that of others may also be due to differences in the concentrations of cocaine employed. Cocaine fatalities have occurred with cocaine concentrations ranging from .8 to 8.2 μg/ml. We examined cocaine at clinically relevant concentrations, ranging from 1.3 to 13.5 μg/ml. The failure of our study to show direct vasoconstriction to cocaine may be explained in several ways: 1) Illicit forms of cocaine are often contaminated with undefined chemicals which may mediate the vasoconstrictive response to cocaine observed clinically. Our study used pure cocaine hydrochloride. 2) Wilkerson et al., have suggested that the cardiovascular effects of cocaine include an important nervous system component which would not be present in our model. Conclusions: 1) Cocaine has no direct vasoconstrictor effect on rabbit thoracic aorta in vitro. 2) Our results suggest that vasoconstriction to cocaine, in vivo, is mediated by another substance such as norepinephrine.

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Myocardial Damage Induced by Chronic Cacaine Administration in the Rat

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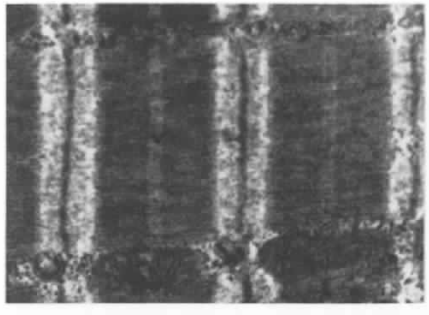
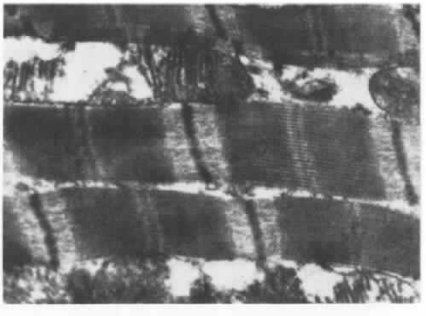
Rats fitted with I.P. osmotic pumps were administered for 7 to 21 days isotonic saline or the same volume of a cocaine solution at the rate of 40 mg/kg/day, dose which was well tolerated. After chronic administration, resting blood pressure and increments of blood pressure caused by challenging doses of cocaine (10, 20, 30 mg/kg) administered via an intracaudal arterial catheter are significantly lower than in saline treated animals. These symptoms are indicative of cardiovascular tolerance. ECG is comparable in all groups. In another series, 4 groups of animals were administered 40 mg/kg of cocaine or saline. They were sacrificed at the end of 7 or 21 days, without any symptom of ill effects. They were bled and their heart removed for light and electron microscopy. Controls present normal cardiac histology. Cocaine administered display breaks of myocytes, oedema, blood stasis and intracytoplasmic vacuolisation. Electron microscopy shows consistent, disseminated lesions of cardiomyocytes with disorganization of myofibrils which present enlargement of their interspace by hydropic infiltration and Z stria out of alignment. Mitochondria are swollen with destruction of the crests and a cloudy appearance. The number and extent of these lesions are dose related and their reversibility is open to question. Chronic cocaine administration procduces fine myocardial lesions without gross cardiac pathology or dysfunction, and may be primarily caused by coronary constriction, hypoxia, catechols and Ca2+ accumulation.



Electron micrograph of normal myofibrils and mitochondria. (x10000)



Electron micrograph of myofibrils and mitochondria damaged by cocaine. (xl0000)

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Ethanol Vasoconstriction is Not Prevented by Diltiazem

A.A. Bove, X.-Y. Zhang and D. Morley

The mechanism by which ethanol constricts large arteries is not known. Ethanol may alter cellular calcium activity to increase the availability of free cytoplasmic calcium for activation of the smooth muscle contractile apparatus. The purpose of this study was to determine whether ethanol's vasoconstrictor effect is to enhance intracellular calcium availability by influencing calcium entry via voltage operated calcium channels. Our hypothesis was that if ethanol enhances calcium entry through these channels, vasoconstriction to ethanol would be inhibited by pre-treatment of arteries with a calcium channel antagonist. We studied the effect of the calcium channel antagonist, diltiazem, on ethanol vasoconstriction in a standard isolated vascular ring model. Twenty thoracic aortic rings from 6 New Zealand White rabbits were studied. One-half of the rings were denuded by gentle rolling over forceps. Endothelial condition was verified by challenge with methacholine. For each ring, dose response curves to norepinephrine (.0l-10 μM) and ethanol (500-2500 μg/ml) were conducted. After adding diltiazem (10 μM) to the bath, the dose response to ethanol was repeated. Both ethanol and norepinephrine caused a dose dependent constriction of the arterial rings. The addition of diltiazem to the bath did not alter the dose response to ethanol in endothelial denuded rings. For endothelial intact rings, the addition of diltiazem shifted the dose response curve to ethanol significantly to the right ($p<.05$), but ethanol still resulted in vasoconstriction equivalent to 35% of the maximum contraction to norepinephrine. The predominant effect of diltiazem is on slow calcium channels to shorten duration of the action potential. We know from previous work that small amounts of ethanol, as used in this study, may cause constriction of coronary arteries. Thus, diltiazem may not prevent coronary vasospasm in those patients susceptible to constriction initiated by moderate consumption of ethanol.
Conclusions: 1. In arteries with significant endothelial damage, diltiazem does not inhibit vasoconstriction to ethanol. 2. In arteries where the endothelium is primarily intact, diltiazem attenuates, but does not abolish, vasoconstriction with ethanol. 3. Ethanol may enhance calcium influx via voltage operated calcium channels. 4. Enhancement of extracellular to intracellular flux of calcium ions is not the primary mechanism of ethanol induced vasoconstriction.

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Low Doses of Ethanol Abolish Nitroprusside Vasodilation

D. Morley, X.-Y. Zhang, A.A. Bove

The mechanism by which ethanol causes direct vasoconstiction of arteries is unknown. Ethanol may facilitate artery constriction by altering cyclic nucleotide regulation. The purpose of this study was to examine the relationship between ethanol and cyclic GMP in vascular smooth muscle regulation by determining the effect of ethanol on vasodilation due to nitrate induced increases in cyclic GMP. We hypothesized that if ethanol caused arterial vasoconstriction by negatively influencing the cyclic GMP pathway for vasodilation, ethanol should diminish relaxation to organic nitrates. To examine the effects of ethanol and nitroprusside on vasomotor tone, we employed a standard isolated vascular ring preparation. Twenty thoracic aortic rings from 6 New Zealand White rabbits were studied. One half of the rings were denuded of endothelium by gentle rolling over forceps. Endothelial condition was verified by challenge with methacholine. For each ring, dose response curves to norepinephrine (10^{-8} to 10^{-5}M), ethanol (500-2500 µg/ml) and nitroprusside (10^{-9} to 10^{-6} M) were done in random order. Ethanol concentrations were equivalent to one to five drinks in the averaged sized man. After adding the approximate ED_{50} dose of ethanol, 1500 µg/m1, to the bath, the dose response to nitroprusside was repeated. Both denuded and non-denuded arteries showed a dose dependent constriction to ethanol. Nitroprusside alone caused marked vasodilation of arteries regardless of endothelial condition. The approximate ED_{50} dose of ethanol abolished the vasodilator response to nitroprusside in both denuded and non-denuded rings. Sodium nitroprusside exerts its vasodilating action by increasing smooth muscle cyclic GMP. Our data demonstrate that ethanol has a negative effect on this process, favoring vasoconstriction.

Conclusions: 1. Moderate concentrations of ethanol abolish vasodilation to nitroprusside. 2. Ethanol in some way alters intracellular processes stimulated by nitroprusside which lead to increases in cyclic GMP and vasodilation. 3. Our results support the hypothesis that one mechanism of ethanol induced vasoconstriction is alteration of smooth muscle guanine cyclic nucleotide regulation.

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Effects of the Mu-Selective Agonist, PL-017, on Brain Temperature, Oxygen Consumption and Heat Flux in the Rat

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Opioid receptors serve several physiological functions in the body, including an active role in temperature regulation. In this study, we focused on the effect of the p-selective agonist, PL-017, and a μ-selective antagonist, CTAP, on brain surface temperature (T_b), oxygen consumption (Vo_2), and heat exchange (Q). Tyr-Pro-N-MePhe-D-Pro-NH2 (PL-017) was injected into the right lateral ventricle of unrestrained, male S-D rats. At an ambient temperature of 20±0.5°C, Tb, Vo_2 and Q were measured three hours post-injection in a gradient-layer calorimenter. Results are reported as change from saline controls. Significance was calculated using Students t-test with pooled variance. Doses were chosen on the basis of prior experiments in which a full dose-response curve was obtained using rectal Tb. A dose of 0.2 μg produced no significant changes in the parameters when compared to saline controls. However, 1.0 μg PL-017 (onset 15 min post-injection) caused an increase in V02 (+1.25 mlO_2/g/hr), a sustained increase in Tb (+1.35°C, 45 min post-injection) and a decrease in Q (-0.39 cal/g/hr) followed by an increase in Q (+1.45 cal/g/hr) over control levels. The changes were blocked by 30-min pretreatment with 1 μg CTAP (cyclic D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2). A higher dose, 6.25 μg, of the agonist caused a small hypothermia (-0.28°C) followed by a hyperthermia of 0.52°C. Vo_2 decreased during hypothermia, then rose, reaching a plateau at control levels until 100 min post-injection, when a further increase occurred, preceding the rise in T_b. Q was essentially unchanged from control levels, with a small increase in heat gain seen during hyperthermia. At this dose, the effect of CTAP on Tb and Vo_2 may be too small to see. The ratio of Q to Vo_2, representing the relationship between the modulatory arms of thermoregulation, decreased sharply during periods of hyperthermia. The hypothermia induced by the higher dose of PL-017 was characterized by an increase in Q/Vo_2. Changes seen in Q/Vo_2 (1 μg PL-017) were absent following pre-treatment with CTAP. We may conclude that PL-017, acting primarily through μ-opioid receptors, alters both Vo_2 and Q, resulting in changes in Tb. The data lend further support to the concept that μ agonists cause hyperthermia by altering set point.

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Role of Opioids in Calcium and Calcitonin Gene-Related Peptide (CGRP)-Induced Hypothermia in Mice

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Besides producing antinociception. the opiates and opioid peptides also alter thermoregulatory processes. Mu receptors predominantly participate in hyperthermic responses, while kappa receptors appear to mediate only hypothermia. Using opiate receptor antagonists and antisera to opioid peptides, we have demonstrated that calcium (Ca^{++}) administered intrathecally (i.t.) blocks thermal nociception by releasing met-enkephalin. Studies where Ca^{++} i.t. blocks p-phenylquinone-induced writhing indicate that Ca^{++} also releases dynorphin. In this study, Ca^{++} at 150,300 and 600 nmol (22 °C ambient) decreased rectal temperatures at 15 min. by -1.7, -2.7 and -3.4 °C, respectively. Hypothermic respoding was eliminated in mice spinalized at t6-t8. The time-course of 600 nmol Ca^{++} i.t. employing ambient temperatures of 4, 22 and 30 °C demonstrated poikilothermia at 4 °C (-14.5 °C at 30 min.), and hypothermia at 22 °C (-5.0 °C at 45 min.) and 30 °C (-2.1 °C at 30 min.). Thus, Ca^{++} appears to alter the hypothalamic set-point in mice. Laser doppler flowmetry demonstrated that Ca^{++} (600 nmol, i.t. at 22 °C) produced significant increases in mouse tail blood-flow at 5-10 min. following injection which disappeared at 15 min.(probably due to activation of homeostatic mechanisms). It was possible that the opioid peptides released by Ca^{++} might have produced hypothermic responding. However, under 22 °C ambient conditions the opioid antagonists naloxone (i.t.: 0.l-1.0 µg; s.c.: 1.0 mg/kg), naltrindole (5-20 µg, i.t.) and nor-BNI (20-40 µg, i.t.) all failed to block the hypothermia produced by 600 nmol it. Ca^{++}.

CGRP (53-1050 pmol, i.t. at 22 °C) produced hypothermia, with the 1050 pmol dose significantly reducing temperatures by -1.8, -2.4, -1.3, -1.3 and -1.4°C at 1, 3, 6, 12 and 15 hrs., respectively. There was a decrease in the number significant time-points as the dose was decreased. CGRP (2-1050 pmol, i.t.) at 3 hrs. produced dose-dependent hypothermia from 2-25 pmol, but increasing the dose from 53-1051 pmol failed to elicit further reductions, indicating a plateau effect of CGRP. Inactive doses of CGRP (5.3 pmol, i.t.) and Ca^{++} (75 nmol, i.t.) were combined to determine whether CGRP produced hypothermia by stimulating the neuronal uptake of Ca^{++}. At a 1 and 3 hr CGRP pretreatment, the combination appeared to synergistically reduce temperatures by -2.3 and -2.6 °C respectively, suggesting that CGRP produced hypothermia through a calcium-related mechanism. No tolerance developed to the hypothermic effects of CGRP (525 pmol, i.t. every 12 hours for 3 days). There were no significant differences between the hypothermic effects of CGRP in morphine-tolerant and non-tolerant mice. Supported by grants F32-DA05415, DA-06031, DA0727 and the Commonwealth of VA Center on Drug Abuse Research).

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The Impact of HIV Testing on Risk for AIDS Behaviors

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INTRODUCTION

The reduction of needle sharing and the increased used of condoms among intravenous drug users (IVDUs) has become a national public health objective and a common goal of substance abuse treatment. As a consequence, many programs of education and treatment outreach have been instituted in an effort to reduce the risk of infection and transmission among IVDUs. This report examines the potential impact on high risk behaviors of HIV testing, a relatively brief and inexpensive activity. The Risk Assessment Project is monitoring the serologic status and high risk behaviors of 255 IVDUs in and out of methadone treatment in Philadelphia. Assessments take place at six month intervals and the project has achieved an 86% retention rate during the first year of the study.

Although the assessment of behaviors associated with the transmission of HIV has become quite common among IVDUs, validation of these assessment techniques has been limited, complicating the interpretation of findings. Any study attempting to understand the changes in high risk behaviors must consider the validity of its assessment techniques.

METHODS

In order to examine the impact of serologic testing on the high risk practices of participants, subjects with complete data were first grouped according to their serologic status over the course of the first year of study. One hundred ninety-five subjects tested negative for HIV at each assessment point during the first year. Thirty subjects with complete data tested positive for antibodies to HIV at our initial assessment. Those individuals who seroconverted to HIV positive at the 6 month or the 12 month assessment points were not included in these analyses.

The Risk Assessment Project has developed and completed preliminary evaluation of a self administered questionnaire known as the Risk for AIDS Behavior (RAB). The RAB consists of 38 closed ended questions that can be answered in approximately 15 minutes by most methadone patients. The RAB offers a more "private" approach to the assessment of high risk behaviors and eliminates the potential biasing effects introduced by the interviewer. In order to test the validity of the RAB, results were compared to the AIDS Initial Assessment, a personal interview developed by NIDA and used extensively in the United States.

RESULTS

In both seropositive and seronegative groups, significant reductions in needle sharing took place between the initial assessment and the six month follow-up. Forty-rive percent of the seronegative group reported sharing needles at baseline and this dropped to 28% six months later. For seropositive subjects, 60% reported sharing needles at baseline and this rate dropped to 24% at the six month follow-up. In both groups, continued though less dramatic changes in needle sharing were observed at the 12 month follow-up point. When these analyses were conducted controlling for treatment status, a more rapid reduction in needle sharing rates was observed among the In-Treatment sample than among the Out-of-Treatment sample although rate for both groups were equivalent by the close of the first year of the study.

With regard to condom use, significant differences were observed between the seropositive and the seronegative subjects by the close of the first year. At baseline, only a minority of both groups were engaging in safe sexual practices. At the 6 month follow-up, 55% of the seropositives were practicing safe sex and this rate increased to 65% by the close of the first year.

The analyses directed at examining the validity of the RAB produced strong correlations on reports of needle sharing and other high risk behaviors. Interview and questionnaire results were also used to generate odds ratios for seroconversion. The results indicate that for those reporting needle sharing on the RAB the odds of becoming HIV positive was 13:1 while for those reporting needle sharing during the interview, the odds of conversion were 8:1. There were no false negatives (HIV positive and reporting no sharing) for the RAB responses while a 2% false negative rate was found for the interview.

CONCLUSIONS

The data reported here suggest that the systematic testing of IVDUs contributes to the reduction of high risk behaviors. We believe that an ongoing testing program provides the subject with an external marker against which they can compare their behavior. The consideration of possible infection can be a motivating experience and when it is repeated every six months its potential for affecting behavior change increases. In our study, both HIV positive and HIV negative subjects showed significant decreases in their rates of needle sharing. While this finding is consistent with the hypothesis that testing reduces risk behaviors, it provides only weak evidence. Stronger evidence is seen in the finding that condom use increased significantly among those who tested HIV positive while changing little among those testing HIV negative.

Our findings also suggest that high risk behaviors can be monitored effectively by the RAB, a brief self administered questionnaire. The results of our initial validation work suggest that the RAB results are not only highly correlated with the results of the ALA interview, but were somewhat better predictors of HIV infection. It is possible that the questionnaire approach is not only a more efficient way to collect this sensitive information but a more acceptable one to subjects.

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Risk Reduction Through Indigenous Outreach to Intravenous Drug Users

W.W. Wiebel, R. Booth, J.T. Brewster and S. Koester

This paper describes the results of interventions in Denver, Baltimore and El Paso designed to modify the risk behaviors of injection drug users. Indigenous outreach workers were recruited in each site to serve as prevention advocates and education specialists. More than 500 pre-post interviews were conducted over a two year period.

In the analysis of overall risk, utilizing a dichotomous risk index, Denver subjects demonstrated the greatest improvement followed by Baltimore, while no significant change occurred in the El Paso cohort. Consistently, in a logistical regression examining factors related to the movement from high to low risk, the only significance associated with change was the residency of the subject. Among high risk subjects at time one, the odds of being high risk at follow-up were 4.50 greater in El Paso than in Baltimore and 2.04 greater in El Paso than in Denver.

In El Paso, the agency responsible for project activities believed in providing prevention materials and education to any IDU appearing at the door. That intervention through outreach could be effective was thus found not only in the significant risk reduction observed among subjects in Baltimore and Denver, but in the relative lack of success in El Paso which, for the most part, lacked the reinforcing presence of indigenous outreach staff. The results of this study suggest that outreach to injection drug users through indigenous prevention advocates working in social networks can be effective and enduring.

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AIDS Risk Patterns Among Los Angeles Drug Users

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This paper reviews recent trends in AIDS risk behavior across successive cohorts of injection drug users in Los Angeles, California. Analyses describe two drug-related and two sex-related sources of risk: needle sharing, use of bleach to disinfect needles, sex with more than one partner, and condom use.

Procedure. The NIJ-funded Drug Use Forecasting project conducts quarterly face-to-face interviews with random samples of incoming felony arrestees at Los Angeles city and county jails. Analyses in this paper are based on 1987-90 subsamples who reported injection drug use (n=1,056).

Analyses. Trends were tested through Bartholomew's Chi square statistic (Fleiss, 1981).

Results. A modest but significant reduction occurred in needle sharing (Figure 1). Bleach use increased marginally (Figure 2). A modest but significant increase occurred in condom use (Figure 3). Sex with multiple partners increased significantly (Figure 3).

Conclusion. AIDS risk reduction has been modest among Los Angeles injection drug users. Preventive education efforts must include an aggressive campaign promoting bleach use and safer sex.

Fleiss, J.L. Statistical Methods for Rates and Proportions. New York: John Wiley & Sons, 1981.

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Shooting Galleries in Alaska: Two Years Experience with AIDS Prevention Outreach Workers

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The purpose of this analysis was to investigate drug injection practices in Anchorage, Alaska. 2556 contacts by ex-IV drug using outreach workers were made during calendar years 1989 and 1990. Half of these contacts were IV drug users (IVDUs) and half were the sex partners of the IVDUs. Women were significantly more likely to be sex partners of IVDUs. Women, whether IVDUs or sex partners, were significantly younger than the men. Most of the individuals contacted were White, followed by Black, Alaska Native, Hispanic, and Asian. A third of the contacts were made at "depots" which are places where the workers can leave information about HIV infection, condoms, bleach and other supplies. There are 47 depots documented. These depots are classified on the basis of whether drugs (heroin or cocaine) are sold there, and whether people inject drugs there. Alaskan outreach workers consider most of the depots to be "dope houses" rather than "shooting galleries" because drugs are sold at almost all the locations where people go to inject. Only two of 47 depots are places where injection without sales occur. There may be several different definitions of the term "shooting gallery" and there appear to be alternative social injection behaviors occurring in Anchorage.

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Low Rates of HIV Infection Among Substance Abusers Reporting High Risk Behavior: Trend or Time Bomb?

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Public health surveillance rates of HIV infection among intravenous drug abusers in the St. Louis Metropolitan area are currently low (3%), far below the 50-60% rate of infection among IVDUs in New York City. Explanations for low HIV rates include: reporting differences, low rates of risk factors, successful prevention efforts, different social structure, and a time bomb/time lag theory. Population surveillance rates of Hepatitis B show that St. Louis rates are similar to those of San Francisco and New York City. As part of a NIDA-funded longitudinal study, we have interviewed 514 substance abusers recently admitted to 6 representative treatment centers and 91 of their sexual partners. Data on the 605 subjects include detailed psychiatric history using the DIS-III-R interview, history of high risk behavior as obtained by the modified NIDA high risk assessment and HIV test results. One half of the sample has reported lifetime IV drug use; 60% have injected drugs in the last 6 months. The findings from the first wave of the study, completed in December 1990, indicate that 60% of both male and female IVDUs have shared needles in the last 6 months, and the drug of choice is heroin (62%) followed by cocaine (25%). Use of heroin and amphetamines by smoking or snorting was rare. Among non-IVDUs, 9% reported having an IVDU sexual partner compared to 38% among IVDUs. Cleaning syringes was reported by 45% of the sample; travel was as frequent among IVDUs as non-IVDUs with most travel occurring in the Midwest. Condom use was reportedly rare in both groups. IVDUs and non-IVDUs were comparable in terms of number of sexual partners, but not other sexual behaviors such as type of sexual behavior. These indicators lead the discussion on where this midwestern city is headed.

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HIV Sero-Conversion and Treatment Status

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INTRODUCTION: To better understand the patient factors associated with high risk behaviors and the role of methadone treatment in restricting the spread of HIV infection, we are conducting a prospective study of seroconversion and high risk behaviors among IVDUs both In-Treatment (IT) and Out-of-Treatment (OT). This study is taking place at the methadone treatment program of the Girard Medical Center, the largest methadone program in Pennsylvania, with an average census of 440 patients. It is located in the North Central part of Philadelphia, a neighborhood with areas of severe poverty, inadequate housing, and high rates of drug use. This paper presents results from the first year of study. From a group of 379 volunteers, 152 patients were randomly selected to participate in a longitudinal study of HIV infection and high risk behaviors. One hundred three OT subjects were identified and recruited through referrals from treatment subjects and community outreach work in the neighborhood surrounding the Girard Medical Center. Since we wanted to assess a sample comparable to that recruited from the treatment program, selection for participation was made after the completion of a brief, semi-structured screening interview conducted by the research staff. To be eligible for study parcipitation, referrals had to have a history that included intravenous opiate dependence and could not have been in any drug abuse treatment during the preceding ten months. Thus, subjects who had only injected cocaine or other non-opiates were excluded as were those who had received any type of substance abuse treatment during the preceding ten month period.

HIV INFECTION RATES: Baseline HIV infection rates reflected the higher rate of involvement in risk behaviors reported by the OT sample. The treatment group had a 10% (15/152) seropositivity rate while the OT sample had a 16% (16/103) baseline infection rate. At the 12 month assessment point, 120 of the 134 IT subjects who had previously tested seronegative were retested. Two additional subjects were confirmed positive increasing the infection rate to 14% (20/138). Among the OT sample 66 previously negative subjects were retested, Setostatus was therefore known for 86 (83%) OT subjects at the 12 month point. Eight previously negative subjects were found to be HIV positive. Thus, the OT sample had an infection rate of 33% (28/86) at the end of the first year of the study. Among the 193 subjects who were tested at each assessment point, infection rates for the IT subjects were 11% at baseline, 13% at the 6 month follow-up and 15% at the one year assessment. Among the OT subjects, 19% tested positive at baseline, 24% were positive at the 6 month assessment point, and 33% were positive at the one year assessment.

DISCUSSION: The HIV infection rates reported here reflect rapid HIV seroconversion, particularly among the out-of-treatment sample. To our knowledge the increase in the infection rate among the out of treatment sample is the highest reported in the United States. The data highlight the importance of monitoring incidence of infection as opposed to simply assessing its prevalence periodically. The data presented here suggests that despite prevention effort., HIV in Philadelphia is spreading at a much higher rate than previously thought. The data also suggest that methadone treatment can effectively restrict the spread of HIV.

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HIV Seropositivity and Effectiveness of Methadone Maintenance Treatment

R.M. Swift, C. Love, R. Marlink and B. Foss

Methadone maintenance (MM) is effective in reducing IV drug use and may decrease the risk of Human Immunodeficiency Virus (HIV) transmission. To assess effects of HIV seropositivity on MM treatment efficacy, 114 clients in a MM clinic (25% of total) underwent voluntary HIV testing, with counseling and AIDS education. The 25 HIV seropositive (SP) subjects found were compared with 25 randomly chosen seronegative (SN) and 25 randomly chosen not-tested (NT) clients and followed prospectively.

SP subjects were less compliant with treatment than the other groups after becoming aware of their HIV status. 72 % of the HIV SP clients used IV drugs within 3 months of becoming aware of their SP status. In contrast, only 40% of the SN group and 44% of the NT group showed IV drug use.

To assess treatment compliance prior to HIV testing, we examined urine toxicological screens of subjects during this earlier period. Of those subjects in treatment 60 days prior to testing, 20 % of SP subjects had positive urine drug screens, as compared to 4 % of SN subjects and 6 % of NT subjects ($p< 0.02$). The mean length of MM treatment for SP subjects (111 days), was shorter than that for SN subjects (529 days) [$F=4.4$, $p < 0.041$.

These findings suggest several possible conclusions. First, the data suggest that MM treatment had a positive effect in decreasing high risk behaviors for HIV infection. Increased time on MM is related to a decreased frequency of HIV seropositivity. Second, subjects entering MM in the past year are more likely to be infected with HIV due to the increased prevalence of the virus. Third, HIV SPs are more likely to continue illicit drug use (including IVDU), while on MM and are therefore more likely to be infected with HIV. Fourth, the differences in groups may be due to selection effects, i.e. subjects remaining on MM for longer periods do so because of their better compliance (others have dropped out or were detoxed) . Finally, knowledge of HIV seropositivity may lead to increases in illicit drug use.

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HIV Risk Status of New Methadone Admissions

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Referral patterns and HIV risk status of 250 consecutive new admissions to methadone treatment were assessed via the AIA interview. All subjects had not been in treatment for a minumum of 30 days prior to admission. Sample demographics were: 61% male; 50% white: 42 % black: 32.8% employed: 52.8% less than high school: age (M = 39.5, $s.d.$ = 7.8). Other clients, friends or relatives referred 59%, counselors, 14%, outreach workers, 4%, needle exchange, 3%, streets or other contacts, 19%. Pharmacies always or usually served as a source of sterile injection equipment for 56%, and the needle exchange for 38%. New needles were used half the time or more by 56%. Sharing of equipment in the prior 6 months was reported by 174 (69.6%) subjects. Among sharers, 39.6% shared with only one other person, 60.3% with more than one person in the prior 6 months. Bleach was used half the time or more to clean needles by 63.2% of sharers. Sixty (24%) subjects had some contact with CHOWs. Those who had CHOW contact were more likely to have used new needles (X^2 = 9.37, p < .01) and more likely to have cleaned needles with bleach (X^2 = 5.96, p < .10) in the past 6 months than were subjects without CHOW contact. These results were confirmed by comparing the needle use behaviors of the current sample to a sample from the same methadone clinic prior to the existence of CHOWs in the community. However, CHOW contact had no impact on needle sharing, whether or not subjects cleaned their needles, and on their use of condoms. These results suggest CHOWs were able to effect changes in some aspects of needle use behavior. However, causal inferences from these data should be interpreted cautiously.

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Sexual Risk Behaviors Among Human Immunodeficiency Virus (HIV) Positive Patients at Methadone Clinics in New York City (NYC)

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While the rate of AIDS cases in the U.S. among homosexual male is leveling off, the proportion of injecting drug users (IDUs) continue to increase. This study investigated high risk behaviors among HIV positive IDUs, focusing on their sexual practices and behaviors in relationship to drug use. The eligibility criteria were patients with documented HIV seropositive infection as well as evidence of injecting drug use. After an informed consent form was signed, a structured interview was administered by a trained research counselor, blood serum were collected and tested to confirm seropositivity. From the end of 1988 to the beginning of 1991, 54% African American, 4% white, and 42% Hispanic comprised a total of 247 participants. Male participants represented 64.4% of the total sample. Sex with a non-IDU were reported by 35% of the respondents; about one-quarter of the respondents had sex with a person from outside of the United States. Casual sex was reported by 45.7% of the respondents. Males reported having twice as much casual sex, compared to females ($p=.001$). Sixty percent of respondents who reported poly-drug use were having casual sex compared with 39% of the respondents who use only one drug. The most popular drugs used in exchange for sex were found to be cocaine and crack. Crack use was highly associated with alcohol consumption ($p=.003$). This data suggests that more effective interventions and education on risk reduction behaviors among IDUs is desperately needed.

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Characterization of Antinociceptive Tolerance and Development of Physical Dependent to Morphine Using Continuous Intracerebroventricular Infusion in Mice

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A procedure for the induction of physical dependence in mice using continuous intracerebroventricular (*i.c.v.*) infusions has been developed. Male, ICR mice (20-30 g) were implanted with permanent, indwelling stainless steel guide cannulae into the right lateral ventricle. After a 3 day recovery, animals received continuous *i.c.v.* infusion of morphine sulfate (MS; 10 µg/hr) for 1, 3 or 7 d via s.c.-implanted osmotic minipumps (Alzet 2001). Control mice were treated in an identical manner though were not implanted with a minipump. Following infusion, withdrawal was precipitated with naloxone hydrochloride (NX; 3 mg/kg, *s.c.*) and the animals were observed for wet shakes, diarrhea, urination and jumping for a 20 min period, as well as weight loss at +1, 2, and 3 hrs. After MS infusion for 1 d, NX challenge produced significant urination, and a marked weight loss at 1 hr compared to sham-infused animals. In contrast, NX precipitated withdrawal in the 3 d infusion group resulted in significant jumping, with no significant weight loss. After a 7 d infusion, the incidence of withdrawal symptoms were not different from those observed in control animals. Tolerance to hot plate (55°C) antinociception was also quantitated in these latter group of animals. Significant antinociception was observed in morphine-treated animals at 1, 2 and 4 hr after the start of infusion, though tolerance developed rapidly to this treatment; by 8 hr post-infusion, the percent antinociception was not different from that in sham-implanted animals. The results demonstrate that antinociceptive tolerance and physical dependence to MS can be produced in the mouse using continuous *i.c.v.* infusions.

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Alteration in In Vivo Opioid Receptor Binding Following Arcuate Nucleus Stimulation in the Rat

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Opioid peptides have been implicated in a large number of physiological and behavioral functions. However, only limited correlation of anatomic localization of opioid action with available behavioral/physiologic data has been established. *In vivo* ^{3}H-diprenorphine (^{3}H-Dpr) receptor autoradiography has been proposed as a useful method for measuring opioid receptor occupancy in freely moving animals, allowing for precise anatomic analysis of the loci of opioid action in the brain during given behaviors or physiologic responses. This method is based on the assumption that stimulus induced, released endogenous opioids will exclude the exogenous ligand from the receptor, resulting in decreases in binding which can then be visualized autoradiographically. This approach has been used to measure opioid release following several behavioral states such as stress, feeding and social interactions (Blake et al., 1987; Panksepp et al., 1981; Seeger et al., 1984; Stein et al., 1990). Questions remain, however, regarding the nature of the events being measured. We have therefore employed this method to visualize the release of opioid peptides following electrical stimulation of the arcuate nucleus in anesthetized rats. Stimulation parameter efficacy was assessed by changes in nociceptive tail-flick latency. Consequently, groups of rats were stimulated at 100 or 500 uA either in the presence or absence of the enkephalinase inhibitor acetorphan. Optical density measurements on 38 brain regions revealed significant drug independent, stimulation induced ipsilateral increases in binding in the lateral and dorsomedial nucleus of the hypothalamus, medial and lateral preoptic areas, bed nucleus of the stria terminalis, corticomedial amygdala and the caudal paraventricular thalamus. Cingulate exhibited bilateral, drug dependent increases in binding. Contralateral decreases were seen in the paraventricular hypothalamic nucleus, basolateral amygdala, suprachiasmatic nucleus, caudal central gray matter, and the substantia nigra pars compacta. Bilateral decreases were seen in the olfactory tubercle and the supraoptic nucleus; the latter exhibiting enhanced depression of binding in the presence of acetorphan. Acetorphan was effective in non-stimulated animals only in the rostra1 paraventricular thalamic nucleus where binding increased. This observation implies a relatively low tonic level of opioid activity in the CNS. Acetorphan alone or combined with stimulation, only affected 8 of 38 regions, suggesting ß-endorphin as the principal released peptide since acetorphan protection of enkephalins should have altered binding more profoundly. Overall, these results indicate that *in vivo* ^{3}H-Dpr autoradiography is indeed useful in detecting changes in binding due to the release of endogenous peptides. However, the somewhat unexpected finding of stimulus induced binding increases in the majority of areas suggests that the opioid system may be more complex than previously anticipated.
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Effect of Pertussis Toxin (PTX) on the Antinociceptive Profile of Buprenorphine (B) and Morphine (M): evidence for Differential Regulation of Pain Transmission

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There is considerable evidence suggesting that the Intensity of a noxious stimulus, that is. the frequency of firing elicited in spinal dorsal horn neurons (DHN). is dependent on factors which include the modality and duration of the stimulus and the degree of tissue damage sustained. This study compares the antinociceptive profiles of B and M against three different intensities of noxious stimulus: **(a)** the 50°C hot-water tail-dip *high* **(b)** the short early phase of the rat paw formalin model - *intermediate* and **(c)** the more prolonged late phase of the formalin test - *low*. In the rat. s.c. B has low antinociceptive efficacy against the tail-dip and typically produces a bell-shaped dose-response curve (DRC). In contrast, B is potent and fully efficacious in both phases of the formalin test. In the early phase, but not the late phase, of formalin the efficacy of B decreases at doses >1 mg base/kg. These three distinct profiles. notably full efficacy in the formalin test and a marked lack of efficacy in the tail-dip test, occur together in the same animal. Morphine is consistently efficacious. A common feature of opioid receptors is their ability to modulate effector function via PTX-sensitive G-proteins and there are several reports that PTX inhibits M antinociception. We wanted, therefore, to determine whether suppression of different intensities of noxious stimulation by either of these test agents involved a PTX-sensitive mechanism. The potency and efficacy of B against all three endpoints is unchanged by pretreatment with i.th. PTX (1 µg. at -96 h). Maximum efficacy against late phase formalin Is not. however, maintained and B produces a bell-shaped DRC. Unlike B, the antinociceptive efficacy of **M** in the tail-dip test Is attenuated by pretreatment with PTX. Indeed, at high doses (≥10 mg/kg). M antinociception is replaced by hyperreflexia which is particularly evident in the presence of noxious stimulation by formalin. In the same animals. there is also a rightward shift in the early and late phase formalin DRC's for M of 10x and 4x. respectively, although full efficacy is still achieved. Thus, against three different noxious stimuli in the same rat, B and M have distinct profiles of antinociceptive activity. The ascending portion of the B DRC's is insensitive to PTX In contrast, the effect of M against high. and to a lesser extent intermediate. intensity noxious stimulation is markedly attenuated by i.th. PTX. These data suggest that: ***(i)*** noxious stimuli eliciting different intensities of DHN firing are differentially processed ***(ii)*** sites coupled to PTX-sensitive G-proteins, while important In down-regulating high intensity signals, are not essential for the regulation of intermediate and low Intensity stimuli ***(iii)*** the contrasting antinociceptive profiles of M and B are related to their interaction with spinal sites coupled to different transduction mechanisms. Since. accumulating evidence suggests that opioid receptors are not exclusively coupled to PTX-sensitive G-proteins. the possibility that this differential activity involves transducer proteins coupled to distinct opioid receptors cannot be dismissed. Moreover, since PTX attenuates M-induced physical dependence, the suggestion that M and B cause antinociception by different post-receptor mechanisms may help to explain the lower dependence liability of B.

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Isobolographic Analysis of Interactions Between (μ) and (δ) Agonists in Rats: Potentiation Depends on μ Agonist and on Ratio of Compounds

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Considerable evidence suggests a potentiative interaction between μ and δ opioid agonists. The present study used isobolographic analysis to study the selective δ agonist, DPDPE, tested in combination with morphine or the selective μ agonist, PL017. Rats were tested for analgesia in the cold water (-3°C) tail-flick test both before and after icv administration of morphine (10-30 μg), PLO17 (0.2-2.0 μg), or DPDPE (20-200 μg). From these dose-effect functions, isobolograms can be constructed, and theoretical additive ED_{50} values can be calculated for any fixed-ratio combination of two agonists. Using our statistical method (Tallarida *et al.*, Life Sci. 45:947, 1989), analysis does not require quantal data or parallel curves. Most importantly, variance and confidence limits can be derived for the theoretical additive ED_{50} values. Morphine, PL017 and DPDPE, administered icv, each produced dose-dependent analgesia (ED_{50} values were 14, 0.78 and 64 mg, respectively) and each was fully efficacious in the cold water tail-flick test. Full dose-effect curves were generated for two fixed-ratio combinations of morphine and DPDPE. The mixture with 20% DPDPE was found to be superadditive (additive ED_{50}=17 μg vs. actual ED_{50}=5.2 μg); the mixture with 40% DPDPE was simply additive (18 vs. 19 μg). Thus, small changes in the ratios of the same two agonists can affect whether potentiation is observed. Dose-effect curves also were generated for two combinations of PL017 and DPDPE (40 and 80% DPDPE). Neither mixture differed significantly from additivity (1.3 vs. 1.2 μg and 3.7 vs. 6.1 μg, respectively). These results challenge the generality of potentiation between μ and δ agonists, and illustrate the importance of conducting isobolographic analysis before concluding superadditivity for individual drug interactions.

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Efferent Projections of the Dorsal Raphe Nucleus and Nucleus Raphe Magnus

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Antinociception is elicited by electrical stimulation or opiate microinjection into the dorsal raphe nucleus (DRN) or nucleus raphe magnus (NRM). These nuclei are innervated by opiocortin-ir fibers derived from the arcuate nucleus and may be important for antinociception mediated by endogenous opioid peptides. The present studies were undertaken to identify efferents from the DRN and NRM that may contribute to antinociception. *Phaseolus vulgaris* leucoagglutinin (PHA-L) was microiontophoresed into the DRN or NRM and visualized immunocytochemically. Following NRM injections, PHA-L-ir fibers with putative terminals were identified in the lateral hypothalamus, parafascicular nucleus, ventral periaqueductal gray (PAG), parabrachial nucleus, locus coeruleus, A7, A5, spinal nucleus of the V nerve and nucleus tractus solitarius. DRN injections resulted in extensive PHA-L fiber labeling in regions including the septum, amygdala. preoptic area, bed nucleus of the stria terminalis, hypothalamus and intralaminar and midline thalamus. PHA-L-ir fibers with putative terminals were also identified in nuclei including PAG, locus coeruleus, parabrachial nucleus, nucleus tractus solitarius and medullary raphe and reticular nuclei. Dual immunostaining after DRN injections demonstrated neurotensin-ir neurons in putative contact with PHA-L-ir terminals in the preoptic area and septum; enkephalin-ir neurons in the lateral septum; and corticotropin releasing factor~ir neurons in the preoptic area, bed nucleus of the stria terminalis and Barrington's nucleus. These anatomical results indicate that the NRM may influence nociception via feedback to the ventral PAG or projections to the locus coeruleus. A7 or A5 The DRN has extensive projections that may affect nociception, including ascending projections to the intralaminar thalamus; local projections to the PAG; and descending projections to brainstem nuclei at the origin of bulbospinal pathways. Because of the similarities in connectivity and the endogenous chemical neuroanatomy of the DRN and ventral PAG, it is possible that these nuclei may act as a functional unit, at least in regard to nociception.

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Tramadol, an Atypical Opioid Analgesic: Opioid and Nonopioid Components

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Tramadol,(1RS,2RS)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)-cyclohexanol is an orally active, clinically effective opioid analgesic with an atypically safe side effect (SE) profile (little or no respiratory depression, constipation or addiction liability). The mechanistic explanation for the low opioid SE's is the subject of the current investigation. Tramadol has low, but preferential, affinity for mu-opioid receptors (K_i: mu=2.1, delta=57.6, kappa=42.7μM) and inhibits NE and 5-HT neuronal uptake (K_i=0.78 and 0.99μM, respectively). Tramadol does not bind to alpha$_2$, 5-HT, NMDA or benzodiazepine sites nor does it inhibit the uptake of adenosine, cAMP, DA or GABA. Tramadol produces dose-related antinociception in acute and tonic (yeast-induced inflammatory pain) tests in mice and rats. Its *in* vivo opioid activity is mu-mediated based on a common pA2 for naloxone against tramadol or morphine and its inactivity i.c.v., but not s.c., in CXBK (supraspinal mu-opioid receptor deficient recombinant inbred) mice. However, unlike pure opioids, naloxone only partially blocks tramadol-induced antinociception in several tests and i.p. administration of yohimbine or ritanserin blocks the antinociception (rat tail-flick test) produced by tramadol (but not morphine) administered i.t., suggesting that aminergic uptake inhibition plays a significant role *in vivo*. These results suggest opioid and nonopioid components to tramadol-induced antinociception. The wide separation between therapeutic activity and SE's of tramadol might result from an advantageous interaction between these mechanisms.

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Lack of Correlation Between Cellular Immunity and Delayed Hypersensitivity in Reaction in Dually HIV and TB-Infected Drug Abusers

L.S. Brown, Jr., J. Bailey, A.L. Smith, T. Nemoto and C.P. Felton

Tuberculosis (TB) represents another cause of the widening spectrum of medical consequences of AIDS and drug abuse. However, HIV associated immunosuppression may impair the capacity to detect latent TB infection. As a part of a secondary TB prevention study (prophylactic isoniazid therapy) among drug abusers, we report about the relationship between cellular immunity and delayed hypersensitivity (magnitude of induration following application of purified protein derivative [PPD]).

Following an informed consent, a standardized questionnaire of demographic, drug and sexual behavior information was administered to 214 patients enrolled in methadone maintenance who were PPD+ on admission or at annual physical examination. Sera was also collected for complete blood count, serum electrolytes, liver chemistries, HIV serology and T-cell subsets.

Of the 214 patients enrolled, 97 were HIV antibody (ab) positive (+). There were no significant demographic, drug, or sexual behavioral differences between HIV ab+ and HIV ab- patients. As compared to the HIV ab- patients, HIV ab+ patients had significantly lower absolute lymphocytes (p=0.001), total T cells (p=0.003). helper T-cells (p=0.00l), and helper/suppressor T-cell ratio (p=0.001). However, the magnitude of induration following PPD application did not differ between HIV ab+ and HIV ab- patients.

Ninety-seven patients, who were HIV ab+ and PPD+. were divided into three groups (5-10mm, 11-l5mm, and greater than 15mm) based upon the degree of duration. The mean level of white blood counts differed among patients in each of these three groups. However, these three groups did not differ significantly in absolute lymphocyte, total T-cell, helper T-cell, or suppressor T-cell counts.

In this study, there was no association between the magnitude of induration (or delayed hypersensitivity) and cellular immunity in HIV ab+ and PPD+ drug abusers. It is possible that our findings may be due to the fact that this population as a group may be very early in their HIV disease and therefore had little HIV-associated immunosuppression at this point. Also, our selection of HIV ab+ and PPD+ patients may have excluded anergic patients with suspected latent TB infection (who exhibit PPD- in HIV ab+ patients). -Nonetheless, these findings do suggest that the delayed hypersensitivity reaction in TB and HIV infected drug abusers may not closely correlate with HIV-associated immunosuppression. This study was supported by a contract (#9013966) from the New York City Department of Health and the Centers for Disease Control.

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Long Latency AERPs in Drug Users With HIV Disease

T.B. Horvath, L. Handeisman and M.M. Schroeder

The P3 latency and earlier components of long latency auditory event related potentials (LLAERP) have been used to analyze cognitive deficits in neuropsychiatric disorders, and are sensitive markers of HIV-I involvement in the CNS in the gay risk group. (1,2). 39 male veterans were stratified HIV- (N=20), HIV+ CDC stage II ("asymptomatic") (N=12), or HIV+ stage IV (AIDS) (N=7). Assignment was arrived at by consensus of a psychiatrist, neurologist, and infectious disease expert, and validated by neuropsychological tests. The stage IV group was characterized by mild HIV dementia by Sloan-Kettering rating. Patients with active substance use, alcoholism, major psychiatric co-morbidity, or on AZT, were excluded from study. An oddball auditory paradigm with a go/ no-go response was administered with 5 tests of linearity planned for the Pl, Nl, P2, N2 and P3 latencies of the waveform generated by the "rare" stimulus. Pl, Nl, and P3 latencies were prolonged as a function of HIV-l staging. (P1: F (1,36) = 11.85;, Nl: F (1,36) = 6.38: P3: F (1,36) = 8.47: all p<.05 after Bonferroni correction.) The P3 latency in the stage 4 group was prolonged compared with that of HIV negatives. There was no effect of HIV infection of LLAERP amplitudes. Pl and Nl prolongation is consistent with a pattern of subcortical dementia. Subsequent LLAERP tests using an alternative "count the rare tones" response condition confirmed the validity of the results of the go/ no-go condition for P3 latency.

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Spect Regional Cerebral Blood Flow in HIV Infected Drug Abusers

C.H. van Dyck, SW. Woods, S.S. O'Malley, B.L. Martini, C.J. McDougle, L.H. Price, J.H. Krystal, P.B. Hoffer and T. R. Kosten

This study aimed to determine whether HIV infected drug abuse patients differ from HIV- drug abuse patients and healthy controls in regional cerebral blood flow (rCBF). 13 HIV+ methadone patients (8M, 5F, 33.2 ± 7.5 yrs), 11 HIV- methadone patients (6M, 5F, 36.0 ± 4.5 yrs), 13 healthy controls (8M, 5F, 32.4 ± 7.9 yrs) underwent SPECT scanning with Tc-99m HMPAO. rCBF ratios were computed for 14 regions of interest. Both HIV+ and HIV- patients showed decreased rCBF in right and left frontal cortex compared to healthy controls (right frontal .968 ± .006 HIV+, .959 ± 010 HIV-, vs .986 ± .006 healthy; left frontal; .954 ± .006 HIV+, .948 ± .005 HIV-, vs .973 ± .005 healthy, $p<.05$, Student's t-test). HIV+ patients also showed increased rCBF in right temporal cortex compared to healthy controls (1.020 ± .007 vs .997 ± .005, $p<.05$), and HIV- patients showed increased rCBF in left thalamus compared to healthy controls (1.130 ± .010 vs. 1.092 ± .009, $p <.05$). No significant differences in rCBF were found between HIV+ and HIV- patients. The fact that the bifrontal decrease in rCBF appeared in both patient groups compared to healthy controls suggests that this finding may be related to drug abuse. However, no significant correlations were found between drug abuse variables and rCBF ratios in this study. Supported by NIDA 5T32-DA07238.

AFFILIATION:

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Peptide T Treatment of Cognitive Impairment HIV+ Intravenous Drug Users

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Peptide T. an analogue of Vasoactive Intestinal Peptide, has improved cognitive function in patients with AIDS Dementia Complex in-open clinical trials. We treated five methadone maintained, cognitively impaired HIV positive patients in a double blind cross-over-study for four weeks with Peptide T, 5 mg intranasally tid, and with placebo for four weeks. The five patients were impaired on at least two tests of neuropsychological function, and had been treated with AZT for at least one month prior to enrollment. The sample was 80% male, with a mean age of 37 and baseline WAIS of 84. verbal IQ of 86. performance IQ of 83.

Our patients showed an average improvement of 4 SD on the following five tests with Peptide T, compared with no change with placebo: Trials B (TMB), the Parker Verbal Learning Test (PVLT), Grooved Pegboard (GPB-N). Stroop Interference Test (STRP-CW). and the Paced" Auditory Serial Addition Test (PASAT). Patients improved 1.1. SD on Peptide T compared with placebo for those tests they were originally impaired on. Our other data suggest improvement in constitutional symptoms with Peptide T.

Methodologic differences between our study and the previous open label trials include; different doses of Peptide T. different routes of administration (IN vs. IV), concurrent administration of AZT, and subject population (methadone maintained substance abusers vs. homosexual men). Further studies will be necessary to determine optimal parameters for Peptide T administration and selection of patients likely to respond to Peptide T.

Traci Tropasso assisted in analysis of data.

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AFFILIATION: Connecticut Mental Health Center, New Haven, CT

Drug and Sexual Behaviors Among Intravenous Drug Users (IVDUs) Who Were Enrolled in Isoniazid (INH) Therapy

T. Nemoto, L.S. Brown, Jr., M.M. Chu, A.F. Chu, J. Bailey and D.C. Ajuluchukwu

To investigate the efficacy of INH therapy among IVDUs, patients in methadone maintenance programs in New York City who had positive response to skin testing with purified protein derivative (PPD) tuberculin were asked to participate voluntarily in the therapy. This study evaluated the relationships among drug and sexual behaviors, environmental factors (particularly living experience in public shelters), and HIV status, using intake data of the INH efficacy therapy. After obtaining informed consent, a standardized intake questionnaire was administered by trained research nurses. Also, blood was collected and tested for HIV antibody. A total 180 participants consisted of 125 blacks (69%), 50 Hispanics (28%), and 5 Whites; 99 males (55%) and 81 females. The average age was 37.8 years. The HIV infection rate was 46%. A significantly higher number of HIV positives used heroin and cocaine intravenously, and speed-ball compared with HIV negatives. Fifty-seven (32%) were currently or had ever lived in shelters. Significantly larger numbers of black and white females were currently or lived in shelters compared with Hispanics. Male IVDUs who lived shelters used cocaine, crack, marijuana, and condoms more frequently than those males who did not. Female IVDUs who lived in shelters reported significantly higher numbers of male sex partners than those females who did not. Specific drug and sexual behaviors among IVDUs who lived in shelters should be targeted by education and prevention programs in shelters.

A C K N O W L E D G M E N T

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A F F I L I A T I O N

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Assessment of HIV Seroprevalence and Behavioral Risk Among Cocaine Users

M.R. Kowalewski, H.K. Khalsa and M.D. Anglin

Research has indicated that cocaine use, particularly injecting or smoking crack, may act as a secondary risk factor for transmission of human immunodeficiency virus (HIV). Users may en age in high risk sexual practices due to the disinhibiting effects of the drug, or may use sex as a barter for cocaine. Additionally, cocaine injectors may inject the drug frequently and may share syringes with other users due to both the short lived effects of the drug as well as its disinhibiting effects. This study examined self-reported HIV related risk behaviors in relation to AIDS knowledge/attitudes, perceived risk of infection and levels of cocaine use among a sample of male cocaine users. The rate of HIV seropositivity was also assessed.

The sample included 244 male veterans with a history of cocaine dependence admitted to a drug treatment program. All subjects received a blood test for HIV antibodies at the time of the follow-up interview. Instruments used in data collection were the "Survey of HIV Risk Among Selected California Populations" and a drug consumption instrument used in a national data collection (National Institute of Justice Drug Use Forecasting Program) of populations in correctional facilities.

The rate of seropositivity in the sample was low. There were five seropositive cases in the sample (2%). Nevertheless, subjects engaged in behaviors placing them at risk for HIV infection. Of the subjects who used IV drugs in the year prior to the interview (n=14), half shared needles. Yet, half of the injection users also cleaned their needles with bleach. In terms of sex practices in the year prior to the interview, few subjects reported having sex with other males (5%), but 58% had sex with more than one female. At the same time, condom use was low for the whole sample (73% never). Perception of personal risk for HIV infection was not found to be significantly related to HIV risk behaviors. Level of cocaine use in the year prior to the interview was related to high risk sexual practices. Subjects who used more cocaine were significantly more likely to have more than one sex partner and pay for sex with drugs than those who used no cocaine.

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Psychiatric Disorders and Drug Use in 100 HIV-infected IVDUs

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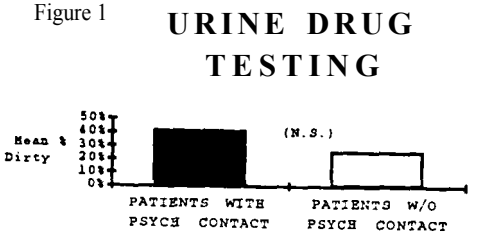
Method: A chart review examined psychiatric consultation in all 100 HIV-infected patients in methadone maintenance treatment (MMT) at SFGH during March, 1990. Sixty-one percent of patients were males; mean age was 40. Approximately two thirds were minorities. Mean length of opiate use was 20 years and mean time in MMT was 17.4 months. At entry into MMT, the secondary drug of abuse was cocaine for 44% alcohol for 1l%, and amphetamines for 3%.

Outcome: Sixty-six of patients had a psychiatric contact. The most prevalent diagnoses were psychoactive substance dependence disorders: cocaine in 25 (38%), alcohol in 16 (25%), and sedative-hypnotic in 11 (17%), dementia in 9 (14%), disorders with psychosis in 10 (15.2%), sleep disorders in 9 (13.6%) and adjustment disorders in 4 (6%). Organic disorders were predominant. Medications were used in 52 (79%) of patients: antidepressants in 28 (42%), non-benzodiazepine antianxiety agents in 12 (18%), benzodiazepines in 13 (20%), antipsychotics in 8 (12%) and disulfiram in 7 (11%). Urinalysis (Figure 1) revealed that patients with psychiatric contacts had 42% dirty urines, while those who had no psychiatric contact had only 26%.

Figure 1



Patients who had psychiatric contact tended to have more drug-positive urines for all categories for drugs, including morphine, cocaine, and amphetamine.

Conclusion: Psychiatric disorders in these HIV-infected IVDUs were common, were predominantly organic, and were associated with higher rates of drugs use. Supported by NIDA Grant #18 06097 and NIDA Grant DA 01696.

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A Model Program for Primary Care of Intravenous Drug Users With HIV Infection

P.G. O'Connor, S. Moide, S. Henry, W.L. Shockcor and R.S. Schottenfeid

Introduction: Intravenous drug users (IVDUs) often have significant medical problems and are in need of comphrehensive primary care (PC) services in addition to drug treatment (DT). To improve access to PC, we developed an on-site PC clinic -- The Central Medical Unit (CMU) -- for substance abusers in DT. Here we report on outcomes those with HIV infection.

Methods: The CMU is located within walking distance to each program. Affiliated programs include DT for opioid, cocaine, and alcohol addiction. Goals for PC focused on 7 objectives. Four included the prevention and management of common infections associated with IVDU and HIV infection including: tuberculosis (TB), syphilis, hepatitis, and pneumococcal vaccination. Three included providing HIV-specific PC: T-cell testing, antiretroviral therapy, and pneumocystis prophylaxis.

Results: In 1990, 1,399 patients made 5,008 visits. Of these patients, 509 were known to be IVDUs. There were 112 patients known to be HIV infected -- comprising 22% (112-509) of IVDUs. On admission, 60% had no source of PC. The mean age was 38 years, 59% were male, and 55% were minority group members. All patients were offered screening for TB, syphilis, and hepatitis. Of those tested, 11% were PPD positive, and 92% (10/l1) accepted INH prophylaxis. Syphilis screening revealed that 2% of our 112 patients had evidence of active syphilis. Both patients completed treatment. Following screening, 12% (13/l12) of our patients were eligible for hepatitis B vaccine but only 2 accepted. Pneumococcal vaccination was accepted by 66% of patients. T cell testing was accepted by 97% (109/112) of patients. Of those tested, 44% had counts over 500, 34% were 200-499, and 22% were under 200. Antiretroviral therapy was accepted by 91% of the 66 patients eligible for it. Pneumocystis prophylaxis was offered to 29 eligible patients, 97% of whom accepted therapy.

Conclusions: By combining PC with DT, the CMU model program appears to be effective in providing PC for HIV infected IVDUs. The high cost of hepatitis B vaccine remains a barrier to its use. We believe that successful management of HIV infection in IVDUs may require unique approaches. The CMU model program of providing PC in conjunction with DT is one approach that may have merit.

AFFILIATION: Yale University School of Medicine, New Haven, CT

The Myth of Polydrug Abuse Among Opiate Addicts in the US.

J.C. Ball

The term "polydrug abuse" is fraught with semantic problems: it is ambiguous as to specific combinations of drugs referred to; it does not differentiate frequencies of use (i.e., ever, occasional, daily); it does not denote route of use; and it does not indicate whether such use is transitory or persistent. Therefore, the term polydrug abuse is of limited meaningfulness. Instead of referring to polydrug abuse, in general, it seems efficacious to delineate and measure specific combinations of drugs which are simultaneously used and to do so within designated populations of drug abusers.

With regard to opiate addicts, the proposition has repeatedly been advanced that most opiate addicts are persistent polydrug users; that is, that they are more or less indiscriminate users of numerous illicit drugs. This myth of polydrug use is found not to be supportable.

Based on detailed drug abuse histories obtained from 617 male methadone maintenance patients, it was found that 98 percent had been intravenous drug users: 97 percent had regularly used heroin, and only 30 percent had regularly used other opiates. Thus, their regular opiate use was largely restricted to heroin.

With regard to non-opiate drugs, 47 percent of the patients had a history of regular cocaine abuse (i.e., one or more years of regular abuse), 33 percent had used sedatives, 24 percent barbiturates, 24 percent amphetamines, 15 percent hallucinogens, and less than 5 percent inhalants; regular marijuana smoking obtained for 69 percent. Thus, with the exception of marijuana smoking, most addict patients were not regular abusers of other illicit drugs or they only used one such drug on a regular yearly basis.

AFFILIATION: Addiction Research Center, Baltimore, MD

Predictors of Relapse Levels in Cocaine-and Methamphetamine Abusers

F.G. Castro, E.H. Barrington, E.V. Sharp and MA. Walton

This prospective study examined the rates of relapse to four illicit drugs and the predictors of overall level of relapse in a sample of 100 outpatients and 120 inpatients admitted into treatment with a diagnosis of cocaine abuse or methamphetamine abuse. Assessment interviews were conducted within two weeks of admission, followed by assessments at 1, 7, 13, and 19 months following treatment entry. In-depth data from a six-month calendar of recovery milestones was evaluated by two independent raters to generate reliable measures of relapse on an I-level Relapse Index: abstinence, lapse (3 levels) and relapse (4 levels). In 34 cases, self-reports of drug use were cross validated via hair samples that were analyzed by radioimmunoassay (RIAH). Concordance rates of hair test validation and self-reports regarding the use of cocaine, methamphetamine and marijuana respectively were: 85%, 79% and 85%. At the seven-month assessment, data for 132 cases revealed rates of (1) abstinence, (2) lapse, and (3) relapse to any drug of: 36.4%, 38.6%, and 25.0% respectively. In this total sample, abstinence rates for cocaine, methamphetamine, marijuana and alcohol respectively were: 77.3%, 75.0%, 72.0% and 50.4%.

Three regression analyses were then conducted to examine the antecedents of relapse status as measured by the 8-level Relapse Index at the 7-month post-test. Variables in these analyses included the scales of: Self-efficacy in Avoiding Relapse, Disinhibition, Internal Health Locus of Control, Tempting Stressors, Distressing Stressors, Cravings, Anxiety, Depression, Somatization, Paranoia and Hostility. Amounts of cocaine and of methamphetamine used at the time of heaviest usage were also included as predictors, as were age and level of education. Each of these predictors were examined at the Pretest, some at the Interim (1 month interview), and all at the Post-test (7-month interview). Heaviest methamphetamine use (beta= -.28) was the sole predictor of the Relapse Index at the Pretest period. By contrast, Self-Efficacy (beta= -.29) was the sole predictor at one month, and at seven months, Self- Efficacy (beta= -.49) and Disinhibition (beta- +.34) operated as predictors. These results offer implications regarding temporal considerations in self-efficacy enhancement as a clinical strategy for promoting relapse avoidance.

AFFILIATION: San Diego State University and MATRIX Center
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Inpatient vs. Outpatient Study: A Patient-Treatment Matching Design

R.M. Pettinati, K. Meyers, B.D. Evans, J.M. Jensen, F.N. Kaplan, C.R. Reutsch and J.I. Tracy

Empirical support for inpatient alcohol treatment is completely lacking; yet, the clinical community maintains that specified patient populations need inpatient treatment for substance dependence. Prior research, which has exclusively promoted outpatient treatment, has typically used random assignment which excludes certain patients, such as psychiatrically-complicated patients, due to ethical concerns.

Given that these may be the patients who need inpatient treatment, we initiated a study that uses matching rather than randomization. The matching criteria to inpatient treatment involves high psychiatric severity and/or lack of social support. We predict that patients with either of these profiles (or both) will have a better outcome in an inpatient than in an outpatient program. We also predict that patients with profiles of low psychiatric severity and/or good social support will do well in an outpatient program, without incurring the higher costs of inpatient treatment.

Our preliminary results have focused on early attrition and treatment failure. Outpatients were 4 times more likely than inpatients to leave treatment early (35% vs 9%, $p < .001$). Also, those who failed treatment were overrepresented among those mismatched to outpatient treatment ($p<.05$). In addition, mismatched outpatients experienced significantly more legal, social, employment, and psychological problems while also feeling more troubled by family problems (ASI composite scores used, $p<.01$). Mismatched outpatients who failed early in the treatment program reported spending more time with substance users ($p<.05$) and had more days of employment problems ($p<.05$) in the 30 days prior to entering treatment. These results suggest a high potential for outpatient treatment failure and attrition compared to an inpatient program. Also, selected matching criteria may be useful to predict early treatment failure.

AFFILIATION:
Carrier Foundation, Belle Mead, New Jersey

Arousal Responses to Cocaine Cues: Factors of Variance and Clinical Significance

J.C. Negrete and S.G. Emil

Cocaine addicts are believed to be sensitive to external cues which - by virtue of their association with the drug or the circumstances in which it was used - are capable of evoking in them memories that stir up cocaine cravings and urges to repeat the experience. Such sensitivity is thought to be particularly strong during the first 4 months following cessation of use (withdrawal or "anhedonic" phase). The present study tested the possible variance in responses to cue exposure as a function of the addicts' age, sex, severity of cocaine dependence, length of cocaine use history, time elapsed since last episode of use and the degree of overall psychological distress they were experiencing at the time of the experiment. Follow-up data (12 months) served to assess the predictive value of such observations with respect to therapeutic response.

Data: Thirty-six cocaine abusers in treatment and 16 non-using controls were exposed to visual cocaine cues; measurements included changes in skin conductance and a rating of psychological arousal (persisting cocaine thoughts and images during the 24 hours following the experiment). The GSI score of the HSCL-58 was used as an indicator of psychological distress.

Findings: Probands showed arousal responses significantly higher than the controls. Skin conductance readings correlate positively with HSCL-58 scores and with severity of craving in the week prior to the trial. Unexpectedly they correlate negatively with duration of cocaine use history and do not vary as a function of the severity of cocaine addiction or the duration of cocaine abstinence prior to the test. Neither cocaine addiction measurements, nor arousal responses were found to predict cessation of use (a minimum of 3 months of sustained abstinence) one year after the test.

AFFILIATION:

McGill University, Montreal, Canada

Cue Reactivity in Heavy and Light Drinkers

J. Greeley, W. Swift, J. Prescott and N. Heather

The level of desire for alcohol reported by drinkers in the presence of alcohol-related cues differs for heavy and light drinkers. Fifty male drinkers between 18 and 50 years of age were asked to rate their desire for a drink of alcohol when presented with the sensory properties (i.e., sight, smell and taste) of their preferred alcoholic beverage (Alcohol Cue) or a nonalcoholic lemon drink (Neutral Cue). The cues were presented in a counterbalanced order and each was presented for 20 minutes preceded by a 5-minute pre-cue baseline period. Desire for Alcohol, heart rate (HR) and skin conductance level (SCL) were monitored continuously while blood pressure, stress and arousal levels were measured before the first cue and at the end of each cue presentation. Light Drinkers (n=29 drank on average 1.6(±0.9) standard drinks per day and Heavy Drinkers (n=21) drank 14.6(±9.4).

Planned orthogonal polynomials were used to test the Cue x Time x Group interaction and to test for interactions with Order of Cue Presentation. There were no significant effects of Order for any of the measures. There was a statistically significant interaction between linear trend and Group in the presence of the Alcohol Cue $F(1,46)=11.16$ ($p<.05$). Heavy Drinkers showed a linear increase in Desire for Alcohol from pre-cue to the end of the 20-minute presentation of the Alcohol Cue. The Light Drinkers showed an initial increase in Desire for Alcohol but this dissipated over time and was not statistically significant. Neither the Heavy nor the Light drinkers showed any change in Desire for Alcohol when presented with the Neutral Cue. HR showed a significant Group x Time interaction $F(1,45)=8.83$ ($p<.05$) but only when the Alcohol Cue was presented first. Alcohol intake over the last 30 days and depression level on the day of testing predicted increasing desire for alcohol in the presence of the Alcohol Cue.

AFFILIATION: National Drug and Alcohol Research Center
Sydney, Australia

Depression and Smoking Treatment: A Clinical Trial of an Affect Regulation Treatment

S.M. Hall, R. Munoz and V. Reus

Individuals with a history of Major Depressive Disorder (MDD) are overrepresented among smokers seeking treatment. They may experience more severe withdrawal symptoms and are likely to fail at quitting. A promising mechanism linking smoking and MDD is dysphoria, or poor mood. An intervention to prevent dysphoria would be likely to increase abstinence rates among smokers with a history of MDD. We designed such an intervention and compared it with a standard treatment control. We hypothesized that the depression prevention intervention ("Mood Management") would be differentially effective for smokers with a history of MDD. We used as a control a successful treatment adapted from earlier studies. The condition ("Health Motivation") used group support, individualized plans, and commitment to abstinence. Subjects in both conditions received nicotine gum.

The subject were 149 smokers between the ages of 18 and 65 who smoked ten or more cigarettes per day. They were assessed at baseline, at the end of treatment (8 weeks), and at 12,26, and 52 weeks after the end of treatment on smoking and mood measures. At baseline, lifetime and current depression and dysthymia diagnoses were obtained using the Diagnostic Interview Schedule (DIS).

Subjects were predominantly well educated and white (88%). M age = 41 years; SD = 9.2 years. The sample has slightly more women (52%) than men. M number of cigarettes at pretreatment = 25; SD = 10.8. M number of years smoked = 25; SD = 9.5. Here we present preliminary data for Weeks 8, 12, and 26. Of these 149 subjects, 46. or 31%, reported a history of MDD. The abstinence rates, computed from CO-verified self-report, supported the hypothesis. At Week 26, abstinence rates were: Mood Management, MDD history positive = 52%; Health Motivation, MDD history positive = 18%; Mood Management, MDD negative = 36%; Health Motivation, MDD negative = 38% ($p < .05$). Parallel trends were observed at assessments at Weeks 8 and 12. These data suggest that affect regulation treatment may be specifically efficacious for smokers with a history of MDD. They also suggest that the low abstinence rates reported in smoking cessation treatment clinics may be influenced by the large proportion of smokers with a history of MDD treated in those settings.

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AFFILIATION: University of California, San Francisco and San Francisco Department of Veterans Affairs Medical Center

Increase in Current Perception Threshold After Cranial TENS

D. Taylor and J. Katims

In a double-blind protocol, 72 healthy volunteer subjects were administered 30 min. of cranial transcutaneous electrical nerve stimulation (TENS) (biauricular electrode placement) of 5 Hz, 100 Hz or 2000 Hz frequency and sub-threshold intensity, or placebo TENS (no current was passed between the electrodes). The four groups were compared on pre- to post-treatment changes in perception of a transcutaneous electrical stimulus, assessed using a two alternative forced-choice examination. All three active TENS groups showed significant hypoesthesia ($p < .05$) after 30 min. as compared to the placebo group, with low frequency TENS (5, 100 Hz) resulting in greater hypoesthesia ($p < .05$) than high frequency TENS (2000 Hz). No significant change was observed in the placebo group. These findings may reflect a cranial TENS effect on endogenous opiates, suggesting possible application of this technique to the management of opiate dependence.

AFFILIATION: Narcotic and Research Institute, New York, NY

A Neurostimulator Device for Opiate Detoxification

E.A. Elmoghazy, B.D. Johnson and F.A. Alling

A study was conducted on 124 subjects at an opiate detoxification clinic in Manhattan to assess the effects of a Neuro Stimulator Device (NSD) vs. Methadone on the severity of opiate withdrawal and craving symptoms. The major research question was whether *the* NSD is as effective as methadone in alleviating a variety of opiate withdrawal and craving symptoms. A triple blinded study was conducted with two experimental and two control groups. In the two experimental groups, clients were randomly assigned to an NSD group that received an active device and placebo methadone while the Methadone group received declining dosages of methadone and a placebo NSD device. Self-reports were collected on the severity if twenty-seven items which were combined into five measures of the withdrawal syndrome: sleep discomfort, cramp discomfort, objective discomfort, psychological discomfort, and drug hunger (craving discomfort). An analysis of variance showed that. except for the psychological symptoms, the NSD group was not significantly different from the other groups at the alpha level of .05.

Table 1. Mean severity scores of the two experimental groups on composite indices of the withdrawal syndrome

Symptoms Domain	No	NSD methadone N=10	Methadone Sham-NSD N=27	T Value	Sig. Level
Sleep Discomfort		10.9	8.3	1.71	.20
Cramping Discomfort		10.1	5.5	5.86	.02
Objective Symptoms		7.2	5.5	.98	.33
Psych Discomfort		10.2	7.9	1.04	.32
Drug Hunger		6.5	5.2	.55	.46

However, the pretest scores for these items, used as covariates, had much larger and statistically significant share in the explained variances for all measures
of the withdrawal syndrome.

DISCUSSION

The evidence suggests that the NSD may be as effective as methadone during detoxification from opiates, and in alleviating various domains of the withdrawal symptoms for patients with moderate chemical dependence. The small sample sizes and the differential attrition of subjects limit the validity of the study results. A large study needs to be undertaken.

AFFILIATION: Narcotic and Drug Research, Inc. and
St. Luke's-Roosevelt Hospital Center, New York, NY

Reduction of Tobacco Withdrawal Symtoms By Transcranial Electrostimulation Therapy (TCET)

D.R. Jasinski, J.T. Sullivan, M. Testa and K.L. Preston

The objective of this study was to determine if TCET reduced the discomfort of the tobacco withdrawal syndrome with measures utilized by others to show the antiwithdrawal effect of nicotine polacrilex gum. Highly dependent male and female cigarette smokers (N=147) were randomly allocated to TCET or SHAM treatment for one hour daily for five consecutive days. Subjects were to quit smoking the night before the first treatment. self reports of smoking, daily CO levels, and change in urine cotinine indicated equal levels *of* tobacco deprivation throughout the treatment period. Fifty-two subjects completed treatments with SHAM and 48 with TCET. Prior to treatment (Day l), both groups showed similar withdrawal scale scores (WSS) and bradycardia. on Days 2 and 3, WSS increased for the SHAM group followed by decreases on Days 4 and 5. In contrast, WSS decreased on Days 2, 3, 4 and 5 for the TCET group. WSS on Days 2 and 5 were significantly less for TCET in comparison to SHAM. The WSS calculated as AUC for Days 2, 3, 4 and 5 were also significantly less for TCET than SHAM treatment. No effect of TCET on bradycardia or reports of craving were seen. It is concluded that TCET reduces the same discomforting symptoms of tobacco withdrawal reduced by nicotine polacrilex gum.

AFFILIATION

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Delineation of Central Opioid Receptors Regulating Natural Killer Cell Activity In Vivo

L.C. Band, A. Pert, W. Williams, M. Seggel, B.R. de Costa, D. Thomasson, K.C. Rice and R.J. Weber

Opiates have well-documented immunosuppressive properties. Evidence has been presented that the inhibition of natural killer cell (NK) activity produced by morphine *in vivo* is centrally mediated and primarily involves the periaqueductal grey area (Weber & Pert, Science, 245, 1989). In order to determine the CNS opioid receptor subtypes responsible for opiate effects on NK cytotoxicity, agonists selective for the major classes of opioid receptors were microinjected into the lateral ventricle of Fischer 344N male rats. Three hours after injection, splenic NK activity was determined using a 5-hour chromium release assay (Williams et al., *NIDA Res. Mon.,* 105, 1990). Dose ranges of 20-200 nmol of (S,S)-U50,488 and and 60-200 nmol of [D-Pen^{2,5}]-Enkephalin (DPDPE), selective for kappa and delta receptors, respectively, did not affect NK cytotoxicity. The lowest DPDPE dose, 20 nmol, increased this measure. In contrast, 60-200 nmol of the selective mu agonist [D-Ala^2, NMe-Phe^4, Gly-ol]-Enkephalin (DAGO) caused a reduction in NK activity, blocked by pretreatment with naltrexone (5 mg/kg, i.p.). These findings indicate that opiate-induced immunosuppression is mediated primarily through central mu-receptors. DAGO (60 nmol, i.c.v.) had no effect on plasma corticosterone or ACTH levels three hours postinjection, suggesting that central mu binding does not reduce NK activity through activation of the hypothalamic-pituitary-adrenocortico-axis. The fentanyl derivative mirfentanil produces analgesia in rats without respiratory depression and has mixed agonist-antagonist effects at mu receptors. Mirfentanil (40 mg/kg, s.c.) did not affect NK activity; however, pretreatment with mirfentanil blocked the severe reduction in NK activity induced by sufentanil (0.06 mg/kg, s.c.), a pseudoirreversible mu agonist. These preliminary data provide evidence that analgesia and immunosuppression produced by mu agonists are mediated by distinct binding sites or binding states. Mirfentanil is an example of new possibilities in the design of analgesics having neither respiratory-depressive nor immunosuppressive effects.

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The Effects of Dynorphin Peptides on Human Natural Killer Cell Activity In Vitro

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Recent studies suggest a modulatory effect on some immunological indices by neuropeptides released by the central nervous system. Special attention has been focused on the endogenous opioids: enkephalins, endorphins and dynorphins. Natural killer cells (NK) are large lymphocytes which provide the first line of immune defence against many tumor and viral invasions. Many studies have reported specific saturable sites on lymphocytes, which respond to endogenous opioids, but evidence is conflicting whether these sites are classical opioid receptors, or novel sites of action. The effects of endogenous opioids on NK activity have been studied, with conflicting results: some have found increase, others have shown decrease, and yet others have found no effect on NK. Dynorphin A (dyn), is a 17- amino acid natural peptide containing Leu-enkephalin as its N-terminus, and has a selective kappa opioid activity. In this study we report the effects of a wide range of concentrations (10^{-13}-10^{-3}M) of dyn(1-10)[a natural peptide processed in vivo. but breaks down easily]; dyn(1-10)-amide[a synthetic stabile analogue which does not exist in vivo]; dyn(1-13)[a peptide which has the natural amino acid sequence of dyn, but does not exist in vivo] and dyn(2-17)[a non opioid peptide with the N-terminus removed]. Using peripheral blood mononuclear cells from 13 normal volunteer subjects, NK activity was measured by the ^{51}Cr release assay with K562 target cells and an effector;target ratio of 50:1. There was no change in NK activity after dyn(l-10) was added to the assay in all concentrations used. When dyn(l-10)-amide was added a significant reduction in NK activity was found at concentrations above 10^{-4}M. (Similar results were shown by our group, studying the effects of the opiate agonist methadone, and the opiate antagonist naloxone on NK activity in vitro). In the dyn(l-13) assay, activity was significantly increased at concentrations of 10^{-9}M and 10^{-3}M. When dyn(2-17) was added, a significant increase in NK activity was observed at concentrations of 10^{-4}M and higher. These findings suggest that amino acids near the carboxy-terminus of dyn may provide the active component in enhancing NK activity, and that a non opioid mechanism is involved.

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Morphine Causes the Selective Loss of CD4+/CD8+ Lymphocytes in the Murine Thymus Through the Induction of Apoptosis

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Several investigators have reported that subcutaneous implantation of a 75 mg timed release pellet of morphine will cause a rapid thymic atrophy in the mouse. This study seeks to define the mechanism through which morphine induces thymic atrophy. B6C3Fl mice were subcutaneously implanted with a 75 mg morphine pellet at time zero. 48 hours post implant a loss in thymus weight (-70%) and cellularity (~80%) is observed. Both effects were statistically significant at the $P \leq 0.01\%$ level. Flow cytometric analysis of the lymphocytes remaining in the thymus at this time demonstrates a specific loss of the CD4+/CD8+ subpopulation (>90%) while other thymic subpopulations were much less dramatically affected. The CD4+/CD8+ thymic subpopulation is especially sensitive to death by apoptosis. Apoptosis is a form of cell death, distinct from necrosis, in which a cell synthesizes a specific endonuclease and fragments its own DNA. Although glucocorticoids effectively stimulate apoptosis in thymocytes, apoptosis is also believed to occur physiologically in thymocytes undergoing the stringent selection process occurring during T-cell maturation. An assay to determine the percentage of DNA fragmentation in thymocytes reveals that 12 hours after implantation of the morphine pellet DNA fragmentation is increased 3-4 fold over placebo implanted controls ($P \leq 0.01\%$). Kinetic studies revealed that peak DNA fragmentation (at 12 hours post-pellet implant) occurs prior to the previously noted alterations in either thymus subpopulations or cellularity. When separated on an agarose gel, the size of the DNA fragments observed corresponds to the multiples of 180 base pairs (180, 360, 540, etc.) characteristic of apoptosis. When a 10 mg naloxone pellet is implanted along with the morphine pellet the increase in DNA fragmentation is blocked indicating that this effect is mediated through an action at an opiate receptor. The morphine induced DNA fragmentation of thymocyte DNA could also be blocked by the glucocorticoid antagonist RU38486 at a dose of 30 mg/kg or higher. However, when morphine was added directly to thymocyte cultures at doses of as high as 10^{-4}M, no increase in DNA fragmentation was observed. We conclude that morphine causes thymic atrophy through induction of a process known as "programmed cell death" or apoptosis. Both opiate and glucocorticoid receptors appear to be involved in this process and *in vitro* studies suggest that the opiate receptor is not located on the thymocytes themselves. (Partially supported by NIMH grant R29MH45931-02.)

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The Effect of Interferon-Alpha on Opioid Ligand Binding to Rat Brain Membranes

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Interferon alpha (IFNα)has been reported to modulate naloxone-precipitated withdrawal in morphine-dependent rats, analgesia in rats, cognitive behavior in humans and electrical activity of temperature- and glucose-sensitive neurons. The ability of human recombinant interferon alpha (hrIFNα) to alter the binding of radiolabeled opioid ligands was assessed in a rat whole brain (without cerebellum) membrane preparation. Nonspecific binding was determined in the presence of various unlabeled opioid ligands. Preliminary data indicate that ^{3}H-naloxone binding was inhibited by hrIFNα in a concentration and temperature-dependent manner; this inhibitory effect was observed at 37°C but not at 25°C. In contrast, stimulation of naloxone binding by hrIFNα and rat IFNα/β was observed at 25°C. Saturation experiments with 1000-5000 U/ml hrIFNα revealed an increase of naloxone K_D values from 3.37 nM to 6.9 nM while BMAX values did not change significantly from 117 fmol/mg protein. These results indicate competitive inhibition, perhaps at a particular subset of receptors which bind naloxone. Other experiments utilized membranes pre-labeled to equilibrium with ^{3}H-naloxone. Addition of 3000U/ml of hrIFNα promoted release of naloxone; steady state was reached within 5-10 minutes with inhibition of 40-50% of naloxone-specific binding. This effect was not observed when incubations were done at 25°C. Control incubations with bovine serum albumin did not inhibit naloxone binding, which suggests that the observed inhibition was not due to a non-specific protein effect. Dose-dependent inhibition of ^{3}H-DADLE binding was also observed at hrIFNα concentrations of 30-3000 U/ml; maximum inhibition was approximately 30% of control binding. The difference in the ability of hrIFNα to inhibit naloxone and DADLE binding suggests that this cytokine may bind a particular subset of opioid receptors. These data support the hypothesis that certain biological properties of IFN are mediated via opioid systems. *This work is supported by NIDA (05723) and a USF Research Council grant (6118-932). NRSH is supported by an RSDA (DA00158), and SER is supported by a NIDA training grant (07245).*

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Morphine Attenuates IL-1 Levels in Transected Spinal Cord

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Interleukin-1 (IL-l) has been found to induce hyperthermia, slow-wave sleep, turnover of norepinephrine and release of certain neuropeptides. Previous work in this laboratory has revealed that IL-1 is also present in the spinal cord and that the levels increase over time following spinal cord transection. Administration of morphine has now been found to reduce the transection-induced rise in IL-1 levels. The spinal cord at the vertebral T5-T6 level was exposed through a mid-thoracic incision and laminectomy in deeply anesthetized, male Sprague-Dawley rats ranging in age from 40 to 50 days. Following transection with a scalpel, a 75 mg pellet of morphine was implanted subcutaneously in the region of the left scapula. These animals were compared to: 1) rats that were transected, but did not receive morphine, 2) sham animals that were subjected to the same surgery, but omitting transection and morphine, and 3) unhandled control rats. Sections of spinal cord adjacent to the injury were removed from each rat 72 hours following surgery and were sonicated and stored at -70 C until assayed for IL-1 content. IL-1 was assayed using the IL-l-dependent D10(N4)M mouse T cell line. IL-1 levels in transected spinal cord were more than three times those of either sham or unhandled control animals ($p<.05$). However, while still elevated compared to sham and unhandled control rats, IL-1 levels were significantly less in transected animals that received morphine than in those that did not ($p<.05$). Mean thymus weight (± SD) was also significantly less in the transected plus morphine (174 ± 47 mg) vs. transected alone rats (315 ± 65 mg) ($p<.05$). Splenic cell proliferation in response to Con-A and LPS, and NK induced cytolysis were not affected by the administration of morphine. Nonetheless, the trend was in the direction of suppression. By reducing IL-1, morphine may reduce the number of reactive astroglia and the extent of neovascularization following spinal injury. *This work is supported by a grant from N/DA (05723). NRSH is supported by an RSDA (DA00158) and SR is supported by a NIDA training grant (07245).*

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Interleukin-6 (IL-6) Reverses Morphine-Induced Suppression of the Antibody Response

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Previous studies in our laboratory have shown that morphine treatment *in vitro* (Taub *et al.,* Proc. Nat. Acad. Sci. 88:360, 1991) and *in vivo* (Eisenstein *et al.,* Ann. NY Acad. Sci. 594:377, 1990) suppresses the secondary antibody response in mice. In this study, the primary antibody response was measured *in vitro* by the plaque-forming cell (PFC) assay, following implantation of morphine pellets *in vivo*. Morphine suppressed the primary antibody response in C3HeB/FeJ mice, which was blocked by co-implantation of a naltrexone pellet. This suppression of the PFC response did not occur until 24 hrs, peaked at 48 hrs after implantation, and returned to control levels by 96 hrs. Cells from morphine-treated mice co-cultured with control cells did not show a suppressed response, suggesting that morphine does not cause immune suppression through the production of suppressive factors. Addition of IL-6 *in vitro* reversed the suppression of the PFC response in cells from morphine-treated animals in a dose-related manner. Lower doses of IL-6 (1-10 U/ml) attenuated the suppressive effect of morphine treatment, while higher doses (100-10,000 U/ml) completely restored the PFC response to control levels. Addition of IL-1 ß to cultures reversed morphine-induced suppression only at higher doses ($\geq$100 U/ml). At these doses, IL-1ß is most likely acting through the induction of IL-6. Addition of IL-2 or IL-4 to cultures had no effect on morphine-induced suppression. These data indicate that morphine may cause immunosuppression by blocking production of IL-6 from macrophages, which is necessary to generate a normal antibody response, by inducing final differentiation of B cells to antibody-producing cells.

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Morphine, Cytomegalovirus, and HIV-1 Growth

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Morphine (Peterson et al., 1990) and human cytomegalovirus (HCMV) (Schooley, 1990) each have suggested to promote the progression of AIDS. Given the high incidence of HCMV infection in drug abusers, we investigated in the present study the interaction between morphine and HCMV in a peripheral blood mononuclear cell (PBMC) co-culture assay which is used as in *in vitro* model of infection due to HIV-l, the primary etiologic agents of AIDS. PBMC derived from healthy donors were treated with varying doses of HCMV AD 169 strain for 3 hr prior to culturing for 72 hr. These cells were then cocultured with cryopreserved PBMC, which had been infected with an HIV-l isolate derived from an asymptomatic patient. The release of p24 antigen into coculture supematants was used as in index of HIV-l replication. HIV-l replication was augmented ($P<0.05$) when PBMC from HCMV-seropositive donors were exposed to HCMV (123±41 and 563±204 pg p24 antigen in control and HCMV-stimulated PBMC, respectively, n=3). This effect was dependent upon the dose of HCMV. In contrast, PBMC from HMCV-seronegative donors exposed to HCMV failed to foster HIV-l growth. Pretreatment of PBMC with morphine (10^{-15} M) for 3 h prior to exposure to a suboptimal concentration of HCMV amplified ($P<0.01$) HIV-l replication (1120±103 vs 147±136 pg p24 antigen/ml at day 6 of coculture in morphine vs control groups, respectively). This concentration of morphine did not stimulate HIV-l replication in the absence of HCMV. Furthermore, morphine-related enhancement of HIV-l growth was dose dependent with a bell-shaped dose-response relationship. Taken together, these studies suggest that HCMV infection promotes HIV-l replication and that morphine amplified the stimulatory effect of HCMV.

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Detection of HIV in CNS Tissue and Cells and Effects of Cocaine and Metabolities, In Vitro

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SUMMARY AND INTRODUCTION

Drug abuse, a major risk factor for AIDS, shows little sign of abatement. Users of cocaine may be at risk for accelerated progression of AIDS disease. This laboratory is investigating HIV in the CNS and the effects of **cocaine,** its metabolite, **coaethylene,** CE, and **ethanol** on HIV replication. CE may have profound physiological effects and is produced after ingestion of both cocaine and alcohol (Hearn et al., 1991). HIV load in AIDS CNS tissue is determined using three techniques: virus production in explant cultures of post-mortem brain, *in situ* hybridization (ISH) and peroxidase immuno-histochemistry (IHC) to detect HIV gp41 viral antigen in sections of formalin fixed paraffin embedded tissue. HIV was detected in AIDS CNS including drug abusers. Prior studies showed that cocaine stimulated HIV replication at nM concentrations (Peterson et al., 1991). We found cocaine/CE/ethanol stimulated HIV replication at physiological concentrations.

RESULTS AND DISCUSSION

HIV was detected from 17 of 27 (63%) AIDS cases. HIV was detected in macrophage/monocytes. microglial nodules, and multinucleate giant cells in CNS tissue from 9 of 25 (36%) AIDS cases by *ISH* with appropriate controls. Gp41 was detected in 8 of 29 (28%) cases. Possible correlations of these parameters and drug toxicology am being analyzed. The effects of cocaine/CE/ethanol on normal donor peripheral blood mononuclear cells (nPBMNCs), neural, and lymphoid cell lines were examined. Cocaine ($\leq$1l00μM), CE ($\leq$100μM, and ethanol ($\leq$1.7M) showed no toxic effects. Cocaine ($\geq$ 100μM) was toxic (60% viability) for a lymphoid cell (MT2) line persistently infected with HTLV-1. Stability of cocaine and CE were quantified using gas-liquid chromatography, confirmed by mass spectrometry, (Hearn et al., 1991) in culture supernatants of MT-2, neuroblastoma, and nPBMNCs. Preliminary experiments were as follows. Cocaine cleared from neuroblastoma and nPBMNC cultures slowly whereas CE was cleared more rapidly from nPBMNC cultures (t 1/2 = 8 hours). Cocaine (10μM)) stimulated HIV replication in H-9 cells, CE and ethanol stimulated HIV replication in nPBMNCs which were pre-treated followed by infection. Infected nPBMNCs followed by treatment with cocaine, CE. and ethanol resulted in increased HIV production.HIV is stimulated more by CE than the other compounds used. Ethanol effects were a surprise. This work is supported by grants DA04787 and DA06227 from the National Institute of Drug Abuse. We thank Dr. S. Rose for toxicology and Dr. M. Sotomayor for epidemiological information.

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Preliminary Evidence that CBR12909 is Less Effective at Elevating Mesolimbic Dopamine Function than Cocaine

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Administration of dopamine (DA) uptake inhibitors to rats stimulates locomotor activity via elevation of mesolimbic DA levels. In the present study, rats received i.p. injections of doses of cocaine (COC, 20 mg/kg), GBR12909 (GBR, 20 mg/kg), nomifensine (NOM, 5 mg/kg), WIN-065-2 (WIN, 1 mg/kg) or saline (SAL) which preliminary studies indicated produce the same level of locomotor stimulation and stereotopy. These parameters were measured for 30 min. At the end of the 30 min test period, the subjects were sacrificed, and the brains removed and kept frozen at -70° C. The brains were homogenized in 10 ml/gm wet weight ice-cold 10 mM TRIS-HCI, pH 7.0. The homogenates were then centrifuged at 30,000 xg for 20 min, and the supernatants kept frozen at -70° C. The next day, aliquots of the supernatants were serially diluted, and assayed for inhibition of [^{3}H]DA reuptake by a striatal synaptosomal preparation. Only supernatant prepared from GBR-injected rats strongly inhibited [^{3}H]DA reuptake (IC50 about 100 μ1). The rank-order of potency was GBR>>NOM>WIN=COC. The brain level of GBR was calculated to be about 1 μM. Control studies determined that brain supernatant did not metabolize these agents. In vivo binding studies using [^{3}H]BTCP to measure occupancy of the DA transporter resulted in the following rank-order of occupancy: GBR>>NOM>WIN=COC. Since COC produces the same level of response as does GBR, but at lower receptor occupancy, these data support the hypothesis that GBR is less efficacious than is COC at increasing mesolimbic DA function, and are consistent with our previous finding that GBR attenuates the ability of COC to elevate extracellular levels of DA (Rothman et al, 1989).

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Effects of Intra-A10 Administration of the Dopamine D2 Agonist Quinpirole on Psychostimulant-Induced Motor Activity

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The mesolimbic dopamine (DA) system arises from the A10 region to innervate the nucleus accumbens (NA). Amphetamine (AMP) and cocaine (COC) both stimulate motor activity, at least in part, via their actions within this DA system. The motor-stimulant response to AMP and COC is associated with an increase in extracellular dopamine in the NA. Dopamine neurons in the A10 region are under the inhibitory control of GABAB receptors and D2 autoreceptors. In a previous study, we demonstrated that injection of the GABAB agonist baclofen into the A10 region blocked the motor-stimulant response to both AMP and COC. In this study we examined the effects of injection of the D2 agonist quinpirole (QUIN) on AMP- and COC-stimulated motor activity. One week after bilateral implantation of guide cannulae above the A10 region, animals received intra-A10 injections of saline or QUIN (0.15 nmol/side) 5 min before peripheral injections of saline, AMP (0.5, 1 .0 or 2.0 mg/kg) or COC (7.5, 15.0 or 30.0 mg/kg). Separate groups of rats were used at each dose of COC or AMP, and all rats received each of the 4 possible combinations of treatment. QUIN pretreatment blocked AMP-stimulated motor activity for at least the first 30 min after injection at all doses tested, but did not block COC-stimulated motor activity at any of the doses tested. In vivo microdialysis revealed that QUIN pretreatment did not block the AMP-induced increase of DA in the NA despite blocking the behavior. COC inhibits neuronal firing primarily via long loop, GABAergic feedback while AMP does so via both long loop and short loop, somatodendritic DAergic feedback. Thus, it is possible that baclofen which blocks both AMP- and COC-stimulated motor activity is mimicking long loop feedback, while QUIN which only blocks AMP-stimulated motor activity is mimicking short loop feedback.

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Differential Effects of Cocaine and Dopaminergic Agonists on Hypokinesia Induced by Dopaminergic Antagonists

P. Terry and E. Tirelli

The effects of direct and indirect dopamine agonists on the hypokinesia induced by dopamine receptor-subtype agonists was examined in male C57/BL6 mice. Locomotor activity was measured over one hour in automated (photobeam) activity chambers. Both the D-l antagonist SCH23390 and the D-2 antagonist spiperone dose-dependently reduced activity when injected (IP) directly before test. For drug interaction studies, we selected doses of SCH23390 and spiperone which produced submaximal hypokinesia: in each case, 0.1 mg/kg. Pretreatment 10 min before testing with nonexcitatory doses of cocaine (0.6 - 2.5 mg/kg, SC) dose-dependently reversed the hypokinesia induced by either spiperone or SCH23390. Nonexcitatory doses of the specific D-l agonist SKF38393 (SC, also 10 min before test) were also able to attenuate the hypokinesia produced by SCH23390. However, SKF38393 pretreatment was unable to reverse the hypokinesia resulting from administration of the D-2 antagonist spiperone. Neither inactive nor active (hypokinetic) doses of the specific dopamine D-2 agonist RU24213 (SC, 10 min pretest) were able to reverse spiperone-induced hypokinesia. Perhaps less surprisingly, the drug was also without effect on the hypokinesia produced by SCH23390. Finally, behaviorally-inactive doses of the mixed D-1/D-2 agonist apomorphine were without effect in combination with either of the two receptor-subtype antagonists.

The fact that cocaine was injected at nonexcitatory doses suggests that its effect was specific and not ascribable to an overall behavioral activation. However, the lack of effects of the specific D-2 agonist RU24213 suggests that the present interaction between cocaine and the D-2 antagonist may not be mediated solely by D-2 receptors. Moreover, the inability of apomorphine to reproduce the effects of cocaine further demonstrates the marked differences in pharmacology between direct and indirect dopaminergic agonists that can result in different behavioral effects.

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Behavioral Hypersensitivity to Apomorphine Upon Acute Cocaine Withdrawal in Mice

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Reports of cocaine withdrawal symptoms in cocaine abusers have been difficult to model with animals in the laboratory. In this preliminary study, we tried to tackle this question by probing a potential change of dopaminergic function after an acute injection of a relatively high dose of cocaine. The behavioral responsivity to the D1/D2 agonist apomorphine was used as a probe in male C57BL/6J mice. Mice were injected ip with 30 mg/kg cocaine 1456 (24 hrs + 16 min), 736 (12 hrs + 16 min), 256 (4 hrs + 16 min) or 16 min before testing them with apomorphine injected at 4, 8, 16, 32 or 64 mg/kg ip. At this dose range, apomorphine alone typically induces climbing and dose-dependent gnawing-while-climbing (directed towards the wire of the cage). These behaviors were quantified with a time-sampling technique (4 periods of 2 min separated by 14 min and organized into 24 point-samples; maximal possible score: 96). Injected alone, cocaine did not produce significant climbing or gnawing-while-climbing. None of the cocaine (30 mg/kg) pretreatments changed apomorphine-induced climbing. Intense gnawing-while-climbing was induced by apomorphine in mice receiving cocaine 256 or 16 min before; dose-response functions for apomorphine were significantly shifted upward and to the left. Cocaine did not influence these behaviors when injected 736 and 1456 min prior to apomorphine test. The fact that cocaine desappears from the rodent brain within 2 hours (e.g. Benuck *et al*., 1987) suggests that the changes seen in mice treated with 256-min-apart cocaine may be due to a transient change of postsynaptic dopamine functioning, and not to a direct potentiation. Such an effect may lead to the development of an animal model of withdrawal after high acute injections of cocaine.

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Classically Conditioned Rotation: Tests with Cocaine and L-DOPA

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It has previously been shown that administration of apomorphine (APO) to rats with unilateral 6-hydroxydopamine lesions of substantia nigra can result not only in an acute episode of rotation (circling), but also in rotation conditioned to the environment in which the APO was administered (Silverman and Ho, 1981). The conditioned rotation can be demonstrated months after even a single APO administration. Here cocaine, an indirect-acting dopamine agonist, and L-DOPA, precursor to the direct-acting agonist, dopamine, were tested to determine if their administration would also result in rotation conditioned to the drug-associated environment. Lesioned rats were placed in the rotation environment and rotation before and after drug administration was recorded. Cocaine treatment resulted in ipsilateral rotation. When subsequently placed, undrugged, into the test environment, rats that had been treated on one or more occasions with cocaine continued to show the slight ipsilateral bias that is characteristic of this lesioned preparation. While conditioning rotation with cocaine may well be possible under other conditions, the striking conditioned rotation seen after similarly limited APO treatment was not seen here after cocaine. L-DOPA, after carbidopa pretreatment, induced the acute contralaterally directed rotation typical of direct agonists. Two weeks after L-DOPA treatment most animals exhibited a brief epoch of rapid contralateral rotation upon being placed in the drug environment. As was the case with APO, rotation could be conditioned with a single L-DOPA treatment, and a small dose was more effective than larger doses in conditioning in one trial. When administered L-DOPA on three consecutive days, a very large increase in response was seen (i.e., sensitization was apparent). Animals treated three times with L-DOPA exhibited rapid conditioned rotation when placed in the drug-associated environment months later.

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Blockade of the Locomotor Stimulant Effects of Cocaine by Potential Antipsychotic Agents Active at Sigma Binding Sites

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Current antipsychotic compounds block some effects of psychomotor stimulants but their utility as therapeutic entities is limited by their potent behavioral activity, their potential for enhancing sensitivity to cocaine and the propensity for extrapyramidal side-effects upon repeated administration. We evaluated the ability of the sigma ligands BMY 14802, rimcazole and NPC 16377 to block the locomotor stimulant effects of cocaine in male, SW mice. The effects of haloperidol and (+)-3PPP which have both dopamine and sigma receptor affinity, and the novel non-sigma antipsychotic clozapine were studied for comparison. Test compounds were given (ip), followed in 15 min by saline or cocaine (ip) and activity levels were evaluated for 30 min.

When given alone, the compounds produced dose-related decreases in activity with a rank order of potency of haloperidol > clozapine > (+)-3PPP > BMY 14802 = rimcazole > NPC 16377. Behaviorally-inactive doses of BMY 14802 (10 mg/kg), rimcazole (10 mg/kg) and NPC 16377 (40 mg/kg) decreased the locomotor stimulant effects of cocaine and shifted the dose-response curve to the right without reducing maximal effect. In contrast, behaviorally-active doses of haloperidol (0.1 mg/kg) or clozapine (1 mg/kg) were required to dampen the stimulant effects of cocaine; higher doses of (+)-3PPP were still ineffective.

The attenuation of the stimulant effects of cocaine by BMY 14802, rimcazole and NPC 16377 suggests a novel approach to the development of effective cocaine abuse treatments. The present results suggest that these compounds might function to block the psychotropic effects of cocaine without interfering with ongoing behavior. The lack of blockade of cocaine stimulation by behaviorally-inactive doses of the non-selective compounds supports the idea that the greater selectivity of BMY 14802, rimcazole and NPC 16377 for sigma binding sites may mediate their unique cocaine blocking effects.

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Regulation of Mu-Opioid Receptors Following Exposure to Cocaine

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Several studies implied that the opioid system may play a prominent role in the neurochemical effects of cocaine. Repeated exposure to cocaine induces elevated levels of plasma beta-endorphin and an increase in dynorphin A(1-8) concentration in limbic structures of rat brain. The findings that the partial opioid agonist, buprenorphine, attenuates cocaine-self administration by monkeys and partially protects against the lethal effects of the drug in mice, further support the involvement of the opioid system in the reinforcing and toxic effects of cocaine.

The present study was undertaken to determine whether repeated exposure to cocaine regulates the mu-opioid receptors. In one set of experiments, male Sprague Dawley rats (230-300 g) were treated (i.p.) with either saline or cocaine 40 mg/kg/day for 7 days, sacrificed 24 h following the treatment, and whole brains (minus cerebellum) were prepared for opioid receptor binding assays. Saturation binding experiments, utilizing the selective mu-opioid ligand [^{3}H]DAGO, revealed 40% reduction in the total number of mu-opioid receptors in membranes derived from cocaine-treated rats (control (n=lO), Bmax=106±7; cocaine (n=lO), 63±4 fmole/mg protein). No apparent change in the Kd value of [^{3}H]DAGO was observed (control, Kd=0.9±0.06; cocaine, Kd=1.03±0.07 nM). In a second set of experiments, the binding properties of [^{3}H]DAGO were examined in various brain regions derived from rats treated with either saline (n=6) or cocaine (n=6) 30 mg/kg (t.i.d) for 7 days. A reduction in the number of mu-opioid receptors was observed in the following brain regions of cocaine-treated rats: hippocampus, amygdala and cerebral cortex (40, 59 and 68% of control, respectively). In the hypothalamus, however, no apparent change in the Bmax or Kd of [^{3}H]DAGO was noted. These results indicate that cocaine induces down-regulation of mu-opioid receptors in critical brain regions of the rat. It is postulated that this phenomenon may be associated with cocaine-craving and the psychological addiction to cocaine.

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Behavioral and Respiratory Effects of Buprenorphine in Monkeys

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Three different preparations were used to determine the receptor types contributing to the behavioral and respiratory effects of buprenorphine, a mixed-action opioid with high affinity for μ and K receptors. In squirrel monkeys trained to discriminate the x-agonist, U-50,488 (0.1 mg/kg IM), from saline, buprenorphine does not substitute for U-50,488; rather, discriminative stimulus and response-rate decreasing effects of U-50,488 were antagonized by buprenorphine. In rhesus monkeys responding under a fixed-ratio schedule of food presentation, buprenorphine, like the opioid antagonist, quadazocine, shifted dose-effect curves for the μ-agonist, levorphanol, to the right. Respiratory frequency *(f)*, tidal volume *(V_T)* and minute volume *(V_E)* were also measured in awake rhesus monkeys breathing air or a mixture of 5% CO_2, in air: Unlike quadazocine, which shifted dose-effect curves for levorphanol-induced decreases in *f*, V_T and V_E to the right, buprenorphine alone decreased *f*, V_T and V_E limiting its ability to antagonize levorphanol over the dose range studied in this preparation. These data are consistent with buprenorphine having actions at both *K* and μ receptors.

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The Effects of Nalbuphine on Cocaine- and Food-Maintained Responding in Rhesus Monkeys

J.B. Kamien and N.K. Mello

An opioid mixed agonist-antagonist, buprenorphine, reduced cocaine self-administration by rhesus monkey by 72 to 93% whereas food self-administration decreased by 28 to 34% (Mello et al., 1989, 1990). We now report the effects of nalbuphine, an opioid mixed agonist-antagonist with a different profile of receptor affinities on responding maintained by cocaine and food. Saline or nalbuphine (0.1 - 3.0 mg/kg/day in 5 ml) were infused over 50 min through one catheter lumen each day. Ten days of saline treatment preceded 10 days of each of four doses of nalbuphine, followed by a saline recovery period. Cocaine (0.05 or 0.10 mg/kg/inj) or food (1 gm banana pellet) were available for 1 hr each on a FR 4 (VR 16:S) schedule of reinforcement, 4 times each day. Nalbuphine reduced cocaine self-administration by all 4 monkeys in a dose-dependent manner. The highest dose of nalbuphine studied (3.0 mg/kg/day) decreased cocaine self-administration by an average of 58% (range=25-96%) in four monkeys, but also reduced food-maintained responding by an average of 69% (range=36-90%) in 3 of 4 monkeys. Nalbuphine increased food-maintained responding in one monkey. Nalbuphine reduced cocaine self-administration most during session 1 (100 min post-nalbuphine), but its effects diminished by session 4 (21.5 hrs post-nalbuphine). Food- and cocaine-maintained responding by all 4 monkeys returned to near base-line levels within 10 to 30 days post-nalbuphine. These results suggest that although nalbuphine (1.0 and 3.0 mg/kg) decreases cocaine self-administration, the effect is not selective since food-maintained responding also decreased. Moreover, these data indicate that nalbuphine, a morphinan, affects cocaine and food-maintained responding differently than buprenorphine.

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Actions of (+)-Buprenorphine on Cocaine and Opiate-Mediated Effects

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The binding profile of (+)-buprenorphine at classical opioid receptors has already been determined (Grayson, et al., *Problems of Drug Dependence 1990,* L.S. Harris (Ed.), NIDA Research Monograph, Washington, DC., 380-81.). Because this isomer exhibits no affinity for opiate receptors, (+)-buprenorphine is useful as a control in studies of antinociception and "kappa abstinence syndrome", a behavior precipitated collectively by (-)-buprenorphine, naloxone, and nor-BNI. Our studies in these areas reveal that the antinociceptive properties of (-)-buprenorphine can be effectively measured using the rat paw formalin test and that the (+)-isomer exhibits no activity in this model. In addition, (+)-buprenorphine does not induce kappa abstinence syndrome under conditions where the (-)-isomer precipitates various stereotypic behaviours associated with this sydrome.

The role of classical opiate receptors in cocaine addiction is now being intensely studied. Recently, (-)-buprenorphine has been found to produce dose-dependent protection against the lethal effects of cocaine in mice. (+)-Buprenorphine does not protect up to doses over 100 times greater than the lowest effective dose of its (-)-enantiomer. Low doses (0.3-1.0 mg/kg) of naltrexone blocked the protective effects of the (-)-isomer and protection was not observed in CXBK mice, a recombinant inbred strain relatively devoid of μ-opioid receptors. These results indicate that the lethal effects of cocaine appear to be mediated through the μ-opioid receptors. Future clinical studies will be necessary to more fully evaluate the utility of buprenorphine in the treatment of cocaine addiction.

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Chronic Buprenorphine Attenuates Cocaine Place Preference

T.A. Kosten, D.W. Marby and E.J. Nestler

Previous research has shown that chronic treatment with the mixed opiate agonist-antagonist, buprenorphine (BUP), reduces cocaine (COC) use among opiate addicts (Kosten et al., 1989) and suppresses COC self-administration in primates (Mello et al., 1989). We investigated the effects of chronic BUP administration on the development of COC conditioned place preference (CPP). CPP is a drug abuse model in which rats exposed to COC in a distinctive place show enhanced preference for this place after training. CPP is based on classical conditioning; self-administration is based on operant conditioning.

Rats were injected with BUP (0.5 mg/kg s.c. 2x/day) or vehicle for 1 wk prior to and during CPP training. After baseline assessments, rats were trained by pairing either saline (SAL) or COC (15 mg/kg i.p.) injections with side 1 for 4 days. CPP was assessed by comparing time spent on this side after training to baseline (change in min).

Vehicle treated rats (n=12) trained with COC showed an 8.6 ± 1.0 min CPP compared to the 0.7 ± 0.6 min shown by rats (n=9) trained with SAL. BUP treated rats (n=8) trained with COC showed significantly less CPP at 3.9 ± 0.8 min and BUP treated rats trained with SAL (n=6) showed a 1.9 ± 1.1 min CPP, F's(1,33)=23.0 (COC) and 5.8 (COC X BUP); p's<0.05. CPP training with BUP (0.5 mg/kg, s.c.) without chronic treatment did not lead to CPP (BUP: -1.6 ± 1.6 vs SAL: 1 .0 ±. 1.5 min).

These results with CPP extend the finding that chronic BUP diminishes the reinforcing effects of COC to an animal model based on classical conditioning. The mechanism of chronic BUP action is unknown. One possibility is that chronic BUP influences signal transduction pathways in the mesolimbic dopamine system believed to be involved with drug reinforcement (Terwilliger, et al, 1991).

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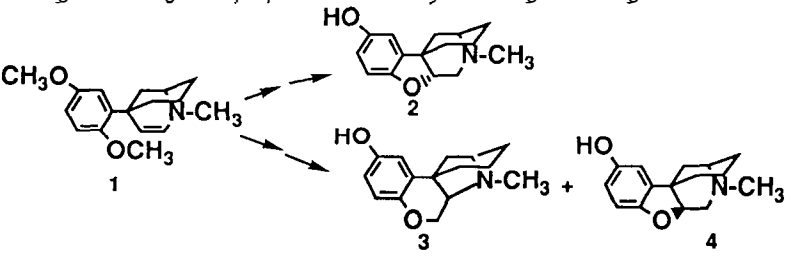
Epimeric Oxide Bridged 5(m-hydroxphenyl) Morphans as Probes for Narcotic Receptor-Mediated Phenomena

D. Tadic, J.T.M. Linders, S. Mirsadeghi, R.B. Rothman, H. Xu, A.E. Jacobson and K.C. Rice

5-(m-Hydroxyphenyl)morphans are strong analgesics which have unusual properties. Structurally they differ from "classical" opioids (e.g., morphine). These phenylmorphans have phenyl ring in the equatorial position with respect to the piperidine ring. Both of their enantiomers exhibit opioid activity, in contrast to the phenyl axial opioids, where analgesic activity is expressed in one antipode. Moreover, the (+)-isomer is a good suppressor of morphine abstinence phenomena while the (-)-isomer precipitates withdrawal in morphine dependent monkeys. As part of our study of the opioid receptor system, we have undertaken the synthesis of a series of conformationally restricted, oxide-bridged 5(m-hydroxyphenyl)morphans in order to relate their conformation to the agonist/antagonist properties shown by nonbridged analogs.



Here we report the approach to the synthesis of the epimeric compounds **2** and **4**, through the common intermediate enamine **1**. Construction of the 5-phenylazabicyclo[3.3.1]nonene **1** was accomplished by the application of the method of Evans and Zimmerman. Bromination of **1**, followed by the reduction of the double bond afforded a single bromo epimer, which was converted to **2** by O-demethylation with BBr_3 and base-mediated epoxy ring closure.

Also, the nucleophilic displacement of the bromide obtained from **1** was accomplished by treatment with potassium benzoate which afforded benzoate ester with inversion of configuration. Hydrolysis to the corresponding alcohol followed by esterification gave the unstable mesylate. O-Demethylation with BBr_3 followed by work-up with aqueous KOH resulted in the predominant formation of the tetrahydropyrane derivative **3** and traces, in variable yields, of a compound whose spectroscopic properties suggest **4**. Work is in progress on the improvement of the reaction conditions required for the formation of the desired compound **4**.

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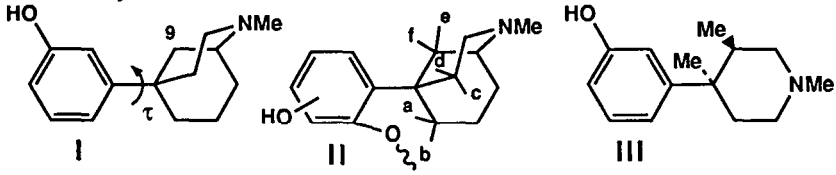
Computer-Assisted Molecular Modeling of Phenylomorphans

J.T.M. Linders, A.E. Jacobson and K.C. Rice

5-(*m*-Hydroxyphenyl)-2-methylmorphan (**I**) and derivatives show an interesting spectrum of activities ranging from potent analgesia to moderate narcotic antagonism, depending both on the absolute configuration and on substituents at C-9. In part, these different activities may be linked to the occurrence of different conformations. Restriction of the rotation by a bridging unit, such as the oxygen in the oxide-bridged phenylmorphans **II**, defines the spatial relationship between the orientation of the nitrogen lone pair, the phenolic OH, and the aromatic ring. These structural data can be used to describe a pharmacophore and relate receptor affinity and selectivity to a conformation.



I **II** **III**

Molecular mechanics calculations on **I** show that the rotational barriers and the differences in energy between the conformers of **I** are too low to be biologically relevant. It is noteworthy that the X-ray conformation is significantly different from any of the minimum-energy conformations of **I**. Receptor points, representing hydrophobic interaction with the aromatic ring and a hydrogen bond to the nitrogen, were built onto the minimum-energy conformations of **I**, **III** (a potent antagonist with an SAR pattern similar to **I**), and all isomers of **II**. When overlayed, the f-isomer (a weak antagonist) and also the, yet unsynthesized, c-isomer show an excellent fit with energy minima of **III** and **I**. The a- and d-isomers, which have low affinity for opiate receptors, show close similarity to high-energy conformations of **I**.

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Structural and Electronic Requirements for Binding at the Mu-Opioid Receptor in the Fentanyl Class of Compounds

C. Cometta-Morini, P. Maguire and G.H. Loew

A combined theoretical and experimental study of selected flexible and conformationally constrained fentanyl analogs with varying μ-receptor affinity and selectivity was undertaken to characterize the molecular requirements for recognition of this family at the μ-receptor. An extensive conformational search of the parent compound, three flexible (R30490, lofentanil and carfentanil) and two rigid derivatives was carried out using a) Molecular Dynamics simulations at different temperatures (CHARMm potential energy function) and b) nested rotations searches (AM1 semiempirical quantum mechanical method) with full optimization of all geometrical variables. Combined analysis of the results of the conformational search and of our own competitive binding studies, led to the characterization of a proposed bioactive form. In a subsequent innovative step we computed structural indicators, environmental indices and a series of electronic properties (charges, dipole, polarizabilities, proton accepting and donating capabilities, $^{E}_{HOMO}$, $^{E}_{LUMO}$ and frontier orbital charges) for all considered analogs. As a result of these studies we were able to obtain a specific set of steric and electronic criteria for recognition common to all high affinity analogs. Four key regions of the ligand are essential for recognition in the fentanyl class of compounds: 1) a protonated center involved in electrostatic interaction with an anionic site on the receptor, 2) a polar proton accepting function involved in hydrogen bonding with a proton donating site on the receptor, 3) one aromatic moiety involved in lipophilic interaction with a similar site on the receptor and 4) a second aromatic moiety involved in electron transfer interaction with an aromatic site on the receptor. In the proposed μ-pharmacophore two angles strictly define the spatial relationship between the four recognition points. Further validation of the model is taking place in ongoing studies of other u-selective analogs from different families of opioids.

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Mechanistic QSAR of δ-Selective Cyclic Opioid Peptides

H.O. Villar, C. Chew and G.H. Loew

Theoretical studies have been carried out on twelve cyclic analogs of [D-Pen2,D-Pen5] enkephalin to identify the bioactive form and common molecular determinants for recognition at the δ--opiate receptor. The compounds studied included high affinity ligands DPDPE, DPLPE, DPDPE-NH2, DCLPE, DPLCE, DCLCE, DCDCE, 3R-Methyl-DCDCE and DCDPE as well as low affinity analogs LCLCE, [D-Phe^4] DPDPE and [SAR^3] DPDPE. The criteria used to identify this bioactive form was its accessibility and similarity for all high affinity analogs and its absence for low affinity ones. Another unresolved question addressed was the most appropriate environment i.e. low or high dielectric for formation of the bioactive form. As a first step, conformational studies were made using a search strategy that allowed broad sampling of conformational space and assured the identification of accessible energy domains for each peptide. The strategy consisted of systematic generation of initial conformers by a combination of nested rotations and ring closing algorithms, followed by cycles of high (900K) and low (312K), molecular dynamics simulations which allowed a broad sampling of conformational space and local refinements of geometries respectively. This procedure was repeated for low (ε = r) and high (ε = 80) dielectric media, since peptide conformation is highly susceptible to environment. As a result of this step, 8-20 low energy (Δ]E < 5Kcal/mol) conformers were obtained in each environment for each analog. The bioactive form was selected by specific comparison among all low energy forms among all 12 analogs. These comparisons included both steric and electronic components, such as rms of the disulfide ring, average distances between the aromatic rings of Tyr and Phe, patterns of hydrogen bonding and globularity. After extensive and systematic comparison, a unique conformer in a dielectric of 80 but not in low dielectric was found to fulfill all the criteria. This bioactive form can now be used as the basis for design of δ--selective peptide surrogates and peptidomimetics.

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Synthesis and Evaluation of Conformationally Unrestricted Ethylenediamine Derivatives as Sigma Receptor Ligands

L.A. Radesca, W.D. Bowen, L.J. DiPaolo, and B.R. de Costa

Sigma receptors were first studied for their ability to bind psychoactive drugs such as SKF10,047, haloperidol, phencyclidine, dextromethorphan. Very little is known about the functional role of these receptors due to the shortage of compounds that bind not only potently but also selectively.

We recently reported that (+) and (-) cis-N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl)cyclohexylamines are very potent and specific sigma ligands. They exhibited affinity constants (K_i of 6.0 and 1.3 nM respectively when tested against [^{3}H]-(+)-PPP in guinea pig brain. Removal of the cyclohexane ring from either of these diamines gave rise to the corresponding ethylenediamine derivative which exhibited an increase in affinity (K_i=0.34 nM). With these results in hand an extensive structure-activity relationship (SAR) study was performed in order to determine the requirements for high sigma affinity.

Twenty two compounds were synthesized and tested and the following conclusions were drawn: 1) N-methyl is the optimal N-substituent for high affinity; 2) two tertiary amines are required: 3) any substitution on the ethylene moiety lowers the affinity; 4) the affinity decreases by opening of the pyrrolidine ring; 5) substitution of pyrrolidine by either piperidine or homopiperidine rings increases the affinity; 5) both chlorine atoms are necessary: although the meta is slightly more important than the para; 6) the optimal distance between the nitrogens is two carbon atoms; 7) there is no change in the affinity when the distance between the nitrogen and the phenyl group is changed (for example one carbon atom). In this study nine of the twenty-two compounds exhibited an affinity of less than 1 nM; these subnanomolar affinity compounds showed no affinity for kappa-opioid, PCP and D_2-dopamine receptors, those which commonly cross-react with sigma ligands.

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The Agonist Pharmacophore of the Benzodiazepine Receptor, Synthesis of a Selective Anticonvulsant/Anxiolytic

H. Diaz-Arauzo, P. Skolnick and J.M. Cook

The benzodiazepines exhibit a wide range of pharmacological actions which include anxiolytic, anticonvulsant, sedative/hypnotic and myorelaxant effects mediated by specific binding sites in the central nervous system. The benzodiazepine receptor (BzR) is one constituent of a supramolecular complex which also contains discrete, but allosterically coupled recognition sites for GABA and barbiturates. The pharmacological properties of BzR ligands appear to be a continuum, ranging from a complete mimicry of 1,4-benzodiazepines (such as diazepam) to substances termed inverse agonists that produce actions best described as oppostie to the benzodiazepines. Despite advances at the molecular level, the search continues for selective anxiolytics which are devoid of the other effects typical of the 1,4-benzodiazepines. Recently, a computer-assisted analysis of the pharmacophore for the agonist domain of the BzR has been developed based on the SAR of agonist ligands and molecular modeling (E/S-390, SYBYL). Based on this model, the selective agonist 6-(n-propoxy)-4-(methoxymethyl)-ß-carboline-3-carboxylic acid ethyl ester was synthesized and screened in mice for efficacy at BzR. This agonist bound tightly to BzR (IC_{50} = 8nM) and demonstrated potent anticonvulsant (ED_{50} = 1.6 mg/kg)/anxiolytic effects *in vivo* Moreover, this agent was devoid of myorelaxant/ataxic activity at a dose of 20 mg/kg and completely antagonized the muscle relaxant/ataxic effects of diazepam. The ß-carboline, 6-(n-propoxy)-4-(methoxymethyl)-ß-carboline-3-carboxylic acid ethyl ester is, therefore, a selective anxiolytic/anticonvulsant devoid of the other properties common to 1,4-benzodiazepines.

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Small-Angle X-ray Diffraction Study of Interactions of Cannabinoids with Model and Biological Membranes

D.-P. Yang, T. Mavromoustakos and A. Makriyannis

Small angle X-ray diffraction has been used to study the topography of (-)-Δ^8-tetrahydrocannabinol (Δ^8-THC) and its pharmacologically inactive methoxy analog (-)-O-methyl-EΔ^8-THC (Me-Δ^8-THC). The membrane preparations included partially hydrated bilayers of synthetic phospholipids (DMPC, DOPC, OPPC, sphingomyelin), bovine brain phospholipid extract with added cholesterol, bovine brain total lipid extract and natural membrane bilayers of bovine synaptosomes. Our partially hydrated membrane preparation exists as a stack of lamellae which allows us to obtain coherent Bragg-like diffractions. The diffraction patterns were analyzed to provide us with the total period repeat distance (d-spacing) and the electron density profiles of the bilayer. Parallel experiments were carried out using membrane preparations in the absence and presence of Δ^8-THC or Me-Δ^8-THC. Comparisons between electron density profiles from drug-containing and drug-free membranes showed that Δ^8-THC resides near the interface of the bilayer as a result of amphipathic interactions of Δ^8-THC with the membrane. Me-Δ^8-THC is distributed in two distinct sites in the hydrophobic region of the membrane bilayer. A fraction of the Me-Δ^8-THC molecules intercalate between the acyl chains of contiguous lipid molecules near C_7 and are located deeper than the parent Δ^8-THC; the remaining Me-Δ^8-THC in all likelihood exists as small aggregates between the two leaflets of the membrane bilayer. This observation agrees with our recent data from solid state ^{2}H-NMR and DSC experiments of Me-Δ^8-THC in model membranes. Our results point out that these two structurally-related, but pharmacologically very different cannabinoids interact with membrane in strikingly different manners and are consistent that amphipathic molecules (Δ^8-THC) engage in different interaction with the membrane bilayer than structurally similar non-amphipathic ones (Me-Δ^8-THC). This may lead us to a better understanding of the molecular features of amphipathic drug-membrane interaction and the structure-activity relationships of cannabinoids.

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Interactions of Cannabinoids with Model and Biological Membranes Studied by Solid State ^{2}H-NMR

A. Makriyannis, D.P. Yang and T. Mavromoustakos

Specifically ^{2}H-labeled (-)-Δ^8-tetrahydrocannabinol(Δ^8-THC) and its pharmacologically inactive methoxy analog (-)-0-methyl-Δ^8-THC (Me-Δ^8-THC) have been used in solid state ^{2}H-NMR experiments with model and biological membranes. The ^{2}H-labels on the cannabinoid tricyclic ring system gave rise to the quadrupolar splittings which were used to determine their preferred orientation and location in the membrane bilayer as functions of drug concentration and temperature. We have found that, when incorporated in the bilayer up to 20 molar percent, the biologically active amphipathic cannabinoid Δ^8-THC completely intercalates between the acyl chains of the contiguous lipid molecules. On the other hand, the pharmacologically inactive and non-amphipathic analog Me-Δ^8-THC distributes in two sites in the membrane according to an equilibrium which is concentration and temperature dependent. For Me-Δ^8-THC concentrations up to 5 molar percent, all of the Me-Δ^8-THC molecules intercalate between the lipid acyl chains in the bilayer. When more Me-Δ^8-THC is added, it forms small aggregates in the center of the bilayer which produce pseudo-isotropic ^{2}H-NMR spectra. The formation of such aggregates is enhanced at lower bilayer temperatures. When intercalating between the lipid acyl chains, the two cannabinoid analogs assume very different orientations. Δ^8-THC, which has a free phenolic hydroxyl, assumes an orientation with its long axis perpendicular to the lipid chains such that the OH group is pointing towards the polar side of the membrane in order to maximize the amphipathic interaction. Conversely, Me-Δ^8-THC orients with long axis parallel to the rotation axis. Our results emphasize the key role played by the polar group (OH) of the amphipathic cannabinoid during its interaction with the membrane. Absence of such a group transforms the molecule into one having strongly hydrophobic properties and interacting very differently with the bilayer. We have extended the current methodology used to calculate the orientations of molecules in anisotropic environments so that it now has general applicability.

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Methadone Dosing Level: Effects on Treatment Outcome

E.C. Strain, M.L. Stitzer, G.E. Bigelow and I. Liebson

While the current medication of choice in the treatment of opioid dependence is methadone, the optimal dosing of methadone remains an issue with some programs still committed to low dose regimens. The purpose of this study was to compare the relative efficacies of 50 mg and 20 mg of methadone, to methadone-free treatment, in a contemporary population of opiate addicts. Participants were 212 opiate-dependent addicts (70% male; 51% black, 49% white) enrolled in a 182 day short-term double-blind methadone treatment program. Subjects were stratified by gender and race, and randomly assigned to one of three fixed-dose treatment conditions. High dose methadone (HDM) patients had dose increases until they were on 50 mg per day in week 6, middle dose methadone (MDM) patients were maintained on 20 mg of daily methadone starting in week 6, and low dose methadone (LDM) patients were detoxified from 25 mg at a rate of 5 mg per week. Daily doses of 50, 20, and 0 mg were maintained during a stable dosing period of 14 weeks (through week 20); HDM and MDM subjects were detoxified over the last 6 weeks of treatment. Results show treatment retention was directly related to the size of methadone dose (survival analysis, $p<.00l$). Mean days in treatment were: HDM, 134 days; MDM, 110 days; and LDM, 85 days (ANOVA, $p<.001$); each group differed from the other groups in post-hoc testing. 63% of patients in the HDM, 48% of patients in the MDM, and 24% of patients in the LDM group remained to week 20. Attendance (percent of doses ingested while actively enrolled in treatment) was 87% for the HDM, 83% for the MDM, and 81% for the LDM groups. Urine testing results for opiates and cocaine, summarized in two week blocks during the stable dosing period, were analyzed for patients retained through week 20. The percentage of opiate positive urines differed significantly between the three groups, (LDM>MDM>HDM). There was a trend for the HDM group to have a lower percentage of cocaine positive urines. The HDM group had a significantly lower percentage of urines simultaneously positive for opiates and cocaine; they did not have a compensatory increase in cocaine positive urines which were negative for opiates. Thus, high dose methadone decreased opiate, and mixed opiate/cocaine use. Furthermore, these orderly dose-related results suggest that clinical efficacy may continue to increase at doses higher than 50 mg of methadone. These results illustrate the importance of methadone dose in decreasing opiate and mixed opiate/cocaine use, and in maintaining patients in treatment. (Supported by USPHS grant DA05792; conducted at Francis Scott Key Medical Center, Johns Hopkins School of Medicine, Baltimore, MD.)

A Repeated Treatment Design with Longitudinal Self-Report Data: Cumulative Versus Stabilizing Effects of Methadone Maintenance

K.I. Powers and M.D. Anglin

Research studies based on longitudinal self-report data have been criticized for two major reasons, (1) low reliability and validity, and (2) "self selection" phenomenon due to lack of random sampling. Because of these problems, many researchers recommend applications of experimental designs with random sampling and control groups to drug abuse studies. The present paper reviews strengths and weaknesses of survey and experimental approaches and suggests that survey approaches, if combined with appropriate research design and statistical analysis, can provide sound and valuable information on various sociological and psychological aspects of drug abuse. This argument is supported by reviewing previous research studies which discuss reliability and validity of longitudinal self-report data and those which have successfully integrated this type of data with appropriate research design and analytical approaches. Finally, an application of a repeated treatment design to self-report data of narcotics addicts was demonstrated to assess the cumulative versus stabilizing effects of methadone maintenance. The analyses examined the nature of methadone maintenance effects on narcotics use, related crime, and other behavioral variables for addicts experiencing more than one treatment episode. Data were based on retrospective self-report information collected from approximately 1,000 narcotics addicts who were admitted to methadone maintenance treatment in Southern. California during 1974-1978. The pattern of behavioral changes across treatment episodes on both mean percentage of time using narcotics daily and abstinence from narcotics clearly indicated stabilizing effects by methadone maintenance. Other behavioral variables, e.g., dealing, employment, property crime, also showed stabilizing effects of methadone maintenance, but with a smaller degree of effectiveness. On the other hand, although a slight trend in improvement over multiple episodes was observed for the narcotics use and crime variables, the trend was not statistically significant. No indication of cumulative effects was observed for the other variables.

AFFILIATION: University of California, Los Angeles

Buprenorphine vs. Methadone for Opioid and Cocaine Dependence

T.R. Kosten, R.S. Schottenfeld, C.H. Morgan, J. Falcioni and D. Ziedonis

Recent work in humans (Kosten et al, 1989) and primates (Mello et al., 1989) has suggested that buprenorphine may reduce cocaine abuse/self-administration. Buprenorphine (B) at 2 mg and 6 mg sublingually was compared to methadone (M) at 35 mg and 65 mg during a 24 week trial in 127 opioid dependent patients. In preliminary analyses the average retention was 18 ± 7 weeks with lower retention in the B than in the *M* groups (15.5 vs. 20 weeks). Urine toxicologies for opioids averaged 68% in the B group and 43% in the M group, which was significantly different. Cocaine abuse was also detected in 47% of the patients with no significant difference across treatment groups at week one. Maximum reduction of cocaine abuse was greater for the 6 mg B than the 2 mg B group (71% vs. 28%) and the M groups had an intermediate rate of reduction at 40%. Overall rates of cocaine abuse were not significantly different between the M and B groups. Among the cocaine abusers, the average amount of money spent per week on cocaine averaged $30 and did not significantly differ between the two treatment groups. Thus, B does not appear to have better efficacy than M in the treatment of cocaine abusing addicts, although higher dosages of B appear to be more efficacious than lower dosages of B in reducing cocaine abuse.

AFFILIATION: Yale University School of Medicine, New Haven, CT

Pilot Study with Low-Dose Buprenorphine

P. Banys, D.J. Tusel, H.W. Clark, K. Sees, L. Mongan, J. Nathan and E. Callaway

Buprenorphine (BPN) is a prescription analgesic with mixed opiate agonist and antagonist properties. Although normally injected for pain relief, it is also effective in a sublingual route of administration. A prior pilot study (Mongan and Callaway, 1990) with psychiatric inpatients revealed that some subjects are highly responsive to very small sublingual doses of BPN. We designed a pilot study with methadone-dependent patients to determine whether low doses (0.15-0.3) of sublingual BPN would relieve opiate withdrawal symptoms. We wished to identify characteristics of responders and non-responders.

Eight male subjects from a methadone maintenance program were recruited for a 46 hour study carried out on the Substance Abuse Inpatient Unit. All experienced mild to moderate withdrawal 26-31 hours after their last methadone dose (35-50 mg.) Once in withdrawal, the subjects were given a unit dose (0.15 mg.) of BPN administered sublingually. A second unit dose could be repeated in an hour, and a two-unit (0.30 mg.) dose could be given in two hours if the subject obtained no relief of withdrawal.

Withdrawal was measured by subject self-report, research staff observation, and several withdrawal scales including an analog scale, a modified Himmelsbach withdrawal scale, and the Addiction Research Center Inventory (ARCI) Opiate Withdrawal Scale (List 116). Vital signs were recorded hourly.

In five cases, the subjects responded to a low dose of 0.15 to 0.3 mg sublingual BPN within a time period ranging from 15 minutes to 2.5 hours. “Responded” in this context means that the subjects reported that their withdrawal symptoms has completely disappeared. Typically they remained comfortable for about eight hours following initiation of BPN. Three subjects failed to experience relief of withdrawal symptoms even after three hours and a total of 0.6 mg BPN. These three were considered non-responders and were given no more BPN.

There were no obvious factors distinguishing responders from non-responders, however, all three non-responders were subjects of Hispanic heritage. All other subjects *were* of European, British, or Black African descent. The two Caucasian subjects required 1-2 hours to respond to BPN The one African-American subject required 20 minutes. This is consistent with what was found in the previous pilot study with psychiatric inpatients, most of whom had substance abuse histories, in which 9 out of 12 subjects responded to BPN with an increased sense of relaxation, comfort, and talkativeness. However, response times for Black-Americans and Caucasians differed: Black responders noted changes within 2 to 40 minutes. All Caucasian responders required at least 2 hours.

VA Medical Center, San Francisco; Univ. of CA, San Francisco and San Francisco NIDA Treatment Research Unit

Assessment of Abuse Liability of Transnasal Butorphanol vs. Placebo in Treatment of Chronic Pain

G. Gribkoff, G. Chu and R.E. Pyke

Transnasal butorphanol tartrate (TNBT) was tested in 303 patients with chronic pain of non-malignant origin in a double-blind placebo-controlled multi-center clinical trial of up to 6 months duration. Patients with arthritis pain or lumbar musculoskeletal pain were treated with TNBT 0.5 to 16 mg/day at 18 centers. Ninety-nine patients were treated with transnasal placebo solution. Codeine was allowed as an adjunct analgesic. One week after discontinuation, a post-study assessment for withdrawal symptoms was made. Retention in study for one month or more was achieved by approximately 65% of the TNBT treated patients. Only two cases of moderate withdrawal syndrome from TNBT were diagnosed (0.7%) and one case of problematic euphoria (0.3%). Both cases of withdrawal followed abrupt discontinuation in patients who exceed the maximum dose of 16 mg/day. Data searches disclosed additional possible mild withdrawal symptoms at a maximum incidence of 1% each, vs 0% with placebo. Patients who use more than 10 mg TNBT per day for 4 weeks or more should undergo tapering of medication over approximately one week. The low abuse liability of TNBT makes it a promising alternative to oral opioids for chronic pain of non-malignant origin.

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Fluoxetine and Behavioral Factors in Treatment of Cocaine Dependence

J. Grabowski, R. Elk, K. Kirby, C. Cowan and H. Rhoades

Early open studies have reported efficacy of desipramine and other drugs in treatment of cocaine dependence (e.g. Kosten 1991; Gawin, 1991). Subsequent blind studies have indicated limitations and suggested that efficacy may reside in careful matching of patient characteristics and the pharmacological agent (Kosten, 1991). The need for carefully controlled double blind studies is clear.

This ongoing study is examines the joint action of fluoxetine and clinic visit frequency in cocaine treatment (3x2; 6 grps, 26 Ss per group). The intake reviews all major areas (AS1/Beck/POMS/Hamilton), medical status including HIV and TB, and psychiatric evaluation (SCID/DSM IIIR). Patients are assigned and begin the two week stabilization phase within three days. Fluoxetine doses are 0 mg, 20 mg and 40 mg. Medication effect is examined in the context of patients receiving either 2 or 5 take home doses per week (clinic visits either 5 or 2 times per week). One intensive individual counselling session each week includes review of major areas of function. Specific behaviorally based recommendations are made to prevent drug use. Urine drug screens are conducted twice each week and paper and pencil measures are obtained weekly.

A planned interim analysis of the medication effects on cocaine dependence has been delayed. Results are available to' describe the groups in terms of visit frequency and patient characteristics. Drop out has averaged 50% within the first two weeks across all groups for these cocaine dependent patients. On average patients with Low Frequency Take Homes (LFTH) of placebo or fluoxetine (required five days per week visits) for the brief "pharmacy visit", have had lower drop out rates than those for whom only two visits per week are required. Patients in LFTH, have had a lower percentage of cocaine positive urines screens but have had much higher marijuana positive urines. There have been only two cases where the blind has been broken due to untoward presumed side effects; both were found to be active drug patients at the high dose. One had pervasive rash and the other had persistent nausea and vomiting. In a separate double blind study of fluoxetine in methadone patients suggestive but *transient* group differences (placebo v. fluoxetine) emerge early in treatment but then diminish.

Fluoxetine was selected due to putative neurochemical advantage, efficacy as an antidepressant, and side effects profile. Visit frequency is thought to be an important feature of treatment although little attention is given to intensity. In this study, high frequency brief pharmacy visits in the clinic setting conveyed advantage in cocaine dependence. Data collected to date on fluoxetine v. placebo suggest no major differences that necessitate or commend breaking the blind.

AFFILIATION: University of Texas Hlth Sci Ctr-Houston, Houston, TX

Differential Effects of Serotinin Uptake Inhibitors on Addictive Behaviors in Alcoholics

K.E. Kadlec and C.A. Naranjo

Serotonin uptake inhibitors have been postulated as pharmacological adjuncts to treat addictive behaviors. To test their true pharmacologic effects we conducted randomized, placebo-controlled, double-blind trials, with serotonin uptake inhibitors: zimeldine 200 mg/day (n= 13), citalopram 40 mg/day (n=19), viqualine 200 mg/day (n=14) and fluoxetine 60 mg/day (n = 11), in non-depressed moderately-dependent alcoholics. Behaviors were monitored by self-report and objectively, but no formal advice was given. Serotonin uptake inhibitors decreased alcoholic drinks by averages (%) of 14.2 (zimeldine), 16.2 (citalopram), 20.5 (viqualine), 17.3 (fluoxetine) and all placebos by <5%. Daily nonalcoholic beverages, monitored in 2 studies, increased during fluoxetine to 5.6±0.3 ($\bar{x}$±SEM) from 5.0±0.4 during baseline ($p<0.05$), but viqualine had no effect. Smoking (cigarettes/day in daily smokers, serotonin uptake inhibitors vs placebo) was not affected by zimeldine (25.9±3.9 vs 27.1±3.1, n=.5), citalopram (19.1±4.4 vs 19.6±4.2, n=8) or viqualine (33.1±7.6 vs 36.3±6.6, n =6) (all $p>0.05$), but increased with fluoxetine (n=7) to 26.9±4.5 from baseline (25.1±4.6) (p 0.05). Body weight (kg) decreased with serotonin uptake inhibitors vs placebo: 4 weeks citalopram (80.4±2.4 vs 81.7±2.5), 2 weeks viqualine 76.8±3.2 vs 77.7±3.2), 4 weeks fluoxetine (79.2±2.5 vs 81.4±2.6 baseline)), (all $p<0.05$) and one week zimeldine (76.2±3.1 vs 76.8±3.1, $p<0.l$). Decreases in weight and alcoholic drinks did not correlate. Appetite loss was frequent during citalopram and fluoxetine. In another study (n=16) desire to drink decreased during one week citalopram 40 mg/day vs. placebo ($p<0.05$), and correlated ($r=0.5$, $p<0.01$) with change in alcoholic drinks (4.6±0.6 (citalopram) vs 5.7±0.8 (placebo), $p<0.05$). These findings indicate that serotonin uptake inhibitors differentially alter addictive behaviors in alcoholics. The have no consistent effect on smoking or nonalcoholic beverage intake, but consistent decreases in alcohol intake, urges to drink and body weight are clinically important.

AFFILIATION: University of Toronto, Toronto, Canada

Waiting for Drug Treatment: A Naturalistic Study

K. Holloway, L. Handelsman and M. Aronson

Little is known about the behavior and treatment response of drug addicts on waiting lists for inpatient treatment. One hundred eight cocaine dependent veterans were enrolled in a 2 week waiting list prior to admission to a detox ward. Two to three days after initial presentation, they were screened medically and psychiatrically. Amantadine 200 mg qd was offered; disulfiram 250 mg qd was also offered when alcohol use was believed to trigger cocaine use. Patients were asked to visit the clinical 5x/wk to complete a drug use and craving form. Of the 108 patients, 32 received amantadine, 18 patients were treated with both medications, 37 patients received no medication. The medicatedgroup used less daily cocaine than the group that refused medication, [$3.92±7.34 vs. $11.32±3.92, t=2.45, p<.01 (one-tailed)]. This was due mainly to a decline in cocaine use during the first week. Craving and retention were not strongly related to taking medication. The differential response to taking medication was not related to demographic factors or drug history. Self-selection of medication was related inversely to age and prior lifetime history of polysubstance use. There were no untoward effects of medication.

AFFILIATION: Psychiatry Service, Veterans Administration Medical Center, Bronx, NY

Depresssion as a Treatment Matching Factor in Cocaine Dependence Treatment

D. Ziedonis and T. Kosten

The role of pharmacotherapy in the treatment of cocaine dependence may be important in specific sub-populations of cocaine abusing patients. In this study we compared the pharmacotherapy response in depressed (n = 20) versus non-depressed (n = 74) cocaine abusing methadone maintenance patients in a twelve week randomized, double blind trial using amantadine 300mg daily (n=33), desipramine 150 mg daily (n=30), and placebo (n=31).

In comparing the outcomes of the two placebo groups, the non-depressed patients had significantly better treatment outcome compared to the depressed patients. The non-depressed patients had better retention (92% versus 67%), had more of a decrease in the amount of money spent on cocaine (43% versus 17%), a decrease in Beck Depression Inventory scores by 55% compared to the 76% increase in scores by the depressed patients, and far more patients had at least two weeks of cocaine-free urines (32% versus 0%).

In comparing the outcomes of the depressed patients, the medicated depressed patients reported significantly less cocaine usage than the placebo depressed patients by week three. At week twelve the medicated depressed patients (in treatment) reported a 84% decrease in cocaine usage (versus 17% decrease) and a 48% decrease in cocaine craving (versus a 29% increase). During the last two weeks of treatment, 42% of the urine toxicologies of the medicated depressed patient's were cocaine free compared to only 6% of the placebo treated "depressed" patients.

These results indicate that relapse prevention alone can be somewhat effective for non-depressed patients, but not for depressed patients. "Depression" appears to be an important predictor of a good response to pharmacotherapy in cocaine abuse treatment, and thus an important factor in matching patients to specific cocaine abuse treatments.

AFFILIATION: Yale University Sch of Med., New Haven, CT

Are All Placebos Created Equal? An Evaluation of Nicotine Polacrilex Placebo Doses

S.J. Leischow, D.P.L. Sachs and N.L. Benowitz

Placebo doses of nicotine polacrilex should have similar sensorial effects to treatment doses, but dissimilar pharmacologic effects. In most drug trials, the placebo includes no pharmacologic agent; however, this has not been the case with nicotine polacrilex. Three different formulations have been used as placebos (containing 0, 0.5, and 1.0 mg nicotine), though direct comparisons between the 3 have not been made. This study evaluated whether there are sensory differences between these 3 placebo doses of nicotine polacrilex relative to treatment doses (2 or 4 mg nicotine), and whether either the 0.5 or 1 .0 mg (unbuffered) doses are pharmacologically active (i.e. produce significant changes in serum nicotine or cotinine levels). With this information, an investigator can make a rational choice of which placebo dose to use in clinical trials.

Twenty three healthy smokers (13 ♂, 10 ♀) participated in the 5 day within groups study, where all subjects received, on different days and in random order, 5 pieces of each of the 5 doses of polacrilex. Subjects visited the clinic each day in the morning and afternoon, and chewed the polacrilex between visits while maintaining smoking abstinence. Serum nicotine and cotinine were assessed at each visit, and a sensory questionnaire was administered at and between visits.

After 5 hours use, the 0, 0.5, 1.0, 2.0, and 4.0 mg doses resulted in serum nicotine levels of 1.3, 1.8, 3.5, 6.3, and 13.7 ng/ml, respectively - a significant overall dose effect. In addition, the placebo doses alone resulted in a significant linear trend in serum nicotine. Of the sensory effects, overall dose effects were found in spicy flavor, peppery flavor, nicotine flavor, burning and tingling in the mouth, and saliva production. When linear trends were assessed in the placebo doses, the only trend was in nicotine flavor, with the greatest flavor found in the 1.0 mg placebo dose. The placebo doses were equivalent on all other sensory characteristics.

Since the 0 mg placebo achieves, with one exception, sensory effects that are comparable to the nicotine-containing placebo doses, it is recommended over the 0.5 and 1.0 mg doses as the nicotine polacrilex placebo of choice in clinical trials. The 0 mg placebo has sensory effects that are quite similar to the placebo doses which contain nicotine, yet has pharmacologic effects that are least similar to the treatment doses.

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AFFILIATION: Palo Alto Center for Pulmonary Disease Prevention and San Francisco General Hospital, San Francisco, CA

Nicotine Transdermal Patch, Smoking Cessation, and Gender

D.P.L. Sachs, U. Sawe and S.J. Leischow

Clinical trials over the last 15 yrs have clearly shown that nicotine replacement therapy using nicotine oolacrilex (Nicorette). clearly improves tobacco dependence treatment results. We now report the 6-mo results using a 16-hr, nicotine transdermal patch (NTP) (manufactured by Kabi Pharmacia Therapeutics AB & Cygnus Research Corp.), with results stratified by gender and also by level of nicotine dependence. The trial, involving 220 subjects (Ss) (90 males and 130 females), was randomized, double-blind, placebo-controlled. All Ss were motivated to quit smoking and were not paid for oarticipation. Each set an individual Target Quit Date (TQD) and developed an "Action 'Plan" to help 'cope with cigarette urges. They were instructed to stop smoking and began patch use on TQD. Ss were seen the day before TQD. and then 1, 2, 3, 6. 12. 15, 18, & 26 wks after TQD. Ss received no group counseling or psychological treatment; they did. however. receive brief (5-10 min) common sense advice from a project nurse. designed to supplement the tips learned from the self-help audio book they we're provided 2wks before TQD. Ss used their assigned 30 cm^2 patch for the first 12 wks. reducing to a 20 cm^2 patch between Wks 12-15, reducing further to a 10 cm^2 patch for the final 3 wks of treatment, 15-18. No further patches were worn after Wk 18. (All Ss will continue to be followed for 1 yr after patch use ceased, at Wk 18.) Self-report of nonsmoking status was confirmed by exhaled air carbon monoxide (CO) < 10 ppm and, after Wk 18, serum cotinine < 50 ng/ml. 113 Ss received active NTP treatment and. 107 placebo patch, with the active NTP consistently producing better results than placebo at all time points (Survival Curve Difference [SCD] p = 0.0003). At the end of treatment with the 30 cm^2 patch (Wk 12). 47% of active and 31% of placebo patch Ss were sustained nonsmokers. (Once a Ss relapsed back to smoking that Ss was classified as a permanent failure, even if the relapse episode was short-lived.) During the 6-wk tapering ohase. there was minimal further relapse for active patch Ss. down to 44%. while placebo Ss dropped more, to 22%. Six mos from TQD. or 1½ mos after stopping patch use, 35% of active NTP Ss were still sustained nonsmokers, compared to 17% placebo Ss. Women did substantially better than men, whether treated with active or placebo patch. Of the 130 women enrolled in the trial, 67 received active and 63 placebo patch. At the end of 30 cm^2 patch treatment, 48% of active v. 29% of placebo patch women were sustained nonsmokers from TQD. By Wk 26, 1 ½ mos after the Tapering Phase had finished, 37% of active v. 17% of placebo patch women were still sustained nonsmokers. (SCD for women was p = 0.001, power = 0.95.) For men, at the same time points, the results were, respectively, 46% v. 34% (active v. placebo) and 30% v. 16% (SCD p = 0.1022, power = 0.45). Low nicotine dependent smokers (collapsed by gender), defined as Fagerstrom [nicotine] Tolerance Questionnaire (FTQ) score $\leq$ 6 + cigarette smoking (baseline) serum cotinine < 253 ng/ml. showed excellent treatment results: At Wk 12, 58% v. 41% (active v. placebo patch) low nicotine dependent Ss were sustained nonsmokers, and at Wk 26, 49% v. 15% were sustained nonsmokers (SCD p = 0.0119). High nicotine dependent smokers (FTQ $\geq$ 7 + serum cotinine $\geq$ 253 ng/ml) did less well than low dependent smokers. but still showed a significant treatment effect (SCD p= 0.0453). Wk 12 & Wk 28 results for active v. placebo, all respectively. were 25% v. 10% and 15% v. 3%. 80 of 113 Ss on active and 95 of 107 Ss slipped at least once during the trial. By Wk 26, only 8% of those on active patch had not yet relapsed, while 6% on placebo were still not smoking. There was no difference between survival curves by treatment condition; that is, whether the Ss received active or placebo, if the Ss had even 1 slip, the odds were 92-94% that they would subsequently relapse by Wk 26, 1½ mos after tapering off their assigned patch. In conclusion the 30 cm^2 transdermal nicotine patch with a 16-hr delivery time period produced excellent abstinence results during treatment and tapering for women and for subjects with low nicotine dependence. 30 cm^2 NTP was less effective for high dependent smokers and for males. Future studies should investigate the utility of a higher nicotine delivery patch or of combination therapy with other nicotine replacement agents for these two subgroups. Such studies should also explore longer treatment periods with longer tapering phases. Finally slippers were loosers; 92-94% who had smoked even 1 cigerette during treatment ultimately relapsed by wk 26, 1½ mos after stopping patch use. Affiliation: Palo Alto Ctr. for Pulmonary Disease prevention. Palo Alto, CA, Kabi Pharmacia Therapeutics AB, Helsingborg, SWEDEN Karolinski Institute, Stockholm Sweden

The Combined Use of Modecular Mechanics and NMR Spectroscopy for the Conformational Analysis of U50,488

C.M. DiMeglio, M.Froimowitz and A. Makriyannis

The conformational preferences of the κ-selective opioid agonist U50488 have been studied by MM2-87 calculations and NMR spectroscopy. A systematic search found 72 distinct stable conformers with certain consistent conformational preferences for some of the important dihedral angles. The preferred conformers proved to be compact structures stabilized by intramolecular attractive van der Waals' interactions, although some of these have unfavorable electrostatic energies. Conformational analysis by one and two dimensional high resolution ^{1}H NMR spectroscopy involved the interpretation of vicinal ^{1}H-^{1}H coupling constants, phase sensitive 2-D COSY, 1-D NOE and phase-sensitive 2-D NOE spectra. Three x-ray crystal structures were also examined. Although there was generally good agreement among all three methods of conformational analysis, the calculations and NMR studies suggest a *gauche* conformation for the phenyl ring while all three crystal structures show a *trans* orientation. There appears to be reasonable geometric agreement between a gauche conformer of U50488 and the relatively rigid κ-agonist (-)-ketazocine.

ACKNOWLEGEMENTS. This work was supported by a grant from the National Institute on Drug Abuse (DA 04762).

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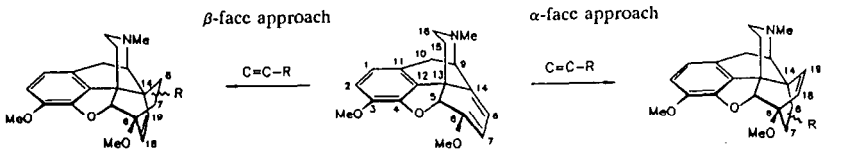
Synthesis of C-Ring-Substituted Etheno(iso) Morphinans

L. Maat and R.H. Woudenberg

The baine has been proven to be an effective starting material for the synthesis of CNS biologically active compounds. Diels-Alder reaction of thebaine with different dienophiles all yield 7α-substituted 6α14α-ethenoisomorphinans, as a result of ß-face approach of the dienophile. A more accurate analysis of some conversions show that a-face approach is possible, although only in small amounts.



6α,14α-ethenisomorphinan thebaine 6β,14β-ethenomorphinan

The aim of this study was to investigate the Diels-Alder reactions of thebaine analogues that would allow a-face approach of the dienophile to a larger extent. For this purpose, we studied the influence of a substituent at the 5ß-position or the 7-position in connection with the 6-methoxy group. Firstly, we prepared some 5ß-substituted thebaine analogues via the thebaine anion. All cycloadditions took place still through ß-face approach yielding the usual 7a-substituted 6α,14α-ethenoisomorphinans.

Secondly, we synthesized 5ß-methyl-6-demethoxythebaine. Deprotonation of the 6-demethoxy analogue of thebaine followed by methylation gave mainly the non-conjugated 7ß-methylmorphinan,5,8-diene, with the wanted 5ß-methyl-6-demethoxythebaine as minor product. The latter compound was, therefore, prepared indirectly from 5ß-methylthebaine in six steps and an overall yield of 40%. Diels-Alder reaction of the compound with ethyl acrylate showed both a-face and ß-face approach of the dienophile in a ratio of about 1:1.

Thirdly, we introduced a chlorine atom at position 7 of 6-demethoxy-thebaine to force the cycloaddition electronically to g-substituted products. Three products were formed (4:3:3). The main product, however, was still the 7α-substituted ethenoisomorphinan, the two others being indeed 8-substituted isomers.

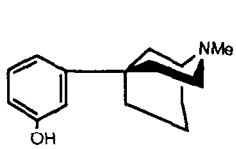
From the present results, it can be concluded that a single 5ß-substituent in thebaine is not capable of inversing the approach of the dienophile. A 5ß-substituent or a 7-chloro substituent in combination with the absence of the 6-methoxy group gives rise to both a-face and ß-face cycloaddition. making a new class of ethenomorphinans available for biological studies.

AFFILIATION: Delft University of Technology, Delft, The Netherlands

An Efficient Synthesis of the 'ortho and para A-Isomer' of Oxide-Bridged 5-(3-hydroxyphenyl)-2-methyl-morphan

K. Yamada, A.E. Jacobson and K.C. Rice

In our continuing study of ligands for the opioid receptor-endorphin system, we have examined conformationally rigid derivatives of the potent narcotic agonist, 5-(3-hydroxyphenyl)-2-methylmorphan (1).



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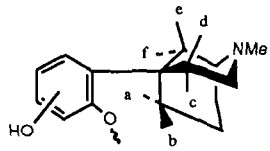
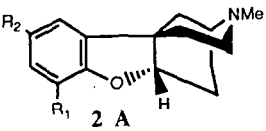


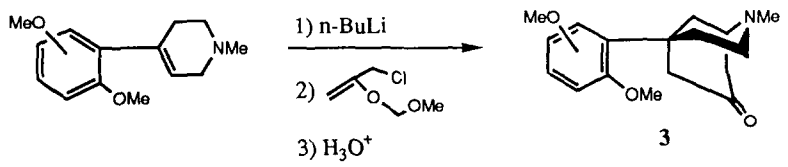
2 Isomers A-F



2 A

"ortho isomer" R_1=OH,R_2=H
"para isomer" R_1=H,R_2=OH

We now present a novel, expedient synthetic approach to the racemic "ortho and para A-isomer" (2) of oxide-bridged 5-(3-hydroxyphenyl)-2-methylmorphan. A key step in the approach utilized an intramolecular Mannich-type cyclization of a 4-acetonyl-4-aryltetrahydropyridine intermediate to generate the 2-azabicyclo[3.3.1]nonane system (3).



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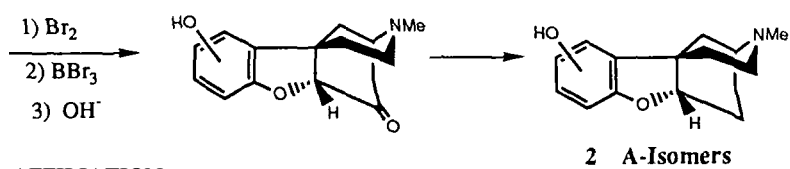

2 A-Isomers

AFFILIATION

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The Modulatory Roles of Spinal Corticotropin-Releasing Factor and Dynorphin A on Acute Morphine Tolerance

Z.H. Song and A.E. Takemori

Previously, we have demonstrated that intrathecally (i.t.) administered corticotropin-releasing factor (CRF) produces antinociception and modulation of morphine-induced antinociception by mechanisms involving spinal kappa opioid receptors. Recently, we also have found that CRF releases immunoreactive dynorphin A, a putative endogenous kappa opioid receptor agonist, from superfused mouse spinal cord *in vitro*. Dynorphin A administered intracerebroventricularlly to mice has been shown to modulate the expression of morphine tolerance. Therefore, in the present study, the possible modulatory effects of i.t. administered CRF as well as dynorphin A on morphine tolerance were studied in an acute tolerance model.

Subcutaneous administration of 100 mg/kg of morphine sulfate (MS) to mice caused an acute tolerance to morphine-induced antinociception. The antinociceptive ED_{50} (95% confidence interval) of MS was increased from 4.4 (3.5-5.4) mg/kg (naive mice) to 17.9 (14.3-22.2) mg/kg (4 hours after the injection of 100 mg/kg MS). In order to study the modulatory effects of spinal CRF and dynorphin A on the expression of morphine tolerance, CRF and dynorphin A were injected i.t. at 15 min and 5 min. respectively, before tail-flick testing in the acute tolerant state. The antinociceptive ED_{50} (95% confidence interval) of MS in tolerant mice was decreased to 8.8 (7.0-10.9) mg/kg and 7.1 (5.6-8.8) mg/kg, respectively, after i.t. administration of CRF (0.1 nmol) and dynorphin A (0.2 nmol). In contrast, 0.5 nmol of alpha-helical CRF (9-41). a CRF antagonist and 0.4 nmol of nor-binaltorphimine, a highly selective kappa opioid receptor antagonist. when administered i.t. at 15 min before the tail-flick test in the tolerant mice, increased the autinociceptive ED_{50} (95% confidence interval) of MS to 56.6 (44.9-70.2) mg/kg and 88.8 (71.6-110.6) mg/kg, respectively.

These data confirmed the modulatory of effect dynorphin A on morphine tolerance and suggested that CRF, which releases dynorphin A in several brain regions, also plays a modulatory role in the expression of morphine tolerance.

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Regional Levels of Met-Enkephalin-Arg^6-Phe-^{7} in Rat Brain Following Chronic Administration

E.M. Unterwald, J. Horne, F. Nyberg, L. Terenius and M.J. Kreek

Although cocaine has a variety of pharmacological actions, it is thought that the reinforcing properties of cocaine are mediated by its ability to inhibit the reuptake of dopamine tbereby increasing the concentration of dopamine at the synapse. In animal models of drug reinforcement. dopamine receptor antagonists attenuate cocaine-induced reward supporting the role of dopamine in cocaine reinforcement. In addition, the opiate antagonist naloxone also blocks the rewarding effects of cocaine suggesting an interaction between central opioid and dopaminergic systems in cocaine reinforcement. Moreover, chronic exposure to cocaine has been shown to alter opioid receptor density and (ß-endorphin levels. To further investigate the interaction of cocaine with the endogenous opioid system, the levels of Met-enkephalin-Arg^6-Phe^7 were determined in various brain regions of rats chronically exposed to cocaine. Sixty day old male Fischer rats were injected daily at 9:30, 10:30. and 11:30AM with cocaine HCl(10 or 30 mg/kg/day. i.p.) or saline for 14 days. After 14 days of treatment. the animals Injected with 30 mg/kg/day of cocaine weighed significantly less than either of the other two treatment groups, whereas food consumption was not altered by any treatment. Peptide levels were determined by radioimmunoassay using an antibody raised against the sulfoxide heptapeptide Met-O-enkephalin-Arg^6-Phe^7. Results demonstrate that in control animals Met-enkephalin-Arg^6-Phe^7 had a heterogenous distribution in the brain with the highest level found in the nucleus accumbens. Moderate to high concentrations were detected in the hypothalamus, caudate putamen. and central grey. Low levels were measured in the pituitary. frontal cortex, hippocampus, and cerebellum. Chronic administration of cocaine under these conditions did not alter the expression of Met-enkephalin-Arg^6-Phe^7 in any of the brain regions tested including the frontal cortex, nucleus accumbens, caudate putamen. hypothalamus, pituitary, hippocampus. central grey. or cerebellum.

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Presynaptic Dopamine Effux is Enhanced by 5-HT_3 Receptor Activation in Medical Prefrontal Cortex of Freely Moving Rats

J. Chen, W. Paredes, H.M. van Praag and E.L. Gardner

The mesocorticolimbic (A10) dopamine (DA) system is heavily implicated in the reinforcement induced by addicting drugs (Wise & Rompre, Annu Rev Psycho1 40:191-225,1989). Serotonin ($5HT_3$) receptors are found in high density in A10 DA loci (Kilpatrick et al, Nature 330:746-748,1987), and it has been suggested that 5-HT_3 antagonism might be a useful approach for treating drug addiction (Costall et al, Br J Pharmacol 92:881-894,1987). However, little is known about the modulatory function of these 5-HT_3 receptors on A10 DA pathways. In the present *in vivo* microdialysis study, we studied the effect of the selective 5-HT_3 agonist 1-phenylbiguanide on DA efflux from the medial prefrontal cortex of freely moving rats. At concentrations of 0.1-1.0 mM in the perfusate, 1-phenylbiguanide caused a dose-dependent increase in presynaptic DA efflux; this effect was antagonized by co-perfusion of the selective 5-HT_3 antagonist zacopride (2mM). These data are similar to our previous findings in the nucleus accumbens, another reward-relevant A10 DA terminal region (Chen et al, Brain Res 543:453-357, 1991), and suggest that 5-HT_3 receptors have a potent modulatory interaction with the A10 DA system which could form the basis for novel treatment strategies for drug abuse.

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The Effects of Cocaine and Other Monoamine Uptake Inhibitors on Cultured Mesencephalic Dopamine Neurons

B.A. Bennett and J.E. Clodfelter

Cocaine is known to have a potent effect on brain reinforcement mechanisms and this is thought to be mediated throughout brain dopamine (DA) systems. There is now substantial evidence that indicates there is a strong correlation between the ability of cocaine to inhibit DA uptake and its reinforcement property. The purpose of these studies was to assess the changes induced by cocaine and other neuronal uptake inhibitors on dopaminergic function using a tissue culture model.

The neuronal uptake inhibitors examined were cocaine (COC). methamphetamine (METH), and mazindol (MAZ), a serotonin and DA uptake blocker. Drugs were added to cultures of rat ventral mesencephalon and various indices of dopaminergic activity were assessed. The cultures were prepared from fetal rat brains (19-20 days gestation) and the ventral mesencephalon collected and enzymatically dispersed. COC, METH, and MAZ were added to the cultures daily for 5 days and experimental procedures performed 24 hours later. Indices of neurotoxicity were included to assess the effect of the drugs not only on dopaminergic neurons, but also on other neuronal cell types. These indices included immunostaining for tyrosine hydroxylase (TH) and neuron specific enolase (NSE) which yielded data on cell survival. Also, the level of lactate dehydrogenase (LDH) activity in the media, which is an indicator of cell death, was determined. The effect of chronic exposure to these agents on high affinity DA uptake was measured using 50 nM [^{3}H]-dopamine. The sodium dependence of DA uptake was established by replacement of NaCl with choline chloride.

The results show that mesencephalic cultures chronically exposed to METH 10^{-4}M had reduced DA cell survival and deceased dopaminergic uptake. The data suggest that factors in addition to DA cell loss are responsible for the reduction in DA uptake. COC ($10^{-}4$, 10^{-5}M) had little or no effect on either DA uptake or cell number. Chronic exposure to MAZ (10^{-6}M) reduced DA uptake while not affecting DA cell survival. LDH activity was not significantly increased over the time period examined after exposure to either COC, METH, or MAZ. This would indicate that the neurotoxicity induced by METH and MAZ may be specific for dopaminergic neurons and may not significantly affect other cell populations. In these experiments, the lack of an effect of COC in reducing DA uptake or affecting the TH (+) cell population would suggest that COC is not a neurotoxin and does not permanently alter the cells ability to transport DA.
This research was supported by a grant from NIDA #05073

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Photoaffinity Labels for the Cannabinoid Receptor

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In an effort to probe the endogenous cannabinoid receptor we synthesized 5'-N3-Δ^8-THC (1) and 2-I-5'-N_3-Δ^8-THC (2). Compound 1 was prepared by reacting 5-Br-Δ^8-THC with TMGA. Compound 2 was obtained by reacting 1 with NaI, mCPBA and 18-crown-6. The molecules were evaluated in male ICR mice for their abilities to produce sedation, catalepsy, lower body temperature and antinociception. The results showed 1 to be 4.5-19 times more active than Δ^8-THC in all tests while 2 was shown to be equipotent to the parent molecule. The binding affinities of the analogs for cannabinoid receptors present in rat cortex membranes were evaluated from their abilities to displace [^{3}H] CP-55,940. The IC_{50} values obtained were 31 and 490 nM for 1 and 2, respectively. Compound 1 was tested for its ability to inactivate the cannabinoid receptor present in rat cortex membranes in which, after equilibration and irradiation, prevented [^{3}H] CP-55,940 binding by 80%. Compound 2 was tested for its effects on WI-38 cell cultures, where after equilibration and irradiation it inhibited Δ^9-THC stimulated synthesis of PGEs.

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Alcohol's Effects on Estradiol and LH in Female Rhesus Monkeys

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Alcohol enhanced synthetic LHRH stimulation of LH in both normal and ovariectomized female monkeys (Mello et al., 1986a and b). Since alcohol consistently increases estradiol levels in women (Mendelson. et al., 1988), we postulated that an alcohol-induced increase in estradiol levels could have sensitized the pituitary and augmented the LH response to LHRH stimulation in monkey. We re-examined the effects of alcohol (2.5 or 3.5 g/kg) and an isocaloric sucrose control solution on LH and estradiol before and after administration of synthetic LHRH (100 mcg/i.v.) in follicular phase female monkeys (days 4-7). Alcohol (2.5 g/kg) augmented LHRH-stimulated LH in 4 of 6 females ($P<.01$) and this was accompanied by an antecedent increase in estradiol (11 to 30 percent) in 3 monkeys. The magnitude of the estradiol increase from base-line ranged from 9 to 15.2 pg/ml, an increase comparable to that measured in follicular phase women (19.5 ± 4.1 pg/ml). After a higher dose of alcohol (3.5 g/kg), an augmentation of LHRH-stimulated LH ($P<.01$) occurred in 4 of 6 subjects and estradiol increased by 12 and 16% in 2 monkeys. Estradiol remained elevated after alcohol (2.5 and 3.5) + LHRH ($P<.05$) in comparison to placebo. These data suggest that an increase in estradiol is often associated with an alcohol-related enhancement of LHRH-stimulated LH and that estradiol may modulate pituitary sensitivity to synthetic LHRH. However, augmentation of LHRH-stimulated LH also occurred in the absence of estradiol increases in 3 of 8 observations.

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Binding and Functional Responses of Different Chemical Families of μ Opioid Agonists in SH-SY5Y Cells

E.M. Costa and G.H. Loew

The human neuroblastoma SH-SY5Y cell line was used to compare the receptor recognition and activation properties of p-selective agonists. Receptor affinities were determined by using [^{3}H]-DAGO. Inhibition of PGE_1-stimulated cAMP accumulation was better characterized in retinoic acid-treated cells (10μM/6 days), while the increase in GTPase activity did not change with cell differentiation. The Table below summarizes our results:

Agonist	Ki (nM)	GTPase stimulation[a] ED_{50}(nM)	Inhibition of cAMP[b] ED_{50}(mM)
R30490	0.11	4.5	.14
Carfentanil	0.15	<0.1	<0.01
Fentanyl	0.39	1.3	0.42
Oxymorphone	2.3	3.7	13
Metazocine	2.9	2:0	18
DAGO	3.6	8.5	22
PLO17	4 1	5 6	100
Meperidine	650	60	1000

a - ED_{50} values calculated from the average of 3-5 experiments with S.E. < 18%. (Koski et al, J.Biol.Chem.257:14035'1982)

b - Indicated ED_{50}, calculated at 40 % inhibition using the average of 3-5 experiments with S.E<17%. (Yu V.C. & Sadee W, J.Pharm. Exp. Therapeutics, 245:350'1988).

The three fentanyl compounds had highest affinity, followed by oxymorphone, metazocine and DAGO, with similar K_i and finally by PLO17 and meperidine. The same rank order was observed in the inhibition of PGE_1-stimulated cAMP accumulation, with all compounds reaching ~80% inhibition. By contrast, the variation in ability to stimulate low Km GTPase activity was much smaller distinguishing only compounds with major differences in receptor affinity. Maximum stimulation was comparable for all compounds (~50%), except for the lowest affinity analog, meperidine 30% at 10^{-5}M). The very potent carfentanil showed a shallow dose-response in both biochemical end-points, reversed by naloxone. This cell line may be a suitable system for further characterization of the signal transduction pathways elicited by the μ-receptors.

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Affinity Labeling of Mu Opioid Receptors in Rat and Bovine Brain Membranes Using a 14ß-Bromoacetamido Derivative of Morphine

J.M. Bidlack, R.A. Kaplan, R. Subbramanian, A. Seyed-Mozaffari and S. Archer

Previous studies have demonstrated that after reduction of a critical disulfide bond at the μ opioid binding site, 14ß-bromoacetamido derivatives of morphine and morphinone irreversibly inhibited μ opioid binding to rat brain membranes (Bidlack et al., 1990). This study shows that after reducting the disulfide bond at the μ opioid binding site, [^{3}H]14ß-bromoacetamido-7,8-dihydromorphine (H_2BAM) alkylated this binding site in rat brain and bovine striatal membranes. To determine the molecular weight of the affinity-labeled proteins, rat and bovine [^{3}H]H^{2}BAM-labeled membranes were separated on sodium dodecyl sulfate polyacrylamide gels, followed by either slicing the gel and extracting [^{3}H]H_2BAM-labeled proteins, or fluorography. With both rat and bovine membranes, a major protein with a molecular weight of 54,000 was labeled, and two minor proteins with molecular weights of 51,000 and 31,000 were affinity-labeled. The 54,000 dalton protein contained at least 75% of the total [^{3}H]H_2BAM incorporated into membranes. Opioids, known to bind to the μ binding site, blocked the labeling of all three proteins, while ligands specific. for the δ and κ opioid binding sites did not alter the incorporation of [^{3}H]H_2BAM into membranes. The degree of labeling of all three proteins was dependent on the concentrations of the disulfide bond reducing reagent, dithiothreitol, and [^{3}H]H^{2}BAM. The time, temperature, and pH of the incubation of membranes with [^{3}H]H_2BAM also influenced the amount of [^{3}H]H_2BAM incorporated into membranes. By affinity-labeling membranes with [^{3}H]H_2BAM, the purification of a ^{3}H-labeled μ opioid binding site should now be possible. (Supported by grants DA03742 and DA01674 from NIDA.)

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A Study of Oligonucleotides for Use as Reagents in a Drug-of-Abuse Immunoassay

D.G. Deutsch and E.R. Tombler

An immunoassay for analytes, such as drugs-of-abuse, has been described which utilizes hybridized oligonucleotide chains as reagents (U.S. patent #4,921,788). To determine the ideal composition and length of these oligonucleotides, we describe a spectrofluorometric solution assay using ethidium homodimer (ETDi), a bifunctional intercalator, to measure chain hybridization. The fluorescence of several short chains were compared to calf thymus DNA at room temperature (all 0.2 μM nucleotide). The complementary 20-mer chains $pd(A)_{20}.pd(T)_{20}$ yielded 86% as much fluorescence as calf thymus DNA while pd(A)10.$pd(T)_{10}$10-mer chains did not fluoresce significantly. No apparent hybridization occurred with $pd(G)_{10}.pd(C)_{10}$, $pd(G)_{10}$ and $pd(C)_{10}$. Furthermore, the oligonucleotides $d(A)_{20}$, $d(T)_{20}$, and $d(C)_{20}$ yielded little fluorescence above background. Surprisingly, $d(G)_{20}$ yielded a fluorescence nearly as great as calf thymus DNA and nearly twice that observed for $d(G)_{20}d(C)_{20}$. The self-complementary 24-mer $d[C_{12}G_{12}]$ demon strated 47% as much fluorescence as calf thymus DNA while $d[T_{12}A_{12}]$ did not demonstrate significant fluorescence. Linearity of $pd(A)_{20}$,$pd(T)_{20}$, $d(G)_{20}$, and $d[C_{12}G_{12}]$ was demonstrated from 0.024 μM to 0.23 μM nucleotide. These studies show that complementary oligonucleotide chains with twenty base-pairs form stable complexes which intercalate ethidium homodimer and that the minimum chain length for hybridization is greater that ten base-pairs. Furthermore, it appears that certain homopolymers ($d(G)_{20}$) and heteropolymers ($d[C_{12}G_{12}]$) are self-annealing and many of these oligonucleotides may be useful in an immunoassay employing oligonucleotides as reagents.

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Possible Role for Serotonin in Discriminative Stimulus Effects of the Kappa Opioid U50,488

M.E. Bronson, K. Burchett, M. Picker and L. Dykstra

A growing body of evidence suggests that the analgesic action of kappa opioids, as well as the development of tolerance to kappa-induced analgesia, may be mediated through serotonergic mechanisms (Vonvoigtlander et al. 1984; Ho and Takemori 1989). Further studies indicate that the kappa agonist U50,488 releases serotonin from brain slices and spinal cord synaptosomes in mice (Ho and Takemori 1990); that the anti-tussive activity of kappa agonists can be antagonized by a serotonin antagonist (Kamei et al., 1990) and that kappa inhibition of morphine-induced Straub tail is attenuated by $5HT_3$ antagonists (Hasegawa et al., 1990). The purpose of the present study was to examine serotonergic involvement in the discriminative stimulus properties of U50,488. Pigeons were trained to respond differentially on one of two keys depending on whether they received a dose of 5.6 mg/kg U50,488 or water. Various doses of U50,488 were then substituted for the training dose of U50,488, and additional tests were conducted with various serotonin (5HT) agonists and antagonists, both alone and in combination with U50,488. The dose effect curve for U50,488 atone was relatively steep, with 5% responding on the drug-appropriate key at 1 mg/kg and complete generalization at the training dose of 5.6 mg/kg. When given in combination with the training dose of U50,488, the $5HT_{1A}$ agonist, 8-OH-DPAT, dose-dependently antagonized the discriminative stimulus effects of U50,488, and this antagonism was blocked by pretreatment with the $5HT_{1A}$ antagonist, NAN190. NAN 190 neither antagonized the training dose of U50,488 nor potentiated a lower dose of U50,488. The $5HT_{1A}$ partial agonist buspirone attenuated but did not completely block the discriminative stimulus effects of U50,488. The $5HT_{1B,C}$ agonist, mCPP, did not antagonize the discriminative stimulus properties of U50,488, nor did it potentiate the effects of a lower dose of U50,488. The $5HT_2$ antagonist, ketanserin, attenuated but did not completely block the discriminative stimulus properties of U50,488. When administered alone, none of the serotonergic compounds occasioned drug-appropriate responding, with the exception of buspirone, which produced partial generalization at the highest doses tested. These results suggest that $5HT_{1A}$ A and possibly $5HT_2$, but not $5HT_{1B}$ or $_{IC}$, receptors are involved in the discriminative stimulus properties of U50,488 in pigeons.

REFERENCES: References available upon request from Maureen E. Bronson

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What is Learned in Conditioned Tolerance Experiments?

V.W. Rees, J.D. Greely and R.F. Westbrook

In experimental designs in which hot-plate exposure accompanies tolerance training, evidence for contextual control of drug tolerance may be compromised. In Experiment 1, rats were taken to a distinctive environment (E1), given morphine (Group EXP) or saline (CONT & NAIVE), and placed on an ambient (23°C) hot-plate, once daily for 12 days. They were treated 2 hr later in the home room with saline (Groups EXP & NAIVE) or morphine (CONT). On the first test in El, half of the rats were given morphine (5mg/kg) while the other half were given saline, before testing on a 54°C hot-plate. There was no evidence for contextually controlled tolerance, nor a hyperalgesic response, since Groups EXP & CONT did not differ in paw-lick latency when tested with morphine or saline. On the second test, half of the morphine-tested rats were again given morphine in El, while the other half were given saline, before exposure to a 52°C floor. The same procedure was applied to those rats previously tested with saline. Rats from group NAIVE paw-licked with longer latencies than rats from groups EXP and CONT, when trained and tested with morphine [$F(1,64)=78.7$, $p<.001$] or trained with saline and tested with morphine [$F(1,64)=68.1$, $p<.00l$]. There was no difference between NAIVE and EXP groups when rats were trained with morphine and tested with saline, nor when trained and tested with saline, suggesting that the effect of exposure to the 54°C floor was similar despite differences in prior exposure to morphine. In Experiment 2, rats were trained as above over four training sessions but were exposed to one of three hot-plate temperatures (23, 52 or 54°C). When tested at 52°C in El with either morphine or saline, rats expecting morphine showed shorter paw-lick latencies than did rats expecting saline [$F(1,108)=25.6$, $p<.001$]. However, rats previously trained on 54°C showed longer paw-lick latencies than those trained on the two lower temperatures [$F(1,108)=14.6$, $p<.001$), indicating the acquisition of conditioned hypoalgesia by those rats as a result of the association between thermal nociception during training, and El. This suggests that differences observed between control and experimental groups in experiments of conditioned tolerance may be due to differences in conditioned hypoalgesia. These results have implications for Pavlovian models of drug tolerance. Affiliation: National Drug & Alcohol Research Ctr. & School of Psychology, Univ. of New South Wales, Sydney, Australia

Drug/Drug Discrimination Learning Within the Conditioned Taste Aversion Procedure

M.A. Kautz, S.T. Smurthwaite and A.L. Riley

Recently, our lab and others have reported the rapid acquisition of drug discrimination learning within the taste aversion procedure. In this procedure, animals are typically injected with a drug prior to a specific taste-toxin pairing and the drug vehicle prior to a nonpoisoned exposure to the same taste, i.e., they are presented with a drug vs. vehicle discrimination. The present study extends the types of discriminations that can be trained within this procedure to include a conditional two-drug discrimination in which neither the drug alone nor the taste alone conveys information about subsequent toxicosis. Specifically, in the present experiment following amphetamine (1 mg/kg) rats were given a sodium chloride-toxin pairing or a nonpoisoned exposure to saccharin, whereas following pentobarbital (10 mg/kg) they were given a saccharin-toxin pairing or a nonpoisoned exposure to sodium chloride. Subjects acquired the discrimination, displaying differential decreases in consumption of the two solutions depending upon the drug given prior to solution access. Further, on subsequent generalization tests ethanol given prior to access to either saccharin or sodium chloride produced selective dose-dependent decreases in saccharin consumption, i.e., the pentobarbital-appropriate response.

The demonstration of a conditional two-drug discrimination within the taste aversion procedure may offer more than an extension of the types of discriminations that can be acquired within this baseline. It may also offer a baseline to assess the similarities and differences among related compounds as well as the bases for these similarities and differences.

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The Reinforcing Versus Other Pharmacologic Effects of Chronic Cocaine Administration on Serotonin Turnover in the Rat Brain

S.I. Dworkin

The effects of cocaine on serotonin turnover in rats that either self-administered or were exposed to response-independent administration of the drug were evaluated. Male Fischer-344 rats, continuously housed in triad-operant chambers on a reversed light/dark cycle, received either response-dependent (self-administering or SA), yoked-cocaine administration (YC) or yoked-saline infusions (YS). Cocaine (0.33 mg/infusion) was available for six hours each day from 900 to 1500 hours for at least 30 days. The triads were pulse labelled, 24 hours after their last infusion, with *L*[^{3}H]-tyrosine, *L*|[G-^{3}H]-tryptophan and *D*|[U-^{14}C]-glucose. The differences in serotonin turnover between rats exposed to yoked-cocaine and yoked-saline administered is suggested to be the result of the pharmacologic actions of cocaine on neuronal systems. Whereas, the differences observed between the self-administration and the response-independent administration of the drug is considered to be directly related to the reinforcing actions of the drug. The pharmacologic effects of cocaine (YC vs YS) on serotonin turnover included a decrease in 5HT turnover in the frontal cortex and inferior colliculus and an increased turnover in the pre-optic/diagonal band region. The self-administration of the drug ISA vs YC) augmented the decreases observed in the frontal cortex and attenuated the increases seen in the diagonal band. In addition, exposure to the reinforcing effects of cocaine resulted in increased 5HT turnover in the nucleus accumbens and pyriform cortex and a decreased turnover in the motor somatosensory cortex. Serotonergic neuro-transmitter systems appear to be involved in the reinforcing effects of cocaine.

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Peripheral Corticosterone Increases in Male Rats During Repeated Infusions of Cocaine

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Little is known about the endocrine responses to repeated injections of cocaine. We employed a model of passive administration in which each rat received an i.v. infusion of 0.15 M NaCl (saline, 1 ml/kg) or cocaine (1 mg/kg) at 12-min intervals for 2 hr daily for 10 days. On Days 1, 5, and 10 of this regimen, we measured the concentration in plasma of the adrenal steroid corticosterone in rats before and after the 2-hr session. Corticosterone was measured in blood from other rats on Day 5 one min after each of the 10 sequential i.v. infusions of saline or cocaine throughout the session. Blood was withdrawn from a separate group of animals at 30-min intervals for the 4 hr preceding passive administration and at 12-min intervals during the infusion period. Concentrations of corticosterone in the plasma of saline- and cocaine-treated rats were low (ca. 65 ng/ml) and indistinguishable from each other when measured either immediately before or after the period of passive infusion. Corticosterone was higher in cocaine-treated rats relative to the saline controls (cocaine, 302 +/- 68 ng/ml; saline, 167 +/- 63 ng/ml, respectively) after the first infusions. Corticosterone decreased in saline-treated rats by mid-session but remained elevated at all times during the cocaine infusion, suggesting that these rats did not habituate to the infusion procedure as did the controls. However, corticosterone in control animals increased to levels comparable to cocaine-treated rats by the end of the session, indicating that repeated blood withdrawal alone could increase corticosterone. This was supported by the observation that repeated withdrawal of blood over a 6-hr period increased corticosterone similarly in both saline-and cocaine-treated rats. Finally, the acute administration of cocaine (0, 10 or 30 mg/kg, i.p.) to hypophysectomized rats failed to increase corticosterone, suggesting that the cocaine-enhanced release corticosterone occurs via the central stimulation of corticotropin-releasing hormone.

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Gonadal Factors and Ethanol's Actions on Hepatic Drug Metabolism: Effects on Cytochrome P450 CYP 2C11

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Alcohol impairs testicular function and results in significant changes in circulating hormones and metabolic processes regulated by gonadal factors. We hypothesized that gonadal factors are important determinants of ethanol's effects on the hepatic monooxygenase system in the rat, especially on those isozymes such as CYP 2C11 that are thought to be regulated by growth hormone. CYP 2C11 hydroxylates testosterone (T) at positions 2α and 16α. Adult male rats (n=16) were infused via an intragastric cannula with a diet that promotes normal growth rates in rats. Eight of these rats were infused diets containing ethanol (35% of the total calories) isocalorically substituted for carbohydrate. After receiving these diets for 35 days, 4 rats from each group were bilaterally castrated and continued on their respective diets. All rats were killed 10 days postcastration and selected hepatic cytochrome P450 isozymes were studied in vitro using Western blot analysis and metabolism of prototype substances such as ^{14}C-T. Our results demonstrate that: 1) The total parenteral nutrition (TEN) system used in these experiments provided excellent nutrition that supported daily weight gains that were equal to those of the control group fed standard rat food (even in ethanol-treated groups); 2) Chronic ethanol treatment of male rats in a system utilizing TEN produced a demasculinization of hepatic drug metabolism that in some respects resembles the effects of castration; 3) In combination, the effects of chronic ethanol treatment and castration were additive on CYP 2C11-dependent testosterone 2α- and 16α-hydroxylase activities and steroid 5α-reductase activity, suggesting that ethanol may act via a mechanism independent of gonadal factors; 4) Gonadal factors appeared not to affect the regulation of CYP 2E1 by ethanol or diet, or the ethanol-induced suppression of testosterone 6ß-hydroxylase activities and 5) In some cases, one treatment interfered with the effects produced by the other. For example, ethanol treatment prevented the castration-induced rise in testosterone 7α-hydroxylase activity (CYP 2A1); while castration blocked the ethanol-induced suppression of EROD activity (CYP 1A1). Supported by NIAA-AA08213 and NIAA-AA08645.

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Alcohol Attenuates the E_2B Stimulated LH Surge in Ovariectomized Monkeys

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Examination of the acute effects of alcohol on the peri-ovulatory LH surge has been difficult because of the problems in accurate temporal prediction of ovulation. But estradiol benzoate (E_2B) can stimulate an LH surge in OVX monkeys, 42-52 hours after acute administration and the LH increase appears to be associated with increased LHRH pulse frequency and amplitude (Levine et al., 1985). We studied the effects of alcohol (2.5 and 3.5 g/kg) and an isocaloric sucrose control solution on LH and FSH secretory activity in 5 ovariectomized monkeys, 41 to 51 hours after E_2B administration (42 mcg/kg/i.m.). Integrated plasma samples were collected at 20 min intervals. Under sucrose control conditions, a surge in LH (445 and 584 ng/ml) occurred in 2 monkeys within 46 to 49.3 hrs after E_2B administration and high amplitude LH pulses were evident in 3 monkeys. After *2.5 g/kg alcohol,* peak blood alcohol levels averaged 195 mg/d1 within 200 min. There was no LH surge or detectable LH pulses in 4 of 5 m&keys. In one monkey, LH gradually increased to a peak of 1036 mg/ml at 48 to 50.6 hrs after estradiol but there were no discemable LH pulses. After *3.5 g/kg alcohol,* peak blood alcohol levels averaged 274 mg/dl within 230 min. No monkey had an LH surge and pulsatile LH release was reduced in comparison to control conditions. E_2B did not stimutate an increase in FSH and FSH levels remained stable across alcohol and control conditions. These data suggest that alcohol disrupts hypothalamic-pituitary gonadotropin release stimulated by exogenous estrogen in this model. These findings are consistent with menstrual cycle disruptions (e.g. anovulation, luteal phase dysfunction) observed during chronic alcohol self-administration in the primate model and in alcohol dependent women.

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Pharmacological Evidence for More Than Two Benzodiazepine Receptor Types

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Receptor binding studies in different brain regions have thus far provided evidence or two subtypes of benzodiazepine BDZ/$GABA_A$ receptors. Cerebellum have been reported to contain only one (Type I) and spinal cord only the other (Type II) subtype. Results of studies presented here however, indicate more extensive heterogeneity: three binding sites in spinal cord distinct from Type I. These sites were detected by inhibition of [^{3}H]Ro15-1788 binding (0.5 nM, 0°C) by a selected set of compounds (Ro15-1788, flunitrazepam, ß-CCE, ß-CCP DMCM, zolpidem, alpidem, CL218872 and AHR11797) in rat cerebellar and spinal cord membranes. In cerebellar membranes, each compound displaced [3H]Ro15-1788 from a single receptor (Hill slope = 1). Evidence for receptor heterogeneity was found in the spinal cord. Hill slopes <1 were observed for flunitrazepam (.88), AHR11797 (.87), CL21887 (.82), ß-CCP and ß-CCE (.74), zolpidem (.62) and alpidem (.50). Algidem was unable to displace -10% of the specifically bound [3H]Ro15-1788 from spinal cord membranes. Using LIGAND analysis, the best fit was to a 3-site model, with relative receptor densities of 61%, 31%, and 8%. These 3 sites, designated Type IIa, IIb, and IIc, appear to be pharmacologically distinct from the Type I receptor. Finally, thermodynamic analysis of the binding (from binding studies performed at 0°C, 20°C an d 37°C) of each ligand to each receptor subtype provides additional evidence for unique receptors. Below are binding affinities at 0°C and thermodynamic parameters of two compounds previously designated "Type I-selective".

	Type I	Type IIa	Type IIb	Type IIc
CL218872				
K_i (nM,0°C)	45.8	541	47.9	4300
$\Delta H°$(kcal/mol)	-10.7	-6.94	-12.4	-7.57
$\Delta S°$(cal/mol K)	-5.46	+3.13	-12.1	-3.27
Zolpidem				
Ki (nM,0°C)	10.5	31.7	1200	46900
$\Delta H°$(kcal/mol)	-11.9	-7.14	-9.18	-4.47
$\Delta S°$(cal/mol K)	-6.22	+8.01	-6.68	-17.0

Clearly, further studies of BDZ ligand selectivity must include these additional binding sites.

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Alteration of Barbiturate Dependence by Benzodiazepine Inverse Agonists

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INTRODUCTION

The GABA-A receptor mediates the action of CNS depressants and, possibly, the development of dependence (Olsen, 1981, Tallman and Gallager 1985 Benzodiazepine inversee agonists have been used to further define the interactions which occur at the GABA-A receptor complex (MacDonald et al., 1986, Gardner, 1988). Both Ro 15-4513 and FG 7142 bind reversibly and with high affinity to the benzodiazepine binding site on the GABA-A receptor. They have been shown to inhibit hypothermia as well as various behavioral activities induced by pentobarbital (PB) (Bonnetti et al, 1984, Weinger et al., 1990; Hoffman et al., 1987). They also inhibit the PB enhancement of GABA-activated chloride ion flux (Harris et al, 1988). These studies were designed to determine the effect of Ro 15-4513 and GF 7142 on PB-induced dependence.

METHODS

Male, Sprague-Dawley rats were prepared with indwelling, ip, cannula allowed several days of recovery and were continuously infused with either saline (Contol) or with escalating doses of PB for 12 days. There following an abstinence peroid during which all rats received aline. In Study 1, rats were treated, once daily for 12 days, with Ro 15-4513, 15 mg/kg,ip while in Study 2, rats received FG 7142, 15 mg/kg,ip, once daily for 12 days. In Study 3, rats were treated with either Ro 15-4513 or FG 7142, at 5, 10, or 15 mg/kg, ip, at 0,4,8,12,16,24,28,32 or 36 hours of abstinence. Body weight was determined every 2 hrs up to 16 hr and at 24,28,32,36 and 48 hr.

RESULTS AND DISCUSSION

Once daily administration of Ro 15-4513 significantly attenuated withdrawal signs in PB dependent rats while FG 7142 had no effect. Neither compound had a significant effect on body weight. In Study 3, only Ro 15-4513 had a significant effect on withdrawal scores. It appears that the administration of Ro 15-4513 during the PB infusion period inhibited the development of dependence. It is proposed that the action of Pb and Ro 15-4513 on the GABA-A receptor results in opposing actions on chloride ion flux through the chloride ion channel.

REFERENCES: Available upon request.

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Pentobarbital-Like Discriminative Stimulus Effects of Direct $GABA_a$ Agonists

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Male Sprague-Dawley rats (n=10) were trained using a 2-lever operant procedure to discriminate pentobarbital (PB) (5.0 mg/kg) from saline vehicle. Responding was maintained under an FR-32 schedule of food reinforcement. Stimulus control was assessed under drug (PB) and saline conditions prior to initiation of generalization tests. Substitution tests were conducted with the direct GABA agonists muscimol (0.1-3.0 mg/kg i.p.), THIP (1.0-30 mg/kg i.p.) and progabide (30.0-560 mg/kg i.p.) and the indirect GABA agonist diazepam (0.3-5.0 mg/kg i.p.). Muscimol yielded partial substitution for PB with a maximum of 60% drug-lever responding. THIP similarly produced a maximum mean of 50% PB-lever responding. Dose-dependent rate decreasing effects were noted following the administration of muscimol and THIP. Progabide produced a maximum of 40% PB-lever responding at a dose (560 mg/kg) that significantly decreased response rate. Doses below 560 mgkg failed to alter responding. Diazepam (0.30-3.0 mg/kg) substituted completely for pentobarbital in all rats tested without altering response rate. The data provide additional evidence for differences in the in vivo effects of direct and indirect-acting GABAergic drugs. (Research supported by NIDA grants DA-01442, DA-03112 and DA-07027).

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Further Evidence That Nicotine Exerts Discriminative Stimulus(DS) Control of Behavior in the Rat Via an Action at Select Central Nicotinic Acetyl-Cholinergic Receptors (n-AChR's)

R. James, H. Villaneueva, J. Johnson, S. Arezo and J.A. Rosecrans

Current concepts concerning nicotine's CNS mechanism(s) of action suggest that this drug is producing its effects via an interaction at nicotinic-chohnergic receptors (n-AChRs) which open a membrane cation channel. Following initial opening of the channel, nicotine appears to induce a rapid desensitization (DZ) of the n-AChRs, closing the channel and resulting in a cessation of nicotine's effects. Research presented here provides evidence of this secondary desensitization process in vivo by demonstrating nicotine's ability to induce acute tolerance in the discriminative stimulus (DS) paradigm. It appears that we have found a means of investigating cellular mechanisms in vivo using operant behavior.

The ability of nicotine to elicit DS control of behavior was significantly reduced via a challenge dose (800 ug/kg. s.c.) of nicotine administered 15-180 minutes prior to the training dose (400 ug/kg, s.c.). Initial experiments showed that select rats exhibit nicotine-induced DZ of this DS; 13 of 21 rats demonstrated this phenomena, and the time to develop acute tolerance varied, suggesting that these effects may be contingent upon the individual rat studied. Only 39% of the rats studied were unable to exhibit DZ to nicotine.

Further experiments provided additional support for this in vivo DZ and suggested that this effect was associated with acetylcholine (ACh). When rats were tested for generalization to physostigmine, non-DZ rata generalized to physostigmine and DZ rats responded to physostigmine as if it were saline. Of special importance was that mecamylamine and scopolamine were observed to attenuate the physostigmine generalization. Thus it is postulated that nicotine is inducing desensitization via receptor sites analogous to that of the endogenous neurotransmitter ACh, and that the nicotinic cue can be mediated and/or modulated via multiple receptors, contingent upon the individual rat studied.

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Physical Dependence Exhibited in Mice Continuously Exposed to 1 ,1 ,1 -Trichloroethane

E.B. Evans and R.L. Balster

The halogenated hydrocarbon 1,1,1-trichloroethane (TCE) is a common commercial solvent that is contained in products that are voluntarily inhaled for their psychoactive effects. It is not known whether TCE can produce dependence. TCE was continuously administered by placing male CFW mice in a 20.8-1 glass exposure chamber through which a nominal concentration of 500 to 4000 ppm TCE vapor was passed at a rate of 10 liters/minute. TCE vapor chamber concentrations were continuously monitored by passing the air exiting the chamber through an IR spectrometer. After TCE exposure mice were evaluated hourly for signs of withdrawal. The withdrawal reaction was primarily measured by scoring convulsions elicited when either lifting a subject by the tail or lifting and gently spinning the subject. Two hours after cessation of 4 days of continuous exposure to 2000-4000 ppm TCE, most subjects exhibited tonic or tonic-clonic convulsions. Tonic convulsions continued for 10-12 hours post exposure with the peak withdrawal reaction occurring 3-6 hours post exposure. TCE withdrawal signs were reversed by re-exposure to TCE. Cross dependence was obtained between TCE and pentobarbital and ethanol but not chlorpromazine. These data provide evidence that an extended continuous exposure to an abused solvent may result in a physiological dependence. (Research was supported by NIDA grant DA-03112 and NIEHS grant ES-07087.)

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Behavioral and Neurochemical Responses to 4-Methylaminorex; A New Stimulant of Abuse

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4-Methylaminorex (4-MAX) is a pemoline-like amphetamine analog which recently has gained attention due to its potential as a stimulant of abuse and the ease with which it is synthesized from readily available drugs, such as phenylpropanolamine. As a drug of abuse, 4-MAX has been masqueraded by drug dealers as methamphetamine or cocaine. Even though 4-MAX was recently classified as a Schedule I substance (April, 1989), little is known about its pharmacological effects. Behaviorally, 4-MAX induced locomotor and stereotypic activity much like other amphetamine analogs, but a unique property of this drug was its potent epileptogenic action. With i.c.v. doses as low as 82 µg/kg, 50% of treated mice experienced all-limb clonic seizures. In rats, an i.p. dose of 15 mg/kg caused 50% of the animals to experience seizures. The neurochemical response by rats to 4-MAX appeared to be comparable to other amphetamine derivatives, especially methylenedioxymethamphetamine (MDMA; ecstasy). Specifically, 10 mg/kg 4-MAX caused early and persistent declines in striatal tryptophan hydroxylase activity and changes in the dopaminergic systems which suggest that this drug causes (1) some toxicity to serotonergic neurons and (2) substantial dopamine release. In addition, 4-MAX administration dramatically increased nigral neuropeptide levels (neurotensin, dynorphin A and substance P) much like other amphetamine-related drugs: these peptide changes were most likely a result of drug-induced increases in activity of dopaminergic pathways. (Supported by grants DA 00869 and DA 04222).

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Evaluation of the Reinforcing Effects of Mazindol in Baboons

C.A. Sannerud and R.R. Griffiths

Mazindol is an imidazoisoindole anorectic agent used to treat obesity. Although mazindol has pharmacologic activity similar to prototypic anorectics, it differs from other psychomotor stimulants in its site and mechanism of action. Mazindol produces amphetamine-like discriminative stimulus effects and CNS stimulation in animals and humans, as well as reinforcing effects and stimulant-like stereotypies in animals. Recently, mazindol was used as a radio-labelled ligand for a cocaine-sensitive receptor site mediating dopamine reuptake and it has been proposed as a potentially useful psychotherapeutic agent in the treatment of cocaine abuse and dependence. The ability of mazindol to maintain self-injection was examined in 3 baboons using a standard i.v. substitution procedure. Responding was maintained under a fixed-ratio 160-response schedule of cocaine delivery (0.32 mg/kg/inj). Each drug injection produced a 3 h time-out allowing a maximum of 8 injections per day. Vehicle or mazindol (0.001-0.1 mg/kg/inj) was substituted for cocaine for a period of 15 or more days. Mazindol doses (0.001-0.032 mg/kg/inj) maintained self-injection within the range of vehicle control. The highest mazindol dose (0.1 mg/kg) produced a cyclic pattern of self-injection and disrupted food-maintained behavior, suggesting psychomotor stimulant toxicity. The present study found that mazindol can serve as a reinforcer and can produce behavioral toxicity in the baboon, thus confirming and extending previous reports of mazindol's behavioral effects. Supported by NIDA Contract No. 271-89-8146.

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Ritanserin, A New Therapeutic Approach for Drug Abuse

T. Meert, G. Clincke, Y. Gelders and G. De Smedt

The serotonin antagonist ritanserin is clinically observed to markedly increase slow wave sleep and to improve mood and drive in dysthymic and depressed patients. Occasional observations over the last five years indicated that ritanserin may be of value in subjects withdrawing from drugs of abuse. Such patients may experience similar problems in coping with daily life as dysthymic/depressed patients. Different experiments were conducted to evaluate a possible role of ritanserin in drug abuse. In an oral drinking test in rats, ritanserin was demonstrated to reduce drug intake and drug preference in animals given the choice between the drug of abuse and water after a period of forced drug exposure. Adaptations of the test procedure by prolonging the drug exposure time, by building in a withdrawal phase and by changing treatment schedules, did not essentially change the activity of ritanserin. Ritanserin was found active against three pharmacologically different agents: alcohol, cocaine and fentanyl. The strong preference for a non-addictive substance. sucrose, was not attenuated by ritanserin. At no time did ritanserin interfere with total fluid intake or any consummatory physiological processes. Additional drinking experiments indicated that ritanserin did not create aversion for the various substances tested. Also a direct interaction between ritanserin and the drugs of abuse could be ruled out on the basis of interaction studies.

Further experiments indicated that ritanserin was not self-administered and had no discriminative stimulus properties in a drug discrimination test procedure. Ritanserin also did not substitute for various drugs of abuse. In a conditioned place preference test, ritanserin selectively attenuated the place preference for various drugs.

With regard to drug withdrawal, ritanserin was demonstrated: i) to regulate sleep disturbances after cessation of chronic cocaine treatment: ii) to block naloxone-induced response rate reductions in opioid trained rats: iii) to reduce naloxone-induced withdrawal tics in chronic morphine-dependent mice and iv to overcome exploratory inhibition after stopping a chronic cocaine treatment.

Because of the apparent lack of any direct interaction with substances of abuse, the evidences presented here indicate that ritanserin might be a good candidate for treatment of problems related to drug abuse. The first open trials with ritanserin in drug addicts appear to confirm this idea.

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Pharmacogenetic Assessment of the Use of Carbamazepine for the Treatment of Cocaine Addiction and Toxicity

R.J. Marley and S.R. Goldberg

There are genetic differences in the development of increased susceptibility to the convulsant effects of cocaine following its repeated administration (cocaine kindling). The repeated administration of cocaine results in the development of tolerance or sensitization to the convulsant effects of cocaine depending on the genetic background of the individual being examined. The present studies used inbred mouse strains to evaluate genetic differences in the modulation of the epileptogenic and convulsant effects of cocaine by carbamazepine. Chronic carbamazepine markedly attenuated the development of cocaine-kindled seizures. There were, however, differences among the strains of mice in the degree to which carbamazepine inhibited these seizures and while carbamazepine slowed down the rate of cocaine kindling, the inhibition of the kindling process diminished over time. Results from cocaine challenge experiments in which the chronic cocaine/carbamazepine-treated mice were administered a convulsant dose of cocaine 3 days after the end of the treatment period suggested that chronic carbamazepine may be effecting the development of tolerance, but not sensitization, to the convulsant properties of cocaine. These studies also revealed that there are genotype-specific lethal effects associated with the combination of cocaine and carbamazepine. The concurrent administration of cocaine and carbamazepine was lethal among C57, but not among BALB and SJL mice. In fact, carbamazepine may have protected SJL mice from the lethal, anorectic, effects of chronic cocaine observed in this strain. Chronic carbamazepine also appears to increase the threshold for acute cocaine-induced seizures. BALB and C57 mice, but not SJL mice, chronically-treated with carbamazepine for 10 days were substantially less susceptible to a convulsant dose of cocaine than their controls in spite of the fact that carbamazepine treatment had been stopped 72 hrs earlier. These studies indicate that C57Bl/6J, BALB/cByJ and SJL/J mice can serve as useful animal models for elucidating the mechanisms underlying some of the effects of chronic cocaine and/or carbamazepine.

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A Non-thermal Nociceptive Model in Dogs

P. Lloyd and M.H. Ossipov

We are presenting a novel non-rodent model for assessment of analgesia. A hand-held pressure device was used to deliver a noxious stimulus to beagle dogs. Pressure was steadily increased, pushing a metal peg (2 mm diameter) against the forelimb of the dog. The lifting of the limb 1 cm was taken as a nociceptive response. The following analgesics were injected intravenously: sufentanil, fentanyl, hydromorphone, methadone, and morphine. Fore-limb withdrawal pressure was measured before (control) and several times after (post) drug injection. A maximum pressure of 100 psi was used as a cut-off to prevent tissue damage. Data were converted to % maximal possible effect (%MPE) by: 100 x [(post - control)/(100 psi - control)] and a graded dose-response curve (DRC) was generated (n=4 dogs per dose). The A_{50} (50 %MPE dose) for each DRC was determined by linear regression. The A_{50} values (95% confidence limits) for sufentanil, fentanyl, hydromorphone, methadone, and morphine were 0.0008 mg/kg (0.0007 - 0.00l), 0.0027 mg/kg (0.001 - 0.005), 0.06 (mg/kg (0.02 - 0.18), 0.36 mg/kg (0.357 - 0.364), and 0.50 mg/kg (0.36 - 0.71) respectively. The rank order of potency in this model is the same as the clinical rank order of potency. This model provides for reliable, non-thermal assessment of nociception in dogs.

AFFLIATION:

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A Study of Opiate Abuse During Pregnancy Using a Rat Model

J.E. Olley, G.K.L. Tiong and T.L. Pierce

Rat and human development show many features in common but parturition occurs earlier in the rat, approximately equivalent to 26-30 weeks; and so an animal model of drug exposure in utero should not only have even medication in place before conception, but this should be continued until the equivalent degree of neural development in the human at birth has occurred, approximately postnatal day 18 in the rat (Dobbing and Sands, 1979; Hutchings 1990). To avoid artefacts related to fluctuating narcosis and withdrawal (Lichtblau and Sparber 1984). uniform medication of virgin female rats with opioids was achieved by administration in the drinking water (Badawy et al., 1982. Maintenance concentrations of morphine, methadone (0.2 - 0.8 mg/ml) and buprenorphine (0.001 0.1 mg/ml) were achieved 4 days prior to mating then continued through pregnancy until parturition. Litters (4-12) were fostered within 24h of birth to lactating females drinking either plain water or the maternal opiate solution. Rat Pups were assessed on survival, physical and behavioral development to postnatal day 21. Morphine and methadone (0.4-0.8 mg/ml) treated mature animals, although physically dependent appeared healthy, conceived and produced litters at doses which reduced survival of their pups, those fostered to water drinking nurses being most affected. Growth of the pups was unimpaired except for those nursed by high dose morphine or methadone users. All opiate exposures delayed development of the righting reflex. There were minimal or no significant effects of morphine or methadone on eye opening, incisor eruption or auditory startle; however, buprenorphine from 0.1 mg/ml markedly retarded (by at least 1 day) eye opening, incisor eruption and auditory startle. This rat model concurs with human experience that opiates are more toxic to the fetus than the mother and that the consequences of abrupt withdrawal in the equivalent of the third trimester are severe. It should be noted that indicators of deranged development were greatest for buprenorphine-exposed rat pups. Of particular concern was the effect on auditory startle, possibly indicating brainstem dysfunction and potential for vulnerability to SIDS (sudden infant death) in the human infant. Entirely appropriate medication of the postnatal rat pup has still not been achieved but this rat model provides a basis for further studies to evaluate the effects of controlled opiate exposure in utero and various degrees of withdrawal after parturition.

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RTI-4614: A Highly Selective Ligand for, and A Pseudoirreversible Inhibitor of, the Mu Opioid Receptor

H. Xu, G.A. Brine, F.I. Carroll, A.E. Jacobson, K.C. Rice and R.B. Rothman

RTI-4614 ((±)-cis-N-[1-(2-hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-N-phenylpropanamide HCl) is an analog of 3-methylfentanyl, and is a mixture of the 4 possible diastereomers. The apparent Ki values (nM±SD) of RTI-4614 for mu, delta, kappa,, kappa, and kappa, binding sites were 0.0055±0.0006, 148±11, 84.9±12.1, 2275±147 and 22.3±2.2 nM, respectively, indicating a mu/delta selectivity of about 27,000-fold, and a mu/kappa, selectivity of about 4000-fold (Rothman et al., 1991). In other experiments, rat brain membranes were preincubated with concentrations of RTI-4614, washed extensively by centrifugation, and wash-resistant inhibition measured using [^{3}H]DAMGO. Residual drug in the aqueous phase of the membrane suspension was measured by centrifuging the membrane suspension, and assaying the supernatant for inhibitory activity. Receptor inhibition was calculated as wash-resistant inhibition minus supernatant inhibition. The results demonstrated an IC50 for wash-resistant inhibition of 18.7 nM, and a maximal receptor inhibition of about 40%. Saturation binding studies using control and RTI-4614-pretreated membranes demonstrated a decrease in the Bmax and no change in the Kd. The ability of RTI-4614-4 to partially decrease the [^{3}H]DAMGO Bmax could be interpreted as preliminary evidence for mu receptor subtypes. Future studies will examine the individual properties of its 4 diastereomers as well as the use of RTI-4614-4 to define subtypes of the [^{3}H]DAMGO binding site.

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Naltrindole Retards Tolerance Development to Morphine-Induced Effects on EEG and EEG Power Spectra

H. Stamidis and G.A. Young

Recent evidence suggests the involvement of delta opioid binding sites in the development of tolerance to morphine in mice, as measured by the tail flick test for analgesia (Abdelhamid, 1990). In the present study, EEG and EEG power spectra were used to assess the effects of naltrindole, a selective *delta* opioid antagonist, on the development of tolerance to morphine. Adult female Sprague-Dawley rats were implanted with cortical EEG electrodes and permanent indwelling i.c.v. and i.v. cannulae. Twice daily for 7 days, rats were pretreated with either i.c.v. naltrindole (20 nM) or i.c.v. water, 30 minutes before i.v. morphine (10 mg/kg) injections. The treatments produced EEG slow-wave bursts and associated behavioral stupor. The amount and duration of these effects decreased more rapidly over the 7 days in the naltrindole pretreated rats than in the water pretreated rats. EEG data were further analyzed on a Pathfinder II computer. The development of tolerance was reflected by decreases in the total absolute EEG spectral power (1-50 Hz) over the 7 day period. Rats that were pretreated with i.c.v. water displayed an approximate 60% decrease in total absolute EEG spectral power by the 7th day, whereas those rats that were pretreated with i.c.v. naltrindole (20 nM) showed a slower rate of decrease in total absolute EEG spectral power over the 7 days. Furthermore, a delayed qualitative change in the EEG power spectra was observed in rats pretreated with i.c.v. naltrindole. On day 1, EEG slow-wave bursts were associated with increases in EEG spectral power over the 1 to 10 Hz range. However, by day 3, EEG slow-wave bursts were associated with a predominant EEG spectral peak in the 4-6 Hz band. These results further implicate the *delta* opioid binding site in the development of tolerance to morphine. (Supported by NIDA Grant DA-01 050.)

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Agonist-Antagonist Properties of Partial Mu Opioid Agonists in the Drug Discrimination Procedure: The Role of Training Dose

S.R. Mattox, S.S. Negus, M.J. Picker and L.A. Dykstra

The drug discrimination procedure has been very useful in evaluating the discriminative stimulus properties of opioid. Several studies have demonstrated that training dose is an important factor in the pattern of substitution seen with many agonists in this procedure. The purpose of this study was twofold: a) to investigate the degree of substitution of partial opioid agonists for the discriminative stimulus properties of a high and low training dose of morphine (MS) and b) to evaluate the degree of antagonism produced by partial agonists of the discriminative stimulus properties of a high and low MS training dose. Rats were trained to discriminate either 3.0 mg/kg (low TD group; N=6) or 10.0 mg/kg (high TD group; N=6) MS from saline under an FR 20 schedule for food reinforcement. When a stable discrimination had been established, dose-effect curves were obtained for levallorphan (LEVL: 10-56.0 mg/kg), (-) pentazocine (PENT: 0.1-56.0 mg/kg) (+) and (-) cyclazocine (CYCL: (+): 1.0-30.0 mg/kg; (-): 0.01-3.0 mg/kg) and (-) N-allylnormetazocine (NANM: 0. 1-10.0 mg/kg). In addition, dose-effect curves for (-) CYC (0.03-1.0 mg/kg) and (-) NANM (0.3-17.5 mg/kg) in combination with each training dose were also obtained. LEVL, (-)PENT, (-)CYCL, (+)CYCL and (-)NANM all produced greater substitution for the low MS TD than for the high MS TD. In addition, (-)CYCL and (-)NANM produced greater antagonism of the high MS TD than of the low MS TD. These data indicate that the weak agonist properties of partial opioid agonists can be detected by the use of a low training dose whereas the antagonist properties of the partial agonists can be revealed using a higher training dose. Thus, training dose is important in characterizing the degree of agonist and antagonist properties of partial opioid agonists.

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Determination of an Apparent Dissociation Constant for Buprenorphine *In Vivo* Relative to That of Morphine

K.A. Freeman, H. Wheeler-Aceto, A. Cowan and R.J. Tallarida

Complete dose-response curves (DRC's) of two drugs with different efficacies can be used to determine the dissociation constant (**K**) for one of the drugs provided that the **K** for the other drug is known (Freeman and Tallarida, 1991). This method was used to determine an apparent **K** for buprenorphine (BUP), the mu opioid partial agonist, by comparing its effects to those of morphine (MOR) in rats in the hot water tail-dip test and gastrointestinal (GI) transit test. Both agents were administered S.C. to male Sprague Dawley albino rats and their antinociceptive or GI transit effects were recorded over a range of doses. BUP was more potent, but less efficacious, than MOR in both assays. The DRC's for BUP were bell-shaped; however, only the ascending portion of the curve was used in computations. The administered doses of each drug were converted to brain (cortex) concentrations from which DRC's were reconstructed and subsequently analyzed. The brain unit conversions significantly altered the relative potencies of BUP and MOR. These curves and a formula from the reference above were used to determine the **Kp** for BUP. $\mathbf{K_A}$ values of 17×10^{-7}M and 5.1×10^{-7}M (tail-dip and GI transit tests, respectively) for MOR were previously determined in these assays by the method of partial irreversible receptor occlusion (Tallarida and Cowan, 1982; Raffa et al., 1982). We calculated the apparent Kp for BUP to be 3.75×10^{-7}M and 3.15×10^{-7}M (tail-dip and GI transit tests, respectively), suggesting a greater affinity relative to MOR. An alternate computation based on reciprocal equieffective concentrations confirmed the values reported by this method. We conclude that our technique of analysis is useful for obtaining K values for opioids from functional studies. (Supported by DA 03945 and DA 07237 from NIDA).

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The Effects of Buprenorphine Alone and in Combination with Cocaine on Self-Stimulation Thresholds

P.Z. Manderscheid, S. Panicker, and R.A. Frank

Buprenorphine (BUP) has recently been suggested as a pharmacotherapy for cocaine abuse. The abuse liability of BUP and its ability to antagonize the reinforcing effects of cocaine (COC) were evaluated by assessing the effects of BUP alone, and in combination with (COC) on self-stimulation (SS) thresholds. It has been demonstrated that a drug's ability to lower SS thresholds is indicative of its abuse liability. Male Long-Evans rats implanted with ventral tegmental area electrodes served as subjects. SS thresholds (using a curve-shift paradigm) were measured 15 min following saline or BUP (0.004, 0.02, 0.09, & 0.2 mg/kg IP) treatment. BUP failed to produce any changes in SS thresholds or in maximum response rates. In the next phase, BUP (0.02, 0.09, & 0.2 mg) was injected 10 min prior to COC HCl (25 mg/kg IP). Animals were tested 15 min following COC administration. COC produced a significant decrease in SS thresholds, which was not antagonized by BUP pretreatment. Since the dose of COC may have been too high to detect any possible changes produced by BUP on SS thresholds, 0.9 mg/kg BUP was combined with 15 mg/kg COC. As previously demonstrated, COC significantly lowered SS thresholds. However, the combination of BUP and COC did not produce any significant changes in SS thresholds when compared to COC alone. These experiments indicate that, in a SS paradigm, BUP shows little evidence of abuse liability. Results from the co-administration studies indicate that, at the dosages tested, the reinforcing properties of COC are neither blocked or attenuated by BUP pretreatment. However, 0.2 mg/kg BUP in combination with 25 mg/kg COC did produce a significant reduction in response rates. This suggests that BUP and COC can interact synergistically, and that further study of BUP/COC interactions is warranted. This research was supported by NIDA grant DA04483.

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Chronic Administration of GBR1 2909 Partially Attenuates Cocaine-Induced Locomotor Activity in Rats

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INTRODUCTION

GBR12909 is a high affinity inhibitor of dopamine (DA) reuptake (Van der Zee et al. 1980) which partially attenuates the ability of cocaine to elevate extracellular levels of DA (Rothman et al, 1989). The present study examined the effect of chronic administration of GBR12909 on cocaine-induced locomotor activity. Four groups of male Sprague-Dawley rats were used: VEH/SAL, VEH/COC, GBR/SAL and GBR/COC [COC=cocaine, GBR=GBR12909, VEH=vehicle, SAL=saline]. Rats received daily injections of GBR (10 mg/kg i.p.) or vehicle for 13 days between 9 and 10 AM, followed by administration of either COC (20 mg/kg, i.p.) or SAL 15 min later. Baseline locomotor activity produced by administration of COC or SAL was determined on day #-2 (2 days prior to administration of GBR). Locomotor activity was also measured on day #4 and #13 (after 4 and 13 days of administration of GBR or VEH). On day #3, the VEH/COC, GBR/SAL and GBR/COC groups showed essentially the same locomotor activity, which was not significantly different from the baseline measurements. The locomotor activity present in the GBR/COC group was not different from that of the VEH/COC group. On day #13, however, the locomotor activity of the GBR/COC group was significantly reduced by 43 % relative to the VEH/COC group. Intense stereotypy was not observed in any of the 4 groups. Interestingly, administration of COC 15 min after GBR did not produce any additional increase in locomotor activity or stereotypy. In a related study, administration of COC (20 mg/kg i.p.) 15 min after administration of COC (20 mg/kg i.p.) also did not produce increased locomotor activity. These data suggest that chronic treatment with GBR may act to attenuate the locomotor effects of COC. Future studies will examine this point using s.q. implantation of GBR pellets.

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Available from the last author upon request.

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Neurobehavioral Consequences of Gestational Cocaine Exposure

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Numerous neurobehavioral alterations were observed in offspring of Sprague-Dawley rat dams exposed chronically to 40 mg/kg cocaine hydrochloride administered subcutaneously from gestational days 8-20 when compared with offspring from pair-fed, nutritional control, and ad lib control dams. No differences in pup body weight at any age or in physical or reflex maturation have been observed. However, young cocaine-exposed offspring exhibit: (1) deficits in cognitive function; (2) a number of signs of alterations in dopamine function such as increased D2 receptor binding and an increased responsiveness to the D2 agonist quinpirole; and (3) alterations in other neural systems including increases in opiate receptor binding along with an increased responsiveness to morphine. Early cocaine exposure may also alter the later reinforcing efficacy of cocaine: attenuations in conditioned olfactory preferences for cocaine are seen in 8-day-old cocaine-exposed pups, and cocaine offspring as adults do not show conditioned place preferences to either 2 or 5 mg/kg cocaine, conditioning that is observed in offspring of control dams. Taken together, these data provide strong evidence that cocaine is a neurobehavioral teratogen in this model system.

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Nonspecific Effects of Carbamazepine on Cocaine Self-Administration in Rats

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Previous case reports suggest that carbamazepine (CBZ) suppresses cocaine-induced rush and craving, whereas in examinations under more rigorous clinical-trial conditions, such claims were not supported (CPDD, 1990). In the present study, rats were trained to self-administer i.v. cocaine in daily 2-hr sessions where every 10th lever press delivered 1 mg/kg cocaine. After two days of stable responding with drug-vehicle pretreatment, CBZ was injected (i.p.) on the 3rd and 4th days before the session. Cocaine-maintained responding was unaffected by CBZ at 7 mg/kg, whereas 15 mg/kg produced significant suppression of responding for cocaine on the 2nd, but not the 1st day of CBZ treatment. When pretreated with the 15 mg/kg dose of CBZ for 4 consecutive days, responding was slightly, but significantly decreased when compared with the vehicle-treatment group. In another group of animals trained to lever-press with food reinforcement, CBZ (15 mg/kg) significantly suppressed rates of responding by 75% suggesting that suppression of cocaine-maintained responding was due to nonspecific effects.

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Discriminative Stimulus Properties of Cocaine: Effects of the Novel Dopamine Autoreceptor Antagonists (+)-AJ 76 and (+)-UH 232

P.M. Callahan, M.F. Piercey and K.A. Cunningham

Recent evidence suggests that the putative dopamine (DA) autoreceptor antagonists, (+)-AJ 76 [cis- (+)-1S,2R-S-methoxy-1-methyl-2-(n-propylamino) tetralin HCl] and (+)-UH 232 [cis-(+)-1S,2R-5-methoxy-1-methyl-2-(di-n-propylamino)-tetralin HCl], share some neurochemical and behavioral effects with both psychostimulants and neuroleptics. Similar to cocaine, (+)-AJ 76 and (+)-UH 232 induce reserpine-sensitive locomotor stimulation and enhance extracellular levels of DA in an impluse- and calcium-dependent manner. On the other hand, their ability to block apomorphine- and amphetamine-induced behaviors and elevate DA synthesis and turnover are characteristic of classical DA postsynaptic antagonists.

The present experiment investigated whether (+)-AJ 76 and (+)-UH 232 mimicked or antagonized the stimulus properties of cocaine. Rata were trained to discriminate cocaine (5 or 10 mg/kg; N=8/group) from saline in a two-lever, water-reinforced, drug discrimination task. Administration of (+)-AJ 76 (2.5-20 mg/kg) engendered only a partial substitution for cocaine (< 60% drug responding). (+)-AJ 76 (2.5-40 mg/kg) given in combination with cocaine (10 mg/kg) failed to attenuate the cocaine cue. Additionally, a fixed dose *of* (+)-AJ 76 (2.5 or 10 mg/kg) plus various doses of cocaine (1.25-5 mg/kg) did not alter the cocaine dose-response curve. When given alone, (+)-UH 232 (2-16 mg/kg) produced saline-appropriate responding. (+)-UH 232 (2-16 mg/kg) given in combination with cocaine (5 mg/kg) was also unable to block the cocaine state. When administered in combination with different doses of cocaine (0.625-2.5 mg/kg), (+)-UH 232 (2 or 8 mg/kg) did not alter the discriminability of cocaine.

Results of the present study suggest that neither (+)-AJ 76 or (+)-UH 232 are perceived as identical to cocaine nor do they antagonize the stimulus effects of cocaine as might be expected of classical neuroleptics. Additionally, administration of these aminotetralins in combination with several doses of cocaine did not result in a potentiation of the discriminability of low doses of cocaine. Taken together, these findings, along with the partial cocaine-like effect and the long duration of action, suggest that (+)-AJ 76 may provide a novel agent to assess in the pharmacotherapy of cocaine abuse in humans.

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Interaction of the Discriminative Stimulus Properties of PCP and Delta^9-THC in Rats

P. Doty, M.J. Picker and L.A. Dykstra

Reports indicate that the most widely used illicit drug, marijuana, is often used in combination with other drugs of abuse, including phencyclidine (PCP). Although the behavioral effects of Δ^9-tettahydrocannabinol (Δ^9-THC) and PCP when administered alone have been fairly well characterized, few studies have evaluated the interactive effects of these drugs. In the present study, a drug discrimination procedure was used to evaluate combinations of Δ^9-THC and PCP.

Using a standard two-lever drug discrimination procedure, two groups of rats were trained to discriminate PCP (1.75 mg/kg) or Δ^9-THC (3.0 mg/kg) from their respective vehicle. Substitution tests were begun once the mean percent of injection-appropriate responses prior to the first food reinforcer was at least 80% over 10 consecutive days.

In PCP-trained rats, Δ^9-THC produced a maximum of 42% PCP-appropriate responding. When administered in combination with PCP, Δ^9-THC produced marked variability of stimulus control and decreased PCP-appropriate responding. In contrast, in these rats, morphine produced a maximum of 30% PCP-appropriate responding, but when administered in combination with PCP, did not alter PCP-appropriate responding. In Δ^9-THC-trained rats, PCP produced a maximum of 57% Δ^9-THC-appropriate responding. When administered in combination with Δ^9-THC, PCP produced marked variability of stimulus control and decreased Δ^9-THC-appropriate responding. In these rats, morphine produced a maximum of 32% Δ^9-THC-appropriate responding and, with the exception of the highest dose, morphine did not alter Δ^9-THC-appropriate responding. In these rats, ethanol produced a maximum of 58% Δ^9-THC-appropriate responding, but when administered in combination with Δ^9-THC, did not alter Δ^9-THC-appropriate responding.

Thus, combinations of PCP and Δ^9-THC produced a clear attenuation of drug-appropriate responding in rats trained to discriminate either PCP or Δ^9-THC. Other drugs such as morphine or ethanol generally did not alter drug-appropriate responding. In contrast with previous reports that combinations of PCP and Δ^9-THC produce effects that are greater than those produced by either drug alone, the present data showed no potentiation of the discriminative stimulus effects in rats trained to discriminate either PCP or Δ^9-THC. (Supported by NIDA contract 271-87-8126).

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Nicotine and Age-Associated Decrease in the Tail-Flick Latency

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In a number of studies, the antinociceptive effects have been demonstrated in rodents and dogs. In humans, during aging and in several neurodegenerative disorders, a decline in ^{3}H nicotine binding has been reported. A reduction in cholinergic activity is also associated with the aging rat. There is evidence that tobacco smoking increases the density of ^{3}H nicotine binding sites. Similarly, in rats chronic nicotine treatment leads to changes in nicotine binding in the brain. The nicotine-induced antinociception in rodents is known to correlate with nicotine brain levels. In the current study, the aged rats (2 years) were not sensitive to the painful stimuli when compared to young controls or to aged-matched rats receiving low-dose nicotine in their drinking water. Therefore, the extent of the contribution of aging and the circulating nicotine to the sensitivity of the old rats in the tail-flick procedure was determined.

The tail-flick latencies following the continuous nicotine intake (0.5 and 1.0 mg %) in the drinking water of male Fisher rats were evaluated in comparison to the young and aged-matched controls using the reaction time to a heat stimulus. The rats were trained to flick their tails and were retested after habituation to the test procedure. Young rats also received the nicotine in their drinking water subchronically and the tail-flick latencies determined during and following withdrawal from nicotine consumption.

The aged rats demonstrated a significant decrease in pain sensitivity which was rendered indistinguishable from young adult rats by the continuous two year low-dose nicotine consumption. The young adult rats showed increased sensitivity to pain during the subchronic nicotine consumption and upon withdrawal, the tail-flick responses returned to control levels. We therefore concluded, that the circulating low-doses of nicotine at up to 1.0 mg% increase sensitivity to nociceptive stimulation regardless of age. Supported by LSU - Pennington Biomed. Res. Center.

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Pharmacological Characteristics of NIH 10443, An Irreversible Opioid Receptor Antagonist, in the MouseTail-Withdrawal Assay

S.D. Comer, T.F. Burke, J.H. Woods and J.W. Lewis

NIH 10443, 14ß-(p-Chlorocinnamoylamino)-7,8-dihydro-N-cyclopropylmethyl normorphinone mesylate, an irreversible opioid receptor antagonist, was evaluated in mice using the warm-water (55° C) tail-withdrawal assay. Up to a dose of 32.0 mg/kg administered intraperitoneally, NIH 10443 failed to produce an increase in tail-withdrawal latency. The antagonist action of a single injection of either 3.2 or 32.0 mg/kg NIH 10443 given in combination with 32.0 mg/kg morphine administered 1 hour, 1, 2, 4 and 8 days after NIH 10443 were 2 and 8 days respectively. When given one hour prior to cumulative injections of morphine (32.0, 100.0, 320.0 and 1000.0 mg/kg), 32.0 mg/kg NIH 10443 produced an unsurmountable antagonism of morphine; 3.2 mg/kg NIH 10443 resulted in a shift down in the morphine dose-effect curve. In contrast, when the competitive opioid antagonist naltrexone was administered prior to the same doses of morphine, an unsurmountable antagonism was never seen, even at the highest dose of naltrexone tested (100.0 mg/kg). Using the method of partial irreversible blockade (Stephenson 1956), the dissociation constant (K_A) was calculated for morphine using 3.2 mg/kg NIH 10443 as the irreversible antagonist. The value of K_A for morphine in this assay was 2.0 x 10^{-5} mg/kg, a value similar to those obtained by other investigators using buprenorphine as the antagonist and measuring the inhibition of gastrointestinal transit and the analgesic effects produced by morphine in rats (Raffa et al., 1982; Tallarida and Cowan 1982). (Supported by NIDA Grant DA00254).

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Effect of Mu, Delta and Kappa Opioid Agonists on Heroin-Maintained Responding in the Rat

S.S. Negus, M.B. Weinger, S.J. Henricksen and G.F. Koob

INTRODUCTION: Current evidence suggests that the reinforcing effects of most opioid agonists are mediated by mu opioid receptors (see Negus and Dykstra, 1989 for review). However, delta opioid receptors also appear to be capable of mediating the reinforcing effects of some opioid agonists. Kappa agonists do not produce positive reinforcing effects, but they can serve as punishers or negative reinforcers, raising the possibility that kappa agonists might block the positively reinforcing effects of other opioids. Therefore, the purpose of the present experiment was to investigate the effect of mu, delta and kappa agonists on responding for the most widely abused opioid agonist, heroin.

METHODS: Male Wistar rats were trained to self-administer heroin (0.06 mg/kg/infusion) during daily three-hour sessions. On test days, rats were injected with either heroin (0-0.4 mg/kg SC), morphine (0-20.0 µg/kg ICV), DAMGO (0-1.0 µ-g ICV), DPDPE (0-20 µg ICV) or U50,488 (0-1.0 µg) one hour into the three hour session. The effects of heroin (0-l.6 mg/kg) were also evaluated on responding maintained by food.

RESULTS: Heroin, morphine and the highly selective mu agonist DAMGO all produced a pause in responding and a resulting decrease in the number of injections during the hour following their administration. In addition, heroin produced a pause in food maintained responding and a resulting decrease in the number of food reinforcements earned during the hour following its administration. However, heroin was approximately twice as potent in reducing heroin-maintained responding as in reducing food-maintained responding. Thus, low doses of heroin (0.2 and 0.4 mg/kg) selectively decreased heroin-maintained responding without affecting food-maintained responding. The selective delta agonist DPDPE and the selective kappa agonist U50,488 did not affect responding for heroin.

CONCLUSION: The ability of low doses of heroin to reduce heroin-maintained responding without affecting food-maintained responding demonstrates that an opiate agonist can selectively reduce heroin-maintained responding without producing a global deficit in the rat's ability to respond. This finding suggests that the present assay (eg. administration of a test drug one hour into a heroin self-administration session) can be used to evaluate pharmacological mechanisms responsible for heroin reinforcement with minimal interference from drug-induced motor impairment. The finding that the prototype mu agonist morphine and the highly selective mu agonist DAMGO produced a decrease in heroin-maintained responding suggests that the reinforcing effects of heroin in the rat are mediated by mu opioid receptors. Delta receptors do not appear to play a critical role in heroin reinforcement, and activation of kappa receptors does not appear to modify the reinforcing effects of heroin. Supported by NIDA Grant DA-04043

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Genetic Variation in Opiate Receptors and Its Relationship to Opiate Self-Administration

G.I. Elmer, J.O. Pieper, S.R. Goldberg and F.R. George

INTRODUCTION

The present study investigated the influence of opiate receptor density and genetic variation in opiate reinforced behavior using inbred strains widely divergent in CNS opiate receptor density; CXBK/ByJ (BK), CXBH/ByJ (BH) and C57BL/6J (C57) mice. The BK mice demonstrate a 40% decrease in CNS mu-receptor subtypes while the BH mice have an elevated opiate receptor concentration relative to other inbred mouse strains. In addition to opioid reinforced behavior, opioid induced-analgesia, -stimulation and -respiratory depression analyzed in order to determine the extent to which multiple opiate-related drug effects are influenced by common genes.

METHODS AND RESULTS

Opioid reinforced behavior was investigated using an FR 8 schedule of reinforcement for etonitazene. Etonitazene induced analgesia, stimulation and respiratory depression were assessed using the hot-plate, open field and Columbus respiratory monitors, respectively. Results show that all strains will drink pharmacologically significant amounts of etonitazene and that etonitazene served as a reinforcer even in a mouse strain deficient in CNS mu receptor concentration (BK). The BK mice were significantly less and the BH mice relatively more sensitive to the above opiate-related drug effects. Drug intake during operant sessions correlated with opiate receptor concentration and analgesic sensitivity but not with opioid-induced stimulation or respiratory depression.

DISCUSSION

Since all strains acquired etonitazene self-administration behavior, there was no relationship between sensitivity to the stimulant, analgesic or respiratory effects of etonitazene and the acquisition of self-administration behavior. However, there appears to be a relationship between the amount of drug taken during self-administration sessions, the analgesic effects of etonitazene and CNS mu-receptor populations.

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Behavioral Evidence for an Interaction Between 5-HT_3 Receptors and Opioid Systems

G.A. Higgins and E.M. Sellers

Historically, the opioids have been a widely abused class of drug; a feature due both to the subjective feelings of euphoria that these drugs produce and the induction of physical dependence leading to adverse withdrawal reactions on termination of their intake. The development of pharmacological strategies to alleviate either of these effects may thus prove beneficial in the treatment of opioid addiction.

Experiment 1. Morphine Place Preference Conditioning

Selective antagonists at the 5-HT_3 receptor (e.g. MDL72222, ondansetron) have been reported to block place preference conditioning to morphine (Carboni et al, 1989). In the present study we re-examined this effect using the unbiased method (Mucha and Iversen, 1984) in order to test the robustness of this antagonism. Male Wistar rats were used and place conditioning to morphine (1.5 mg/kg SC: 4 conditioning trials of 45 min duration) was reliably observed. Thirty minute pre-treatment with ondansetron (0.01 mg/kg SC) and MDL72222 (1 mg/kg SC) prior to morphine conditioning, significantly ($p < 0.05$ Neuman-Keuls test) antagonized morphine place preference.

Experiment 2. Naloxone Precipiated Opioid Withdrawal

Rats were made dependent on morphine by the subcutaneous implantation of a 75 mg morphine base pellet. Three or 4 days later withdrawal was precipitated by naloxone (0.002-1.5 mg/kg SC) and the rats were conditioned to associate this response with distinct environmental cues (Mucha, 1987). The place aversion produced by naloxone (0.05 mg/kg SC) was antagonized by MDL72222 (1 mg/kg SC) and ondansetron (0.1-1 mg/kg SC) ($p < 0.05$ Neuman-Keuls test). Overt signs of withdrawal precipitated by a 0.5 mg/kg dose of naloxone (e.g. wet dog shakes, startle, hypothermia) were unaffected, except weight loss which was attenuated by both drugs.

We conclude that 5-HT_3 receptor antagonists may modify certain subjective stimuli associated with morphine reward and withdrawal.

References available on request to G.A. Higgins.

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Development of Behavioral Procedures for the Repeated Assessment of Drug Effects

T.H. Kelly, R.W. Foltin and M.W. Fischman

Effective procedures for the repeated assessment of drug effects should engender stable measures over multiple observations with minimal training and practice, establish baselines that are differentially sensitive to a variety of drugs, and require little completion time. This study investigated the relative sensitivity of behavior to drug effects during four brief (<10 min) drug assessment procedures. Six healthy adult male subjects gave written consent and participated in 4.5hr sessions, consisting of five 20-min trials (1/hr) over thirteen days. During each trial, subjects completed the Multiple-Performance Assessment Battery (MPAB), the Number Recall task, (NR), the Alluisi task and a Pupil Screen. Subjects were paid for accurate performance. A single active drug dose was administered during a 10-min interval immediately preceding the second trial of each session. Subjects consumed a 455 ml beverage, containing AMPH (0, 5 and 10 mg/70 kg), DZP (0, 5 and 10 mg/70 kg) or ETOH (0, 0.3 and 0.6 g/kg), during a 5min interval, and took 5-signaled puffs on a MJ cigarette (0, 2.0 or 3.5% Δ^9-THC) during the remaining 5 min. Active doses were administered in random order on a single occasion, interspersed with five placebo doses. Performance on the MPAB task was altered by all drugs, except DZP. Performance on the NR task was altered by MJ and AMPH. Alluisi task performance was altered by MJ and ETOH, and Pupil Scan measures were altered by MJ, only. Individual differences in behavioral sensitivity to drug effects were observed.

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Psychomotor Functioning During Alcohol and Cocaine Withdrawal: A Comparative Analysis

L. Bauer

As part of an ongoing study comparing the neuropsychological effects of alcohol and cocaine withdrawal, 20 patients with DSM-III-R diagnoses of alcohol (N =8) or cocaine (N=12) dependence were recruited. Patient were tested on four separate occasions during their recovery, viz, at 1-4 days, 7-10 days, 18-21 days, and 94-100 days after cessation. A group of 14 nonpatient controls were recruited, and were tested at comparable intervals. The three subject groups were comparable in age, gender, racial composition, educational level, and in the prevalence of DSM-III-R ASP disorder. Subjects with major medical or psychiatric illnesses, head injuries, or a recent history of opiate use were excluded.

The neuropsychological test battery included measures of body sway, hand tremor (action & resting), smooth pursuit eye movements, saccadic eye movement latency, and simple and choice reaction times, among others. Preliminary analyses have thus far focused on data collected during the second and third laboratory sessions.

The results of these analyses suggest that alcohol- and cocaine-dependent patients manifest qualitatively different neuropsychological test profiles during the early stages of their recovery. Alcohol-dependent patients exhibited more body sway, longer saccadic eye movement latencies, and smaller smooth pursuit eye movement amplitudes than normal controls. In contrast, cocaine-dependent patients exhibited less body sway, normal saccadic eye movement latencies and larger smooth pursuit eye movement amplitudes. Alcohol- and cocaine-dependent patients also differed in the type of tremor exhibited during the early stages of recovery. Alcohol-dependent patients exhibited more tremor (action tremor) of the type associated with cerebellar dysfunction. In contrast, cocaine-dependent patients exhibited a type of tremor (resting tremor) more characteristic of basal ganglia dysfunction. In neither instance was the tremor so severe as to mimic that typically associated with cerebellar or basal ganglia disease.

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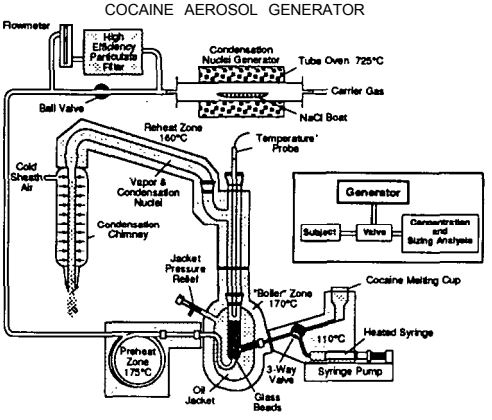
"Crack Smoke" on Demand

R.W. Wood, J.F. Graefe, C.P. Fang, J. Shojaie

Heating "crack" produces a saturated vapor of cocaine base; as the vapor stream cools, the atmosphere becomes supersaturated and forms a condensation aerosol. Electron microscopy suggests that the particles are droplets as they deposit in lung. Species differences in airway distribution and uptake of drug aerosols may be minimized by controlling the particle size distribution. Last year we reported the development of specialized condensation aerosol generators that rely on condensation nuclei ("cloud seeding") to achieve control of particle size. We have now examined their operating characteristics, and can produce test atmospheres upon demand. The particle size distributions range from 0.6 to 1.5 u, with a geometric standard deviation of 1.2 to 1.3; the number concentrations are as high as 10^7/ml; and the airborne mass concentrations are as high as 15 mg/l (ug/ml;g/m^3). Sodium chloride particles (< 100nm diameter, 5-6x10^7 particles/ml, <.05 mg/l) are mixed with the cocaine vapor, reducing the resultant size, and increasing the number of cocaine droplets. Particle size is measured optically; optical systems are calibrated aerodynamically with an inertial impactor. Airborne mass is measured optically, by filtration, and by gas chromatography. Chromatography allows us to detect and prevent pyrolysis. The vapor is generated by passing a heated carrier gas through a reservoir of molten cocaine; this generates stable performance (hours) at low concentrations (< 1 mg/l). At higher concentrations, the cocaine is depleted more rapidly, and reduced concentration is associated with smaller particles. To achieve stable high concentrations required continuous replenishment of the reservoir, with a heated syringe pump. Consumption is 3-5 g/hr; significant amounts are recycled from filters. Loss of vapor to tubing walls during condensation is minimized by "sheathing" the hot vapor concentrically with cold air passed through a porous tube. We are commencing bioassays with these techniques in hand.



Support: K02-DA0017, R0l-DA05080.

AFFILIATION: NYU Medical Center, NY, NY

Marijuana Smoking: Acute Effects on Aggressive, Escape and Point-Maintained Responding

DR. Cherek, R. Spiga, R.H. Bennett and K.A. Cowan

Male subjects with histories of marijuana use smoked 0.00, 1.75, 2.57 or 3.55 w/w delta-9-tetrahydrocannabinol cigarettes. Sessions were 0.5 hrs prior to smoking and 0.0, 0.5, 2.0, 4.0 and 6.0 hrs after smoking. Responding on lever A was maintained by a fixed-ratio (FR) 100 of point presentation. Following point subtractions, completion of an FR 10 on either lever B, aggressive responses, or C, escape responses, initiated a 125 or 250 sec interval free of point subtractions. Subjects were instructed that, i) points were subtracted by another person, ii) lever B responses subtracted a point from their partner, and iii) lever C responding protected their counter for some period of time. Lever B, aggressive responses were increased 0.0-0.5 hrs post-smoking, while effects on escape responding varied depending on provocation conditions. Our results with urban drug users are in marked contrast with reports of decreased aggressive responding following acute administration of marijuana to college students.

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Behavioral Economics of Drug Self-Administration: Effects of Enriched vs. Improverished Environments on Human Drug consumption

W.K. Bickel, R.J. Degrandpre, S.T. Higgins and J.R. Hughes

Two studies were conducted to assess the effects of concurrently available reinforcers vs. the absence of those reinforcers on human cigarette smoking. In Study 1, the fixed-ratio (FR) requirement to obtain two cigarette puffs was increased (FR 100, 200, 400, 800, 1,600, and 3,200) when money ($0.25 or 0.50) was concurrently available or when no money was available. Study 2 was identical to Study 1, except that the concurrent reinforcer was access to a side of a room containing numerous activities (e.g., movies, computer games, reading material, etc) or the absence of those activities. Each FR values was in effect for a single day. The concurrent reinforcer (money or numerous activities) was available according to a constant FR 400. Five cigarette-deprived subjects participated in each study. Across all conditions, smoke consumption decreased in a positively decelerating fashion (in log-log plots) as FR value increased. The presence of the money or alternative activities, produced the same shaped curve as when they were not available except that the curve obtained in the presence of alternative reinforcers were displaced downward. These data indicate that drug consumption is reduced by the cost to obtain that drug and as a function of alternative reinforcers that enriched the environment. This implies that decreases in drug consumption could be accomplished with less of an increase in drug cost when alternative reinforcers are present. (Supported by Grant DA 06626.)

AFFILIATION: University of Vermont, Burlington, VT

Cardiovascular Reactivity and Risk for Substance Abuse Disorders

R.E. Tarter, M. Kabene and H.B. Moss

As part of the NIDA funded Center for Education and Drug Abuse Research (CEDAR) devoted to the elucidation of risk factors in the development of substance abuse disorders, this study investigates the physiological reactivity of sons of DSM-III-R diagnosed substance abusers (n=27;age 10-12 yrs) in comparison to sons of normal individuals (n=34;age 10-12 yrs). The cardiovascular reactivity of these two groups was measured at baseline (in which the subject had to observe an empty TV screen) and in response to three different visual stimuli: 1) during a stressful movie; 2) viewing alcoholic beverages; and non-alcoholic beverages. After each condition, subjects viewed an empty TV screen to allow for a return to baseline. The sons of substance abusers consistently showed tendencies of a higher heart rate at baseline, and through the four conditions but no differences between conditions suggesting non-specific hyperarousal. The heart rate was for the sons of substance abusers and the sons of normals respectively: Baseline=69.8+/-10.0, 65.3+/-10.2; Movie: 77.8+/-11.7, 72.1+/-11.7; Alcoholic Beverages: 77.7+/-11.8, 72.1+/-10.9; Non Alcoholic Beverages: 77.5+/-11.3, 72.0+/-11.5. These findings support previous research suggesting an association between cardiovascular reactivity and risk for substance abuse disorders.

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Endogenous Vasopressin (AVP) and Intranasal Des-glycinamide AVP (DG-AVP) in Nicotine Craving

M.J. Kelley and S. Lightman

While AVP and related peptide fragments have been studied in respect to effects on opiate and alcohol dependence/tolerance, there has been no work on nicotine dependence - a fact which is surprising given nicotine is an agonist for AVP release. Prior to attempting to quit smoking, subjects were given a nicotine challenge (increased CO of >4 ppm) and post-challenge levels of AVP were assayed in plasma samples collected at 2, 10 and 20 min after the challenge. The Shiffman-Jarvik (SJ) and London Addiction-Research Unit (ARU) psychometric measures of craving were obtained after one and eight days of nicotine deprivation. There was no influence of daily intranasal DG-AVP or placebo (1 mg/day, double blind, randomized) on these measures of craving, although the drug had a positive influence ($p<.01$) on attention during deprivation in the subjects with low-post-challenge AVP. Cohort differences in correlations at one Day of nicotine deprivation may reflect literacy problems with the subjects in Cohort One, particularly with the more complex S-J craving questions.

CORRELATIONS BETWEEN POST-CHALLENGE AVP AND CRAVING AT DAY 2

	COHORT ONE (n = 19)	COHORT TWO (n = 30)
ARU-CRAVING		
2-MIN AVP	+.01	-.15
10-MIN AVP	-.04	-.61**
20-MIN AVP	-44*	-.51**
SJ-CRAVITING		
2-MIN AVP	.16	-.18
10-MIN AVP	.10	-.48**
20-MIN AVP	-.13	-.38*

* $p<.05$ ** $p<.01$

CORRELATIONS BETWEEN POST-CHALLENGE AVP AND CRAVING AT DAY 8

	ARU-CRAVING (n = 22)	SJ-CRAVING (n = 22)
2-MIN AVP	+.15	+.19
10-MIN AVP	-44*	-.44*
20-MIN AVP	-.51**	-.60***

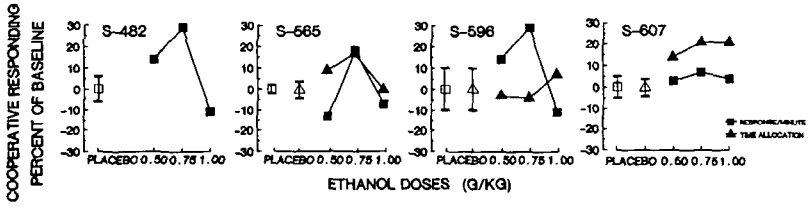
* $p<.05$ ** $p<.02$ *** $p<.01$

AFFILIATION: Westminster Medical School, London

Effects of Ethanol on Human Cooperative Responding

R. Spiga, D.R. Cherek, J. Grabowski, R.H. Bennett and K.A. Cowan

The effects of ethanol (0.5, 0.75 and 1.0 g/kg) on cooperative responding in a laboratory setting was examined in male human volunteers. Subjects participated daily in five 30 min sessions. Ethanol or placebo was administered following the first session. Expired air alcohol content was measured before and after each session. Two periods alternated during a session. During the first points exchangeable for money were presented on a random interval (RI) 60 sec schedule. During the second period subjects could work with or independently of a fictitious other person. Working with the person, the cooperative response, was maintained by points added to the subject's and fictitious other person's counter on an RI 60 sec schedule while working alone only added points to the subject's counter on a RI 60 sec schedule. The figure shows that administration of 0.50 g/kg ethanol increased cooperative response rate for 3 of 4 subjects. For 2 of 3 subjects time allocated to the cooperative option also increased. Responding during the first period was unaffected by ethanol.



Supported by NIDA DA-06633

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An Assessment of the Potential Reinforcing Value of Privileges in a Methadone Maintenance Program

J.M. Schmitz and J. Grabowski

The potential reinforcing value of methadone program privileges was assessed in 12 patients (8 males, 4 females; mean + SD age 38.1 +3.56; education 10.9+1.86 years; 76% white) attending a behaviorally-based outpatient methadone program. Mean history of methadone treatment was 69.8 mos., with an average of 12.36 mos. in the current treatment program. The method of multiple paired comparisons (MPC) was used to design the reinforcer menu. Fifteen items were arranged in pairs so that every item was paired with every other one, resulting in 105 comparison pairs. The reinforcer menu was also completed by treatment counselors (N=4), instructed to respond according to how they perceived patients would respond. An interval scaling model was used to order privileges in a quantitative continuum from least to most preferable. The advantage of this scaling model is that distances between items can be meaningfully interpreted. Free methadone was the most preferred privilege, followed by free dental service and more take-homes, with small differentiation among those preferred privileges. Dose decreases were least preferred. Treatment counselors did identify the privileges most preferred among patients. These findings are generally consistent with previous research (Yen, 1974; Stitzer & Bigelow, 1978). Preference as measured on the reinforcer menu provides an approximate indication of potential reinforcer effectiveness, but actual effectiveness remains an empirical clinical issue.

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Yen, S.: Availability of activity reinforcers in a drug abuse clinic: A preliminary report. Psych Reports 34:1021-1022, 1974.

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Antagonist Sensitivity Time Course in Humans: Effects of Morphine-Naloxone Interval

H.L. June, M.L. Stitzer and I.A. Liebson

Withdrawal signs and symptoms can be reliably precipitated in humans following single dose exposure to an opioid agonist drug such as morphine. These antagonist-precipitated responses after brief opioid exposure may represent the incipient stages of physical dependence development. The purpose of the present study was to further characterize the time course of precipitated withdrawal after pretreatment with a single dose of morphine. Subjects were 10 adult males reporting regular use of opioid drugs but not currently dependent or seeking treatment. Subjects participated in 10 experimental sessions during which they received naloxone (10mg/70kg i.m.) at 0, 1, 3, 6, 12, 18, 24, 30, 36 or 42 hours following a single i.m. dose of morphine (18mg/70kg). Each interval was tested independently in a randomized sequence under double-blind conditions. Opioid agonist effects including constricted pupils and subjective high were apparent from 1-12 hours post-morphine. Following naloxone challange, subjective withdrawal symptoms were significantly elevated at 3,6, and 12 hours post-morphine. Highest average subjective withdrawal scores were seen at 6 hours post-morphine. Observer rated signs and subjective reports of yawning were still significantly elevated at 24 hours. These findings are generally consistent with previous reports from our laboratory. They extend previous findings by further clarifying the onset and offset time course of precipitated withdrawal effects as well as identifying time of peak effects.

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Stability of Immunologic Markers During Acute Cocaine Withdrawal

J.T. Christmas, S. Ruddy, J.S. Knisely and S.H. Schnoll

We monitored immunologic parameters in eight cocaine-dependent pregnant women after they were admitted to a Clinical Research Center for abrupt withdrawal of cocaine and compared these with results obtained in three normal pregnant women matched for age and gestational age and in normal non-pregnant controls. All subjects tested negative for HBsAg; one was positive for antibodies to HIV. Gestational ages ranged from 23 to 36 weeks. Fetal monitoring during withdrawal showed marked change in fetal activity and increased umbilical arterial resistance, a marker of fetal compromise.

Plasma levels of immunoglobulins G, A and M; complement components C3 and C4; and CRP were within the normal range and did not change during the study period, Production of interleukins 1 and 2 and TNF α by cultured peripheral blood mononuclear cells in response to PHA was within normal limits. Cell surface markers monitored by flow cytometry included CD3, CD4, CD8; Fc receptors II (CDw32) and III (CD16); complement receptors I (CD35), II (CD21) and III (CD11b); and the IL-2 receptor (CD25). There was a trend toward higher levels of CD25 in the pregnant women that did not reach significance. With the exception of the HIV-positive patient, all of the other markers did not differ from normal. We conclude that stress associated with cocaine withdrawal, reflected in abnormal fetal parameters, is not associated with perturbations in multiple aspects of the immune system.

Supported by NIDA grant no. DA06094.

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A Comprehensive Postmarketing Surveillance (PMS) Method for Assessing the Abuse Potential and Dependence Liability of Psychotropic Drugs

C.A. Naranjo, U. Busto and K.L. Lanctot

The evaluation of the abuse potential and dependence liability of psychotropic drugs is complex. Preclinical and clinical testing have good concordance, however the postmarketing predictive validity of current methodologies has not been fully determined. We have developed a comprehensive and systematic approach for prompt postmarketing evaluation of the a use potential and dependence lability of psychotropic drugs. The system serves as an early warning procedure to detect abuse and dependence of psychotropic drugs using 4 components. The first of these involves systematically collecting and analyzing case reports of suspected abuse and dependence and determining the probability of a causal relationship between the drug and the reported abuse or dependence problem. Analysis of case reports of withdrawal reactions is done using a Bayesian-based method of differential diagnosis. The Bayesian diagnostic instrument for withdrawal (BDIW) calculates the odds in favour of a particular drug causing the problem compared to alternative causes, referred to as the posterior odds. Secondly, monitoring yearly trends of psychotropic drug use provides the context to interpret abuse and dependence data. The third part of the system involves systematically collecting data on drug dependence-related morbidity and other associated problems (e.g. admissions to hospitals). Data on use and abuse/dependence can be used to determine epidemiological differences in the relative abuse of different psychotropic drugs. Fourthly, monitoring cross-cultural variations in drug use, abuse and dependence is used to enhance early detection of problems. Since drugs are marketed at different times in different countries, new cases of abuse and dependence can be detected early and evaluated. Data collected to date Indicate the utility of this PMS method (e.g. early and better analysis of individual case reports, detection of variations in morbidity and relative abuse of different psychotropic drugs). Our results suggest that the PMS of drug abuse and dependence may be an essential component in the overall evaluation and interpretation of the abuse potential and dependence liability for psychotropic drugs.

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Spontaneous EEG Changes During Tobacco Abstinence are Reversed by Caffeine

C. Cohen, W.B. Pickworth, E.B. Bunker and J.E. Henningfield

Cigarette smoking and nicotine abstinence is influenced by the administration of other drugs, including caffeine. Nicotine abstinence causes EEG alpha slowing and increases theta power. We examined the EEG effects of caffeine and nicotine administration in abstinent smokers. Following 12-hr tobacco and caffeine abstinence, six male volunteers who smoked cigarettes and drank coffee were treated with combinations of 0, 150, or 300 mg caffeine and 0, 2, or 4 mg nicotine polacrilex, 30 minutes later. On one study day, subjects were not deprived of tobacco and caffeine and were not given the capsues or gum. At the end of the session, subjects smoked two cigarettes of their own brand. Two-min periods of spontaneous EEG were recorded several times during the sessions. During EEG recordings subjects relaxed with their eyes closed. EEG was recorded from F_z, C_3, C_4 and P_z electrode sites. The peak frequency and spectral power in the delta (.5-4H2), theta (4-8Hz), alpha (8-13Hz) and beta (13-25Hz) EEG bands were recorded. The instrument automatically eliminated epochs with eye movement or other artifacts. Tobacco and caffeine abstinence significantly decreased alpha frequency and increased theta power. The decrease of alpha frequency was largest at the P_z electrode, whereas increases in theta power were most evident at the F_z electrode. Smoking a single cigarette reversed these withdrawal signs. Caffeine at both doses fully suppressed the increase in theta power but the 150 mg dose was less effective than the 300 mg dose in blocking the decrease in alpha frequency. Caffeine effects were maximal 50 min after the capsules. Nicotine, 4 mg, reversed the increase in theta power, however, the effect was not statistically significant. The observation that caffeine can reverse some EEG signs of the nicotine withdrawal syndrome may have clinical implications for the treatment of tobacco dependence.

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Tramadol Hydrochloride Revisited-Analgesic Efficacy in Post-operative Pain

A. Sunshine and F.L. Minn

Tramadol hydrochloride is a synthetic opiate agonist. It derives its activity from attachment to the mu-receptor and blockage of norepinephrine and serotonin re-uptake. The results of a clinical oral analgesic study presented to CPDD in 1969 by Sunshine et al reported this compound to have analgesic activity. Study #1 (1969) compared the efficacy of tramadol HCl (IRA) 50mg, l00mg, or 150mg to propoxyphene hydrochloride (PRO) 65mg and 130mg, and placebo (PLA) in 160 male or female patients with moderate or severe postoperative, postfracture, or musculoskeletal pain. In 1990, we conducted a single dose double-blind study (Study #2) to determine the efficacy of tramadol HCl 75mg or 150mg compared to acetaminophen 650mg with dextropropoxyphene napsylate 100mg (APAP/PRO) or placebo administered orally to 161 patients with severe postoperative cesarean section pain.

RESULTS: Study #1 - All active treatments were significantly superior to placebo for many hourly PID scores. There was a separation between the two dose levels of propoxyphene based on mean effect, with a significant difference only at hr 1. The three tramadol doses showed separation based on mean effect with the 50mg dose being the least effective and the 150mg dose the most effective; however, statistical significance was seen only at hr 6 between the 100mg and 150mg dose levels. Tramadol 150mg was significantly more effective than propoxyphene 65mg for PID at 1 and 2 hr, and for SPID. Tramadol 100mg was significantly better than low dose propoxyphene for PID at 1 hr. There were no other significant differences between active treatments. One patient treated with tramadol 150mg complained of dizziness and sweating. No other adverse reactions were reported.

Study #2: All active treatments were significantly superior to placebo for many hourly measures for analgesic efficacy and for SPID and TOTPAR. A dose response was achieved for the two doses of tramadol. Tramadol 150mg was significantly more effective than the 75mg dose for SPID, TOTPAR, and many hourly variables. Tramadol 75mg was similar to APAP/PRO during the first two hours, thereafter tramadol 75mg had a more sustained effect and these differences were significant at hr 4 and hr 6. Tramadol 150mg had a more sustained effect than APAP/PRO after hr 1; these differences were significant from hr 2 to hr 6, and for SPID and TOTPAR. No adverse reactions were reported with placebo and APAP/PRO; 1 patient treated with tramadol 75mg and 4 with tramadol 150mg complained of dizziness; 1 patient treated with tramadol 150mg complained of sweating.

CONCLUSION: Based on the results of both studies we conclude that tramadol hydrochloride is an effective oral analgesic with increasing response from 50mg to 150mg. Tramadol 50mg was similar in effect to propoxyphene 65mg. Tramadol 75mg and 150mg was more effective than APAP/PRO after hour 2. Dizziness was noted more frequently with tramadol 150mg. No serious adverse effects were observed. AFFILIATION: NYU Med. Ctr., NY, NY
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Effects of Commonly Abused Drugs on Dynamic Pupilometry, Subjective and Physiologic Measures

W.B. Pickworth, E.B. Bunker and J.E. Henningfield

Static (pupillary diameter) and dynamic (constriction and dilation velocities of the light reflex) measures have been used to quantify the effects opiates. However, the usefulness of pupillary measures as an index of the effects of other classes of abused drugs has not been systematically studied. Me compared pupillary and other physiologic effects with subjective and performance measures after: amphetamine (10 and 30 mg), ethanol (0.3 and 1 Gm/Kg), pentobarbital (150 and 450 mg), hydromorphone (1 and 3 mg), marijuana (1.3 and 4% THC) and placebo. The experiment was conducted in eight residential volunteers using a double-blind, triple dummy, crossover design. Ethanol, pentobarbital, marijuana and hydromorphone decreased pupil size whereas amphetamine gradually increased ft. Constriction velocity was reduced by hydromorphone, marijuana, pentobarbital and ethanol. Dilation velocity decreased after marijuana and hydromorphone (slightly). The drugs caused dose related increases In ratings of "high", drug "liking" and appropriate scales of the ARCI (eg. MBG). Measures of performance (cognitive and motor) were reduced by ethanol, marijuana and pentobarbital but were less affected by amphetamine and hydromorphone. Generally, the pupillary effects followed the time course of the performance and subjective changes. There were no significant correlations between the Individual subject's pupillary measures and his subjective and performance measures. The results indicate that pupillary measures, like subjective and performance effects, are sensitive indicators of drug effects. However, between-subject variability may limit their drug detection application. The lack of correlation between measures suggests pupillary assessment does not predict performance or subjective effects.

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A Human Model for Studying Drug-Induced Concomitant Variations in Alcohol Consumption and Desire to Drink

C.X. Poulos, C.A. Naranjo and K.E. Kadlec

Despite the profusion of models used in animal studies to identify drugs to decrease alcohol intake, there is no widely accepted standard paradigm. Therefore, the predictive validity of the results of animal studies for humans is uncertain. A simple, economical and efficient model is needed to rapidly evaluate promising findings from animal studies and to test drug effects on alcohol consumption in humans. Therefore, we compared the efficiency of: a) a brief intensely-controlled outpatient trial and b) an experimental bar session, to detect variations in alcohol intake and "urge to drink" under medication and placebo conditions. We conducted placebo-controlled, double-blind, randomized, crossover outpatient trials (1-2 weeks) in which alcohol-dependent subjects monitored daily alcohol intake and rated their desire to drink. In one such experiment, citalopram (40 mg/day), a serotonin uptake inhibitor, decreased alcohol intake by 17.5% compared with placebo, from (x±SEM) 5.7±0.8 to 4.6±0.6 drinks per day ($p < 0.05$). Desire to drink also decreased compared with placebo ($p<0.05$), and correlated with changes in alcohol intake ($r = 0.5$, $p<0.01$). In an experimental bar session, the same subjects were offered a series of up to 18 mini-drinks (l/3 of a standard drink) and rated their desire for each. There was some indication that citalopram reduced desirability for alcohol early in the session; however, it had no significant effect when the entire experimental session was analyzed. The potential sensitivity of the method may have been reduced by time of day for testing and restricted beverage choice. Accordingly, a more powerful experimental bar paradigm is being developed. Short-term outpatient trials are simple and efficient methods to study drug effects on urges to drink and alcohol consumption.

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The Fast Action Pharmacodynamics of Marijuana Smoking

M. Huestis, A. Sampson, B. Holicky, J. Henningfield and E. Cone

During marijuana smoking tetrahydrocannabinol (THC) penetrates the central nervous system producing behavioral and physiologic effects. Characterization of the absorption phase of THC during smoking is difficult due to the rapidly occurring changes. Peak plasma THC levels have been reported to occur during the smoking process. We utilized a continuous blood withdrawal system to obtain blood samples before, during and after acute marijuana smoking trials in 6 healthy male, drug-free subjects. Simultaneous physiologic and behavioral measures also were obtained. Each subject smoked a single marijuana cigarette (0% THC, 1.75% THC, 3.55% THC, double blind randomized order) on three occasions under a computerized paced smoking procedure which lasted 11.2 min. Plasma levels of THC, 11-HO-THC and the 11-nor-acid were determined by GC/MS. After the first puff of a 1.75 or 3.55% THC cigarette, mean THC plasma levels were 8.4 and 16.3 ng/mL, respectively. Mean peak levels occurred 8.7 min after onset of smoking, whereas 11-HO-THC and the 11-nor-acid peaked at 14.5 and 114 min. Behavioral and physiologic effects generally peaked at 15-60 min after smoking. This lag between plasma THC and drug induced-effects suggests that THC levels in the CNS initially are lower but equilibrate with plasma levels shortly after smoking.

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Vegal Tone and Attention in 8 to 12-year Old Males Exposed to Opiates In Utero: A Preliminary Report

J.E. Hickey, P.E. Seuss, L. Spurgeon, D.B. Neslin and S.W. Porges

We assessed cardiac vagal tone changes during an attention demanding Continuous Performance Task (CPT) in 12 boys (mean age 9 years, 8 mos) exposed prenatally to opiates, 12 boys (mean age 10 years, 1 mo), whose mothers began using illicit substances after the child's birth, and 12 control boys (mean age 9 years, 8 mos). The three group design of this study was intended to isolate in utero effects from environmental and genetic influences. The mothers participating in our study were primarily single, and of lower income. Groups did not differ significantly on mother's education, income, marital status, or race. Vagal tone was measured pre- and post-baseline and during the 3 tasks of the Gordon Diagnostic System. Vagal tone is a heart rate variability measure that quantifies parasympathetic inhibition of the heart. Results indicated that opiate-exposed boys failed to suppress vagal tone compared to both control groups, when distracters were added to a vigilance task. In normal children and adults, vagal tone is suppressed during tasks requiring sustained attention. These preliminary results indicated that normal physiological responses to increased attentional demand may be impaired in boys exposed in utero to opiates, in this age range. These physiological response patterns could not be accounted for by prenatal alcohol, nicotine, or marijuana exposure in these samples.

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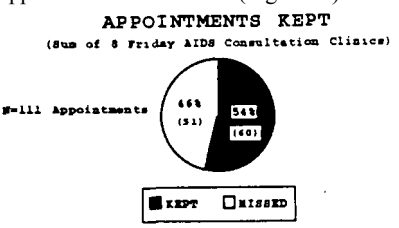
Medical Care of HIV Disease in Methadone Maintenance Treatment

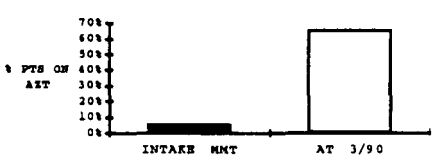
E. Goosby, S.L. Batiki, J. London, S.J. Ferrando, C. Ryan, R. Hartwig, and C. Abbott

A retrospective chart review examined medical care for HIV-infected IVDUs in Methadone Maintenance Treatment (MMT) at SFGH. Analysis of two eight-month study periods in 1988-89 and 1989-90 revealed the following patterns: Mean HIV prevalence among the 190 patients in treatment was 32% and 46%, respectively, for the two study periods. Medical contacts increased from 1200 to 1600 between the study periods. Of the total medical contacts, the proportion used by HIV-infected patients increased from 66% to 81%, and the proportion of contacts that focused on medical, rather than on psychiatric or drug-related complaints, increased from 57% to 86%. By the end of the second study period, 80% of HIV-infected patients were receiving AIDS medical services through MMT. Compliance with appointments was 54% (Figure 1).



By the end of the second study period in March, 1990, 100 HIV-infected patients were in treatment. There was a dramatic increase in the proportion of patients who had ever received AZT, from 5% before MMT to 65% during MMT (Figure 2).

AZT TREATMENT PRE & DURING MMT



HIV medical services were well utilized and may be highly effective when provided on-site through MMT programs. Support: NIDA Grant R18 06097 and NIDA Grant DA 01696

Affiliations: UCSF Department of Psychiatry, San Francisco General Hospital Substance Abuse Services and San Francisco Treatment Research Unit

NIDA Announces New Opiate/Stimulant Drug Testing Program for Medications Development

A.A. Reid, L.R. Toll, I. Berzetei-Gurske, W.E. Polgar S.R. Brandt and G.T. Pryor

NIDA's Medications Development Division (MDD) is in the process of establishing several testing projects which will aid in the discovery and evaluation of potential new medications for treatment of drug and alcohol abuse and mental disorders. These new projects are being conducted in coordination with other established testing programs such as those administered by the CPDD and National Institute of Mental Health (NIMH).

Two NIDA contracts have been established to conduct preliminary "first-level" tests on a structurally diverse group of compounds (~100 compounds/year/ contract). These compounds will be obtained from academic, government and industrial research investigators in the mental health and drug abuse fields. Tests included in the opiate/PCP contract consist of opiate, PCP and sigma receptor binding analyses and smooth muscle preparation bioassays for opiates. Tests included in the stimulant contract consist of biogenic amine receptor binding analyses, bioassays, uptake studies and release determinations as well as in vivo mouse ataxia, locomotor, rearing and stereotypy assessments in the absence and presence of cocaine and amphetamine.

The results generated from these tests will be entered into a NIDA structure-activity database (described in adjacent communication), which will be available to research scientists. Access to information on proprietary compounds will be maintained within restricted files of the database. This database will serve as a resource to assist in the design and discovery of novel compounds for potential use as medications for the above mentioned disorders.

Please contact Ms. Audrey Reid at 301-443-5280 for additional information regarding the testing programs.

AFFILIATION: NIDA, Rockville, MD and SRI International, Menlo Park, CA

NIDA Launches a New Structure-Activity Database for Medications Development

G. Barnett, G. Daly, K. Groover, M. McLoudrey and A.A. Reid

NIDA's Medications Development Division (MDD) has awarded a contract to support development of an extensive structure-activity database, which will serve to facilitate in the discovery and design of new medications for treatment of drug and alcohol abuse and mental disorders.

The NIDA/MDD database will contain a collection of chemical and biological information on a structurally diverse group of compounds. These compounds will be selected from a variety of sources including NIDA's newly established testing program for opiates and stimulants (described in adjacent communication), the National Institute of Mental Health's (NIMH) and CPDD's drug testing programs, and published and patent literature. The chemical information, which will be stored in a MACCS (Molecular Design Ltd) database system, will include fully searchable 2-dimensional molecular structures, molecular formulae and weights as well as other physical parameters. It is anticipated that 3-dimensional structure capabilities will be incorporated in the future. The biological information, which will be stored in an ORACLE database system, will consist of data from biochemical, pharmacological, immunological, pharmacokinetic and behavioral studies. A user-friendly graphical interface, which integrates the chemical and biological databases, will be provided to enhance structure-activity investigations.

The MACCS/ORACLE system is installed on a dedicated MicroVAX 3300, which will be available on a dial-up basis to interested researchers such as medicinal chemists, pharmacologists and industrial scientists. It is anticipated that the database will be operational by January 1992.

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Tracing the Bridges: Social Network Characteristics and Risk Behavioral Among Drug Abusers and Their Significant Others

R.K. Price, L.B. Cottler, D. Mager and S. Keating

IVDUs who maintain close contacts with non-IVDUs arc potential network "bridges" for transmitting HIV between the IVDU and non-IVDU populations. Using the data from an on-going NIDA-sponsored epidemiological HIV study of drug abusers, we examined correlates of IV drug use concordance of proband and his significant others with his risk behaviors and social network characteristics.

The subjects for this paper consist of drug abusing probands (N=514) recruited from several treatment centers in St. Louis. The probands were classified into three groups by their IV drug use history and that of their ascertained or reported partners, or two other reported steady friends: the IVDU-concordant (N=64) defined as both the proland and all of his network members being IVDUs; the non-IVDU concordant (N =236) defined as both the proband and all of his network members being non-IVDUs; and, the IVDU-discordant (N=214) who reported at least one discordant partner or steady friend.

The three groups were found to be distinctively different in high-risk sexual and drug-using behaviors as well as in demographics. While drug-related high-risk behaviors were most prevalent among the IVDU-concordant, they were the least sexually promiscuous. In contrast, the IVDU-discordant were engaged in more high-risk sexual behaviors. The HIV seropositivity was the highest among the IVDU-concordant, the second for the IVDU-discordant. The IVDU-discordant probands reported the highest number of significant others and a larger proportion not living with them, indicating that IVDUdiscordant probands, unlike the IVDU-concordant, were not isolated. The multivariate logistic regression analysis confirmed that relative isolation, paraphernalia sharing and sexual promiscuity were differential characteristics of IVDU concordance type independent of demographics and substance abuse and antisocial history.

Our analysis sheds tight on a mechanism of the HIV spread in St. Louis. Subtyping drug users using a network approach, and targeting differential prevention and intervention efforts might be more effective in halting the further spread of HIV into heterogeneous populations in St. Louis and other cities with similar social stratification.

ACKNOWLEDGEMENTS

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Healthstreet: Linking Drug-Abuse Treatment, AIDS Prevention, and Other Public Health Services in St. Louis

D.J. Claverie, L.B. Cottler, R.K. Price and W.M. Compton, III, W.L. Dotson and D. Sharma

As part of a NIDA-funded research demonstration project among drug abusers in St. Louis, two storefront outreach centers HealthStreet) have been opened in collaboration with the St. Louis City Health Department. These centers are the base of operations for eight community health outreach workers (CHOWs) who go into the surrounding neighborhoods which police data show are at highest risk for illicit drug activity, prostitution, and other crime. In these neighborhoods, CHOWs seek to identify those persons at risk for drug use or dependence. CHOWs try to encourage those identified to come to HealthStreet for further evaluation and possible referral to drug treatment. All visitors to HealthStreet are met by a crisis intervention specialist who counsels individuals and refers them to project-funded drug-free or methadone maintenance treatment. Persons who do not want drug treatment are invited to participate in the research as controls. The HealthStreet staff also provide other free public health services including confidential HIV counseling and testing, flu shots, STD testing, TB testing, and lead screening. By providing such varied health services, Healthstreet is thought to be readily accepted in their neighborhoods. Since the opening of HealthStreet in July 1990, CHOWs have referred 1,449 persons to HealthStreet, but only 184 (13%) have subsequently arrived there. Of those 184 arrivals, 142 (77%) accepted referrals to free drug treatment. Of these referrals to treatment, 59 (42%) have arrived at the treatment programs for preadmission screening, and 32 (54%) of those screened have been admitted to treatment. In addition to those drug abusers referred by CHOWs, the concurrent arrival at HealthStreet of other drug abusers enhances the opportunity to explore various outreach strategies and increase the number of drug abusers entering treatment. comparison data from the two groups are presented.
Supported by NIDA DA-06163

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Risk Behavior Relationships Among Heterosexual Injection Drug Users

R. Booth and J.T. Brewster

The present study was designed to estimate the association of risk behaviors to one another in a sample of injection drug users from three cities, Baltimore, El Paso and Denver. It was also intended to assess the extent to which demographic variables could account for variance in the resultant factors. Subjects reporting no sexual partners or only partners of the same sex were excluded from further analysis, leaving a sample of 296, 210 males and 92 females. Behaviors previously found associated with HIV risk in earlier studies were included in a principle components analysis, with those factors retained that had eigenvalues greater than 1.0. The results of these procedures produced 8 factors, suggesting the following:

* For those involved with intervention programs targeting IDUs, the observed independence of risk factors implies that prevention messages need to be highly individualized. General approaches would not appear offer the communication required to relate to those involved in particular risk activities and lead to behavioral change.

* For females, the significance found among male IDUs having sex with non-injecting or casual partners, as well as total sex partners, should serve to encourage the use of protective barriers when sexual partners are relatively unknown to one another.

* For males, the significant association between sex involving prostitution by female IDUs indicates a high risk potential.

* For epidemiologist, the finding that Blacks and Latinos were at significantly greater risk than Anglos on injection frequency, combined with data on the higher rates of infection within these ethnic groups, portends a possible linkage between these variables.

Supported by Contract No. 271-87-8208 and grant DA-06912 from the National Institute of Drug Abuse, USPHS.

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Assessment of Knowledge of AIDS and Risk Behaviors Among Injecting Drug Users (IDUs)

V.A. Gonzalez, N. Siddiqui, L.S. Brown, Jr., C.Y. Veloz, J. Mantell, and A. Hernandez

Six hundred quantitative interviews are being conducted with men and women at high risk for HIV infection in a Methadone Maintenance Treatment program, to assess knowledge/awareness of AIDS and risk behaviors. Preliminary data has been analyzed on 113 participants; 78 females and 33 male four weeks prior to assessment, 68% (n=76) of the total population reported the use of intraveneous drugs. Twenty-two percent (n=17) of the females reported sex for the exchange of drugs and 38% (n=29) reported sex for the exchange of money. Eleven percent (n=12) of the total population reported condom use. Thirty-one males (94%) and 73 females (94%) responded definitely true when asked if HIV infection can be transmitted from men to women during unprotected sex. The assessment of knowledge/-awareness of AIDS in relation to the independent variables sex/gender, age and ethnicity proved to have no relevance in determining risk behaviors. Ethnicity was significant when correlated with the use of cocaine and sexual risk behaviors [(prob.=0.050 and prob.= 0.058 respectivly)]. This information suggests a need for further research which will examine the clients' failure to effectively decrease high risk behaviors regardless of their knowledge/awareness of HIV transmission.

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AFFILIATION: *Addiction Research & Treatment Corporation; +New York City Department of Health, AIDS Program Services, AIDS Research Unit New York;.

HIV Risk Behavior in Drug Users: Increased Blood "Booting" During Cocaine Injection

L. Greenfield, G.E. Biglow and R.K. Brooner

The practice of "booting" or "kicking", in which blood is drawn into the syringe and then injected, was assessed as a possible behavioral mechanism contributing to cocaine's association with increased HIV-1 infection. IVDUs (N=68) demonstrated (with an empty, needle-less syringe) their usual style of injection of cocaine, heroin, and speedball, in random order. The experimenter recorded the injection procedures and the syringe volumes at each step. Blood volumes demonstrated to be drawn into the syringe were 3 times as great during simulated cocaine and speedball use as during heroin use ($p<.01$); similarly, the number of pumps of the syringe was twice as great ($p<.01$). Subjects also described the booting behavior of their needle-sharing partners; the percentage having partners who booted blood was significantly greater during cocaine use than during heroin use ($p<.01$). These findings indicate that cocaine use is associated with a behavioral style of injection (increased blood booting) that is more likely to contaminate the injection equipment with blood. Thus, the practice of booting may warrant special attention in AIDS prevention interventions and risk assessments.

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Patterns of Use Among Cocaine Users

F.R. Levin, J. Hess, D. Gorelick and P.J. Fudala

Subjects for this study were consecutively self-referred cocaine addicts who were interested in outpatient treatment at the Addiction Research Center. Inclusion criteria for subjects were the following: subjects met DSMIII-R criteria for active cocaine dependence, severe; used on average "one gram" (street terminology) or more per week for 12 weeks or longer; and had positive urine toxicological study for cocaine. The intake assessment at the ARC included a complete medical and psychiatric evaluation which is well described in other studies (3). Also, subjects were given an instrument to determine the specific days of the month they used in the month prior to admission and the Addiction Severity Index. Eighty-five subjects were categorized into the four patterns of use: continuous (27), occasional (13%), intermittent, patterned (35%), intermittent, nonpatterned (25%). When subject characteristics were analyzed based on pattern of use significant differences in age {F=2.75,df (3,81), p=.O48} and amount of cocaine use were found (Fisher's Exact Test ~~0038). There was a trend for continuous users to be unemployed (Fisher's Exact Test p=.O80) However, there were no differences in gender, race, education, or route of administration based on the pattern of use. Intravenous users who came for initial evaluation were less likely to choose to enter treatment than subjects who use smoke cocaine or use intranasally (Fisher's Exact Test p=.O4) Pattern of cocaine use did not predict which subjects would choose to enter treatment. We found that specific patterns of cocaine use exist among patients seeking treatment and that these patterns do not necessarily follow a "cyclical" pattern. We were surprised that patients who use cocaine less than four days a month would seek help for cocaine use.

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Changes in Clinical Status of Newly Abstinent Hospitalized Cocaine Users

R. Cambor, A. Ho, G. Bodner, S. Lampert, J. Kennedy and M.J. Kreek

As part of an inpatient study to characterize psychological aspects of compulsive cocaine use we examined mood, depression, drug-craving and related symptoms in newly abstinent chronic compulsive cocaine users. Subjects were 9 HIV seronegative individuals meeting DSM-IIIR criteria for cocaine dependence. The average age of subjects was 29.7±1.7 years. The average duration of **cocaine** use was 5.0 years. All used cocaine by the smoking route; one subject also used intranasally. Six of nine subjects used on a daily basis. Subjects had used 24±1.5 of 30 days prior to admission and had spent $694.00 on cocaine during that time. Subjects averaged 4.4±0.4 days of use in the week preceeding admission. Eight of nine subjects had used cocaine within 24 hours of admission to the study. **Methods:** Subjects were rated for symptoms of major depression at the end of the first inpatient week using the Structured Clinical Interview for DSM-IIIH Diagnosis (SCID). Drug dependence and drug-related problems were assessed with the Addiction Severity Index (ASI). The Beck Depression Inventory and the more general Profile of Mood States were given within 48 hours of admission, then weekly. Drug craving was assessed on a daily basis by means of a visual analog scale. Vital sign measurements (temperature, blood pressure, pulse) were taken at 6 am. on a daily basis with subjects at rest. Daily weights (kg.) were recorded after morning vital signs. Subjects recieved no medications during days 0-7 of the study; during days 8-21 subjects received one placebo capsule per day. Results: ASI scores indicated that our subjects were medically healthy, with severe employment problems and moderate to heavy cocaine use. At admission subjects were mildly to moderately depressed (BDI 14.9±3.6); 5 of 9 subjects satisfied SCID criteria for Major Depression. Mood state indices were relatively elevated for tension, anger, fatigue and confusion. Four subjects had cocaine craving scores of less than 3 (out of a possible 10), while the remaining 5 subjects bad craving scores of greater than 3. During. weeks 2 and 3 of the study subjects showed significant decreases in depression as measured by the BDI ($F=13.85$, $df=3$, $p<0.001$), with mean scores falling to nonclinical levels (BDI 6.5±1.5). POMS ratings of anger ($F= 3.46$, $df= 3$, $p< 0.05$) and confusion ($F= 3.46$, $df= 3$, $p< 0.05$) showed significant decreases by the end of the first week. Changes in self-reported tension were not significant; subjects rated themselves as feeling more vigorous by the end of the first week and througout the remaining two weeks,; this trend was significant ($F= 8.49$, $df= 3$, $p< 0.001$). No significant patterns of increase or decrease in vital sign measurements were observed. Subjects gained weight throughout the study period. Urges for cocaine were low during the second and thrid week in 6 of 9 subjects; however the remaining 3 subjects showed wide variation in the pattern of drug urges over time. Discussion: Results from our study suggest that inpatient cocaine abusers experience transient mild to moderate depressive symptoms during early abstinence. A period of refeeding lasting two or more weeks appears to occur during abstinence. Cocaine craving is variable and may be present in moderate to severe degrees for as long as three weeks after cessation of use. The symptoms seen in abstinent inpatient cocaine users are subtle and may be nonspecific; abstinence symptoms are not similar to those produced in classical withdrawal states induced by alcohol, sedatives and opiates. Psychological and behavioral symptoms following abrupt cessation of heavy cocaine use may vary considerably depending on the setting in which subjects are observed. (1-P50-DA05130; the Aaron Diamond Foundation.) Affiliation: The Rockefeller University, 1230 York Ave., New York, NY 10021

Intensity of Craving is Independent of Depression in Newly Abstinent Chronic Cocaine Users

A. Ho, R. Cambor, G. Bodner and M.J. Kreek

Craving for cocaine was rated daily on a visual analog scale by nine patients in their first week of hospitalization and of abstinence from cocaine. Three rating scales of depression were administered in this first week: the Hamilton Rating Scale for Depression (on which the psychiatrist rated the patient), the Beck Depression Inventory (self-rated by the patient) and the D (Depression-Dejection) subscale embedded within the more general self-rated Profile of Mood States (POMS).

More than half of the patients were mildly to seriously depressed in the first week of hospitalization. Six had a total Beck score of 12 or higher (range: 1-36); four had a total Hamilton score of 10 or higher (range: 2-21); and six scored more than 20 on the POMS D subscale (range: 3-50). Across patients, depression rated by the clinician on the Hamilton Scale was significantly correlated with the scores of the self-rated Beck Inventory: Pearson r=.672, df=7, p<.05.

Individual averages of craving for cocaine in the first week of abstinence varied from 0.05 to 8.33 (on a scale of 0 to 10).

Despite a wide range across patients in each index, the degree of craving reported was independent of the severity of depression: there was no significant correlation of craving with any index of depression examined, r=.228, .232 and .075 with Beck, Hamilton and subscale D of the POMS.

Across individuals in the first week of abstinence from chronic cocaine use, craving is independent of depression.

(1-P50-DA05130 and the Aaron Diamond Foundation.)
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Subjective Symtoms of Cocaine Withdrawal

F. Gawin, H.K. Khalsa and M.D. Anglin

Prior studies on cocaine withdrawal provide limited information about the nature of the syndrome. Data were collected from a sample of 244 male cocaine addicts admitted to a VA inpatient treatment program. The subjects were asked to retrospectively report on the presence or absence of 27 cocaine withdrawal symptoms occurring during the first week of all periods of abstinence lasting at least one week. Factor analysis was used to determine which symptoms were associated. Four factors, or sets of symptoms, were identified and labeled as follows: "anxiety-irritability" set, the "dysthymia" set, the "physical discomfort" set, and the "anergia" set, each consisting of five to seven symptoms. The four factors were not significantly correlated to each other. Variables thought to be potential predictors of each set of symptoms were studied and included, among others, history of alcohol and illegal drug use, severity of addiction to cocaine, route of cocaine administration, lifetime pattern of cocaine use/abstinence, and demographic variables. A linear regression showed that variables related to the intensity (and pattern) of cocaine use and the intensity of alcohol and other drug use were predictors of the intensity of the four symptom factors. Replications of this research are needed, as are extended studies of predictors of which factors predominate in which individuals.

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The Urinary Excretion of Benzoylecgonine: Implications for a Multi-Compartment Model of Cocaine Pharmacokinetics

H.R. Kranzler, S.S. Dellafera, L.D. McLaughlin and S.H.Y. Wong

We used the ADx system (Abbott), a fluorescence polarization immunoassay (FPIA) method, to measure benzoylecgonine (BE) in 41 patients (28 females) admitted to an inpatient drug treatment program. The FPIA method was found to be reliable (rho = 0.96 on split samples) and valid (rho = 0.99 compared with GUMS). Mean (± SD) age of these patients was 32.0 (± 8.6) years. Predominant route of administration was freebase for 56%, intravenous for 29%, and intranasal for 15%. Mean quantity of cocaine consumed during the week prior to admission was 11.5 (± 20.0) g with a mean of 4.6 (±. 2.0) days of use. Mean number of hours that the assay remained positive (i.e. exceeded 300 ng/ml) in urine from the last reported use was 87.7 (± 42.6). Stepwise multiple regression showed that g used, either during the week or during the three days prior to admission, was the only significant predictor of hr of positivity ($R = .48$, $P < .001$). Age, gender, weight and route of administration did not contribute significantly to the variance. The excretion curve, however, was not linear, suggesting that a multi-compartment model may be most appropriate for the description of cocaine pharmacokinetics. In order to provide a measure of BE excretion rate, 4-hour urine samples are currently being collected during the first week of treatment for another group of cocaine users. This method, together with measurement of urinary creatinine, makes it possible to correct for variability in urine output and collection, which may confound the excretion curve. The profile of excretion for the first subject run using this alternative method was also consistent with a multi-compartment model. We expect that subsequent data collected in this manner will help to clarify the pattern of BE excretion following regular, high-dose use of cocaine.

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The Psychopathy Checklist with Women Addicts

M.J. Rutherford, J.S. Cacciola and A.I. Alterman

An alternative to the DSM-III-R diagnosis of APD is Hare's Psychopathy Checklist-Revised (PCL-R). The PCL-R yields a total score and two factor scores. Factor 1, Psychopathic Personality Features, is similar to the DSM-I and DSM-II antisocial criteria, that is, it measures core traits of psychopathy. Factor 2, Antisocial Lifestyle, consists of items that are similar to the behaviorally based criteria of the DSM-III/-R. The PCL-R has been used with women in two studies, both with prisoners. Hare suggests that antisocial behaviors in men and women are expressed differently despite a basic similarity in core personality traits.

Methadone maintained women (n=25) revealed less psychopathy than men opiate addicts on the Total and factor scores of the PCL-R. Data on one month test-retest reliability (n=19) indicated that psychopathy can be reliably rated with women addicts. Total score and Factor 1 reliabilities were comparable in men and women addicts. However, for women lower reliability was obtained on Factor 2. It may be that Factor 2 items are described in terms more typical of men, which suggests a need to elaborate on the item definitions with examples more relevant to antisocial behaviors of women.

The prevelance of APD in this sample of women opiate addicts was lower (4%) than others have reported using the DSM-III or RDC. A high PCL-R score identified the one woman who received an APD diagnosis. The number of positive APD childhood symptoms was correlated with the number of positive adult symptoms for men, but not for women. Furthermore, PCL-R scores were highly correlated with adult antisocial behavior for women, but not with their childhood antisocial behavior. For men, however, PCL-R scores were correlated with both adult and childhood antisocial behavior. The precursors of adult antisocial behavior may well be different in men and women. Further, the DSM-III-R childhood criteria mav not include enough representive behaviors of early antisociality in women,

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Psychiatric Disorders in Addicted Women

J.S. Knisely, D.L. Haller and S.H. Schnoll

Forty-three women were administered multiple measures for diagnosis of psychiatric and addictive disorders prior to beginning treatment for substance abuse. The mean IQ for the group was 87 and the range was 54 to 112. Seventeen percent met criteria for diagnosis of mental retardation and 14% had IQ's in the Borderline range of intellectual functioning. This finding has clear implications for use of a traditional educational approach to treatment. Based on test findings, 64% of subjects had at least one Axis I diagnosis (excluding addictive disorders) while 100% obtained at least one Axis II diagnosis. The most frequent Axis I diagnoses were generalized anxiety (26%), dysthymia (26%), and phobia (20%). The most frequent Axis II diagnoses were antisocial (87%), histrionic (73%), narcissistic (63%), and passive/aggressive (63%). By contrast, clinicians detected non-addiction Axis I disorders in 23% of the sample and Axis II disorders in 20%. The most common addictive disorder was cocaine dependence (82%) followed by alcoholism (55%), and cannabis dependence (48%). Clinicians alone detected cocaine and alcohol dependence at a similar rate (82% and 44% respectively); however, they may be minimizing the role of marijuana. Clinicians thus appear highly sensitive to the presence of addictive disorders though less sensitive to other psychiatric disorders. The high prevalence of psychiatric illness and cognitive deficiency suggests the need to alter standard treatment to meet their special needs. (Supported by NIDA Grant #DA06094).

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Gender Differences in the Antisocial Behavior of Intravenous Drug Abusers

L.J. Felch, R.K. Brooner and G.E. Bigelow

Previous work has shown that antisocial behavior is commonplace among adult intravenous drug abusers and that about 40% of these patients meet DSM-IIIR criteria for antisocial personality disorder (ASP). These studies have largely reported on the antisocial behavior of male drug abusers, with comparatively little information available about the antisocial behavior of females. The present study compares rates of specific adult antisocial behaviors in male and female drug abusers, both with and without ASP.

The sample consists of 288 males and 118 females. The mean age of the sample was 35.9 years. Thirty-four percent of the sample was white and 49% were in methadone treatment. Fifty-three percent of the males and 29% of females were positive for ASP, with an overall prevalence in the sample of 46%. ASP diagnoses were made using a modified version of the Alcohol Research Center Intake Interview (ARC), a semi-structured diagnostic interview based on the DSM-IIIR criteria. Eleven adult antisocial behaviors taken from the demographic and ASP sections of the ARC were included in the analysis.

There were significant gender differences in the prevalence of ASP in male and female drug abusers and the ASP diagnosis was associated with significantly higher rates of adult antisocial behavior in both male and female drug abusers.

Significant gender differences were found in the adult antisocial behavior of both those with and those without a diagnosis of ASP. In non-ASP drug abusers, males reported more violent behavior than females, with higher rates of weapon use ($p<.001$), felonies committed ($p<.001$) and felony arrests ($p<.001$). Non-ASP males also reported a greater number of employers within the past year ($p<.001$) and since the age of 18 ($p<.001$) than did females. No gender differences were found in the number of misdemeanors or in measures of social functioning.

Drug abusers with ASP showed a pattern of gender differences similar to that seen in non-ASP drug abusers. ASP females reported rates of criminality and aggressiveness equivalent to those reported by non-ASP males.

In summary, the ASP diagnosis appears to be associated with an increase in the antisocial behavior of both males and females. Regarding gender differences, males in both ASP and non-ASP groups report higher rates of serious criminal behavior and aggressiveness than do females within the same diagnostic category. Interestingly, the antisocial behaviors of females in the ASP group were comparable, or greater, in reported frequency than those of non-ASP males.

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Personality Correlates of Drug Abusers With and Without Depressive Disorders

E.E. Rousar, III, R.K. Brooner, P.T. Costa, M.W. Regier, J. Herbst and C.W. Schmidt

Depression is a common complaint in drug abusers and studies have shown that most of these patients obtain high scores on measures of general neuroticism. Despite this, drug abusers often fail to meet formal criteria for a depressive disorder. The present study was undertaken to determine if drug abusers with a lifetime (not current) diagnosis of Major Depression or Dysthymia differed in personality structure compared to drug abusers who have never met criteria for either one of these mood disorders.

The personality dimensions of 148 intravenous drug abusers were assessed using the NEO-PI. a standardized self-report measure of the five-factor model of personality structure (i.e., Neuroticism, Extroversion, Openness to Experience, Agreeableness, and Conscientiousness factors). Lifetime diagnosis of Major Depression and Dysthymic Disorder were made on the basis of a structured clinical interview for DSM-IIIR. Twenty-seven percent of the sample (N=37) met DSM-IIIR lifetime criteria (not current) for Major Depression or Dysthymia. The mean age of the sample was 38, the mean years of education were 11, 67% were male, 38% were white; there were no significant differences between those with and without a lifetime depression diagnosis on these variables.

There were significant group differences on the NEO-PI. Drug abusers with a depression diagnosis had significantly higher scores on the Neuroticism factor (p=.004), including higher scores of the Anxiety, Hostility, Depression, Self-Consciousness, and Vulnerability facets. Those with a lifetime depression diagnosis also obtained a significantly lower score on the Extraversion factor (p=.02), including lower scores on the Interpersonal Warmth, Gregariousness, and Assertiveness facets compared to drug abusers without a depression diagnosis. There were no significant group differences on the Openness, Conscientiousness, or Agreeableness factors.

These data suggest that drug abusers with a lifetime diagnosis (not current) of Major Depression or Dysthymic Disorder experience higher levels of demoralization than drug abusers who fail to satisfy criteria for these mood disorders. The relationship between the chronic drug abuse of these patients and their history of depressive disorder was unclear. However, the chronically high level of distress reported by these patients may have treatment implications, including the possibility of a better treatment response to routine drug abuse counseling if specialized forms of psychotherapy are concurrently provided.

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The Difficulties and Implications of Distinguishing Primary and Secondary Drug Abuse

W.M. Compton, III, L.B. Cottler and J. Hudziak

In an ongoing NIDA-funded longitudinal stud 605 St. Louis area drug abusers recently admitted to treatment (both inpatient and outpatient) and their partners were interviewed to determine details about their risk for HIV infection, their histories of psychiatric symptoms and their pattern of substance use. As assessed by the DIS-III-R, the DSM-lII-R lifetime prevalence rates among the drug dependent subjects were 68% for alcohol abuse/dependence, 43% for antisocial personality disorder, 40% for phobic disorders, 28% for major depressive and/or dysthymic disorders, 10% for generalized anxiety disorder, and 4% for panic disorder. A new variable, "antisocial behavioral syndrome, was defined as any subject having conduct disorder or a pattern of adult antisocial behaviors. Of the drug dependent subjects. 85% had antisocial behavioral syndrome. Subjects with both drug and psychiatric diagnoses were stratified by gender. Then, the earlier age of onset of either drug use or psychiatric symptoms was used to classify the psychiatric condition as primary or secondary. We found that antisocial behavior nearly always antedates drug use in subjects with both antisocial behavioral syndrome and drug dependence. Drug use preceded other psychiatric conditions except phobias. No differences were seen between races in age of onset of non-substance use psychiatric disorders but Whites developed drug, alcohol and tobacco dependence earlier than Blacks. Net of other variables, primary alcohol dependence was predicted by older age and absence of antisocial personality disorder; male sex predicted primary antisocial behavioral syndrome; no variables predicted the other psychiatric disorders being primary. In conclusion, determining precise definitions of such items as "age of onset", "primary" and "secondary" is critical is understanding these issues and need to be clearly specified for comparison with other studies. In this study, the importance of antisocial behavioral syndrome in drug abuse is emphasized. This factor complicates treatment and worsens outcome.

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A Preliminary Evaluation of the Personality Traits in a Population of Drug Dependent Professionals Using the Tridimensional Personality Questionnaire (TPQ)

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Seventy-five drug dependent professionals were administered the TPQ (Cloninger, Science 236, 410, 1987) while in treatment. Preliminary findings indicated that the TPQ can be especially helpful to the client by providing feedback about their own individual behaviors. Four different sub-populations were also identified (Table below); HA appeared especially important in relation to discriminating between groups. A similar, but more consistent pattern, was observed in the relation to HA in female professionals; 18.5 ± 7.5 (N=28). Overall, the TPQ was found to be useful as a clinical tool, but did not correlate with other factors such as profession, drugs used, family background, etc. However, it should be recognized that more than 60% of the clients studied were Adult Children of Alcoholics (ACOA).

TPQ SCORES IN MALE DRUG DEPENDENT HEALTH PROFESSIONALS

	Novelty Seeking (NS)	Harm Avoidance (HA)	Reward Dependence (RD)
Control Data[a] (N=106)	16.8 ± 5.2	10.8 ± 6.0	18.1 ± 4.3
Counselors[b] (N=10)	20.2 ± 4.7	11.2 ± 5.2	19.9 ± 4.9
PHP Patients[C]			
Group A (11)	17.4 ± 2.3	17.1 ± 5.8	13.5 ± 3.0
Group B (14)	18.4 ± 2.1	8.0 ± 2.8	21.2 ± 3.8
Group C (10)	10.3 ± 3.0	18.3 ± 7.0	21.4 ± 3.4
Group D (7)	18.7 ± 4.1	19.6 ± 5.2	22.4 ± 2.6

[a] Data obtained from male volunteers MCV/VCU, [b] Professional Counselors in VA, [c] Patients from the Perspectives Professional Health Program, Hampton VA.

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Validity of Patients' Self-Reported Drug Use at Treatment Intake

M.F. Sherman and G.E. Bigelow

The research investigating the reliability and validity of drug use self-reports still appears to be in its infancy. Recent reviews conclude that there is some evidence that drug abusers' self-reports are reliable and valid. However, there are wide variations among studies depending upon the samples and procedures used to obtain the data The current study was conducted to extend the findings in this area. An examination of the intake interviews and same day urinalyses was conducted on 150 patients enrolling for outpatient opioid detoxification or maintenance at a treatment research clinic. As part of an extensive interview patients [106 males/44 females, 72 White/78 Black, Mean age = 33.29 (5.51), median income per month = $212.50, mean education = 10.94 yrs. (1.93)] were asked about the last date they used various drugs (i.e., opiates, cocaine, methadone, benzodiazepines) and provided a urine sample for analysis. A self-report of drug use within three days of the interview was considered positive, while a self-report of no use or use which occurred more than three days before the interview was considered negative. Urinalysis (EMIT) results revealed 92% positive for opiates, 69% positive for cocaine, 10% positive for methadone, and 12% positive for benzodiazepines Validity results using Kappa (degree of agreement beyond that expected by chance), and Conditional Kappa (degree of agreement with a positive urinalysis beyond that expected by chance) revealed the following: Opiates-$\underline{k}$ = .57, $\underline{k}_C$ = .73, sensitivity = .99, specificity = .50; Cocaine-$\underline{k}$ = 59, $\underline{k}_C$ = .45, sensitivity = .75, specificity = .91; Methadone-k= .72, $\underline{k}_C$ = .64, sensitivity = .67, specificity = .99; Benzodiazepines-$\underline{k}$ = .63, $\underline{k}_C$ = .51, sensitivity = 56, specificity = .99. Comparisons among the proportions of patients denying use of drugs for which they tested positive showed that cocaine (17.3%) was the most underreported drug followed by benzodiazepines (4.6%), methadone (3.3%), and opiates (0.7%) $\underline{O}(3)$= 72.70, $\underline{p}$<.0000. These results suggest that there is a fairly high agreement between drug use self-reports and urinalysis for clients entering a treatment research clinic, although this agreement tends to vary as a function of the type of drug. Furthermore, cocaine tends to be the most underreported drug among the clinic's drug addicts at intake. Factors (e.g., previous treatments, number of arrests) related to underreporting of drug use need to be examine

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Topographic Quantitative EEG Finding in Subjects with 15+ Years of Cumulative Daily THC Exposure

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Previously we showed that quantitative EEGs of daily THC users have significant elevations of Absolute Power, Relative Power and Interhemispheric Coherence of EEG alpha over frontal cortex. We suspected that Ss with excessively long term THC exposure may also show increased frontal-central theta. We initiated a pilot study of the EEG, EP, and neuropsychological characteristics of Ss who have used THC for 15 to 30 consecutive years. Fifteen Ss with 16 to 24 consecutive years (mean=19.6 years) of daily THC use (long term THC) were contrasted with 11 Ss (short term THC) using THC for 3 to 6 years (mean=4.1 years), 22 normal non-user controls from our earlier studies (control 1), and non-user controls from our current NIDA investigation (control 2). When contrasted with short term THC users or controls, long term THC users have significant elevations of theta Absolute Power over frontal-central cortex as shown in the following table:

THETA ABSOLUTE POWER: MANN-WHITNEY p VALUES (2-Tailed)
L=Long Term THC, S=Short Term THC, C1=Control 1, C2=Control 2

DIRECTION	F_{p1}	F_{p2}	F_{pz}	F_3	F_4	F_z	C_3	C_4	C_z
L > S	.06	.04	.06	.03	.03	.02	.04	.01	.01
L > C 1	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
L > C 2	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Long term THC users also have significant elevations of Relative Power of theta over frontal-central cortex when contrasted with short term users but not when contrasts are with controls. Finally long term THC users have significant elevations of Interhemispheric Coherence of theta over central-parietal-occipital cortex as contrasted with 3 to 6 year duration THC users. Preliminary analyses of neuropsychological data suggest that a performance gradient may exist with controls showing the best performance followed by short term THC users and with the worst performance occurring among ultra long term THC users. In general, measures of overlearned information suffered least from THC exposure while those dependent on attention, concentration, memory and learning suffered most with long term THC users differentially more impaired than short term THC users. The existence of a significant EEG spectral power increase of slower theta activity along with the preliminary suggestion of neuropsychological sequelae may suggest an organic change with very long cumulative THC exposure.

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Differences in Complex Reaction Time Between THC Users and Non-User Controls

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Complex Reaction Time (CRT) and other measures are secured as part of an ongoing NIDA study of the neurophysiological and neuropsychological effects of chronic THC use in a nonpatient population. Data from 37 completed subjects were divided into controls (never used), "short term" (10 or fewer years) and "long term" (15 or more years) user groups. Subjects are extensively interviewed vis a vis ancillary drug use and psychiatric difficulties. Marijuana use is confirmed through 8 weeks of twice weekly urine screens. A "special interest" group of 12 long term users (15 or more years) who did not qualify for the NIDA study was also examined. These Ss did not receive urine or psychiatric screens but did undergo *a* comprehensive drug interview.

CRT is evaluated using Sternberg's procedure which briefly presents a small set of integers followed by a short delay and then a probe. S decides if the probe was in the set and CRT is measured by the time it takes from the onset of the probe to press a "yes" or "no" key. Each S receives 120 trials and only correct responses are examined. In addition, S also receives tests of mental tracking, short term memory and learning, and "higher level" conceptual and intellectual abilities. Initial results suggest a relationship between duration of use and poorer performance on the test battery. Differences ($p < 0.05$) or strong trends ($p < 0.10$) were found between controls and long term NIDA Ss/"Special Interest" Ss on measures of CRT, complex verbal learning (CVLT), conceptual abilities (Category Test, CLAT), and short term memory (Verbal, Visual, Delayed WMS-R MQ). Special interest Ss also did poorly on complex mental tracking (Paced Serial Addition Test) relative to controls with NIDA long term users trending in the same direction ($p < 0.15$). Small sample sizes dictate caution with the above results perhaps best interpreted as preliminary at this time.

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Joint Action of Methadone Doses and Take Home Doses Frequency in Treatment Compliance

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Methadone dose as a factor in treatment effectiveness has been extensively studied. In general, higher doses have been reported to have therapeutic advantage. Stringent federal regulations exist on take home frequency, particularly in the first three months of a treatment episode.

This ongoing study examines the *joint action* of methadone dose and take home frequency (5-High Frequency Take Home, HFTH, vs 2-LOW Frequency Take Home, LFTH) in opiate dependent patients (2x2; 4 grps, 26 Ss per group). Study duration is 24 weeks. The intake procedure reviews all major areas (ASI/Beck/POMS/Hamilton), medical status including HIV and TB, and psychiatric evaluation (SCID/DSM IIIR). Patients are assigned and begin a two week stabilization phase within three days. Methadone doses are 50 mg and 80 mg. Medication effect is examined in the context of patients receiving the high or low frequency take home doses each week (clinic visits either 2 or 5 times per week). One structured individual counselling session each week includes review of major areas of function. Specific behaviorally based recommendations are made to prevent drug use. Urine drug screens are conducted twice each week and paper and pencil measures are obtained weekly. Cocaine and other concurrent drug use are examined as a function of methadone dose and visit/take home frequency. Patients completing this study who continue to use cocaine as reflected by 50% positive urine screens in the last two months of treatment, enter a double blind placebo controlled study of concurrent fluoxetine administration with 5 visits per week.

The results provide indications of optimal combinations of treatment for patients receiving methadone for opiate dependence. Results to date suggest better retention in treatment for patients receiving high frequency take homes (HFTH) regardless of dose (50 mg or 80 mg). Drug screen results over the six month treatment period reflect decreased use of opiates, marijuana, and nicotine as a function of methadone dose but not take home frequency. High dose is more efficacious than low dose. However, the urine screen results for cocaine use show an opposite trend with greater prevalence of positive screens in the high methadone dose (80 mg) group. This finding did not vary as a function of take home frequency.

These interim study results suggest that optimal combinations of treatment can be identified and may vary depending on patterns of concurrent drug use. The data do not support the need for daily clinic visits as currently required by federal regulation. Final analyses will help clarify these issues.

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The Effect of Rate of Methadone Metabolism on Treatment Outcome Study One: Methadone Metabolism and Craving, Withdrawal, and Dysphoria

D. Tusel, P. Banys, K. Sees, P. Reilly and K. Delucchi

This paper reports on the findings from the stabilization phase of a 180-day methadone detoxification study. We tested one hypothesis: Subjects who metabolize methadone more rapidly will show more craving, signs of withdrawal, dysphoria, and illicit opiate use without regard to methadone dose.

Thirty-eight subjects were randomly assigned in a double blind fashion either to 80mg (HIGH) or 40mg (LOW) of methadone. Twenty-nine subjects supplied sufficient data for analysis in this study. All subjects were stabilized on methadone by the second week of the study and remained on their stabilization dose from week 3 through week 14. The data for this paper are derived from this time period. Bloods were drawn for serum methadone levels at the midpoint **of** the stabilization phase. The "peak" level was drawn 2 hours after the methadone dose and the "trough" level was drawn 22-24 hours later. The Opiate Symptom and Sign Checklist, withdrawal and craving scales, and the Profile of Mood States (POMS) were administered weekly. The Beck Depression Index (BDI) and the State/Trait Anxiety Index (STAI) were administered monthly. Urines were collected randomly twice weekly and analyzed for opiates and cocaine.

We found a positive correlation between increased methadone metabolism rate and craving, signs of withdrawal, and dysphoria (measured by POMS, Beck Depression Index, and the State Anxiety Index), but only for those subjects on the HIGH dose of methadone. The "trough" serum methadone levels alone did not correlate with craving, withdrawal, or dysphoria. No statistically significant relationship existed between methadone metabolism rate and the illicit opiate use in any of the subjects even if the "trough" level fell below 150 ng/ml.

Those patients on higher doses of methadone who feel that their dose is not holding them may be rapid metabolizers. The dose of methadone may be too high rather than insufficient. The data also suggest that it might be useful to obtain both a "peak" and "trough" serum methadone level in order to determine whether the patients' complaints are related to insufficient dose or more rapid methadone metabolism rate.

AFFILIATION: Veterans Affairs Medical Center, San Francisco

This project was supported by NIDA grant #R18 DA06097

Approach to Management of Methadone Patients Using Illicit Drugs

D.H. Tiuseco and R.I.H. Wang

Many patients on methadone maintenance are known to use illicit drugs. Thirty-nine methadone maintenance pts were surveyed from 10/1/89 to 9/30/90. All pts had randomized urine surveillance 2 times weekly. The illicit drugs measured were opiates, cocaine, benzodiazepines, barbiturates, and propoxyphene. When pts show dirty urine, a written notice and intensive counseling are given. After two consecutive weeks of dirty urine, the pt is dropped a phase. For benzodiazepines and barbiturates, phase is dropped and alternative pharmacotherapy is given while the methadone dose remains unchanged. For opiates and propoxyphene, phase is dropped but the methadone dose is usually increased. For cocaine, phase is dropped. Further dirty urine for cocaine will result in 5 mg methadone dose drop as reported previously (CPDD PG. 71, 1988). After two consecutive weeks of clean urines, phase and subsequently the methadone dose will be restored. In this study of 39 pts: 21% had no dirty urine; 26% responded to counseling alone; 31% to counseling plus a decrease in phase; 10% to counseling plus a decrease in phase and an increase in methadone dose; 2% to counseling plus decrease in phase and decrease in methadone; 10% persistently showed dirty urine inspite of all approaches. The average length of time patients remained clean after counseling alone was 5.4 months; after counseling plus a decrease in phase was 3.5 months; after counseling plus a decrease in phase and an increase in dose was 6.5 months; after counseling plus a decrease in phase and decrease in dose was 5 months. It is concluded that all pts on methadone maintenance using illicit drugs should not be ignored and must be managed using various approaches, including counseling, frequent visits, medication dose adjustment and other pharmacotherapy.

References available upon request.

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Preliminary Data from A Comparison of Three Levels of Methadone Services

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This study seeks to test the effectiveness of three models of supportive services for methadone treatment clients and the impact of urinalysis contingency contracting on treatment outcome. New treatment admissions were assigned to one of three treatment formats: (1) "medication only", (2) "standard" counseling, and (3) "enhanced" services: and to one of two modes of managing urinalysis results: (1) no contingencies (NC), and (2) contingency contracting (CC). Results from 187 subjects who are 6 months beyond admission are reported here. Sample demographics: 62.0% male: 52.9% white, 42.8% black: 50.5% employed; 55.6% less than high school education; 3.7% age 18-25, 25.1% age 26-34, 58.2% age 35-45, 12.8% age 46+. No differences were found in treatment retention during the first 6 months of treatment. However, among 106 subjects who were 9 months past admission, more NC than CC subjects (69.8%) remained in treatment the full 9 months (43.4%) ($\underline{x}^2$=7.53, $\underline{p}$=.006). No main effect of treatment format or contingency contracts on opiate or cocaine positive urinalysis at 6 or 9 months were found. However, at 6 months an interaction between treatment format and contingency contracting was significant for opiate and cocaine positives combined ($\underline{F}$=3.29, $\underline{p}$=.04, $\underline{df}$=2,181). NC methadone treatment was more effective at retaining clients. Specific combinations of treatment format and contingency contracts may be most effective in reducing drug use. Six month results are very preliminary. The long term impact of contingency contracts may not be known until 18 months when subjects discharged due to contract failure are readmitted.

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Cannabis Use and Its Relationship to Other Drug Use Among Patients in a Methadone Maintenance Program

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Little research exists on the prevalence of use of Cannabis and its relationship to use of other illicit drugs among, patients enrolled in methadone maintenance treatment programs. THC frequently is not even assayed in routine toxicological screening by many methadone programs. In one of the few studies that have addressed this issue, Harlow & Anglin (1984) found that involvement in a methadone maintenance program was associated not only with a decrease in daily use and an increase in regular employment, but also a slight increase in cannabis use.

Weekly urine toxicology screens were monitored for 70 subjects enrolled in an outpatient methadone maintenance program. To examine the relationship between cannabis use and other drug use, subjects were categorized into four THC groups: Group 1 - no positive screens for THC; Group 2 - greater than 0 but less than 33.3% positive screens; Group 3 - greater than 33.3% but less than 66.6% positive screens; and Group 4 - greater than 66.6% positive screens.

The results indicate that the use of Cannabis is high. Over 78% of the subjects had at least one positive screen for THC, 35.7% of the subjects had greater than zero but less than 33.3% positive screens, 22.9% had 33.3-66.6% positive screens, and 20.0% had more than 66.6% positive screens. Overall, the 4 THC groups did not significantly differ in terms of the number of positive screens for opiates, cocaine, and benzodiazepine. In contrast, those that tested positive for cocaine had more positive opiate screens (M=27.9%) than those that never tested positive for cocaine M = 5.5%).

Overall, we found a very substantial prevalence of Cannabis use among patients enrolled in a methadone maintenance program. However, we did not find that regular THC use was associated with an increase in opiate, benzodiazepine, or cocaine use. The prevailing treatment ideology in drug abuse treatment programs has emphasized abstinence. This stems from the perception that use of any psychoactive drug may lead to further increases in opiate use. Although, this study suggests this may be true for cocaine, it does not appear to hold for THC. Future investigation should further examine the positive and negative consequences of THC use among methadone maintenance program clients.

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Naltrexone in the Treatment of Federal Probationers

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INTRODUCTION: Substance abuse is a common problem among individuals entering the criminal justice system. Naltrexone, an opiate antagonist, has been shown to have few side effects and although it has been proven to be a safe and effective medication in blocking the actions of opiates and preventing dependence. it's clinical utility has been limited by the general lack of *acceptability* among many patient populations. We believe that underutilization of naltrexone is also due in part to limitations in the design of programs using naltrexone. The project described here is evaluating the feasibility and effectiveness of naltrexone when used with individuals at risk of incarceration should they relapse to opiate addiction. The Naltrexone Treatment Program is a project of the Penn/VA Center for studies of Addiction and is located in the Federal Probation Office in Philadelphia. The program has been operational for the past 36 months. This paper presents some of our preliminary findings.

METHODS: The program is being evaluated using an open, randomized control group design. Participation in the research is completely voluntary. Individuals with histories of opiate dependence or who are evidencing early signs of relapse are self-referred or referred by their probation officer. The project staff provide a full description of procedures and potential risks and benefits of participation. Following orientation and informed consent, subjects are randomly assigned to either the medication or control group.

Those assigned to the medication group are first challenged with naloxone to confirm that they are free of opiates. These patients receive naltrexone two times each week in order to provide adequate blockade coverage. Individuals assigned to the control group are also scheduled to visit the program two times each week for counseling and monitoring. All subjects are asked to contact their probation officers at each visit. Drug use (self-report and urinalysis) and drug craving are monitored on a weekly basis as are physical and psychological symptoms, treatment involvements, and social functioning.

PRELIMINARY RESULTS: Fifty-five subjects have now completed the six month study phase of the project. Preliminary results indicate that for probationers randomly assigned and engaged (completing the first week of program requirements) to the nalhexone group, the six month retention rate is *forty-eight* percent. There have been few negative side effects noted by patients taking the medication. Nausea was the only symptom reported for significantly more weeks (16 weeks vs 7 weeks; $p<.05$) by the naltrexone group. Opiate use was low in both groups as measured by urinalysis. Interestingly, 62% of the control group were incarcerated prior to the completion of their six month study period. This compares to 33% of the group assigned to receive naltrexone.

DISCUSSION: Our findings demonstrate that the delivery of services within a probation environment can be a viable model for drug abuse treatment. Many probationers experience significant difficulty with drug use and probation officers will use treatment services available to them. Although our six month retention rate is quite high, the threat of incarceration is clearly not, by itself, a sufficient motivation for probationers to seek help and remain in treatment. Despite similarly low rates of opiate use, subjects assigned to the naltrexone group demonstrated a much lower rate of incarceration than those in the control group. We believe that this differential in the rate of incarceration is a reflection the availability of a treatment option that is seen as effective in the management of opiate abusing probationers. AFFILIATION: University of Pennsylvania/VA Medical Center, Center for Studies of Addiction, Philadelphia, PA

Regional Cerebral Blood Flow (rCBF) Changes in Naltrexone Precipitated Withdrawal from Buprenorphine

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Acute naltrexone precipitated withdrawal from the mixed opiate agonist/antagonist buprenorphine may be different from the withdrawal from methadone. This study proposed to determine the subjective/objective opiate withdrawal symptoms, blood pressure responses, and regional cerebral blood flow (rCBF) changes associated with naltrexone precipitated withdrawal from buprenorphine (with and without clonidine preloading). Methods: thus far, nine opiate addicts have participated following maintenance on buprenorphine (2mg sublingually) for seven days. Naltrexone was then administered:placebo on day 8, 25 to 50mg on day 10, and 50mg with 0.4 mg clonidine preload on day 12. On each of the 3 days, withdrawal symptoms were assessed and blood pressures taken at baseline, 30, 45, 60 and 90 minutes after naltrexone was given and Tc-99m HMPAO was injected 60 to 90 minutes after naltrexone. A clinician completed a rating scale (range 1 to 5) withdrawal symptoms. Single photon emission computed tomography (SPECT) rCBF scans were acquired using an ASPECT cylindrical crystal device. rCBF ratios were computed by dividing regions of interest (ROI) weighted count densities by whole brain weighted count densities. Results: Oral naltrexone treatment produced symptoms of withdrawal in all treated subjects. The mean peak withdrawal severity score was similar in buprenorphine withdrawal compared to our previous study of methadone withdrawal even though a 25 to 50 fold increase in naltrexone was utilized. Clonidine preload significantly decreased the overall withdrawal severity score. While it is difficult to interpret results of this limited sample, rCBF ratio changes were noted in several areas: rostra1 pans/whole brain rCBF ratios were increased during withdrawal while anterior cingulate/whole brain rCBF ratios declined.

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Buprenorphine's Effects on Morphine and Cocaine Challenges in Heroin and Cocaine Dependent Men

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The prevalence of concurrent cocaine and heroin abuse appears to be increasing in the United States. Buprenorphine, a mixed opioid agonist-antagonist may be efficacious in treatment of opioid dependence. More recent data suggested that it suppressed cocaine self administration in rhesus monkeys and lowered incidence of cocaine positive urines in buprenorphine-maintained patients compared to methadone maintained patients. These observations suggested that buprenorphine may be a potential pharmacotherapy for dual addiction to cocaine and heroin. This study assessed the subjective responses to an intravenous challenge dose of morphine (10mg), cocaine (30mg) and saline control solutions in 16 men, concurrently dependent on opioids and cocaine according to DSM-III-R criteria. The challenge doses were carried out before and after 10 days of sublingual buprenorphine maintenance on either 4 or 8mg daily. Eight subjects were maintained on 4mg and eight subjects were maintained on 8mg. Subjective responses included latency to and certainty of detection, drug intensity and drug quality ratings. All subjects correctly identified the morphine challenge doses prior to initiation of buprenorphine therapy. However, following buprenorphine maintenance of either 4 or 8mg daily, twelve subjects identified the morphine challenge doses as placebo and four subjects detected a 'drug effect'. Of these four subjects, only one subject correctly identified the morphine challenge dose, one subject misidentified the challenge dose as cocaine and two subjects subsequently identified the challenge dose as placebo. All subjects correctly identified the cocaine challenge doses before and after buprenorphine maintenance. Both detection and certainty times after cocaine administration were diminished following the 4mg buprenorphine maintenance. However, administration of the 8mg buprenorphine maintenance dose increased the detection time and decreased the certainty time following cocaine challenge. Drug quality was slightly decreased following buprenorphine maintenance on 4mg daily. In contrast, drug intensity and quality of cocaine appeared to be slightly higher and more prolonged in the subjects maintained on 8mg buprenorphine daily. The results of this study confirmed previous reports of buprenorphine blockade of opioid agonist effects. The effects of buprenorphine on subjective responses to cocaine challenges were variable. Further studies to assess the effectiveness of buprenorphine maintenance on subjective responses to cocaine are indicated.

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Preliminary Results of An Open Trail of Buprenorphine in the Outpatient Treatment of Combined Heroin and Cocaine Dependence

D.R. Gastfriend, J.H. Mendelson, N.K. Mello and S.K. Teoh

Buprenorphine (BPN), a mixed opiate agonist-antagonist, offers advantages over methadone for the treatment of opiate dependence. In addition, animal studies suggest that BPN may reduce cocaine self-administration. We conducted an open blind outpatient trial of daily BPN sublingual administration in men with concurrent heroin and cocaine dependence to determine the safety and effectiveness of BPN for these disorders. We now report results from fifteen cases treated for up to 10 months following inpatient induction with BPN.

Following inpatient BPN maintenance (18 days), 47% (15/32) of patients with a diagnosis of concurrent DSM-IIIR cocaine and heroin dependence elected to participate in a BPN outpatient maintenance program. All of these patients had used heroin IV daily for 2 years and also had self-administered cocaine for an average of 5.3 days per week. Treatment retention for this severely ill group was 87% over a mean duration of 20 weeks. Urine screens were negative more than 50% of the time for both heroin and cocaine. This is a conservative index of BPN's effectiveness for reducing cocaine use, since the 7 day half life of the major cocaine metabolites is greater than the time interval between urine samplings (3-4 days). Daily self-reports of drug use revealed a mean decrease from 7 to less than 1 day per week for heroin and from 5.3 to less than 1 day per week for cocaine. Tolerance for the salient effects of BPN maintenance has not occurred to date. Our data suggest that BPN is a safe and effective pharmacotherapy for the outpatient treatment of concurrent heroin and cocaine dependence. *Supported by MDA Grant DA 06116*

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EEG Sleep Architecture in Cocaine- and Heroin-Dependent Subjects During Buprenorphine Maintenance

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Fourteen healthy adult male volunteers with a history of opiate and cocaine abuse and dependence (DSM-IIIR criteria) provided informed consent for participation in this study designed to determine if chronic administration of buprenorphine could reduce their sleep complaints while they are being treated for dual dependence on opiates and cocaine. Many subjects had to be detoxed from methadone before the study began. Subjects resided on a NIDA Treatment Research Unit (TRU) and had a sleep recording four times during their stay. Subjects were prepared with standard scalp and muscle electrodes for polysomnography and slept in their own beds on a the TRU. Subjects were free of all medications during the first 9 days of the 30-day program. On day 10 they all received a 1 mg dose of buprenorphine provided as a liquid for sublingual administration. The dose was increased on consecutive days to 2, 4, 6 and 8 mg per day. Seven of the subjects continued to receive 4 mg per day while the other seven were increased to 8 mg per day. Subjects continued receiving buprenorphine until day 23. EEG activity was recorded on equipment located in the Sleep Disorders Center which is located in the next building. Subjects were connected via coaxial cable and a digital encoding system. All-night EEG sleep recordings were obtained on days 4, 5, 18 and 19. Records were scored blind using a computer-assisted sleep scoring program and verified by visual inspection of the raw data. On days 4 and 5 of the study, all subjects experienced delayed sleep and REM sleep latencies, reduced total and REM sleep time and a markedly reduced amount of slow wave sleep. Measures of sleep latency, total sleep time, REM latency, slow wave sleep and non-REM sleep were all improved after the 4 mg dose of buprenorphine. Only sleep latency, total sleep and non-REM sleep were improved after the 8 mg dose. In fact, REM sleep and slow wave sleep actually *decreased* during the 8 mg dose. These data suggest that buprenorphine reverses many of the sleep-related problems reported by opiate- and cocaine-dependent individuals. The 4 mg buprenorphine maintenance dose appears to be superior to the 8 mg dose in that measures of REM sleep, slow wave sleep and wake time during sleep were superior to the higher maintenance dose of buprenorphine. Supported by Grants DA06116, DA03994, DA00115, DA00064, and DA00101 from the National Institute on Drug Abuse.

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Pre- and Post-Treatment Cue-Reactivity in Cocaine Addicts Treated with Bupropion

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Psychophysiological reactivity to drug-related cues in chronic cocaine users may sustain addictive behavior, and play a role in relapse. Individual differences in reactivity may also predict success in treatment. We examined pre- and post-treatment reactivity (skin conductance level, skin temperature, and self-reported craving) to neutral and drug -related cues in 19 cocaine addicts who participated in an eight-week study of bupropion (10 mg tid) for the treatment of cocaine addiction. Sixty-eight percent of the patients completed the study. Ten of the nineteen patients achieved abstinence, with abstinence defined as no urine screens positive for cocaine metabolites during the last four weeks of the study. Patients who achieved abstinence did not significantly differ in pre-treatment self-reported drug cue elicited craving, however they did differ in self-reported craving subsequent to a relaxation procedure which was administered immediately following exposure to the drug cues. There was a significant decline in post-treatment self-reported craving to drug cues, however physiological reactivity (skin conductance and skin temperature) did not diminish significantly from pre-treatment levels. Pre-treatment predictors and correlates of success in treatment are presented (e.g., pre/post physiological reactivity to drug-cues, changes in self-concept and depression) which suggest that successful psychopharmacological treatments for cocaine addiction may facilitate a cognitive shift away from drug-use scripts, even while leaving physiological reactivity, at least in the short run, relatively unchanged.

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Bromocriptine: Anti-craving and Other Effects in Patients Abusing Cocaine

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Pharmacotherapy for cocaine dependence is in it's infancy. Earlier enthusiastic reports of the effectiveness of desipramine in the treatment of cocaine abusers have not been substantiated in the most recent findings. Dopamine agonists, such as bromocriptine and amantadine, appear to be more interesting. Both agonists have been proven to be effective, but the side effects experienced by the patients are insignificant for amantadine but intolerable to a good majority of patients receiving bromocriptine. The purpose of this report is to present anti-craving and side effects (SE) of bromocriptine noted by the patients at therapeutic dose levels in an open study. The side effects of bromocriptine noted by other investigators include headaches, vertigo, and syncope. In our open study, nine male cocaine abusers were given bromocriptine orally on a total daily dose of 2.5 mg to 10 mg. Cocaine withdrawal/craving ratings and side effects were recorded daily. Vital signs including sitting and standing blood pressure were taken before and daily during bromocriptine treatment. Results showed that the craving for cocaine was reduced in six of the nine patients. One patient noted that the euphoric effect of bromocriptine resembled the use of cocaine. Only one patient experienced orthostatic hypotension at 2.5 mg four times a day and bromocriptine was discontinued. Three patients noted decreased depression. Two patients experienced brief and slight degree of dizziness during bromocriptine treatment. Headaches were noted in two of the nine patients. It is concluded that bromocriptine at 2.5 mg BID to 2.5 mg QID is beneficial to patients experiencing cocaine cravings. Side effects were noted but tolerable. The results of this study warrants double blind study comparing bromocriptine and any other tolerable medications in the treatment of patients abusing cocaine.

References are available upon request.

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Effects of Cocaine Alone and in Combination with Mazindol in Human Cocaine Abusers

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Mazindol is a dopamine reuptake inhibitor that blocks binding of cocaine at the dopamine reuptake site. An open clinical trial indicated that mazindol may decrease craving for cocaine. The present study was conducted to determine whether mazindol modulates the pharmacologic effects of cocaine in humans. The study was conducted in two phases, a dose-ranging phase and a cross-over phase. Subjects were current users of IV cocaine who were not seeking treatment for their cocaine abuse and who participated as inpatients on a research unit. Mazindol was administered orally 2 hours before the cocaine challenge session in which cocaine was administered IV in a single injection. Physiologic and subject- and observer -rated responses to mazindol and cocaine were measured. In the dose-ranging phase, 4 subjects received increasing doses of mazindol (0.5 to 6 mg, PO) given as pretreatments 2 hr before administration of increasing doses of cocaine (12.5 to 50 mg, IV). Based on the results of the dose ranging phase, the following 12 conditions were tested in 8 subjects in the cross-over phase: cocaine 0, 12.5, 25, and 50 mg (IV) alone and in combination with mazindol 0, 1, and 2 mg (given orally 2 hr before cocaine injection). Dose conditions were tested in separate sessions; results were analyzed using a three factor, repeated measures analysis of variance (factors: mazindol dose, cocaine dose, and time). Cocaine alone increased heart rate and blood pressure and increased ratings of drug effect, liking, rush, desire for cocaine, and symptoms indicating stimulant-like effects. Mazindol alone increased heart rate and blood pressure and had mild, stimulant-like subjective effects. There were significant interactions between cocaine and mazindol on heart rate and blood pressure, with combinations producing effects that were greater than cocaine alone. There was no evidence that mazindol substantially altered the magnitude or profile of the subjective effects of cocaine; nor did mazindol decrease cocaine-induced desire for cocaine. The present study does not support the utility of mazindol in the treatment of cocaine abusers through a mechanism of modulation of cocaine's subjective effects, including craving. Furthermore, mazindol treatment may increase the cardiovascular risks of cocaine. (Supported by USPHS grants DA 05196 and DA 06120).

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Cocaine Blocking Effects of Ondansetron

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The selective serotonin type 3 receptor antagonist ondansetron modulates the effects of dopamine in CNS. For example ondansetron blocks hyperactivity resulting from infusion of dopamine into the nucleus accumbens in rats and marmosets. Previous studies have shown that ondansetron alone up to 40 mg IV is without psychoactive or cardiovascular effects. To learn if ondansetron pretreatment blocks effects of cocaine, a double blind, randomized crossover study was conducted in 12 subjects according to two 6 x 6 balanced latin squares (with extra period) design. At 30 minutes following pretreatment with either IV placebo or ondansetron (.25 mg); placebo or cocaine 25 or 50 mg IV was administered. The second latin square used the same design with an ondansetron dose of 2.0 mg. Subjective, behavioral and vital sign measures were taken at fixed intervals before and after ondansetron and cocaine administration. Ondansetron itself had no effect. Ondansetron (0.25 mg) significantly reduced the self reported "rush", "feel the drug" and "cocaine identification" for the 50 mg dose of cocaine. Ondansetron, 2 mg, significantly reduced the self report of "feel the drug", "rush", "cocaine identification", "dislike the effects", "jitteriness", "alertness", "down", "difficulty concentrating", "paranoid for the 25 mg dose of cocaine. The observer responses demonstrated that odansetron 2 mg decreased reports of "feel the drug", "concentration" and "paranoid'. For the vital signs ondansetron reduced supine systolic blood pressure at the 25 mg dose of cocaine only. Other measurers were not affected significantly although there was a trend towards reduction of effects. In summary, ondansetron pretreatment alters the subjective state induced by cocaine administered IV, but is without significant effects on the physiologic effects of cocaine.

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Quantitative Magnetic Resonance Imaging in Opioid- and Cocaine-Dependent Men

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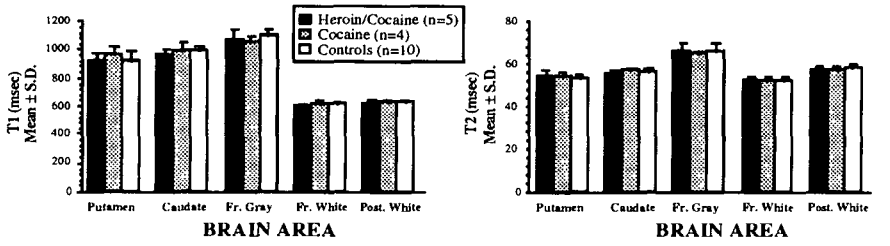
Quantitative magnetic resonance imaging (MRI) of the brain was performed in 14 drug-dependent men (10 heroin/cocaine users and 4 cocaine users) with primary diagnoses of Opioid and/or Cocaine Dependence, and 13 age-matched, healthy, non-drug dependent controls using a commercial 1.5 Tesla Signa whole body imager.

Individuals with evidence of gross neurologic abnormalities (e.g., sulcal or ventricular enlargement or focal signal abnormalities) were excluded from T1 and T2 analyses, in order to determine whether alterations in T1 and T2 relaxation times could be observed in the absence of gross structural changes. The final subject pool consisted of 5 heroin- and cocaine-dependent men ($\bar{x}$ = 33.60 ± 2.07 years), 4 cocaine-dependent men ($\bar{x}$ = 23.75 ± 1.70 years), and 10 non-drug dependent controls ($\bar{x}$ = 30.60 ± 6.37 years). Drug-dependent populations were combined and compared to the controls for the purpose of statistical analyses.

Regional T1 and T2 times were calculated on a single 5 mm thick axial slice positioned just below the caudal margin of the lateral ventricles, passing through the caudate and putamen. A voxel of interest (VOI) cursor was placed bilaterally within the putamen, caudate, frontal grey matter, frontal white matter, or posterior white matter. An iterative chi-square minimization program for a three parameter fit was utilized for determination of T1 at each VOI using five data points (TE 20 and TR 3200, 1600, 800, 400, 200). T2 was determined at each VOI by a similar iterative chi-square minimization program for a two parameter fit using four data points (Te 20, 40, 60, 80 and TR 3200).

T1 and T2 relaxation times did not differ significantly between the subject groups in any brain region studied (Figure 1). These findings suggest that T1 and T2 relaxation times may not identify microstructural CNS alterations resulting from chronic cocaine and opiate abuse. Supported by NIDA grants DA06116 and DA00064.

Figure 1. T1 and T2 Relaxation Times



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Bupropion in the Treatment of Primary Alcoholism

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Bupropion HCl (Wellbutrin), an antidepressant of the aminoketone class, has been shown to be effective in the treatment of depression at doses of 300-450 mg/day. The neurochemical mechanism of bupropion (B) is unknown. Preliminary studies suggest that, unlike tricyclic antidepressants, B does not potentiate the sedative effects of alcohol and may slightly reverse the drowsiness and impairment induced by the the coadministration of alcohol or diazepam. This 6 month study was conducted to assess the efficacy of B in treatment of primary alcoholism, and employed a double-blind, randomized, parallel, placebo-controlled design. An a priori decision was made to include in the analyses only those patients who received ≥21 days of treatment. Fifty-three outpatients were enrolled in the study following hospitalization for alcohol dependence. Thirteen of these were excluded from the analyses because of withdrawal before Day 21. A total of 40 male outpatients, 20 receiving B and 20 placebo (P), were included in the analyses. The average age was 41 years and 78% were Caucasian. 98% of patients were maintained on a 450 mg/day dose of B. No statistically significant differences were found between B and P for the number of days on which alcohol was consumed, the amount of alcohol consumed, or for measures of psychosocial and neuropsychological functioning. A survival analysis using the Kaplan-Meyer method showed no statistically significant difference ($p = 0.094$) in the proportion of patients who relapsed between the B group (12/20) and the P group (6/20). A statistically significant difference was not found when the time to relapse was compared for B (M = 84 days, range: 31-179) vs. P (M = 99 days, range: 22-139). Though the sample size is small, these results suggest that B is not effective for the treatment of primary alcoholism.

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Serotonergic Agents Modulate Weight Gain Following Smoking Cessation

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Smokers who quit or substantially reduce nicotine intake can gain up to 5 kg over the next year. Whether this phenomenon results from changes in preference for specific nutrients, increased overall caloric intake, or metabolic changes is not well understood. Serotonergic agents have been shown to reduce eating and produce weight loss in obese non-smokers. In two recent studies of such agents administered as adjuncts to behavioral smoking cessation treatment, caloric intake, weight, and related variables were monitored to determine whether such agents might help in controlling weight gain. 1) Pomerleau et al. *(Psychoneuroendocrinol,* 1991, 16:in press) examined the effects of 60 mg/day fluoxetine (a 5-HT reuptake inhibitor) on eating behavior in a laboratory setting and on body weight in 37 normal-weight smokers of both sexes undergoing treatment in a behavioral quit-smoking program; only the 11 placebo and 10 active-drug subjects who stringently reduced nicotine intake (plasma cotinine <50% of baseline) were included in the analysis. After 10 weeks, placebo subjects gained significantly more weight (3.3 kg) than fluoxetine Subjects (-0.6 kg). Change in total calories consumed in a standardized test lunch was significantly correlated with weight change. In fluoxetine subjects only, higher initial Body Mass Index (weight/height^2) was associated with greater weight loss. 2) Spring et al. *(Health Psychol,* 1991, 10:216-223) investigated the effects of 30 mg/day d-fenfluramine (a 5-HT releaser and reuptake inhibitor) in 31 overweight female smokers (15 placebo vs. 16 active drug subjects). Four weeks after cessation, placebo subjects gained significantly more weight (1.6 kg) than d-fenfluramine subjects (-0.8 kg). In tests in which a variety of foods was provided over a 48-hour period and intake was directly assessed, placebo subjects, but not fenfluramine subjects, showed a significant increase in caloric intake. This effect, evident 48 hours after cessation and persisting at 4 weeks, was accounted for largely by increases in carbohydrate intake. Change in total calories consumed was significantly correlated with weight change. D-fenfluramine also prevented post-cessation dysphoria. The two studies, taken together, suggest that: 1) Serotonin-enhancing agents effectively prevent the weight gain that accompanies smoking cessation; 2) this effect is accounted for at least in part by differential caloric intake; 3) the increase in intake for placebo subjects is largely due to increases in carbohydrate consumption, an effect suppressed by drug treatment; 4) treatment with a serotonin-enhancing agent also prevents transient increases in dysphoria that characterize the initial week of smoking cessation. For smokers who have shown large weight increases in previous quit attempts or who are daunted by the prospect of weight gain, adjunctive treatment with serotonin-enhancing agents may diminish some of the unpleasantness of the smoking cessation process. This treatment may also help to control negative mood changes during the critical first days of abstinence. Our findings provide a model for the specific use of pharmacological techniques for dealing selectively with key components in the sequence of changes and adjustments that make up the process of giving up smoking.

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Nicotine Patch: Effect on Spontaneous Smoking

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Recent reports of the U.S. Surgeon General indicate that more than 90% of the 17 million persons who attempt to quit smoking each year eventually relapse. The majority of those who relapse do so within the first few weeks of abstinence while in the acute withdrawal phase. At present the only FDA approved medication for smoking cessation is nicotine polacrilex (gum), which has proven useful for many but is not effective for all smokers for whom nicotine replacement therapy is indicated. Our purpose was to assess the subjective and physiological effects, as well as the abuse potential, of a recently developed nicotine patch.

Ten male smokers (mean age, 33.1 yrs; mean Fagerstrom score, 8.1; 5 classified as 'heavy drug users' and 5 as 'light drug users'), who stated no motivation to quit smoking, were studied in a 27-day residential trial. After a 3-day ad-lib smoking period and a 3-day dose run-up safety period, subjects received either a 0, 30 or 60 mg nicotine preparation daily. Administration was via 2 patches which were applied each morning to a new area of the arm. Each dose was held constant for one week; the order of dosing conditions was varied according to a randomized, double-blind, *crossover* design. Subjective and physiological assessments were made as well as recordings of cigarette consumption and smoking topography.

"Liking" for the patches, "Satisfaction" with smoking, as well as other subjective measures, did not vary as a function of dose or drug history group. Cognitive performance was neither consistently impaired nor enhanced. Resting pulse and blood pressure were shown to be affected by the nicotine dose level, however these differences *were* not dose-related and were probably not clinically significant (e.g., mean afternoon pulses were: 74.5.78.3 and 77.5 bpm for 0. 30 and 60 mg, respectively). Nicotine patches significantly reduced the number of cigarettes consumed in a dose-related manner (i.e., mean number of cigarettes smoked daily were: 17.0, 15.3 and 13.4 at 0. 30 and 60 mg. respectively). Afternoon expired CO levels were not significantly reduced by the nicotine patches. Smoking topography measures were not affected by dose level, however light drug users took much longer to smoke cigarettes than heavy drug users (320 seconds vs. 190 seconds).

"Liking" ratings and other subjective effects indicate nicotine patches are of minimal abuse potential; these findings also emphasize the critical contribution of the drug dosage form as a determinant of abuse liability. The effects of transdermal nicotine on physiological function and cognitive performance were minimal.

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EEG Sleep Disturbances in the Elderly and the Use of Passive Body Heating as a Non-pharmacologic Method for Improving Sleep

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Sedative/hypnotic abuse among the elderly is a growing concern. Chronic insomnia occurs in 2530% of 65-79 year olds and survey reports of sleep disturbances in the elderly correspond to a disproportionate use of sleep medication. Polysomnographic changes in the elderly which correspond to subjective sleep complaints include an increase in nocturnal wake-time and substantial decreases in slow-wave sleep (SWS). Three elderly insomniacs, who were otherwise healthy medically and psychiatrically (SCID testing), participated in this 4-night study designed to determine if Passive Body Heating (PBH), a technique previously found to increase SWS in young adults, increases SWS and improves sleep continuity and quality in elderly insomniacs. Core body temperature was recorded continuously using a CorTemp monitoring system. Sleep was recorded on four consecutive nights using technique and scoring criteria standard for polysomnography. Power spectral analysis was done to quantify SWS. Subjective measures of sleep quality were taken before and after sleep. On nights 3 and 4, subjects sat in 40 °C water up to mid-thorax for 30 min. or until core body temperature increased 1.0 °C. Compared to baseline (night 2). measures of sleep continuity and total sleep time improved, and slow-wave sleep increased after PBH treatment. Five of 7 subjective sleep measures reflected improved sleep quality. These data replicate previous findings of increase in SWS after PBH and suggest that sleep in the elderly might be improved by this technique and that sedative/hypnotic use may be reduced. Supported by Grants DA00115 and DA03994 from the National Institute on Drug Abuse.

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Comparing Family Loss in Opiate Abusers and Other Drug Abuseres: Treatment Implications

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Clients entering substance abuse treatment frequently arrive with a varied psychosocial history. Our experience in two large university based clinics (methadone maintenance and outpatient alcohol and drug abuse) serving an inner city population has shown commonalities in the histories of our clients, especially among those who abuse opiates. One common recurrent theme is the high incidence of family loss. Many clinicians and researchers suggest that there may be a connection between substance abuse and the loss of significant others in the lives of addicts. In our clinics we have also been concerned with the counselors' reports of excessive loss in the lives of our clients. For this reason, we sought to systematically survey the following: What is the incidence of loss in the lives of substance abusers? Is there a difference between opiate abusers and other drug abusers? Is loss related to the client's treatment needs and concerns?

We collected preliminary data from 91 substance abusers (36 opiate abusers (mean age = 39) and 55 other drug abusers (mean age = 39)); 69% were black, 27% were white and 4% other; 64% were female and 36% were male. While there were no significant differences between opiate users and other drug abusers, the proportion of losses in the lives of these clients were startling. The average number of total lifetime losses in the clients were: Opiate abusers = 2.39; non-opiate abusers = 2.82. During their lifetime, 57% of the clinic sample had lost one or both parents; 23% had lost a sibling; 16% had lost a spouse or a significant other; 13% had lost a child; and, 91% of the sample had lost one or both grandparents. The incidence of loss in this clinic sample needs to be compared with the incidence of loss in non-clinic samples.

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Methadone Maintenance Outcome: Role of Detox Fear and Its Treatment

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The effect of detox fear treatment on methadone maintenance outcome was examined. A random sample of 271, drawn from Philadelphia and Sepulveda VAMC's and Birmingham in 1983-84, served as subjects. Outcome variables were derived from 235 chart reviews and 102 test/interviews. Control procedures removed and assessed potential confounding effects of experimenter demand in the manifestation of detox fear. Analysis used MANOVA followed by ANOVA with outcomes examined as a function of previous fear status. Interviews discovered 7 nonfear and 6 fear patients obtained nonspecific psychotherapy to help them detoxify, which were compared to their appropriate controls. For months on methadone, the fear-no treatment group averaged 120 mo. and was significantly different from other groups, $p<.005$. Fear-treatment was 72, no fear-treatment 59, and no fear-no treatment was 65 mo. For successful detox attempts both the no fear-no treatment group at .68 attempts and the no fear-treatment group at .57 attempts were significantly different, $p<.02$ from the fear groups: fear-no treatment (.18 attempts), fear-treatment (.33 attempts). Only 5 subjects showed any knowledge of experimenter intent and none were detox fear subjects. Findings suggest experimenter demand does not account for the manifestation of detox fear and early identification followed by treatment may improve outcome. However, effects of treatment may have been confounded by selection factors since subjects were not randomly assigned to treatment.

FOOTNOTE

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Contingency Management Aftercare for Polydrug Abuse of Methadone Maintenance Patients

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Previous studies have demonstrated that contingencies can help initiate abstinence from supplemental drug use of methadone maintenance patients. This study examined the usefulness of contingencies in supporting continued abstinence among maintenance patients who stopped supplemental use of cocaine or benzodiazepines.

METHOD

Twenty-two methadone maintenance patients who demonstrated supplemental use of benzodiazepines and/or cocaine during baseline participated in this study. Subjects provided three urine specimens each week. Specimens were analyzed by EMIT® and thin-layer chromatography. All subjects' treatment contracts required them to stop use of cocaine or benzodiazepines, at least temporarily. Study eligible subjects submitted three consecutive drug-free urines (N=15) or entered a 7-day residential detoxification program (N=7), then were randomly assigned to one of two groups. In the take-home only group, subjects earned take-home medications for evidence of sustained drug abstinence. One or more drug-positive samples in a given week would result in loss of a take-home. Take-homes could be recovered by again showing sustained abstinence. In the combined incentive group, subjects earned take-homes under the same contingency, and could also receive methadone dose increases for each drug-free urine submitted. Dose increases were assessed weekly. Subjects increased in dose if two or more of the three urines were drug-free. Earned doses could be withdrawn if fewer than two urines were drug-free. The maximum allowed increase was 30 mg above the maintenance dose.

RESULTS AND DISCUSSION

Rate of drug-free urines for all subjects was better during the intervention (51.5%) than during baseline weeks (9.8%). and 23% of subjects were still abstinent at the end of 5 weeks. There was no difference between groups. Patients achieving abstinence through outpatient treatment contracts were more successful in reducing drug use during abstinence continuation interventions than those who chose an inpatient detoxification. Also, subjects who had more than 10% drug-free urines during baseline were better able to sustain reduced drug use. This study suggests new strategies focused on sustaining abstinence among polydrug abusing methadone patients.

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Cocaine Abuse: A Comparative Evaluation of Therapeutic Modalities

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Data were collected from 300 cocaine dependent male subjects seeking treatment at the Brentwood VA hospital. Subjects had been either admitted voluntarily to an inpatient program, or randomly assigned to one of three treatment modalities: inpatient, outpatient, or self-help groups. The subjects have been followed at one and two years after the VA treatment. Three major instruments to evaluate treatment effectiveness were used: the "Cocaine Natural History Interview," the "Treatment Evaluation" and the "Treatment Summary" forms. Time-series techniques have been a plied to compare pre- and post-treatment behaviors, including the course of cocaine and other drug abuse and associated behaviors, and to assess treatment effectiveness.

Overall, the three treatment modalities studied, separately or in combination, seem to be effective as they result in a decrease in the level of cocaine and other drug use, a decrease in deviant behaviors, and in an increase in socially desirable behaviors. After treatment,. severe cocaine use dropped considerably and use of other substances declined generally with dramatic decreases in amphetamine and narcotic use; alcohol consumption was initially halved than climbed slightly in a pattern that paralleled occasional use of marijuana. Dealing activity declined considerably as well. The prosocial measures (e.g., employment, relationships) were stable throughout the period from one year before to one year after treatment.

During follow-up, subjects experienced additional episodes of treatment. Six categories defining combinations of treatment modalities were identified. In terms of post-treatment condition, the most dramatic difference among combinations occurred between an Inpatient only group and an Inpatient-with-Outpatient-and Self-Help group. In viewing the range of improvement attributable to each category, more successful outcomes resulted from those combinations that include continued therapy in the form of self-help groups.

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Predicting Completion of Outpatient Cocaine Treatment

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Treatment for cocaine dependence is plagued by high attrition rates. This study sought to develop a model of treatment completion with data from 100 cocaine abusers seeking outpatient treatment. They were 84% white, 64% male, mean age, 30. Primary method of cocaine use was inhaling, 47.7%, smoking, 37.6%, and intravenous, 14.7%. Treatment completion was defined as attendance at 8 or more of 17 sessions; 43.6% of subjects met this criterion. Variables from the following domains were first tested at the bivariate level using t-tests and then entered into a logistic regression equation as predictors of completion: social isolation, affiliation, and stability; motivation; drug use; drug related problems, and criminal behavior, and demographics. Variables in the final model were: living with others; fewer days using multiple substances in the 30 days prior to treatment; legal pressure to enter treatment, and length of time since first initiation of cocaine use. These findings are somewhat consistent with prior research. The relationship of treatment exposure to outcome was also examined. Treatment exposure explained unique variance in cocaine use at 6 month follow-up. It is suggested that future research focus on more proximate (during-treatment or program-related) factors in predicting retention and that strategies designed to reduce attrition must combat the reinforcing properties of cocaine use, reduce social isolation, and provide incentives for lifestyle change and treatment completion.

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Effect of Methadone Dose Contingency Treatment on Cocaine Abuse in a Methadone Program

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Cocaine abuse is a serious problem among methadone-maintained patients. There is evidence that for some patients, cocaine abuse is exacerbated during methadone maintenance. However, the effect of methadone dose itself on cocaine abuse is not ccmpletely understood. We have developed a contingency treatment program in which a methadone-maintained patient's dose is dependent on the patient's cocaine abuse (as determined by urine tox screen). We have studied 2 protocols in 2 sites in the Yale Department of Psychiatry. In one protocol, methadone dose was lowered in response to each cocaine positive urine. Of patients treated under this protocol (n=22), 22% were successfully treated (stopped cocaine use). In another protocol, methadone dose was raised in response to each cocaine positive urine to a maximum dose of 12Omg daily. Six patients have entered this protocol to date and all 6 responded to this treatmant by stopping cocaine abuse. Thus, the increasing methadone dose contingency treatment was more effective (100% vs 33%, p = 0.005, Fisher's exact test). These results suggest that a methadone dose contingency treatment program which increases methadone dose in response to cocaine abuse may be successful treatment for cocaine abuse in methadone programs. This study is limited by its small size and by its design (2 reporting sites, open treatment protocol). Furthermore, both pharmacological and behavioral mechanisms are involved in this treatment and cannot be separated in the current study. A dose contingency protocol with randomized double-blinded assignment to either increasing or decreasing dose is clearly indicated as a next step. A future study should also include measures of cocaine craving and high and opiate withdrawal symptoms.

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Effects of Cocaine and Alcohol, Alone and in Combination, on Human Learning and Performance

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The acute effects of cocaine hydrochloride (4-96 mg/70kg) and alcohol (0-1.0 g/kg), ingested alone and in combination, were assessed in two experiments with human volunteers responding under a multiple schedule of repeated acquisition and performance of response chains. Subjects were intermittent users of cocaine and regular drinkers who were not drug/alcohol dependent. Alcohol was mixed with orange juice and ingested in 6 drinks across 30 min; cocaine was administered intranasally within 1 min at 45 min after completion of drinking. In each component of the multiple schedule, subjects completed response sequences using three keys of a numeric keypad. In the acquisition component, a new sequence was learned each session. Nine sessions were conducted daily: one before alcohol administration and every 15 min for the 1st hr, and every 30 min during the 2nd and 3rd hrs after cocaine administration. In the performance component, the response sequence always remained the same. Alcohol administered alone increased overall percentage of errors and decreased rates of responding in the acquisition component, while responding in the performance component generally was unaffected Cocaine administered alone decreased rates but did not affect accuracy of responding in the acquisition component, and enhanced accuracy of responding in the performance component. The combined doses of cocaine and alcohol. by contrast, did not differ significantly from placebo levels on accuracy or rates of responding in either schedule component. Moreover, the dose combinations were associated with significantly lower error levels in both schedule components and increased rates of responding in the acquisition component compared to the effects of alcohol alone. These results suggest that, under the conditions investigated in this study, (1) alcohol produces greater behavioral disruption than cocaine or cocaine-alcohol combinations, 92) cocaine and alcohol can each attenuate effects of the other, (3) and such attenuation is most pronounced in the case of cocaine attenuating the disruptive effects of alcohol.

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Contingent Reinforcement of Abstinence with Individuals Abusing Cocaine and Marijuana

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Two males seeking treatment for cocaine dependence were diagnosed with cocaine dependence and marijuana dependence/abuse. They received a behavioral intervention comprised of contingency-management procedures and the Community Reinforcement Approach (CRA). During the initial phase of treatment, reinforcement was delivered contingent on submitting cocaine-free urine specimens. Reinforcement consisted of a payment voucher indicating the number of points earned, which increased with consecutive cocaine-free specimens. Points could be exchanged for prosocial goods or services. CRA involved two behavior therapy sessions each week that focused on developing a reinforcing lifestyle to compete with the reinforcing effects of drug use. Almost complete cocaine abstinence was achieved with both subjects, but regular marijuana use continued during this phase. During a second phase of treatment, reinforcement magnitude was reduced, but was still contingent on submitting cocaine-free specimens. Behavior therapy was reduced to a once per week session. Cocaine abstinence and regular marijuana use continued during this phase. Next, subjects were given notice that reinforcement similar to that available during the initial phase would be available, but reinforcement delivery would now be contingent on submitting cocaine- &marijuana-free specimens. This modified contingency resulted in an abrupt increase in marijuana abstinence and the maintenance of cocaine abstinence with both subjects. Initiation of marijuana abstinence corresponded with initiation of the modified contingency for each subject. One- and five-month follow-up assessments indicated that both subjects continued cocaine abstinence, but resumed smoking marijuana. These results indicate that a behavioral intervention involving drug-free contingent reinforcement and CRA is efficacious in achieving and maintaining abstinence from cocaine in cocaine dependent individuals. This intervention also demonstrated efficacy in achieving abstinence from other drug use, in this case marijuana, but that change was not maintained after treatment termination.

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Natural History of Cocaine Abuse: From First Cocaine Use To Two Years After Treatment

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Three hundred male cocaine addicts admitted to a V.A. substance abuse program in West L.A. were studied over a two year period. Data are available on the history of cocaine use; patterns of cocaine use/abstinence; and variables related to the initiation, maintenance and cessation of use, and on the history of drug treatments. Results presented include data from the year prior to the first use of cocaine to V.A. treatment admission (a period averaging 11 years) and one and two year follow-up data. The analyses reveal that as subjects progressed in their cocaine career, the use of more effective methods of delivering the drug (such as smoking crack) increased. Excessive alcohol drinking and other drug use declined as the addiction to cocaine progressed. Surprisingly, in spite of this progression in cocaine use, antisocial behavior (dealing and property crimes) did not increase and the subjects were able to maintain employment and relationships during most of their pre-treatment cocaine using career.

At one year follow-up, the overall performance of the sample can be expressed as 26% remained abstinent from cocaine use for the entire follow-up period, 4% used at a severe level throughout, and 70% relapsed to at least one episode of use.

The second year follow-up is in progress and data are available for a third of the sample (96 subjects). Two years after the VA treatment, during the month prior to interview 77% were abstinent from cocaine use, 8% were using cocaine at a mild level, 1% moderately, and 14% were using cocaine at a severe level.

Pre- and post-V.A. treatment comparisons and post-V.A. treatment history are presented in poster 61.

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Characteristics of Applicants to a Cocaine Research Program

E. Cornell, L. Butler-Weyher, R.W. Foltin and M.W. Fischman

One hundred non-treatment seeking individuals applied to participate in cocaine research during a two-year period beginning October, 1988. Demographic data and drug use histories were collected using telephone screening, psychiatric interviews, and standardized questionnaires. Physiological data were obtained through blood and urine analysis, physical examinations, electrocardiograms, chest radiographs and exercise tolerance tests. Of the 100 applicants, 41 were accepted for participation. Reasons for rejection included illiteracy (7%), depression (12%), aggression (7%), hypotension (17%). abnormal laboratory findings (10%), other medical problem (9%), or subject attrition (40%). The average applicant was an unemployed, single black male, 33 years of age with 11.7 years of education. Applicants denied physical drug dependence, reported use of cocaine by i.v. administration, smoking, inhalation, and in combination with heroin (62%), marijuana (52%). and alcohol (48%). Most applicants (66%) preferred i.v. cocaine, with 28% preferring smoked cocaine. Applicants reported spending $29O/wk on cocaine and $92/wk on heroin, and 81% smoked an average of 18 tobacco cigarettes per day. Seventy-two percent of applicants reported having been arrested, with those who went to jail reporting an average of 32.6 months incarcerated. Seventy-eight percent of applicants were unemployed, 28% had no address, and 37% had previous research experience. Comparing such data with earlier reports provides important baseline data for generalizing to drug-using populations.

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Legal Needle Buying in Lieu of Needle Exchange?

W.M.Compton, III and L.B. Cottler

Needle exchange programs would not seem to be necessary in municipalities where sterile syringes can be purchased over-the-counter. Easy access to sterile syringes may provide a natural primary prevention against the spread of HIV infection. In Missouri, like 38 other states? no ordinance prohibits over-the-counter purchase of sterile syringes. This legal purchase may be one of the reasons why rates of HIV infection are low (3-4%) among IVDU's in St. Louis. The present study was designed to determine if syringes could be purchased over-the-counter in St. Louis metropolitan area pharmacies and to determine if and why barriers to syringe purchase occur. Two research assistants (both male, one African American, the other white) selected 10% of the area's pharmacies (sample N=33) to approach in an attempt to purchase syringes. Purchase was attempted by the individual research assistants on successive days at the same time of day, and the second research assistant was blinded as to the results of the first research assistant's purchase attempt. In 43% (n=15) of the pharmacies, either the purchase of syringes was refused or the required minimum number of syringes to be purchased was so large (100 or greater) that the purchase was discouraged. In 12% the white research assistant was refused and in 24% the African American was refused. Racial bias in rates of refusal and the implications for prohibiting or restricting legal availability of syringes are discussed. Legal needle buying as a substitute for needle exchange is considered.

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Post-Traumatic Stress Disorder Among Subtance Users From the General Population

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The prevalence of Post-traumatic Stress Disorder (PTSD) among substance users in the general population has not been previously evaluated. The St. Louis ECA study, a survey of psychiatric illness in the general population, collected data on PTSD and substance use using the Diagnostic Interview Schedule. Among the 2663 respondents, 430 reported a traumatic event which could qualify for PTSD; however, the rate of PTSD was low, 1.35% overall. To evaluate the relationship of PTSD with substance use, respondents were hierarchically classified into one of 4 substance use categories--ranging from polydrug use to alcohol only. Substance users were compared with persons who did not meet our substance use threshold (controls). Findings indicate that cocaine/opiate users are over 3 times as likely as controls to report a traumatic event, report more symptoms and events, and more likely to meet diagnostic criteria for PTSD than controls. Physical attack, but not combat-related events, was the most prevalent event reported among cocaine/opiate users. Onset of substance use preceded onset of post-traumatic symptoms, suggesting that substance use predisposes to event exposure. Net of other variables, including antisocial behavior, female gender and use of cocaine/opiates predicted PTSD. These analyses warrant the further study of the co-occurrence of these disorders.

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The Relationship Between Substance Use and Area of Practice Among Registered Nurses

A.M. Trinkoff

Recently the author analyzed data from the NIMH Epidemiologic Catchment Area Program and found that nurses had a cumulative occurrence of substance use which was less than or equal to rates for gender and census tract matched non-nurses. Although the nursing profession overall may not have a higher prevalence of substance abuse problems, certain areas of nursing practice have been hypothesized to be high risk settings for either attracting substance abusing nurses, or for creating circumstances which could lead to abuse. These areas include critical care, emergency, and operating room nursing, due to the stress of these work settings and the ready availability of controlled substances.

Data were gathered in 1990 via an anonymous mailed survey of a random sample of registered nurses licensed in Maryland (n=105). To explore the effects of nursing specialty on substance use, the sample was post-stratified into critical care specialty nurses (intensive care, operating room, and emergency) (n=22), vs. non critical care (n=81). Both groups reported the exact same lifetime prevalence of alcohol use (53%). For all other substances, the lifetime prevalence was 82% among critical care nurses vs. only 64% among non-critical care. Access to controlled substances in the workplace was reported to be "easy" for 37% of critical care vs. only 14% of the other nurses. The relationship between access and reported drug use among specialties is an intriguing finding, which deserves further exploration.

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Factors That Influence Physicans' Prescribing of Benzodiazepines

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Disparity has been noted among physicians' knowledge and attitudes, and their subsequent prescribing of benzodiazepines (Gabe, 1990; Chambers et al., 1983). Given that formal courses on prescribing practices are generally not provided in medical school or residency training, and that the literature is not explicit, these disparities should not be surprising. Indeed, little is known about factors that may influence physicians' decisions to prescribe benzodiazepines. In order to design a continuing medical education course on prescribing drugs with abuse potential for primary care physicians, we decided that it was important to identify some of these possible influences. We were most interested in how physicians diagnosed current abuse, and predicted potential abuse of, or benefit from benzodiazepines. A series of ten focus groups was conducted with physicians (general internists and psychiatrists) across the U.S. to elicit factors they considered when prescribing benzodiazepines. A list of patient characteristics identified in the focus groups was combined with additional ones derived from our own clinical experience to design a survey questionnaire. The questionnaire was mailed to 2614 primary care physicians (PC) in Virginia, and 1800 physician experts (ASAM certified) in addiction medicine (ADM). A response rate of 29% yielded 1289 completed surveys for analysis (34% returned for the ADM and 26% for the PC). The first question in the survey asked how 21 different patient characteristics were related (positively, negatively, or not at all) to the likelihood of current abuse. The second question asked how 24 characteristics were similarly related to the likelihood that a patient would become an abuser of benzodiazepines if prescribed. The third question asked how 23 characteristics were similarly related to potential benefit from a benzodiazepine if prescribed. For each question there was considerable disagreement within groups regarding most of the characteristics. In addition, for each question there were statistically significant differences (using a conservative significance criteria of $p<.002$ according to the Bonferroni correction) between groups regarding the importance of several characteristics.

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A Survey of Current and Proposed Drug Testing Policies for Faculty, Employees and Students at U.S. Colleges and Universities

P.J. Fudala, L. Fields, N.A. Kreiter and W.R. Lange

Administrators from 400 colleges and universities were surveyed for information regarding their schools' current and/or proposed policies for the urine drug testing of faculty, non-faculty employees, and students. Preliminary results from that survey are presented here. One thousand eighty-eight questionnaires were sent to various individuals (primarily personnel directors, deans of students, and athletic directors) at the institutions surveyed. An overall response rate of 81% was obtained within three months of the survey's initial mailing. Three hundred seven and 332 schools responded with regard to their testing policies for employees (faculty and non-faculty) and non-athlete students, respectively. Twenty-three schools reported testing one or more of these groups, including applicants for employment. None of the schools reported that testing of applicants for either faculty or non-faculty positions was randomly done and none tested all applicants for all positions. No school reported testing students applying for admission. All schools testing faculty reported doing so for probable cause. Employees other than faculty were most likely to be tested if they occupy certain positions (e.g., police or security personnel). Schools that test students were equally likely to use incident, probable cause, random, or routine scheduled testing. All employees and applicants were commonly tested for amphetamines, marijuana, cocaine, opiates, and PCP; students were less likely to be tested for PCP and more likely to be tested for barbiturates. An immunoassay was used most often as an initial screen followed by a chromatographic method as a confirmatory test. Up to 50% of the schools reported that applicants and employees, respectively, would either not be hired or be dismissed as the result of a positive test. Two schools reported that a positive test could result in a student being expelled or suspended. Over 80% of the institutions that do not currently conduct testing reported that they have decided not to implement a policy for drug testing at the present time, and 90% believed that employees and students would react with either some or strong opposition to urine drug testing.

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Prevalence of Hepatitis C in Substance Abusers

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Intense interest has developed in hepatitis C as a cause of morbidity and mortality related to chronic hepatitis and cirrhosis. One hundred fifteen consecutive patients admitted to the Chemical Dependency Unit of Francis Scott Key Medical Center were tested. Full serology (Hepatitis C antibody; Hepatitis B surface antigen {HBsAg}; Hepatitis B surface antibody {HBsAb}) was obtained for 101 patients. The average age of the group was 34.5 years, of which 62% were male, 65% were black, 35% white, and 69% had a history of intravenous drug use. Serologic testing revealed 67% positive for Hepatitis C, compared to only 35% positive for HBsAb and 1% positive for HBsAg. Of those patients with a history of intravenous drug use, 86% were positive for Hepatitis C, with 1% indeterminate, compared to 43% positive for HBsAb. Ninety percent of these patients positive for HBsAb were also positive for Hepatitis C. Of patients with no history of intravenous drug use, 26% were positive for Hepatitis C. Of patients tested for HIV (n = 43) 30% were positive, of which 90% were also positive for Hepatitis C. In summary, the prevalence of Hepatitis C among substance abusers is extremely high, especially among those with a history of intravenous drug use.

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The Increasing Need for Primary Health Care as an Integral Treatment Component

K. Foster, A. Chu, D. Adjuluchukwu and L.S. Brown

The poor health of the opiate addicted, particularly in historically underserved areas, serves to increase the burdensome task of treatment. The AIDS epidemic has exacerbated this vicious cycle. A chart review of 1168 patients newly admitted to methadone treatment in 1990, was conducted. The patients had a mean age of 36 years, were 95.2% African American and Latino(a), 64% male. A subgroup of 293 (25%) had HIV test results. This group did not differ significantly in race, sex, age, etc. Their seropositivity was 45%. African Americans reported significantly higher rates of hypertension ($p<.0001$) and pneumonia ($p<.001$,) than Hispanics and whites. Whites had significantly higher rates of viral hepatitis ($p<.0001$) and endocarditis ($p=.0455$,) while Latino(a) patients reported higher rates of asthma ($p=.0001$) and chest pain ($p=.0421$). These and other medical conditions are not sufficiently addressed by the standard treatment plan. The use of injectable drugs is clearly a factor common to the HIV and drug abuse crises. These outbreaks often have overlaps and are primary causes for the increased need to provide adequate medical care within the treatment setting. There is a plenitude of anecdotal support for implementing or enhancing primary care as an avenue to increased compliance and program retention. Given the depressed socioeconomic status of these patients, many of who have serological evidence of HIV infection and history of chronic disease, the potential benefits of increased primary care are numerous.

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Toxicology Screens for Cocaethylene in Emergency Department and Trauma Admissions Associated with cocaine Intoxication

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The Drug Abuse Warning Network (DAWN) has identified alcohol in combination with cocaine as the most common substance use pattern found among individuals with substance abuse problems presenting to emergency rooms in twenty four metropolitan areas (NIDA Statistical Series, 1987). A recent national survey indicates that approximately 12 million Americans are using alcohol in combination with cocaine (Grant and Harford. 1990). Morbidity and mortality are exacerbated by concurrent use of these substances (Kreek and Stimmel. 1984). We have demonstrated that in the presence of ethyl alcohol, cocaine is metabolized to its ethyl homolog, cocaethylene (Hearn et al., 1991). The transesterification of cocaine and ethanol to cocaethylene takes place in the liver and represents a novel metabolic reaction. In our previous report, cocaethylene was detected in postmortem blood and liver, and in neuropathological tissues in concentrations equal to and sometimes exceeding cocaine (Hearn et al., 1991). The purpose of the present study was to screen for cocaethylene in blood and urine specimens from patients presenting to the Jackson Memorial Hospital Medical Center (JMH). Patients with suspected cocaine intoxtcation were identified at the JMH Emergency Department, Trauma Center and Crisis Unit. Blood specimens were preserved with sodium fluoride. and both blood and urine were immediately stored at -20° C for subsequent drug testing. Information on injuries and medical treatments was abstracted from the hospital records. Cocaine, cocaethylene and alcohol were quantified in blood and urine extracts by gas chromatography as described previously (Hearn et al., 1991). Across patient groups, there were no significant differences in sex, age or known HIV status. Cocaethylene was reliably detected and quantified in blood and urine specimens from patients with positive blood screens for cocaine and alcohol (N = 58). Toxicology screens performed on patients with suspected cocaine intoxication demonstrated that 62% of the cocaine-positive cases had cocaethylene detected when both blood and urine specimens were available for analysis. Comparisons across the three admission groups demonstrated that a higher proportion (67%) of the patients admitted to the Trauma Center tested positive for cocaethylene. Within this subgroup, we found a significant association between trauma resulting from violence and the presence of cocaetbylene ($X^2 = 4.88$, $p < 0.05$). We are currently examining cocaethylene levels in fatalities to monitor trends in the prevalence of cocaethylene in both cocaine-related sudden death and deaths due to violence.

References available upon request.

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Biological Evaluation of Compounds for Their Physical Dependence Potential and Abuse Liability, XIV. Animal Testing Committee of the Committee on Problems of drug Dependence, Inc. (1991)

A.E. Jacobson

The Animal Testing Committee of the Committee on Problems of Drug Dependence (CPDD) is a subcommittee of the Drug Evaluation Committee (Dr. T. Cicero, Chairman). It is concerned with two programs: 1) the evaluation of analgesics, at both the Medical College of Virginia, Virginia Commonwealth University (MCV - Drs. M. Aceto, E. Bowman, L. Harris, and E. May) and the University of Michigan Medical School (UM - Drs. C. France, F. Medxihradsky, C. Smith, G. Winger, and J. Woods), and 2) the evaluation of stimulants and depressants, at the Medical College of Virginia, Virginia Commonwealth University (MCV - Drs. G. Patrick and L. Harris), the University of Chicago (UC - Drs. M. Nader, W. Woolverton), and the University of Michigan Medical School (UM - Dr. G. Winger). These programs are functioning well. The results which were obtained by the testing groups were discussed at a meeting of the Drug Evaluation Committee in May, 1991, in Richmond, VA.

ANALGESIC PROGRAM

The methodology used to evaluate potential analgesics remained essentially the same over the past year, with the addition of a new antinociceptive assay in mice at MCV, the hot plate assay. This assay was formerly run in NIDDK, NIH. Thus, the rodent assays now include the hot plate, phenylquinone, and tail-flick, as well as tail-flick antagonism vs. morphine.

New compounds are evaluated concurrently at MCV and UM. MCV also carries out single-dose-suppression studies, precipitated-withdrawal, and primary physical- dependence studies in monkeys. Data from substitution for morphine, and primary physical-dependence studies by rat-infusion assays are also obtained from MCV.

The purity and identity of an examined compound is validated from submitted spectra (infrared or NMR), and thin layer chromatograms (TLC). The TLC are repeated by Dr. Everette L. May at MCV. When

major differences are found between the TLC of Dr. May and that of the submitter, and this occurs perhaps two or three times/year, the sample is returned to the submitter for purification.

At UM, displacement assays are run, initially with [^{3}H]etorphine as the radioligand, using a rat cerebrum membrane preparation. On request, μ, δ, and κ opioid assays are carried out using [^{3}H]DAMGO (for μ), [^{3}H]DPDPE for δ, and [^{3}H]U-69,593 for κ opioid displacement assays. Monkey brain cortex membranes are used for this assay. The electrically stimulated mouse vas deferens preparation is also used to distinguish between the opioid receptor sites at which the various drugs interact. When requested, self-administration (SA) and drug discrimination (DD) experiments are carried out at UM, as well as antinociception and respiratory function in monkeys.

STIMULANTS AND DEPRESSANTS

The mouse inverted screen test and a spontaneous locomotor activity assay are carried out at MCV, as are primary physical dependence studies in nondependent rats, and substitution studies in pentobarbital dependent rats. Drug discrimination and self-administration assays are run in the rhesus monkey at UC and at UM, respectively.

STATISTICS

The statistical data on the number of compounds for which data were released, and their source, were in reasonable accord with the compilations obtained in the recent past (Jacobson 1991). I received 30 reports from UM and reports on 42 compounds from MCV (from 5/1/90 to 4/30/91).

About 20% of the compounds for which data were released this year came from domestic and foreign pharmaceutical industry, 65% from U.S. and foreign universities, and the remainder from U.S. governmental sources (NIDA, NIDDK, U.S. Army) or from the deliberate introduction of older drugs for which contemporary data were desired. These percentages are comparable with the mean of the figures obtained over the past 8 years. Although these percentages have remained relatively invariant lately, it might be noted that between 1979 and 1981, 50-60% of our samples came from the pharmaceutical industry.

The 72 reports from UM and MCV which will be published in the NIDA monograph this year are a slightly higher number than those published last year, but somewhat less than the mean over the past 11 years. This may change dramatically next year if we obtain permission to release data on the compounds which have recently been submitted. We have seen a large increase in the number of compounds which have been sent for evaluation in our analgesic program.

COLLABORATION WITH NIDA

Drug Testing

A consortium of groups, including the CPDD's Animal Testing Committee, NIDA, and NIMH, are now involved in the testing of drugs which may have abuse potential, or which might be of value as medications for drug abuse. The Medications Development Division of NIDA has established two programs in which analgesics and stimulants are examined. In vitro assays are carried out at SRI International, Life Sciences Division, Menlo Park, CA, under the direction of Dr. Lawrence Toll. A poster presentation which describes this work will be presented during this Annual Scientific Meeting. Compounds of interest to NIDA are being evaluated in toxicological and other *in vivo* studies by groups with other NIDA contracts. Also, NIMH has a contract with NOVAScreen in Baltimore, MD, for general binding assays with a variety of radioligands. The NOVA/NIMH assays are intended to relate information about the interaction of a compound with just about any known receptor. At this time, the assays are conducted at only one or two concentrations (e.g., 10^{-5} M) of a drug. It is possible that Ki's or IC50's can be obtained for compounds that possess substantial activity at particular binding sites.

A few of our compounds which need further evaluation will be examined through our collaboration with the NIDA/SRI program. There is some, but not a great deal of overlap between programs run under CPDD auspices and the NIDA/SRI program. Conversely, NIDA compounds which appear to be of special interest in SRI's *in vitro* assays will be recommended to us by NIDA for in vivo evaluation under the auspices of the CPDD.

Computerization of Data

For the past year or two NIDA has been involved with the establishment of a database for medications development. NIDA has contracted out this effort to the Biometric Research Institute, Inc., in Arlington, VA, under the direction of Dr. Gene Barnett. A poster will be presented and the computerized database demonstrated at this Annual Scientific Meeting of the CPDD. Most, if not all, of the material which will be seen on the database consists of data gathered under the auspices of the Animal Testing Committee of the CPDD. Eventually, of course, the database will contain work from many other centers. As presently constituted, the database will be maintained on a microVAX 3300 computer at ERC BioServices Corp. (a subcontractor), in Gaithersburg, MD, and two software packages have been implemented for this purpose. These are the Oracle database program, and MACCS from Molecular Design Ltd. The gathered data are being placed in the database so that they will ultimately be accessible to scientists for their research studies from remote MS/DOS systems as well as Apple microcomputers. When the system becomes available as a research tool, answers could be obtained to questions which are difficult or, in some cases, impossible to answer now. For

example, a list could be obtained of all of the compounds with an amide moiety embedded in its structure which effectively displace a radioligand from δ-opioid receptors, or a list could be obtained of all of the compounds which bind to μ, but not to κ opioid receptors and display diuretic activity. The questions which can be answered using a correctly established database is limited only by the imagination of the researcher.

ESPECIALLY INTERESTING COMPOUNDS

Several compounds shown in tables 1-10 are of great interest, and these are discussed below. For detailed information about the compounds which were examined this year, see Aceto et al. (1992) and Woods et al. (1992).

1-(2-Phenylethyl)-4-(N(2-pyrazyl)-2-(furoylamido))piperidine hydrochloride - Mirfentanil - NIH 10647 & 10669

NIH 10647 (or 106691, in table 4, has been given the generic name "mirfentanil". We found it to be from 3-14 times more potent than morphine as an antinociceptive in rodents, depending on the assay, and its actions were antagonized by naloxone. However, in the monkey analgesia assay, its analgesic action could not be blocked by naltrexone or quadazocine in doses sufficient to antagonize the effects of ordinary μ agonists. The relatively high doses required for analgesia in the monkey did not, thus, appear to be mediated by opioid receptors.

Mirfentanil did not substitute for morphine in the single-dose-suppression assay in monkeys. It was somewhat less potent than morphine in displacing [^{3}H]etorphine from rat cerebrum membranes and displayed partial agonist action, which could be blocked by naltrexone, and relatively non-selective, competitive, opioid-antagonist action in the mouse vas deferens preparation. Its respiratory effects in the monkey were similar to those of buprenorphine and nalbuphine. That is, mirfentanil blocked the action of alfentanil in producing apnea.

As a discriminative stimulus, mirfentanil appeared to have μ opioid effects, substituting for a μ agonist but not a κ agonist. Its antagonist activity was shown by its ability to substitute for naltrexone in a drug discrimination assay. A similar pattern of discriminative stimulus effects has been observed with buprenorphine. In monkey self-administration, mirfentanil maintained rates of responding similar to that of codeine.

The mixture of results seen in the various assays, the different results in rodents and monkeys, and the antagonist activity observed, is very unusual for a fentanyl-like compound. The fentanyl series of drugs have not previously been noted to have narcotic antagonist activity.

The ability of mirfentanil to block apnea caused by alfentanil in the monkey is especially noteworthy.

LAAM, norLAAM, and dinorLAAM (NIH 10679, 10652, 10655)

The acetylmethadols, LAAM and dinorLAAM were examined this year (NIH 10679 and 10655, table 6), and norLAAM was evaluated last year (Jacobson, 1991). LAAM was found to have morphine-like antinociceptive potency in rodents, and the N-nor metabolites were as potent as, or more potent than LAAM as antinociceptives. All three compounds completely suppressed withdrawal from morphine in the monkey single-dose-suppression assay, and they were all selective μ agonists in the mouse vas deferens assay. LAAM was unusual in displaying a biphasic concentration-effect curve in the vas deferens. LAAM was a potent and selective μ agonist in binding to the opioid receptors in monkey brain cortex. It was about 100 fold less potent at κ and δ opioid receptors.

The actions of these metabolites of LAAM, norLAAM and dinorLAAM, must be considered when appraising the *in vivo* activity of LAAM.

Naloxone benzoylhydrazone, BOZO, NIH 10656

Naloxone benzoylhydrazone, NIH 10656 (table 2), was found to have potent narcotic antagonist actions in the rodent assay. This compound has been given the acronym BOZO. It was noted by Pasternak and his colleagues (Paul et al., 1990) to "retain its ability to elicit analgesia through a novel and distinct supraspinal κ_3 system". In our hands, NIH 10656 did not display agonist activity. It was equipotent with naloxone in the mouse vas deferens preparation and also acted as an antagonist in single-dose-suppression studies in the monkey. It was found to be essentially equipotent with naloxone in a precipitated-withdrawal study in monkeys. In drug discrimination BOZO substituted for naltrexone and its potency was similar to naltrexone in morphine-dependent rhesus monkeys. In monkey analgesia studies it antagonized the effects of both μ and κ agonists, and shifted the effects of alfentanil three-fold to the right in respiratory function studies.

BOZO was an effective antagonist against both μ and κ agonists and was more potent as a μ antagonist in the behavioral assay. We did not find it to be a particularly selective κ ligand.

(-)-[5*R*-(5α,7α,8ß)]-N-Methyl-N-[7-(l-pyrrolidinyl)-1-oxaspiro[4.5]dec-8 yl]-4-benzofuranacetamide hydrochloride-NIH 10672

NIH 10672, a selective κ ligand, was extensively studied (table 9). This benzofuranacetamide was found to be several hundred times more

potent than morphine in rodent antinociceptive assays. Its antinociceptive action in the tail-flick assay could only be antagonized by a high concentration of naloxone, suggesting κ rather than μ agonist activity. The antinociceptive activity of the drug in rodent assays could not be blocked by norbinaltorphimine. In monkey analgesia studies, the maximum effect was observed at a very low dose, 0.018 mg/kg, and this was antagonized with quadazocine.

NIH 10672 did not substitute for morphine or exacerbate withdrawal in the single-dose-suppression assay in monkeys or in the morphine-dependent rat studies. It was relatively free of physical dependence in rat primary physical dependence studies, but in the primary physical dependence study in monkeys dramatic agonist effects were seen to which tolerance developed, and a withdrawal syndrome was noted on abrupt withdrawal. This syndrome was completely suppressed by NIH 10672. The withdrawal syndrome was unlike that of μ opioids, and similar to that produced by κ opioids. However, naloxone did not precipitate withdrawal.

This drug was more potent in the vas deferens preparation than in binding experiments and appeared to be an opioid agonist selective for K opioid receptors. Unlike most K opioids, however, in the vas deferens the antagonism produced by norbinaltorphimine, a κ opioid antagonist, was surmountable. In drug discrimination, NIH 10672 substituted for ethyketocylazocine (EKC), a prototypic K opioid. It substituted for naltrexone in 2 out of 3 monkeys, but it did not antagonize the effects of morphine. It also failed to maintain self-injection responding in monkeys trained to administer alfentanil, a μ agonist. Respiratory function studies indicated that it would not produce apnea at a maximum dose of 0.032 mg/kg in monkeys.

This drug appears to have an unusual spectrum of action. It is, or will soon be in a clinical trial (Phase 2). From our studies, it is evident that the drug is unlikely to have abuse liability in man. However, there remains the question of whether the subjective effects of the drug, like those of many other κ opioids, will be too adverse for its acceptability as an analgesic in people.

(-)-3-Acetyl-6ß-(acetylthio)-N-(cyclopropylmethyl)normorphine-NIH 10685

Structurally, NIH 10685 (table 2) is a 3-acetyl analog of N-cyclopropylmethylnormorphine, with a thioacetyl group at the C-6 position of the 4,5-epoxymorphinan. Undoubtedly, the 3-acetyl group is readily hydrolyzed *in vivo* to the phenolic compound, but the thioacetyl moiety may be relatively stable. NIH 10685 was about as potent as, or somewhat more potent than morphine in the rodent antinociceptive assays. A high dose of naloxone was needed to antagonize its effect in the tail-flick assay. Its analgesic action in the monkey was

antagonized by quadazocine. NIH 10685 did not show appreciable narcotic antagonist activity in the tail-flick assay vs. morphine. However, it acted like an agonist-antagonist in the single-dose-suppression study in the monkey. It did not substitute for morphine in this study, and it exacerbated withdrawal. It appeared to have a dopaminergic component in its action. NIH 10685 had very high affinity for both κ and μ, and high affinity for the δ opioid receptor in membranes from monkey brain cortex. It showed antagonist activity in the vas deferens preparation against all three opioid receptor subtypes. NIH 10685 did not substitute for alfentanil in drug discrimination studies. It did substitute for, and was equipotent with, naltrexone, and it antagonized the reversal of withdrawal induced by alfentanil, a μ agonist, in these studies. NIH 10685 also substituted for EKC, a κ agonist, in these drug dependence studies. It antagonized the effect of alfentanil on respiratory function, and did not by itself produce apnea. The relatively small effect of NIH 10685 on respiratory function was not clearly antagonized by quadazocine. NIH 10685 apparently exerts its effects through its ability to act as a μ antagonist and a κ agonist.

It is of interest to note the effects of this thioacetyl group on the *in vitro* and *in vivo* action of N-cyclopropylmethylnormorphine (NIH 7952), the parent structure for NIH 10685. NIH 7952 was synthesized by Dr. Marshall Gates over 30 years ago. Drs. G. A. Deneau and M. H. Seevers reported their work with the compound at UM in 1962. From single dose substitution studies in monkeys they concluded that N-cyclopropylmethylnormorphine was slightly less potent than nalorphine as an antagonist, but with a longer duration of action (24 hrs.). Apparently, the introduction of a thioacetyl group at C-6 and an acetyl moiety at C-3 into N-cyclopropylmethylnormorphine were causative factors for the increase in narcotic antagonist potency. In 1962, of course, methodology was not available for examining the interaction of ligands with opioid receptors. It might be of interest to examine the effect of NIH 7952 on subtypes of opioid receptors, to see whether introduction of the C-3 acetyl and the C-6 thioacetyl group alters its pattern of interaction with opioid receptors.

Other compounds examined

All of the seven 4,5-epoxymorphinans which are shown in Tables 1 and 2, are narcotic antagonists. NIH 10663, with a glucuronide moiety on C-3 of the aromatic ring, could represent a metabolic product of the parent 4,5-epoxymorphinan. It might act as a prodrug, where enzymatic hydrolysis of the glucuronide may provide the phenol *in vivo,* thus theoretically leading to a drug in situ with an overall slow onset and long duration of action. The compound showed little activity *in vitro,* and somewhat greater potency *in vivo.*

All of the N-alkyl substituted 6,7-benzomorphans in table 3, and those which have previously been discussed (Jacobson 1991). will be the

subject of a paper by Dr. Everette May (MCV, Richmond, VA). The manuscript will include data from all of the analgesic testing groups and is presently in preparation for publication in *J. Med. Chem.*

Two of the phenylpiperidines in table 4, NIH 10651 and 10681, had some antagonist activity *in vitro.* None of the examined phenylpiperidines substituted for morphine in the single-dose-suppression assay. This is somewhat unusual for phenylpiperidines and fentanyl-like analogs.

As noted in table 5, haloperidol displays fairly potent antinociceptive activity, although it does not seem to interact with opioid receptors *in vitro.* The haloperidol analog, NIH 10671 is considerably more potent than haloperidol in the tail-flick assay. NIH 10671 also substitutes for morphine in the single-dose-suppression assay, and the catalepsy induced in one monkey during that assay was reversed with naloxone.

A considerable number of miscellaneous types of compounds can be seen in tables 7 - 10. Etonitazene (NIH 10665), in table 9, was found to be, as expected, a very potent agonist in antinociceptive assays. It was noted to act as a partial, very potent, agonist in the vas deferens preparation.

ABBREVIATIONS USED IN TABLES 1 - 10

Rounded numbers are used in the tables. For precise values, and details of the procedures, see the MCV and UM reports in this volume (Aceto *et al.,* 1992; Woods *et al.,* 1992).

1) MOUSE ED50/AD50: Antinociceptive Assays (SC injection) Confidence limits are listed in the MCV report (Aceto *et al.,* 1992).

HP = hot plate (morphine ED50 = 0.8 (0.3-1.8)

PPQ = phenylquinone (morphine ED50 =0.23 (0.20-0.25))

TF= tail-flick (morphine ED50 = 5.8 (5.7-5.9))

TFA= tail-flick antagonism vs. morphine (naltrexone AD50 = 0.007 (0.002-0.02); naloxone AD50 = 0.035 (0.01-0.093)).

I = inactive, without a reasonable dose-response relationship, or insufficiently active for statistical analysis.

2) IN VITRO (Data from UM, Woods *et al.,* 1992)

RBH= binding affinity in rat cerebrum membranes (displacement of 0.5 nM [^{3}H]etorphine) in the presence of 150mM NaCl (morphine EC50 = 23.6).

NE= no effect.

NOTE: Contemporary EC50 data cannot be directly compared with those from some previous reports (Jacobson 1984, and preceding years) in which -Na values were quoted.

VD= electrically stimulated mouse vas deferens EC50 values, rounded to one significant figure.

Agonist activity is stated using "E" followed by a negative number: $E = 10^{-x}$ M, where x = the negative number, thus: lE-3 = 1 x 10^{-3} or 0.001 M (1 mM), lE-6 = 1 μM, and 1E-9 = 1 nM. Maximum percent inhibition was previously noted (Jacobson 1991) in parentheses following agonist activity.

SE= slight effect on twitch
NE= No significant agonist or antagonist effect
ANT= Antagonist activity. Selective antagonist activity at μ, δ, and/or κ receptors is noted in parentheses. The antagonist effect may or may not be competitive.

Compounds which suppress the twitch and are not antagonized by naltrexone or other narcotic antagonists are said to be non-opioid agonists (e.g., clonidine can suppress the twitch, but is not antagonized by naltrexone. It is a non-opioid agonist). Compounds which bind with reasonable affinity in the RBH assay and do not suppress the twitch in the VD may have narcotic antagonist properties. The opioid receptor at which the drug exerts its antagonist effect is determined by testing various concentrations of the drug to induce a blockade (antagonism) of the suppression of the twitch in the VD preparation caused by sufentanil (μ), DSLET (δ), or U50,488 (κ) (for these data see Woods *et al.,* 1992).

3) IN VIVO: in the rhesus monkey (from MCV, Aceto *et al.,* 1992; prior to 1988 from MCV or UM).

SDS=single-dose-suppression
NS= no suppression
CS= complete suppression
PS= partial suppression
(Parenthesized numbers = dose range studied, in mg/kg)

Other Studies (noted in the footnotes to the tables)

A) In Rat - RI = rat continuous infusion (data from MCV)

1) SM = substitution for morphine
NS= no substitution for morphine
CS= complete substitution

PS= partial substitution

2) PPD = primary physical dependence

B) In Rhesus Monkey:

1) PPt-W = studies in non-withdrawn monkeys (data from MCV)
PW= precipitated-withdrawal at dose levels, in mg/kg, indicated in parentheses &/or comparison with naloxone [N].
SP= slight precipitation
NP= no precipitation

2) ND = studies using non-dependent monkeys (data from MCV)
M-like = morphine-like effect.

3) PPD = primary physical dependence (data from MCV)

4) SA or SI = self-administration or self-injection (data from UM)
NE= no effect
High = codeine-like
IN = intermediate between saline and codeine
SE= slight effect

5) DD = drug discrimination (data from UM)
NE= no effect
CS= complete suppression

6) MA = monkey analgesia (data from UM)

7) RESP = respiratory function (data from UM)

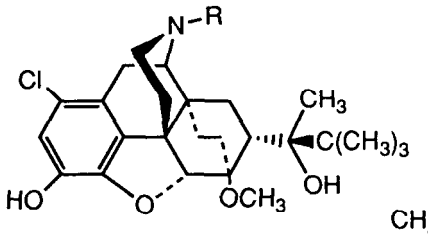
C) *In Vitro*

BIND - binding affinity using monkey brain cortex membranes (selectivity for μ, κ, and δ opioid receptors, using [^{3}H]-sufentanil, -DPDPE and -U69,593, respectively).

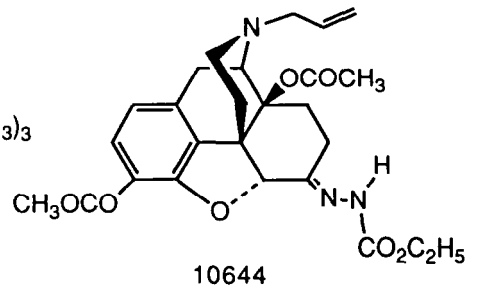
Previous Reports

Previous work on a compound is noted using the year listed in the monograph title (e.g., work cited as "1983" indicates that the work was included in "Problems of Drug Dependence 1983", which was published in 1984). Note that the monograph's publication date may be one year after the titled year of the monograph. Complete details of the original work on a compound can be found in the Annual Report of either Aceto *et al.,* or Woods *et al.*

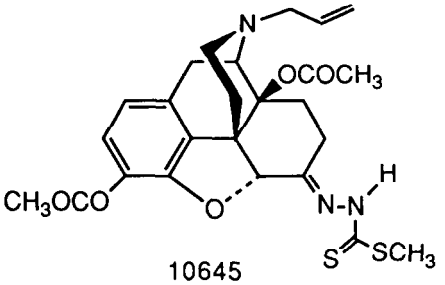
TABLE 1. 4,5-EPOXYMORPHINANS[a]



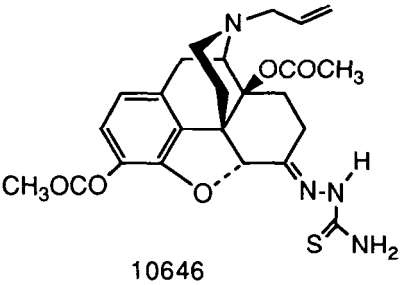
10593: R=Cyclopropylmethyl
10629: R=*n*-Propyl



10644



10645

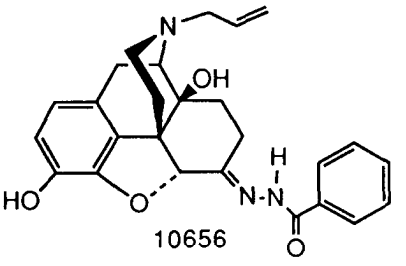


10646

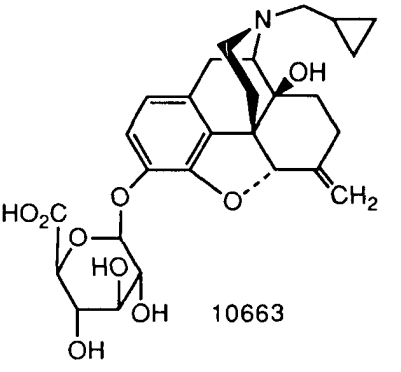
	MOUSE ED50/AD50			IN VITRO		MONKEY
NIH #	PPQ	TF	TFA	RBH	VD	SDS
10593	I	I	4.7[b]	INSOL	INSOL	NS(0.06,0.25)[c]
10629	I	I	I[d]	INSOL	ANT$_{\mu,\delta,\kappa}$[e]	NS(0.5-10)[f]
10644	I	I	03	-	-	-
10645	I	I	0.8	18.3 nM	5.3E-7g	-
10646	_h	_h	_h	17.4 nM	ANT$_{(\mu,\delta,\kappa)}$[e,i]	

a) See text for explanation of column headings and abbreviations.
b) Dependent on vehicle: AD50=4.7 in H_3PO_4/H_2O, and 10.4 in 10% Tween 80, lactic acid and H_2O.
c) PPt-W - severe withdrawal (0.125, 0.5); slow onset, prolonged duration of action.
d) No dose-response - 61% activity at 25 and 40 mg/kg, 74% at 30 mg/kg. However, 2-hr. pretreatment gave AD50=3.8 for naloxone.
e) Noncompetititve antagonist for K receptors.
f) Exacerbated withdrawal. Duration of action > 2.5 hr.
g) Agonist activity not mediated by opioid receptors. Opioid antagonist at μ,δ,κ receptors.
h) Thin layer chromatograpy indicated two spots. Sample not run.
i) Less potent than, but similar to norbinaltorphimine antagonism of κ receptors.

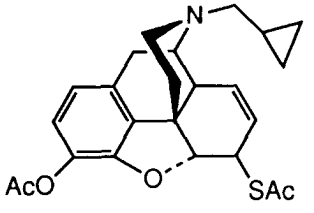
TABLE 2. 4,5-EPOXYMORPHINANS (CONTINUED)[a]



10656



10663

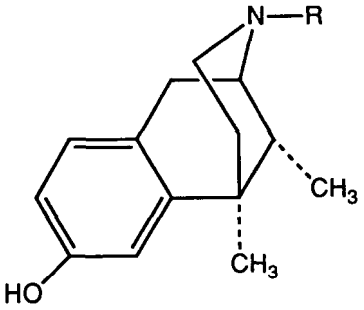


10685

	MOUSE ED50/AD50				IN VITRO		MONKEY
NIH #	HP	PPQ	TF	TFA	RBH	VD	SDS
10656	-	I	I	0.05	6.1 nM	ANT(μ,δ,κ)[b]	NS(0.01,0.04)c
10663	-	I	I	0.55	1.6 μM	ANT(μ)[d]	-
10685	4.6	0.01	2.6[e]	I[f]	-	-	NS(1.5,6)[g]

a) See text for explanation of column headings and abbreviations.
b) Similar to naltrexone in potency and selectivity.
c) PPt-W assay (1991) - PW (equipotent with naloxone).
d) Weak selective μ antagonist.
e) High dose of naloxone needed for antagonism.
f) No dose-effect (50% at 30 and 80 mg/kg).
g) Agonist-antagonist (appeared to exacerbate withdrawal). Some dopaminergic activity.

TABLE 3. 6,7-BENZOMORPHANS[a]

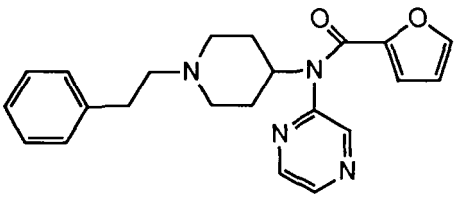


10650: R = BENZYL (+)
10666: R = H (±)
10667: R - METHYL (±)
10673: R = n-HEPTYL (±)
10674: R = n-HEPTYL (±)
10675: R = n-HEPTYL (±)
10686: R = p-METHOXYBENZYL (-)
10691: R = p-METHOXYBENZYL (+)
10694: R = p-HYDROXYBENZYL (+)

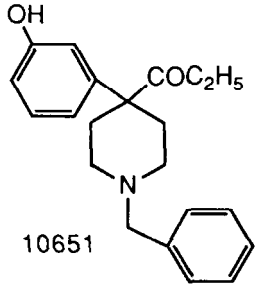
	MOUSE ED50/AD50			IN VITRO		MONKEY
NIH #	PPQ	TF	TFA	RBH	VD	SDS
10650	I	I	I	>10μM[b]	ANT[b,c]	
10666	4.6	I	I	0.664 μM	6.8E-6[d]	NS(1.5,6)
& 7410	0.6	1.5	I	119 nM	1.3E-6[e]	NS(3,6), CS(24)[f]
10673	0.4	3.6	I	128 nM	4.4E-7[g]	NS(2.5,10)
10674	3.5	12.9	I	4.7 μM	1.1E-8[h]	NS(2.5,10)
10675	0.13	1.7	I	89 nM	3.5E-7[i]	NS(1.25,5)
10686	-	-	-	7.0 μM	ANTj	-
10691	-	-	-	6.1 μM	ANT[k]	-
10694	-	-	-	>6μM	ANT[l]	-

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1990.
c) Weak opioid antagonist, competitive at μ, noncompetitive at **κ.**
d) Agonist at μ, **δ,** and **κ** opioid receptors.
e) μ agonist-μ, **δ,** and **κ** antagonist.
f) Previously reported - 1960,1958
g) Agonist at μ opioid receptors.
h) Low efficacy (31% inhibition).
i) Agonist at μ and **κ** opioid receptors.
j) Very weak antagonist, with some **κ** selectivity.
k) μ, **δ,** and **κ** antagonist; insurmountable at **κ,** not simple competitive at μ,**δ** opioid receptors.
l) Antagonist only at high concentrations; slight non-opioid actions.

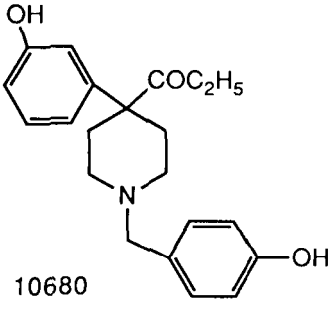
TABLE 4. PHENYLPIPERIDINES AND FENTANYL-LIKE COMPOUNDS[a]



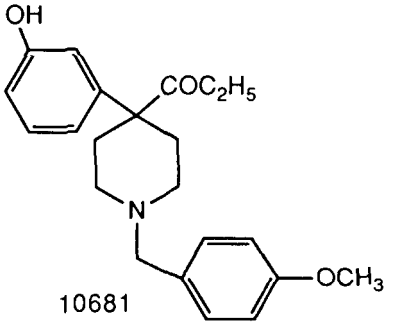
10647, 10669



10651



10680

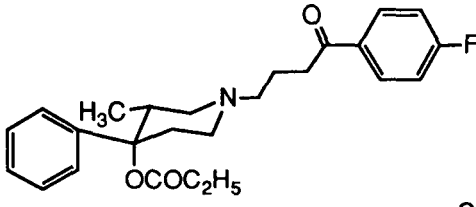


10681

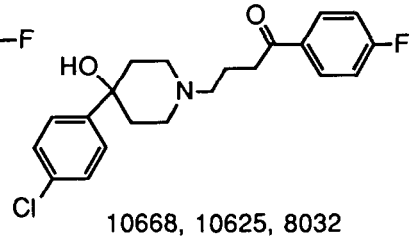
NIH #	MOUSE ED50/AD50 HP	PPQ	TF	TFA	IN VITRO RBH	VD	MONKEY SDS
10647 &10669	0.3	0.08[b]	0.4[b,c]	I[b]	91nM[b]	4.7E-8[d,e,f]	NS(0.05-4)[b]
10651	-	7.5	I	I	1.0μM[b]	ANT(μ,δ,k)[b]	
10680	-						NS(2,8)
10681	-	I	I	I	>6μM	ANT[g]	NS(3,12)[h]

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1990.
c) Reversed by naloxone (before (AD50=0.06) or after ED80 of 10647 (AD50=0.03)).
d) Partial agonist; weak, non-selective, competitive antagonist.
e) Other work (at UM - previously reported, 1990) - SA, DD, monkey analgesia, respiratory depression.
f) Previously reported as antagonist (μ,δ,κ) - 1990.
g) Moderately selective δ antagonist.
h) Non-dose-related exacerbation of withdrawal.

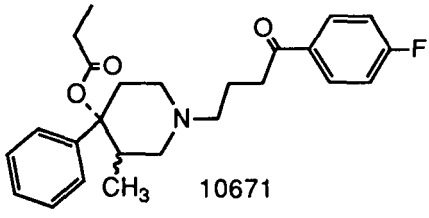
TABLE 5. PHENYLPIPERIDINES RELATED TO HALOPERIDOL[a]



10639



10668, 10625, 8032
HALOPERIDOL

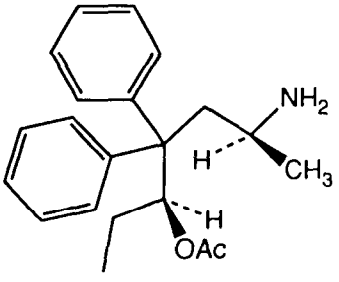


10671

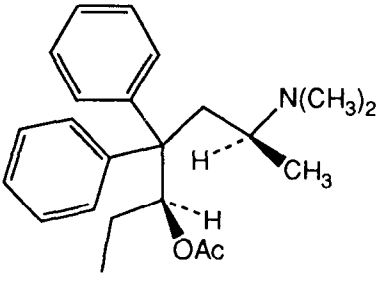
NIH #	MOUSE ED50/AD50 PPQ	TF	TFA	IN VITRO RBH	VD	MONKEY SDS
10639	0.1[b]	0.56[b]	I[b]	41.3 nM	5.6E-8[c]	CS(0.05,0.25)[b]
10668 & 10625 & 8032	0.01[d]	14.6[d]	I[d]	>10μM[d]	SE[d,e]	NS[d]
10671	0.04	0.3	I	-	-	CS(0.006,.025)[f]

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1990.
c) Agonist at μ opioid receptors.
d) Previously reported - 1990,1989 and 1988 as NIH 10625, and in 1963 as NIH 8032. Other work - RI-SM (NS), RI-PPD.
e) Inhibited twitch at 1E-5 concentration (1989), and partially inhibited at 6.1E-8 (1990). Opioid antagonist at μ and κ opioid receptors (1989), and devoid of antagonist activity (1990) in VD. No significant opioid activity in VD or RBH (1990).
f) At 0.5 mg/kg one monkey was cataleptic, unresponsive. Reversed with 0.05 mg/kg naloxone.

TABLE 6. METHADOLS[a]



10655 (dinorLAAM)

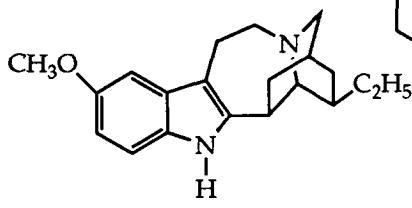


10679 (LAAM)

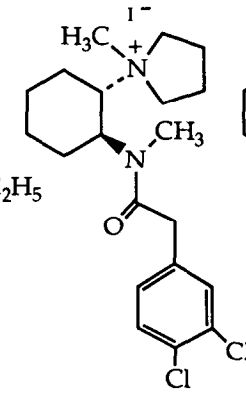
	MOUSE ED50/AD50			IN VITRO		MONKEY
NIH #	PPQ	TF	TFA	RBH	VD	SDS
10655	0.14	5.4	I	22.3[b]	3.4E-7[b,c]	CS(4)
10679	0.4	7.2	I			CS(0.5,2)[d]

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1990.
c) μ Agonist; unusual response to naloxone.
d) Onset slower, duration longer than morphine, equivalent potency.

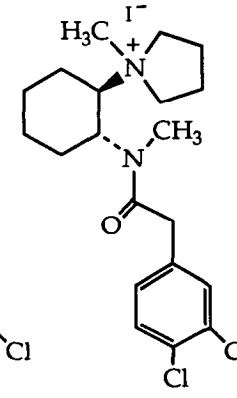
TABLE 7. MISCELLANEOUS[a]



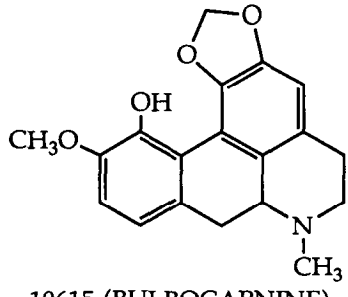
10567 (IBOGAINE)



10577



10578

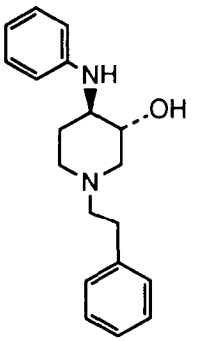


10615 (BULBOCAPNINE)

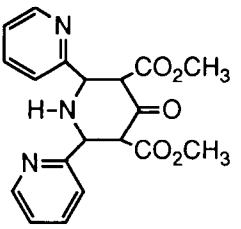
NIH#	MOUSE ED50/AD50 PPQ	TF	TFA	IN VITRO RBH	VD	MONKEY SDS
10567	9.7[b]	I[b]	I[b]	6μM	2.3E-5[b,c]	PS(2,8)[b,d]
10577	4.2	I	I	76 μM[e]	SE[e]	NS(2.5,10)
10578	3.5	I	I	76μM[e]	SE[e]	NS(3,12)
10615	4.5[f]	I	I	-		NS(0.5,2)

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1989.
c) Not antagonized by naloxone.
d) Rat infusion (1990): SM - NS(20), PS(80, possibly non-specific); PPD - None.
e) Very low potency, probably non-opioid.
f) Not antagonized by naloxone (possible D1 antagonist).

TABLE 8. MISCELLANEOUS (CONTINUED)[a]



10637



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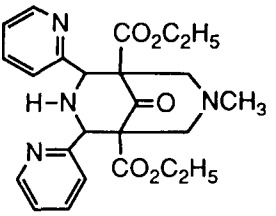

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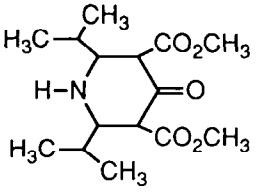
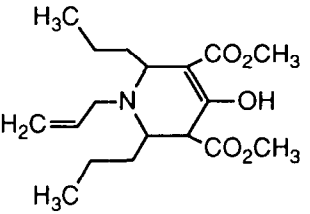


10659

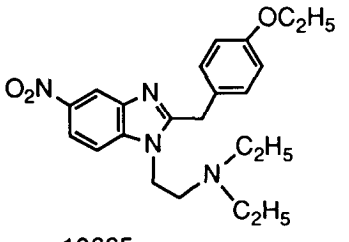


10660

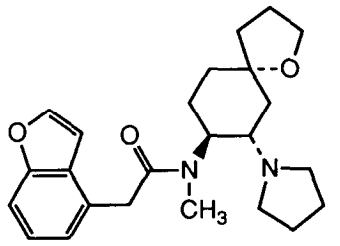
NIH #	MOUSE ED50/AD50 PPQ	TF	TFA	IN VITRO RBH	VD	MONKEY SDS
10637	I	I	I	>10 μM	5.2E-8[b]	NS(3,12)
10657	I	I	I	> 6 μ M	N E	NS(2.5,10)
10658	0.6	I	I	>6μM	4.3E-7[c]	CS(0.125-2)
10659	I	I	I	-	-	-
10660	I	I	I	-	-	-

a) See text for explanation of column headings and abbreviations.
b) Partial inhibition, not mediated by opioid receptors; very weak antagonist at μ and κ opioid receptors.
c) Agonist selective for κ opioid receptors.

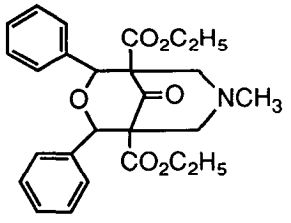
TABLE 9. MISCELLANEOUS (CONTINUED)[a]



10665
(ETONITAZENE)



10672



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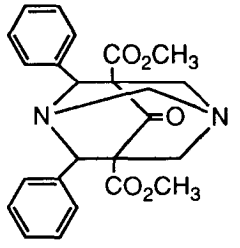

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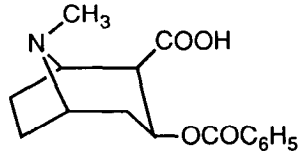
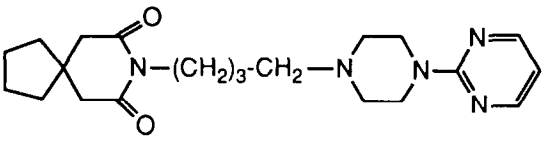


10682

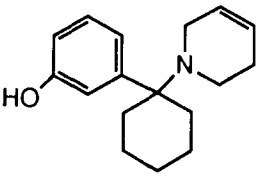
NIH #	MOUSE ED50/AD50 PPQ	TF	TFA	IN VITRO RBH	VD	MONKEY SDS
10665	0.0017[b]	0.005[b]	I	0.51 nM	5.7E10[c]	CS(0.0005,0.002)[d]
10672	0.0015[e]	0.015	I	1.18 µM	1.4E-9[f]	NS00.0002-0.0025)[g,h]
10676	I	I	I	>6 µM	4.5E-7j	-
10677	I	I	I	>6 µM	2.9E-6[k]	-
10682	I	I	I	-	-	NS(2,8,16)[i]

a) See text for explanation of column headings and abbreviations.
b) Blocked by naloxone.
c) Potent partial agonist at µ,δ, and κ opioid receptors.
d) Onset rapid, duration shorter and potency 1500 x morphine.
e) Naloxone prior to ED80 (AD50=0.3). Could not block with norbinaltorphimine in TF or PPQ.
f) Potent agonist, selective for κ. Antagonism produced by norbinaltorphimine is surmountable, unlike other κ agonists.
g) High doses of naloxone needed for antagonism. Potent κ agonist.
h) Rat SM - NS; Rat PPD - No dependence.
i) Intravenous administration.
j) Agonist at µ and κ opioid receptors.
k) Weak partial agonist at µ,δ, and κ, weak antagonist at κ.

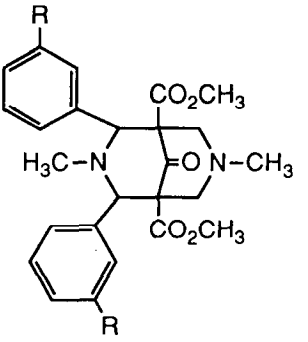
TABLE 10. MISCELLANEOUS (CONTINUED)[a]



10687 (BUSPIRONE)



10700



10713: R=OCH_3
10714: R=CH_3
10715: R=NO_2

	MOUSE ED50/AD50		IN VITRO			MONKEY
NIH#	PPQ	TF	TFA	RBH	VD	SDS
10687	14.6[b]	I	I	-	-	PS(0.2-0.8)[c]
10700	0.3	4.8[d,e]	I	-	-	-
10713	I	I[e]	I	-	-	-
10714	I	I[e]	I	-	-	-
10715	I	I[e]	I	-	-	-

a) See text for explanation of column headings and abbreviations.
b) Not blocked by naloxone.
c) Precipitated withdrawal - NP.
d) Straub tail, ataxia, convulsions.
e) Hot plate assay - I.

STIMULANT/DEPRESSANT DRUG TESTING

Three new compounds were accepted for evaluation this year (5/l/90 to 4/30/91). The report by Winger et al., (this volume) will include the detailed evaluation of five compounds which have been released for publication, CPDD 0020, 0022, 0023, 0032 and 0033. With the exception of CPDD 0032, flunitrazepam, all of these compounds were obtained from pharmaceutical industry. The molecular structures of the compounds and a summary of the work which has been completed on them at UM, MCV, and UC, can be seen in table 11.

2,5-Dihydro-2-(4-methoxyphenyl)-3H-pyrazolo[4,3-c]quinolin-3-one (CPDD 0020)

This diazepam-like anxiolytic did not have discriminative stimulus effects in monkeys and therefore is unlikely to have pentobarbital-like subjective effects in humans.

6-Allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo[4,5-d]azepine dihydrochloride (CPDD 0022)

CPDD 0022 was developed to treat Parkinson's disease. It was not like pentobarbital or amphetamine in drug discrimination. It appeared to decrease cocaine-maintained response rates in the monkey, but further examination indicated that it did not have a selective effect on cocaine, as would be expected if it were acting as a cocaine antagonist.

4-Aminomethyl-1-benzyl-pyrrolidin-2-one fumarate (CPDD 0023)

The compound was indicated to be a nootropic agent (a cognitive enhancer). CPDD 0023 did not have pentobarbital-like or amphetamine-like discriminative stimulus effects in monkeys and therefore would not be expected to exhibit pentobarbital-like or amphetamine-like subjective effects in people.

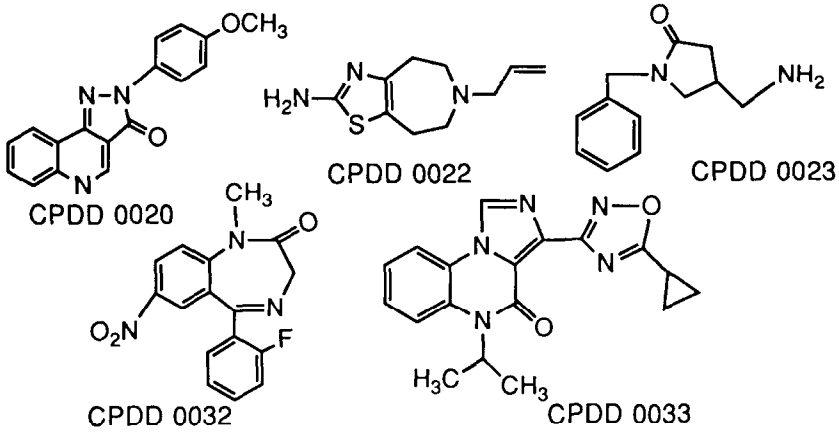
Flunitrazepam (CPDD 0032)

Flunitrazepam was evaluated at the request of the World Health Organization and our work on the drug was completed this year. It exhibited barbiturate-like effects in all of our assays, and would be expected to show pentobarbital-like subjective effects in people.

3-(5-Cyclopropyl-1,2,4-oxadiazol-3-yl)-5-(1-methyethyl)imidazo[1,5-a]-quinoxalin-4(5H)-one (CPDD 0033)

CPDD 0033 is under clinical evaluation as an anxiolytic. This compound did not act as a reinforcer nor did it exhibit pentobarbital-like discriminative stimulus effects in monkeys. From these studies we would not predict that it would have pentobarbital-like subjective effects in humans.

TABLE 11. EVALUATION OF STIMULANT/DEPRESSANT DRUGS



CPDD#	SLA[a]	IS[b]	PD-S[c]	SA[d]	DD[e]
0020	INCONS.[f]	SLIGHT[g]	_h	_i	NO[j]
0022	INCONS.[f]	SLIGHT[k]	NO[l]	NO[m]	NO[j,n]
0023	DEPRESS[o]	DEPRESS	NO[p]	NO[m]	NO[j,n]
0032	DEPRESS	DEPRESS	YES[q]	YES[r]	YES[s]
0033	DEPRESS[t]	DEPRESS[u]	_i	NO	NO[j]

a) Spontaneous locomotor activity (mouse).
b) Inverted screen assay (mouse).
c) Physical dependence - substitution for pentobarbital (rat infusion).
d) Self-administration (monkey).
e) Drug discrimination (intragastric administration, monkey).
f) Inconsistent - mild stimulatory and depressant effects, not dose-related.
g) 20% effect at 300 mg/kg, a dose toxic to 1 out of 6 animals.
h) Observed toxicity precluded procedure.
i) Insufficient solubility for procedure.
j) Discriminative stimulus effects not similar to pentobarbital.
k) Effects not dose-related.
l) Mild, transient sedative effects. No suppression of barbiturate abstinence.
m) Lacks reinforcing effects in methohexital- or cocaine-trained monkeys.
n) Does not share discriminative stimulus effects with amphetamine.
o) Depression
p) No substitution for pentobarbital in dependent animals - no effect on withdrawal signs.
q) Capable of producing barbiturate-like physical dependence in the rat.
r) Acted as a reinforcer in 1 out of 3 monkeys.
s) Shares discriminative stimulus effects with pentobarbital.
t) Erratic, not dose-related.
u) Slightly less potent than pentobarbital, but longer acting.

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Dependence Studies of New Compounds in the Rhesus Monkey and Mouse (1991)

M.D. Aceto, E.R. Bowman, L.S. Harris and E.L. May

All the compounds except morphine·SO_4, codeine·PO_4, meperidine·HCl, bulbocapnine·HCl, NIH 10671 and buspirone·HCI were supplied by Dr. Arthur Jacobson, Laboratory of Medicinal Chemistry, NIDDK, NIH. The identities of all the compounds, except those indicated above, were unknown to us when they were originally submitted. These studies were conducted under the auspices of the Committee on Problems of Drug Dependence.

Dependence Liability Studies in Rhesus Monkeys

Substitution for Morphine (SDS) Test. Male and female rhesus monkeys (M. mulatta) weighing 2.5-7.5 kg were used, and they received 3 mg/kg, s.c., or morphineSO_4 every 6 h. All the animals had received morphine for at least 3 months and were maximally dependent on morphine (Seevers and Deneau 1963). A minimal 2-week recuperation period was allowed between tests. At least 3 monkeys/dose were used. The assay (Aceto and co-workers, 1977 and 1978) was initiated by a subcutaneous injection of the test drug or control substances (morphine and vehicle) into animals in a group that had not received morphine for 14-15 h and showed definite signs of withdrawal. Each animal was randomly chosen to receive one of the following treatments: a) a dose of the compound under investigation; b) morphine control, 3.0 mg/kg; and c) vehicle control, 1 ml/kg. The animals were scored for suppression of withdrawal signs during a 2.5-h observation period. The observer was "blind" regarding the choice of treatments. At the end of the study, the data were grouped according to dose and drug. The mean cumulative score ± SEM was calculated and the data illustrated in figure form.

Precipitated Withdrawal (PPT-W) Test. This evaluation was done under the same conditions as described above, except that the animals were administered a test compound 2-3 h after the last dose of morphine. These animals were not in withdrawal. Naloxone.HCl(0.05 mg/kg, s.c.) served as the positive control.

Primary Physical Dependence (PPD) Study. Drug-naive monkeys were medicated with drug, using escalating dose regimens, periodically challenged with naloxone or placed in abrupt withdrawal. They were observed for overt behavioral signs during drug administration and when they were challenged with antagonist or abruptly withdrawn from the drug.

Rat Infusion Studies

The continuous infusion method was reported by Teiger (1974) and certain modifications are indicated as follows. Rats were anesthetized after which each was fitted with a specially prepared cannula which was passed subcutaneously from the nape of the neck to the lateral side of the lower abdomen and then inserted into the peritoneal cavity. The cannula was anchored at both ends with silk sutures and attached to a flow-through swivel mechanism which allowed the animal to move about in the cage and eat and drink normally. The swivel was connected to a syringe which was attached to a syringe pump. The animals received 7-10 ml of solution every 24 h. Occasionally, when deemed necessary, as with cocaine, infusions were given *via* the right jugular vein.

Substitution for Morphine (SM) Test. The rats received morphine·SO_4 (50 mg/kg/24 h on the first day, 100 mg/kg./24 h on the second day, and 200 mg/kg/24 h from days 3-6). Then, a test drug was substituted for 2 days. The morphine controls received an infusion of water. The animals were observed for changes in body weight and for behavioral-withdrawal signs for 0.5 h at 6, 24, 48, 72 and/or 96 h after stopping the infusion of morphine.

Primary Pphysical Dependence (PPD) Study. The rats received test compound, as specified below, for 6 days and then, were placed in abrupt withdrawal and observed for overt behavioral signs.

Mouse Antinociception Tests

Male mice, weighing 20-30 g, were used. All drugs were dissolved in distilled water or in the vehicle indicated and injected subcutaneously (s.c.). At least three doses were tested, and 6-10 animals per dose were used. When applicable, ED50's were calculated by using computerized probit analysis.

Tail-Flick (TF) and (TF vs *M) Assays.* The procedure and modifications were described (D'Amour and Smith, 1941 and Dewey et al., 1970 and 1971) in the literature. Briefly, the mouse's tail was placed in a groove which contained a slit under which was located a photoelectric cell. When the heat source of noxious stimulus was turned on, the heat focused on the tail, and the animal responded by flicking its tail out of the groove. Thus, light passed through the slit and activated the photocell which, in turn, stopped the recording timer. The heat source was adjusted to produce tail flick of 2-4 s under control conditions. Mice were injected with drug or vehicle and tested 20 m later. In the assay for antagonism of the antinociceptive effect, the potential antagonists were administered 10 m before the agonist, and evaluation occurred 20 m later.

Phenylquinone Abdominal-Stretching (PPQ) Assay. The procedure was reported previously (Pearl and Harris, 1966). The mice were injected with test drugs and 10 m later received 2.0 mg/kg ip of a freshly prepared paraphenylquinone (PPQ) solution. The mice were then placed in cages in groups of two each. Ten m after the PPQ injection, the total number of stretches per group were counted over a 1-m period. A stretch was characterized by an elongation of the mouse's body, development of tension in the abdominal muscles, and extension of the forelimbs. The antinociceptive response was expressed as the percent inhibition of the PPQ-induced stretching response.

Hot-Plate (HP) Assay. The method was also reported previously (Eddy and Leimbach, 1953 and Atwell and Jacobson, 1978). The hot plate was held at 55°C. Mice were placed on the hot plate and activity was scored if the animal

jumped or licked its paws after a delay of 5 s or more, but no more than 30 s beyond the control time.

Table 1

Comparative Data (ED50, mg/kg s.c.) [95% C.L.] of Selected Standards in 4 Mouse Agonist-Antagonist Tests at MCV/VCU

Drug	Tail-Flick	Tail-Flick Antagonist	Phenylquinone	Hot-Plate
Pentazocine	15% at 10.0	(1:826)	(1.0-2.5)	
Cyclazocine	17% at 1.0[a]	0.03 (0.020-0.78)	0.01 (0.005-0.03)	- - - -
Nalorphine.HCl	None at 10.0	2.6 (0.7-10.0)	0.6 (0.03-1.44)	- - - -
Naloxone.HCl	None at 10.0	0.04 (0.01-0.09)	No Activity	- - - -
NaltrexoneHCl	None at 10.0	0.007 (.002-0.02)	No Activity	- - - -
Morphine.SO_4	5.8 (5.7-5.9)	Inactive	0.23 (0.20-0.25)	0.85 (0.39-1.86)
Codeine.PO_4	- - -	Inactive	- - -	6.4 (0.39-16.8)
Meperidine.HCl	- - -	Inactive	- - -	(1.8-11.7)

[a]Mice were ataxic at 3.0 and 10.0 mg/kg but there was no further increase in reaction time.

Table 2

Comparative Data (ED50 mg/kg) [95% C.L.] from the Hot Plate Assay[a]

	Hot Plate s.c./p.o.
Morphine·$S0_4$	0.98 (0.83-1.1) 8.3 (6.0-11.4)
Codeinee·$P0_4$	5.8 (4.5-10.2) 13.5 (9.7-18.7)
Levorphanol Tartrate	0.2 (0.1-0.3)
Meperidine·HCl	5.3 (4.0-7.1)
(-)-Metazocine·HBr	0.6 (0.5-0.9) 10.6 (8.0-14.1)
Dihydromorphinone·HCl	0.19 (0.15-0.25) 0.9 (0.7-1.2)
Nalorphine·HCl	9.9 (5.7-2.1)
Cyclazocine	1.5 (1.1-2.1)
Pentazocine	9.3 (6.7-12.8)
Chlorpromazine·HCI	1.1 (0.9-1.5)

Phenobarbital, amobarbital, oxazepam, flurazepam, meprobamate and mescaline are inactive in the hot plate test.

[a]Data from Table 2 supplied by Dr. A. Jacobson

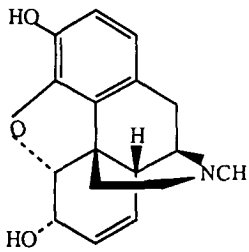
Compound NIH		Chemical Name or Generic Class	MOUSE TF	TF vs M	PPO	HP	RAT SM	PPD	MONKEY SDS	PPt-W	PD
0001	Morphine·$S0_4$	Morphine				+					
0002	Codeine·$P0_4$	Morphine				+					
5221	Meperidine·HCl	4-Phenylpiperidine				+					
10567	Ibogaine·HCl	Indole					+	+			
10577	(-)-1S,2S-U50,488-MeI	Benzeneacetamide quat	+	+	+	+			+		
10578	(+)-1R,2R-U50,488-MeI	Benzeneacetamide quat	+	+	+	+			+		
10593	1-Chlorobuprenorphine	Epoxyethenomorphinan	+	+	+				+	+	
10615	Bulbocapnine	Aporphin-11-ol	+	+	+[a]				+		
10629		Epoxyethenomorphinan	+	+	+				+		
10637		4-Anilinopiperidine	+	+	+				+		
10644		Naloxone Diacetate	+	+	+						
10645		Naloxone Diacetate	+	+	+						
10647	Mirfentanil	4-Aminopiperidine	+[b,c]			+					
10650		6,7-Benzomorphan	+	+	+						
10651		Ketobemidone	+	+	+				+		
10655		Normethadol	+	+	+				+		
10656	BOZO	Naloxone hydrazone	+	+	+				+	+	
10657		4-Piperidone	+	+	+				+		
10658		Diazabicyclononanone	+	+	+						
10659		4-Piperidone	+	+	+						
10660		4-Piperidone (enol form)	+	+	+						
10663	Nalmefene glucuronide	Naltrexone	+	+	+						
10665		Etonitazine	+[b]	+	+[a]				+		
10666		6,7-Benzomorphan	+	+	+				+		
10667		6,7-Benzomorphan	+	+	+						
10671		4-Phenylpiperidine	+	+	+					+	
10672		Benzofuranacetamide	+[b]	+	+	+	+	+	+		+

SUMMARY OF NEW DATA (cont.)

Compound NIH		Chemical Name or Generic Class	MOUSE TF	TF vs M	PPO	HP	RAT SM	PPD	MONKEY SDS	PPt-W	PPD
10673		6,7-Benzomorphan	+	+	+				+		
10674		6,7-Benzomorphan	+	+	+				+		
10675		6,7-Benzomorphan	+	+	+				+		
10676		Azabicyclononanone	+	+	+						
10677		Diazaadamantane	+	+	+	+					
10679		LAAM, Acetylmethadol	+	+	+				+		
10680		Ketobemidone	+	+	+				+		
10681		Ketobemidone	+	+	+				+		
10682		Benzoylecgonine	+	+	+				+		
10685		Morphine	+[b]	+	+	+			+		
10687	Buspirone	Piperazine	+	+	+[a]	+			+	+	
10700		N-Phenyl-N-cyclohexyl-3,4-Dehydropiperidine	+	+	+	+					
10713		Diazabicyclononanone	+	+	+	+					
10714		Diazabicyclononanone	+	+	+	+					
10715		Diazabicyclononanone	+	+	+	+					

[a]Naloxone vs ED80 in PPQ test, [b]Naloxone prior to ED80 in TF test, [c]Naloxone after ED80 in TF test

NIH 0001 Morphine·SO_4

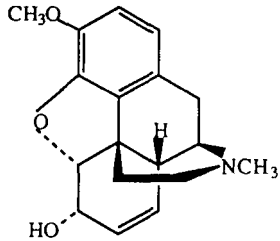


MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF-
2) TF vs. M -
3) PPQ-
4) HP- 1) 0.85 (0.39 - 1.86)
2) 0.72 (0.34 - 1.49)
3) 0.98 (0.83 - 1.1)[a]

[a]Reported by Dr. Jacobson

NIH 0002 Codeine·PO_4

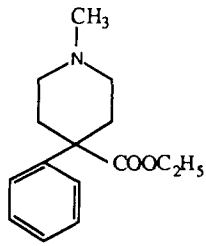


MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF-
2) TF vs. M -
3) PPQ -
4) HP - 1) 6.4 (2.4 - 16.8)
2) 6.8 (4.5 - 10.2)[a]

[a]Reported by Dr. Jacobson

NIH 5221 Meperidine·HCl

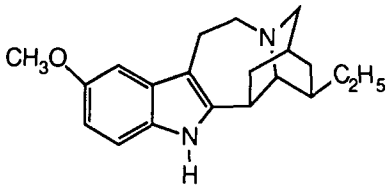


MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF-
2) TF vs. M-
3) PPQ -
4) HP - 1) 4.6 (1.8 - 11.7)
2) 5.3 (4.0 - 7.1)[a]

[a]Reported by Dr. Jacobson

NIH 10567 Ibogaine·HCl



MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 9.7 ((2.8 - 34.0)[a]

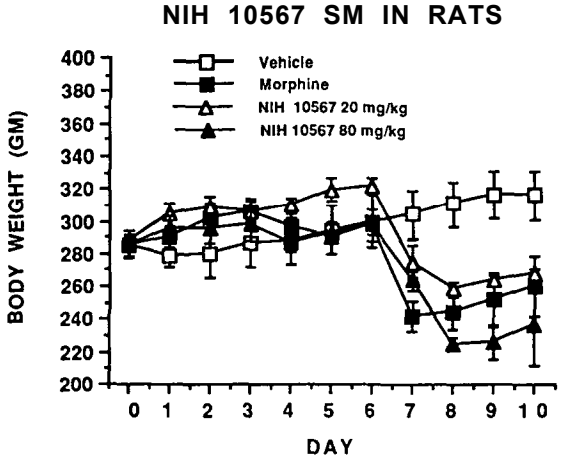
[a]Vehicle - 2% Tween 80 in water

NIH 10567 Ibogaine·HCl (cont.)

Rat Infusion

A. (SM)

As shown in the fig. (designated NIH 10567 SM) and table, NIH 10567 did not substitute for morphine at a dose of 20 mg/kg/day. However, at the high dose, namely 80 mg/kg, some suppression of behavioral withdrawal signs (see table) was noted on day 6 (24-h withdrawal). However, body-weight loss was actually greater than that of the withdrawn morphine-dependent rats (see fig.). In any case, suppression of body-weight loss is considered the most important criterion for substitution for morphine in this assay. These results may simply indicate a non-specific effect on behavior. Additional studies are suggested.



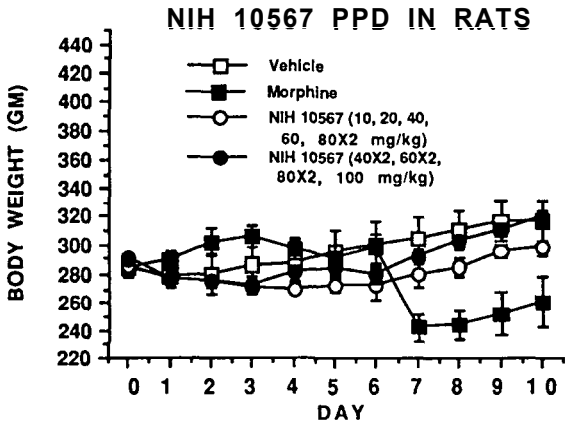
B. (PPD)

As shown in the accompanying fig.(designated NIH 10567 PPD) and table, ibogaine did not produce a significant degree of physical dependence at the dose regimen tested.

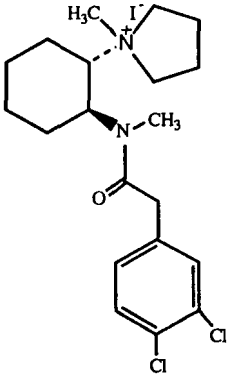
Table (NIH 10567) Primary Physical Dependence (PPD) and Substitution for Morphine (SM) Studies with Ibogaine in Continuously-Infused Rats

Treatment	Hour in Withdrawal					
	2	6	24	48	72	96
	Mean Number of Withdrawal Signs[a,b]					
1. Vehicle + Vehicle Controls[c]	0	0.3	0.3	1.0	0	0.5
2. Morphine[d] + Vehicle Controls	0	0.8	11.3[b]	16.3[b]	11.5[i]	7.5[i]
3. Morphine + NIH 10567[e] (low dose - SM)	0	0	16.5[b,j]	14.0[b,j]	10.0[b,j]	9.8[b,j]
4. Morphine + NIH 10567[f] (high dose - SM)	0	0.3	1.6[i,k]	6.8	8.3[b,j]	17.0[i]
5. NIH 10567 + Vehicles (low dose - PPD)	0.3	2.3	3.5	1.8	3.8[i]	3.0[i]
6. NIH 10567 + Vehicle[h] (high dose - PPD)	0.3	1.8[i]	5.0	4.3	4.0[i]	2.5[i]

[a]Hypersensitivity, squeaking, aggression, wet-dog shakes, rubbing and chewing; bone-tailed test (Mann-Whitney U-test), p = 0.05 or less compared to vehicle controls; [c]Vehicle was Tween 80 + water. Days 1-10; 8 ml/24 h; N = 4; [d]Morphine·SO_4 50 mg/kg on day 1; 100 mg/kg on day 2; 200 mg/kg on days 3-6; N = 4 on day 7, 3 on day 8 and 2 on day 10; Vehicle on days 7-10; [e]Morphine·SO_4 as above on days 1-6 and NIH 10567 at 20 mg/kg on days 7 and 8; N = 4; [f]Morphine·SO_4 as above on days 1-6 and NIH 10567 at 80 mg/kg on days 7 and 8; N = 5 on days 1 and 2, 3 on day 9 and 2 on day 10; [g]NIH 10567, 10 mg/kg on day 1, 20 mg/kg on day 2, 40 mg/kg on day 3, 60 mg/kg on day 4 and 80 mg/kg on days 5 and 6; Vehicle on days 7-10, N = 4, [h]NIH 10567, 40 mg/kg on days 1 and 2; 60 mg/kg on days 3 and 4; 80 mg/kg on day 5; and 100 mg/kg on day 6, Vehicle on days 7-10, N = 4; [i]p value approaching significance when compared to vehicle; [j]Not significantly different when compared to morphine controls; [k]Significantly different when compared to morphine controls p = 0.05 or less.



NIH 10577 (-)-1*S,* 2*S* - *trans* -3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide methiodide [(-)-1*S,* 2*S* - U50,488 methiodide]



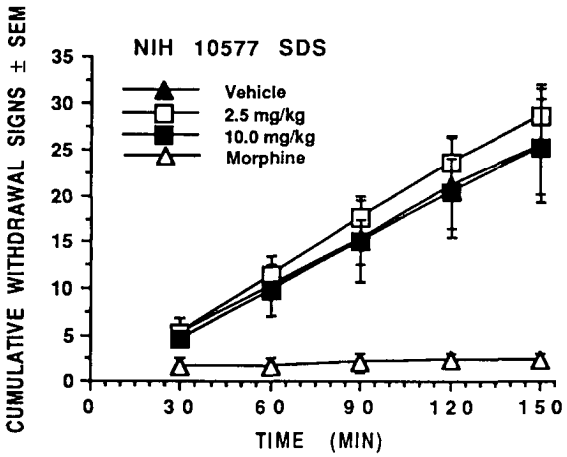
MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 13% at 10.0 and 22% at 30.0
2) TF vs. M - 0% at 1.0 and 1.0 and 20% at 30.0
3) PPQ - 4.2 (1.1 - 15.8)
4) HP - Inactive at 20 mg/kg

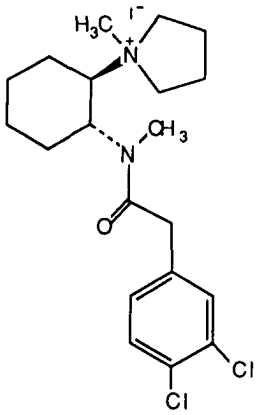
MONKEY DATA
(SDS)

At the highest dose, one monkey retched frequently. Otherwise, as illustrated (NIH 10577 SDS), the drug neither substituted for morphine nor exacerbated withdrawal. Vehicle used was Tween 80, 2-5% DMSO and water.

NIH 10577 (-)-1*S*, 2*S* - *trans* -3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyllbenzeneacetamide methiodide [(-)-1*S*, 2*S* - U50,488 methicdide] (cont.)



NIH 10578 (+)-1*R*, 2*R* - *trans*-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyllbenzeneacetamide methiodide [(+)-1*R*, 2*R* - U50,488 methiodide]



MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

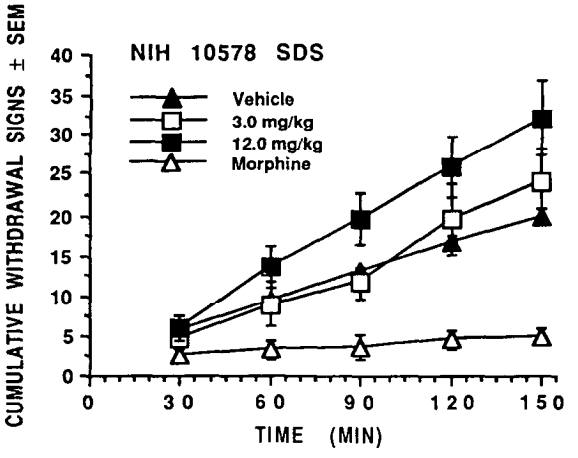
1) TF - 2% at 1.0, 10.0 and 19% at

2) TF 30.0 vs. M - 0% at 3.0 and 30.0 and 28% at 10.0
3) PPQ - 3.5 (1.1 - 11.0)
4) HP - Inactive at 20.0 mg/kg

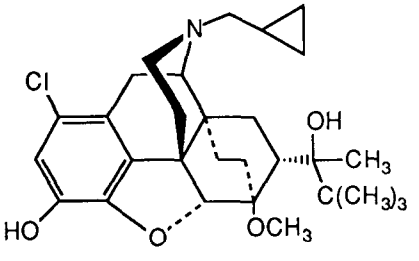
NIH 10578 (+)-1*R*, 2*R* - *trans*-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyllbenzeneacetamide methiodide [(+)-1*R*, 2*R*- U50,488 methiodide] (cont.)

MONKEY DATA
(SDS)

NIH 10578 did not substitute for morphine. The drug seemed to exacerbate withdrawal (see fig NIH 10578). Vehicle used was 10% Tween 80 in water and ultrasound. Drug supply exhausted.



NIH 10593 1-Chloro-17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5 α-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-·α-methyl-6,14-ethenomorphinan-7-methanol (1-chlorobuprenorphine)



MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 6% at 1.0, 0% at 10.0 and 15% at 30.0
2) TF vs. M -
   1) 10.4 (3.7 - 29.2)[a]
   2) 4.7 (1.9 - 11.8)
3) PPQ - 6% at 1.0, 17% at 10.0 and 20% at 30.0[a]

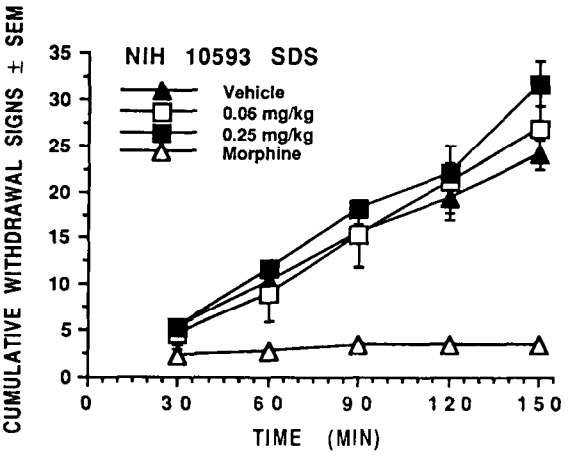
[a]Vehicle 10% Tween 80, lactic acid and water

MONKEY DATA

A. (SDS)

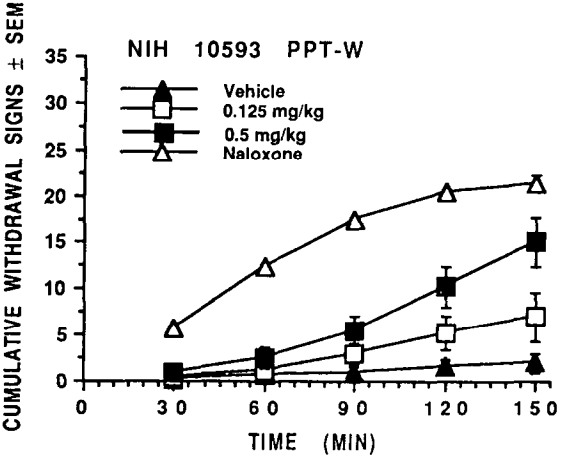
This compound appeared to exacerbate withdrawal in morphine-dependent rhesus monkeys (see fig. NIH 10593 SDS). The action was delayed. Severe vomiting was observed 2.5 h after drug and 2 doses of morphine (3.0 mg/kg) were required to terminate the withdrawal syndrome.

NIH 10593 1-Chloro-17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5 α-epoxy-18,19-dihydro-3-hydroxy-6-methoxy- α-methyl-6,14-ethenomorphinan-7-methanol (1-chlorobuprenorphine) (cont.)

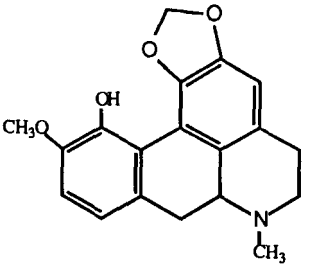


B. (PPT-W)

NIH 10593 precipitated severe withdrawal; its duration was prolonged. Some animals were given an additional dose of morphine 6 h after receiving this drug to terminate withdrawal. The results shown in the figure (see fig. 10593 PPt-W) reflect only the first 2.5 h of the assay only. Vehicle was Tween 80, lactic acid and water.



NIH 10615, Bulbocapnine·HCl



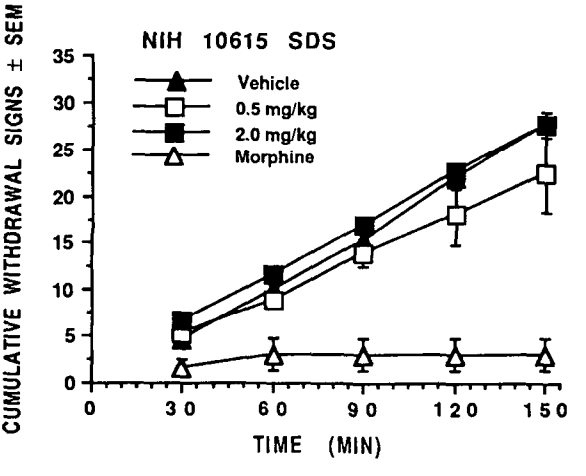
MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 10.0 and 20.0
2) TF vs. M - Inactive at 1.0 and 30.0, 15% at 10.0
3) PPQ - 4.5 (1.6 - 12.8)

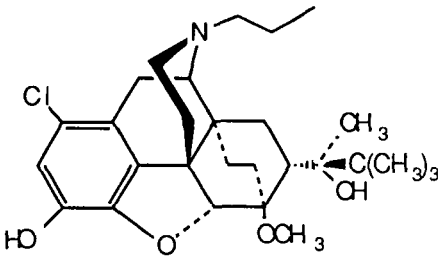
Special Study: Naloxone, at 10.0 mg/kg did not antagonize bulbocapnine's action in the PPQ test.

MONKEY DATA
(SDS)

The aporphine alkaloid, bulbocapnine, a purported dopamine D_1 [TiPS, 11, 233 (1990)] antagonist has also been shown to possess antinociceptive activity as well as antistereotypic effects induced by methylphenidate (Zetler,1988). Because of the renewed interest in compounds with this type of profile, this compound was studied. As can be seen in the fig. (NIH 10615 SDS), bulbocapnine neither substituted for morphine nor exacerbated withdrawal at doses of 0.5 or 2.0 mg/kg. At the high dose, some jaw sag, slowing, piloemction and facial pallor were noted.



NIH 10629 1-Chloro-17-(n-propyl)- α-(1,1-dimethylethyl)-4,5 α-epoxy-18,19-dihydro-3-hydroxy-6-methyl-α-methyl-6,14-etheno-morphinan-7-methanol



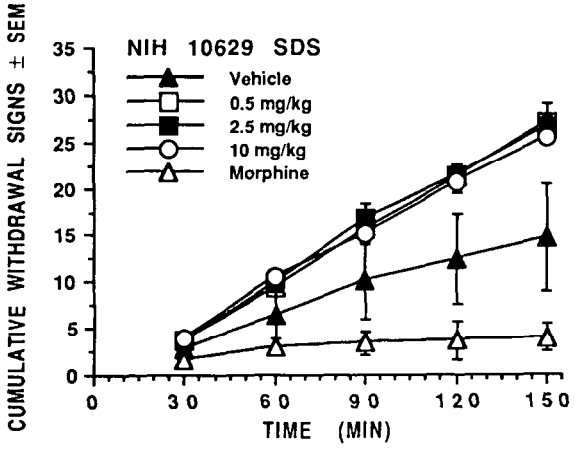
MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - 0% at 10.0, 20% at 20.0, 61% at 25.0, 74% at 30 and 63% at 40.0[a]
3) PPQ - Inactive at 1, 0.1, 10.0 and 30.0[a]

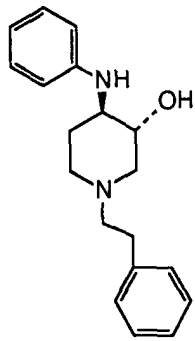
[a]Vehicle 10% Tween 80, lactic acid and water

MONKEY DATA
(SDS)

As shown in the graph, NIH 10629, designated NIH 10629 SDS, this compound did not substitute for morphine. Instead, it exacerbated withdrawal. The drug acted promptly and the duration of action was longer than 2.5 h. Two monkeys at the high dose and one at the low dose still showed signs of withdrawal after receiving additional doses of morphine at 11:00 a.m., noon and 1:00 p.m. Withdrawal abated after another injection of morphine at 1:30 p.m. Vehicle was Tween 80, phosphoric acid and water.



NIH 10637 *trans*-3-Hydroxy-4-anilino-1-(2-phenylethyl)piperidine



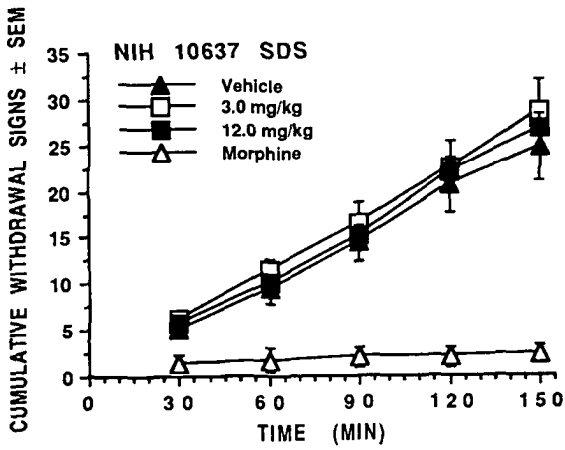
MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 0.1, 1.0 and 10.0, 37% at 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 11% at 1.0 and 10.0, 14% at 30.0

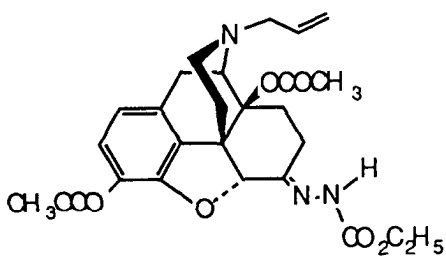
Vehicle - Phosphoric acid and water

MONKEY DATA
(SDS)

NIH 10637 neither substituted for morphine nor exacerbated withdrawal at doses of 3.0 or 12.0 mg/kg (see fig. NIH 10737 SDS).



NIH 10644 Naloxone ethoxycarbonylhydrazone 3,14-diacetate

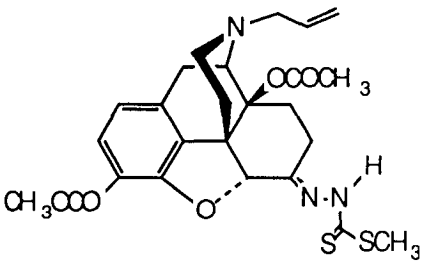


MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 16% at 1.0, 24% at 10.0 and 11% at 30.0[a]
2) TF vs. M - 0.3 (0.1-1.1)
3) PPQ - Inactive at 1.0, 10.0 and 14% at 30.0

[a]Vehicle - 5% Tween 80 aqueous preparation. Vehicle 13% activity.

NIH 10645 Naloxone methyldithiocarbohydrazone 3,14-diacetate

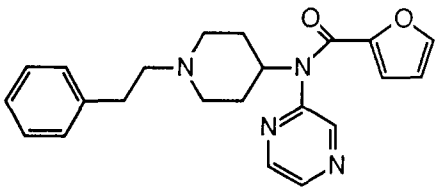


MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - 0.8 (0.2 - 2.5)[a]
3) PPQ - Inactive at 1.0, 10.0 and 30.0

[a]Vehicle - lactic acid and water.

NIH 10647 1-(2-Phenylethyl)-4-[N-(2-pyrazyl)-2-furoylamido]piperidine·HCl
Mirfentanil



MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 0.4 (0.1 - 1.2)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.08 (0.03 - 0.20)
4) HP - 0.3 (0.1 - 0.7)

Special Tests

1) Naloxone given prior to ED80 of NIH 10647 in TF - AD50 = 0.06 (0.02 - 1.84)
2) Naloxone given after ED80 of NIH 10647 in TF - AD50 = 0.03 (0.01 - 0.12)

MONKEY DATA
(SDS)

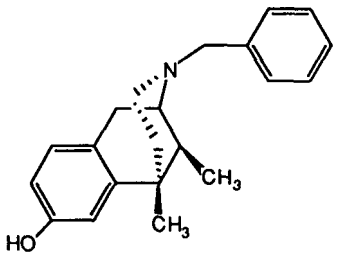
NIH 10647 neither substituted for morphine nor exacerbated withdrawal. Questionable catalepsy, relaxed skeletal muscles and respiratory depression were noted.

COMMENT

NIH 10647 has potent mu-like antinociceptive properties in the mouse. In the monkey, non-opioid effects were observed. This drug may demonstrate species-specific effects.

Note that all data indicated above except HP were reported in 1990. A summary is given in this report so that the drug's profile can be seen.

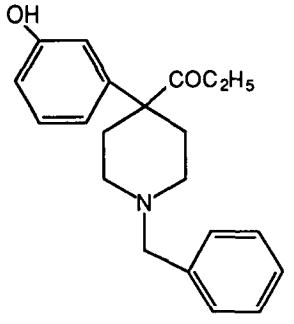
NIH 10650 (+)-2-Benzyl-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan·HBr



MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0 and 30.0, 15% at 10.0
3) PPQ - 28% at 1.0, 19% at 10.0 and 30% at 30.0

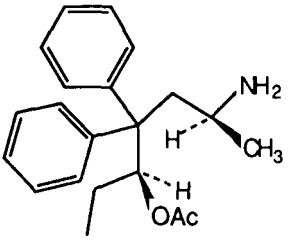
NIH 10651 1-Benzyl-4-*m*-hydroxyphenyl-4-ketoethylpiperidine·HCl



MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 16% at 1.0, Inactive at 10.0 and 30.0
2) TF vs. M - Inactive at 1.0 and 10.0, 17% at 30.0
3) PPQ - 7.5 (2.8 - 19.9)

NIH 10655 (-)-α-Acetyl-N,N-dinormethadol·HCl



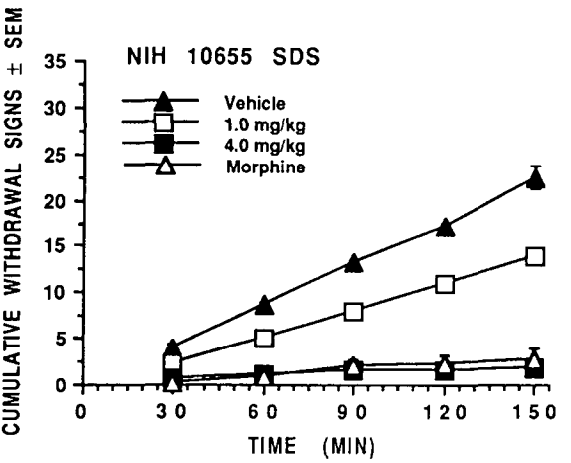
MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 5.4 (1.9 - 15.7)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.14 (0.05 - 0.45)

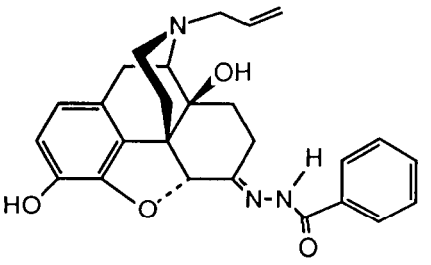
MONKEY DATA
(SDS)

As shown in the accompanying figure below, NIH 10655 dose-dependently substituted for morphine. At 4.0 mg/kg, the drug abolished completely all withdrawal signs. In addition, at this dose, jaw sag, slowing, ataxia, and scratching were observed. Onset of action was prompt and duration was at least as long as that of morphine. Potency is estimated as equal to that of morphine.

NIH 10655 (-)-α-Acetyl-N,N-dinoxmethadol·HCl (cont.)



NIH 10656 Naloxone benzoylhydrazone (BOZO)



MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

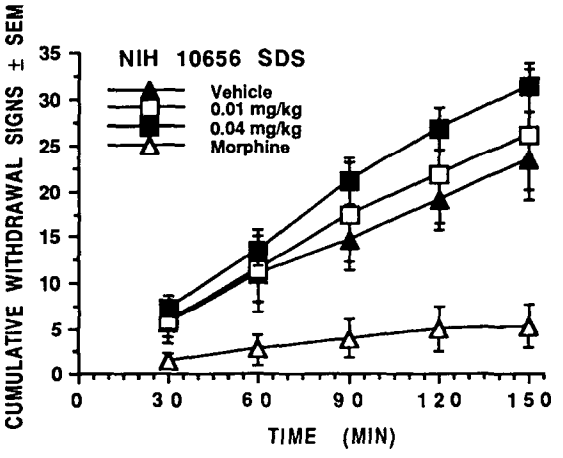
1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 0.05 (0.01 - 0.2)
3) PPQ - 15% at 1.0, 18% at 10.0, 42% at 30.0 and 52% at 60.0

MONKEY DATA

A. (SDS)

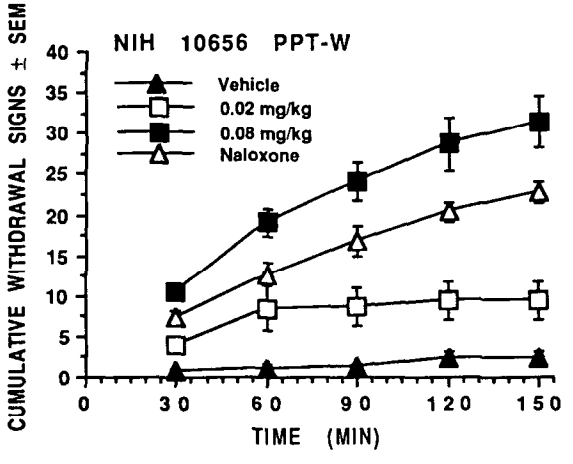
NIH 10656 did not attenuate withdrawal. Instead, it dose-dependently exacerbated this syndrome. In addition, all the NIH 10656-treated animals retched more than vehicle controls. At the highest dose, body jerks, severe tremors and biting of body appendages were noted. The drug appears to be an opioid antagonist. The vehicle was phosphoric acid and water (see fig. NIH 10656 SDS).

NIH 10656 Naloxone benzoylhydrazone (cont.)

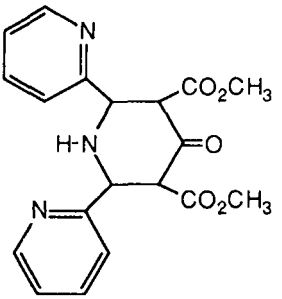


B. (PPT-W)

This compound dose-dependently precipitated withdrawal in morphine-dependent monkeys. Onset was prompt and duration of action was similar to that of the reference compound naloxone. NIH 10656 is approximatley equipotent to naloxone. See accompanying fig (NIH 10656 PPT-W).



NIH 10657 Dimethyl 2,6-di(2-pyridine)-4-piperidone-3,5-dicarboxylate



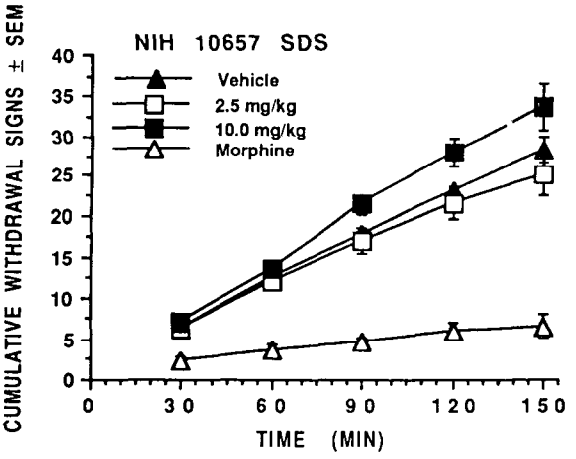
MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a,b]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a,b]
3) PPQ - Inactive at 1.0, 10.0, 26% at 30.0[a,b]

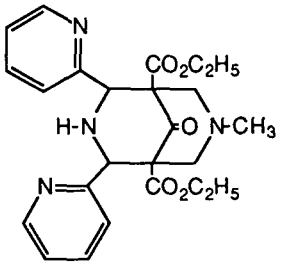
[a]Vehicle - 40% DMSO, 5% propylene glycol and warming
[b]Vehicle inactive.

MONKEY DATA
(SDS)

This compound (NIH 10657) did not substitute for morphine. At the high dose, it appeared to exacerbate withdrawal. In a preliminary experiment in one monkey, severe vomiting and biting of arms were seen after a cumulative dose of 7 mg/kg, administered over a 45-m period. Vehicle ingredients were phosphoric acid, DMSO, propylene glycol, and water (see fig NIH 10657 SDS).



NIH 10658 Diethyl 2,4-di(2-pyridine)-3,7-diazabicyclo[3.3.l]nonane-9-one 1,5-dicarboxylate



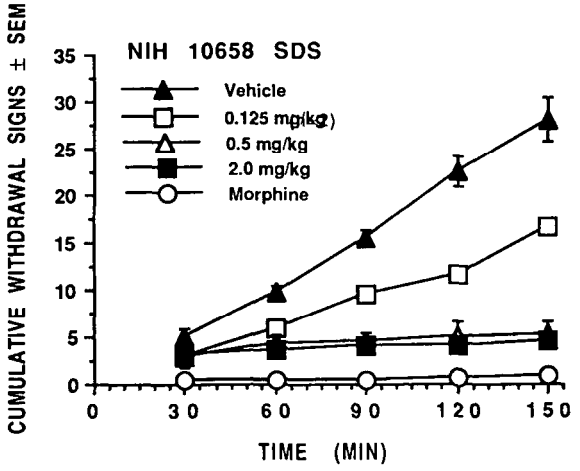
MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 1% at 1.0, 11% at 10.0 and 23% at 30.0[a,b]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.6 (0.2 - 1.7)[a,c]

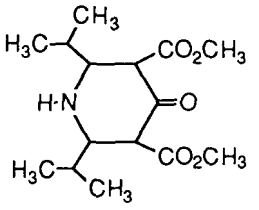
[a]Vehicle - DMSO, propylene glycol and water
[b]Vehicle - 4% activity
[c]Vehicle - 17% activity.

MONKEY DATA
(SDS)

NIH 10658 substituted completely for morphine. The action was prompt and dose-dependent.. Duration of action was as long as that of morphine. In addition, at the highest dose, the signs "cataleptic posture", prostration, body jerks, straub tail, mydriasis, and rubbing face were noted. The drug seems to combine opioid and neuroleptic properties. Vehicle consisted of Tween 80, phosphoric acid and water.



NIH 10659 Dimethyl 2,6-diisopropyl-4-piperidone-3,5-dicarboxylate

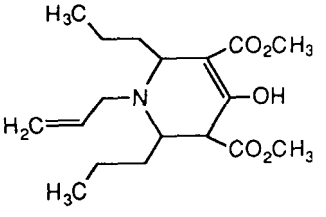


MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0 and 10.0, 14% at and 30.0[a,b]
2) TF vs. M - Inactive at 1.0 and 10.0, 20% at and 30.0[a,b]
3) PPQ - 14% at 1.0, 11% at 10.0 and 20% at 30.0[a,b]

[a]Vehicle 10% cyclodextrin
[b]Vehicle inactive

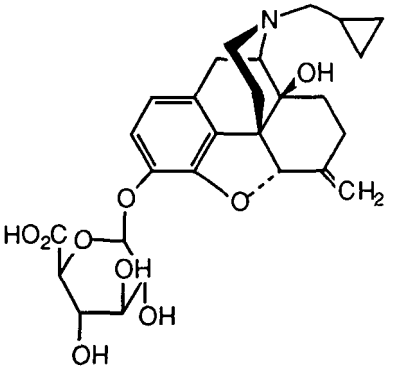
NIH 10660 Dimethyl 1-allyl-2,6-dipropyl-4-piperidone-3,5-dicarboxylate (enolic isomer)



MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 3.0, 10.0 and 30.0
2) TF vs. M - Inactive at 3.0, 10.0 and 30.0
3) PPQ - Inactive at 1.0, 10.0 and 30.0

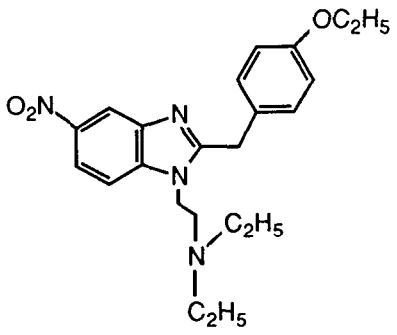
NIH 10663 Nalmefene-3ß-D-glucuronide



MOUSE DATA-ED50 OR AD50
95% C.L.) (mg/kg or % change)

1) TF - Inactive at 3.0, 10.0 and 30.0
2) TF vs. M - 0.55 (0.19 - 1.63)
3) PPQ - Inactive at 1.0, 10.0 and 30.0

NIH 10665 Etonitazene methanesulfonate



MOUSE DATA-ED50 OR AD50 95% C.L.) (mg/kg or % change)

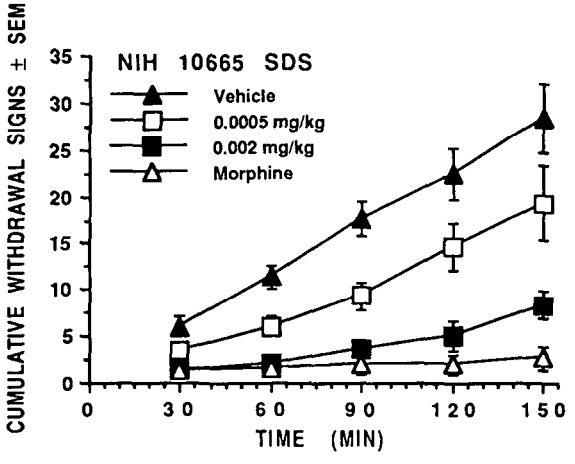
1) TF - 0.005 (0.002 -0.011)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.0017 (0.0005 - 0.005)

Special: Naloxone challenged prior to ED_{80} of NIH 10665 in TF and PPQ Assays

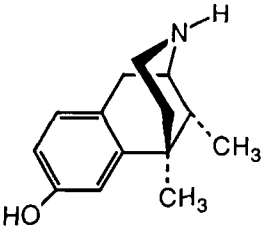
1) Naloxone AD50 = 0.3 (0.2 - 0.5) in TF Test
2) Naloxone AD50 = 0.9 (0.4 - 2.5) in PPQ Test

MONKEY DATA
(SDS)

NIH 10665 substituted completely for morphine in abruptly withdrawn animals. Onset of action was rapid, duration was shorter than that of morphine and potency at peak effect was approximately 1500 x morphine. At the highest dose, the signs jaw and body sag, scratching, and rubbing face were noted (see fig NIH 10665 SDS).



NIH 10666 (±)-5,9α-Dimethyl-2'-hydroxy-6,7-benzomorphan [α-(±)-N-Normetazicine]



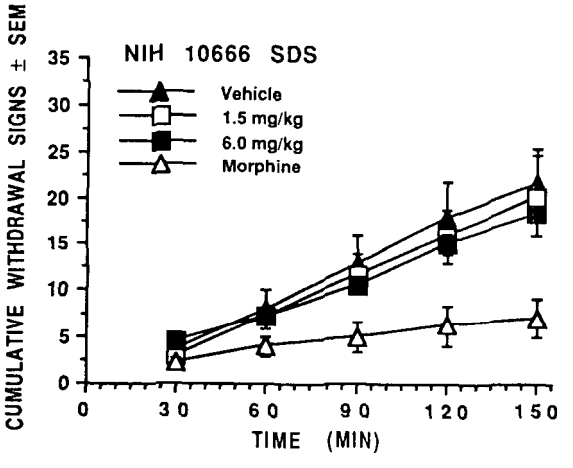
MOUSE DATA-ED50 OR AD50 95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[b]
3) PPQ - 4.6 (0.3 - 74.8)

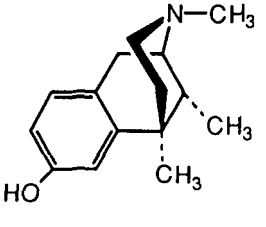
[a]2 mice died at 30.0
[b]5 mice died at 30.0

MONKEY DATA
(SDS)

At doses of 1.5 and 6 mg/kg, NIH 10666 neither substituted for morphine nor exacerbated withdrawal (see fig. NIH 10666 SDS). Salivation, slowing, ataxia and jaw sag were noted, especially at the high dose. Higher doses were not tested because of the lethal effects noted in mice. Vehicle consisted of 1 drop of phosphoric acid in 5 ml of water.



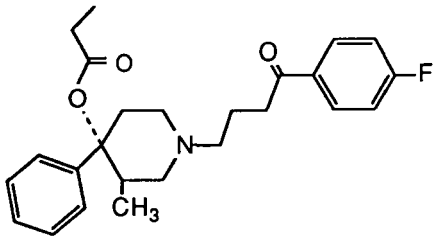
NIH 10667 (±)-2'-Hydroxy-2,5,9α-trimethyl-6,7-benzomorphan·HCl [α-(±)-Metazocine·HCl]



MOUSE DATA-ED50 OR AD50 95% C.L.) (mg/kg or % change)

1) TF - 1.5 (1.0 - 2.5)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.6 (0.4 - 0.9)

NIH 10671, I-184 (±)-N-3-(p-Fluorobenzoyl)propyl-3ß-methyl-4ß-phenyl-4α-propionyloxypiperidine·HC1



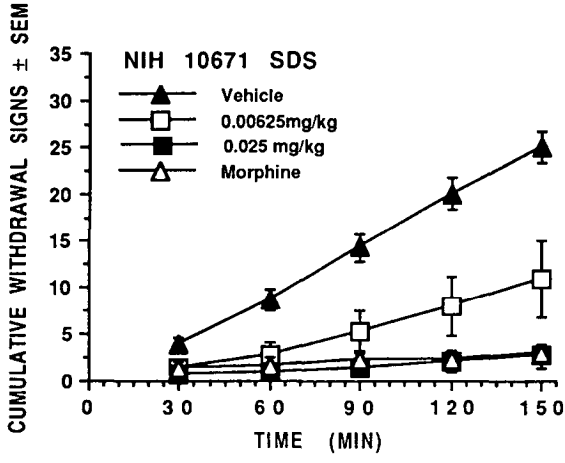
MOUSE DATA-ED50 OR AD50 95% C.L.) (mg/kg or % change)

1) TF - 0.3 (0.1 - 0.6)[a]
2) TF vs. M - Inactive at 1, 10 and 30[a,b]
3) PPQ - 0.04 (0.02 - 0.08)

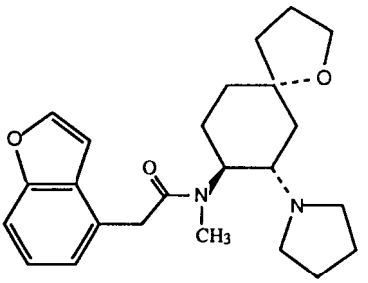
[a]Vehicle - phosphoric acid and water
[b]1 of 6 mice died at 30. The rest were cataleptic.

MONKEY DATA
(SDS)

NIH 10671 substituted completely for morphine (see fig.). The action was dose-related. Onset of action was prompt and duration was at least as long as that of morphine. Jaw sag, ataxia, body sag and scratching were noted and appeared dose-related. One monkey given 0.5 mg/kg became cataleptic, pale and unresponsive ("out cold"). At a dose of 0.05 mg/kg, naloxone reversed this condition in 20 m. The data obtained with this monkey were not included in the analyses.



NIH 10672 [(-)-5R-{5α,7α,8β}]-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro-[4,5]dec-8-yl]-4-benzofuranacetamide·HCl



MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

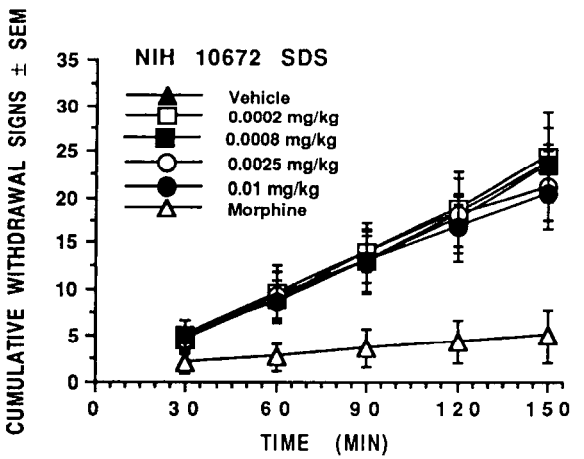
1) TF - 0.015 (0.003 - 0.059)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.0015 (0.0004 - 0.006)
4) HP - 0.01 (0.004 - 0.04)

Special Test: Naloxone prior to ED80 of NIH 10672 in TF test.
AD50 = 0.3 (0.1 - 0.8)

MONKEY DATA

A. (SDS)

As shown in the accompanying figure, NIH 10672 did not substitute for morphine or exacerbate withdrawal. At the highest dose, severe vomiting and catalepsy were noted. Salivation and inability to hold head erect were also noted at the high dose.



B. (PPD)

As indicated in the table (designated PPD in monkeys) below, NIH 10672 produced a wide variety of overt behavioral signs during the administration of the drug and when it was withdrawn. Each time the drug was given, it produced a cluster of signs normally observed after the administration of a mu- and/or kappa-opioid agonist (body and jaw sag, ataxia, drowsiness, salivation, etc.). The drug had a quick onset and very short offset of action (less than 1 h). The degree of tolerance that developed during the course of the study was more than an order of

Table. Primary Physical Dependence Study (PPD) with NIH 10672 in Rhesus Monkeys

DAY	DOSE (mg/kg)	COMMENTS
1	0.002[a]	Four male and female rhesus monkeys (Macaca mulatta) weighing 2.8 - 3.7 kg at the start of the study served as subjects. The dose regimens of NIH 10672 are indicated in the columns. Aqueous solutions of the test substance were given in a volume of 0.25 ml/kg s.c. The signs designated as ataxia, body and jaw sag, eyelid ptosis, slowing, drowsiness and salivation were scored during a 15-min observation period 0.5 h after drug was given. These signs were seen in all the subjects during each injection. As tolerance developed to the overt behavioral effects of the drug, the dose and/or frequency of the injection regimen were increased.
2	0.004[a]	
3	0.008[a]	
4-15	0.008[b]	
16	0.008[c]	Precipitated Withdrawal (Day 16)
17-21	0.01[c]	Three of four animals were injected with 0.25 mg/kg s.c. of naloxone·HCl, the fourth received vehicle. No withdrawal signs were noted.
22-24	0.012[c]	
25	0.014[c]	
26	0.025[c]	
27-28	0.025[b]	
29-30	0.03[c]	
31	0.035[d]	Abrupt Withdrawal (Day 31)
32	0.035[a]	Approximately 12-13 h after NIH 10672 was abruptly withdrawn, a number of unconditioned behaviorial signs were recorded. They included involuntary head, body and perioral movements (chewing, tongue rolling) gnawing, rubbing face on wire mesh, excess grooming (including frequent picking at toes and fingers in all animals) trichotillomania and trichophagy, scratching, yawning, wet-dog shakes and restlessness (increased locomotion). They also were lying on side and rolling. All of the animals appeared alert and oriented. Nineteen h after abrupt withdrawal, NIH 10672 was administered (0.02 mg/kg). The withdrawal behavior promptly ceased and was replaced by that associated with drug treatment. Tolerance seemed lost because this lower dose appeared as effective as the last dose given before abrupt withdrawal.

Table. Primary Physical Dependence Study with NIH 10672 in Rhesus Monkeys (cont.)

DAY	DOSE (mg/kg)	COMMENTS
33	0.04[c]	Precipitated Withdrawal (Day 33)
34-35	0.04[b]	Three of four animals were injected with 0.25 mg/kg of naloxone·HCl, the fourth received vehicle. Naloxone, when given at peak response with 0.04 mg/kg of NIH 10672, appeared to attenuate some of the acute effects of
36	0.04[c]	10672 but did not precipitate withdrawal. The action of naloxone was transient.
37	0.04[d]	Abrupt Withdrawal (Day 37)
		On the abrupt-withdrawal test on day 37 (midnight 3/19/91) signs designated in the 31-day abrupt-withdrawal test were noted. These signs peaked at 16 h and gradually dissipated by 20 h. The animals appeared normal and healthy at the end of the experiment.

[a]Four injections daily at 6 am, 12 noon, 6 pm, and midnight; [b]Six injections daily at 6 and 10 am, 12 noon, 3 and 6 pm, and midnight; [c]Eight injections daily at 6, 9 and 10 am, 12 noon, 1, 3 and 6 pm, and midnight; [d]Four injections daily at 1, 3 and 6 pm, and midnight.

NIH 10672 [(-)-5R-{5α,7α,8β}]-N-methyl-N-[7-(l-pyrrolidinyl)-l-oxaspiro-[4,5]dec-8-yl]-4-benzofuranacetamide·HCl (cont.)

magnitude. In spite of the vigorous dose regimen, the animals appeared healthy, ate well and body weight was not adversely affected.

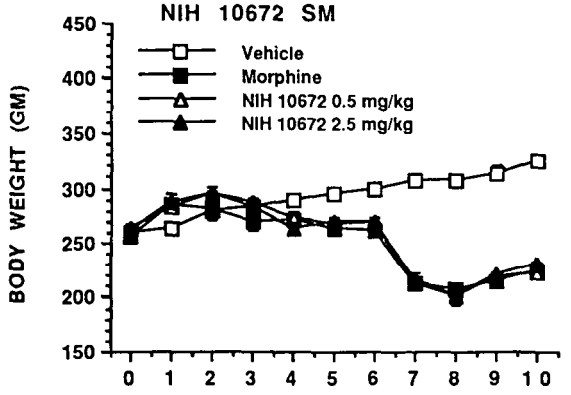
On the other hand, when the drug was abruptly withdrawn, some unexpected signs were noted. One such cluster of behavioral signs was reminiscent of purported doparnine D_1 agonists (grooming and involuntary body and limb and oral movements) and D_2 agonists (increased locomotion). Another cluster of signs typically seen during opioid withdrawal (wet-dog shakes, retching, lying on side, etc.) was also observed. However, two important mu-opioid withdrawal signs were absent, namely, rigid abdominal muscles and vocalization associated with abdominal palpation. In addition, naloxone at a moderately high dose did not precipitate withdrawal although it had an evanescent effect versus agonist signs.

NIH 10672 fulfilled nearly all the criteria normally associated with physical dependence or neuroadaptation. It produced dramatic agonist effects to which tolerance developed. When it was abruptly withdrawn, a withdrawal syndrome was noted. The withdrawal syndrome was completely suppressed by retreatment, and recovery occurred spontaneously. Curiously, some of the withdrawal or rebound signs were not opposite the signs associated with NIH 10672 treatment. However, the withdrawal syndrome was not typically mu-like.

RAT DATA

A. (SM)

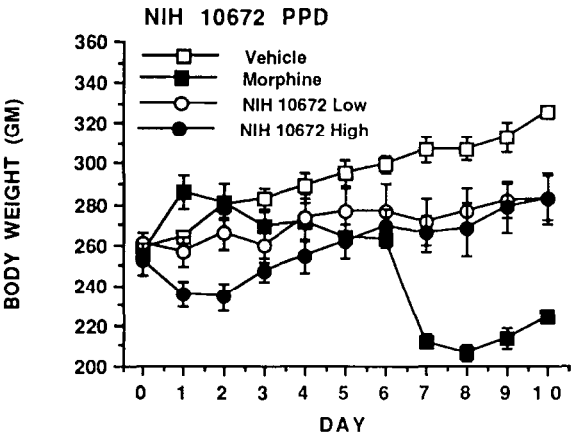
As shown in the accompanying figure (NIH 10672 SM), this compound neither prevented the precipitous loss of body weight nor did it significantly prevent the emergence of overt behavioral withdrawal signs in the withdrawn morphine-dependent rats (see table designated SM and PPD) with rat data.



NIH 10672 [5R-(5α,7α,8β)]-N-[7-(1-pyrrolidinyl)]-1-oxaspiro[4,5][dec-8-yl]-4-benzofuranacetamide·HCl (cont.)

B. (PPD)

NIH 10672 appeared to be relatively free of physical dependence liability in rats. When abruptly discontinued after 6-day, continuous infusion of low- and high-dose regimens, loss of body weight did not occur as it did in the morphine controls. Neither did the abrupt withdrawal of this drug significantly increase the number of overt behavioral withdrawal signs. The statistically significant increase in withdrawal signs on day 8 in the low-dose regimen is considered spurious (see fig NIH 10672 PPD and table designated SM and PPD in rats with rat data).



COMMENT

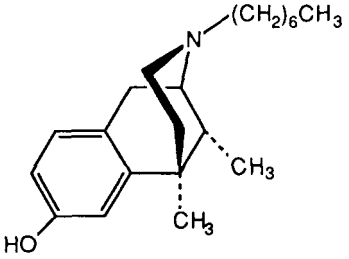
NIH 10672 has potent antinociceptive properties in the mouse which required high doses of naloxone to reverse. The drug neither substituted for morphine nor exacerbated withdrawal in monkeys. Futhermore, it neither substituted for morphine in withdrawn morphine-dependent rats nor did it produce evidence suggesting physical dependence when given continuously for 6 days. NIH 10672 appears to be a potent, non-mu opioid compound and it deserves additional attention. Although the drug produced a withdrawal syndrome in monkeys, it was not mu-like.

Primary Physical Dependence (PPD) and Substitution for Morphine (SM) Studies with NIH 10672 in Continuously Infused Rats

Treatment		Hours in Withdrawal Infusion Day 24 7	48 8	72 9	25 10
		Mean Number of Withdrawal Signs[a,b]			
1.	Vehicle[+] Vehicle Controls	1.8	1.5	0.8	1.5
2.	Morphine + Vehicle Controls[d]	10.3[i]	11.0[i]	10.5[i]	4.3
3.	Morphine + NIH 10672[e] low dose - SM on days 7 and 8 only	6.8[i,j]	16.5[i,j]	5.8[i,j]	7.3[i,j]
4.	Morphine + NIH 10672[f] high dose-SM on days 7 and 8 only	6.0[i,j]	9.5[i,j]	7.0[i,j]	3.0[j]
5.	NIH 10672 + Vehicle[g] low dose-PPD on days 1 thru 6	4.4[j]	6.4[i,j]	5.2[j]	3.6
6.	NIH 10672 + Vehicle[h] high dose-PPD on days 1 thru 6	5.3[j]	1.3	8.0[j]	4.0

[a]Hypersensitivity, squeaking, aggression, wet-dog shakes, rubbing and chewing. [b]One-tailed test (Mann-Whitney U-test), p=0.05 or less compared to vehicle controls. [c]Vehicle was water. Days 1-10; 8 ml/24 hr; N = 4. [d]Morphine·SO_4 50 mg/kg on day 1; 100 mg/kg on day 2; 200 mg/kg on days 3-6; N = 5 on day 5, 4 on days 6-10; Vehicle days 7-10. [e]Morphine·SO_4 as above on days 1-6 and NIH 10672 at 0.5 mg/kg on days 7 and 8; N = 5 on day 3, 4 on days 4-10. [f]Morphine·SO_4 as above on days 1-6 and NIH 10672 at 2.5 mg/kg on days 7 and 8; N = 5 on day 7, 4 on day 8, 3 on day 9. [g]NIH 10672, 0.5 mg/kg on day 1, 1.0 mg/kg on day 2, 2.0 mg/kg on days 3-6. Vehicle days 7-10, N = 5. [h]NIH 10672, 2.0 mg/kg on day 1, 4.0 mg/kg on days 2 and 3, 6.0 mg/kg on days 4 and 5, 8.0 mg/kg on day 6; Vehicle days 7-10, N = 5 on day 6, 4 on days 7-10. [i]P value significance $p < 0.05$ when compared to vehicle. [j]Not significantly different when compared to morphine controls.

NIH 10673 (±)-5,9α–Dimethyl-2-heptyl-2'-hydroxy-6,7-benzomorphan·HCl [α-(±)-N-Heptyl-N-normetazocine.HCl]



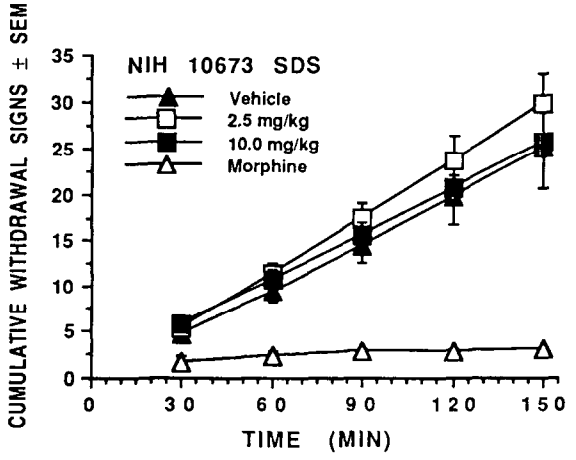
MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 3.6 (1.5 - 8.2)[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.4 (0.1 - 1.4)[a]

[a]Vehicle - 25% propylene glycol and water.

MONKEY DATA
(SDS)

As shown in the fig., NIH 10673 neither substituted for morphine nor exacerbated withdrawal at doses of 2.5 or 10.0 mg/kg.



NIH 10674 (+)-5,9α-Dimethyl-2-heptyl-2'-hydroxy-6,7-benzomorphan·HCl [α-(+)-N-Heptyl-N-normetazocine·HCl]

SEE NIH 10673

MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

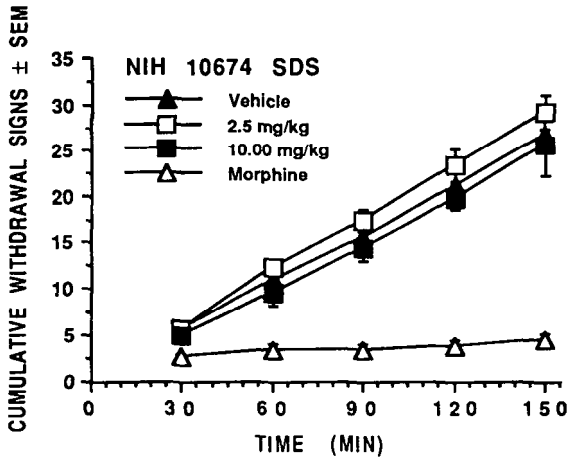
1) TF - 12.9 (6.2 - 26.7)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 3.5 (1.1 - 11.4)[a]

[a]Vehicle - 25% propylene glycol in water.

NIH 10674 (+)-5,9α-Dimethyl-2-heptyl-2'-hydroxy-6,7-benzomorphan·HCl [α-(+)-N-Heptyl-N-normetazocine·HCl] (cont.)

MONKEY DATA
(SDS)

As shown in the fig. below, NIH 10674 neither substituted for morphine nor exacerbated withdrawal at 2.5 and 10.0 mg/kg. Vehicle was 30% propylene glycol in water.



NIH 10675 (-)-5,9α-Dimethyl-2-heptyl-2'-hydroxy-6,7-benzomorphan·HCl [α-(-)-N-Heptyl-N-normetazocine·HCl

SEE 10673

MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

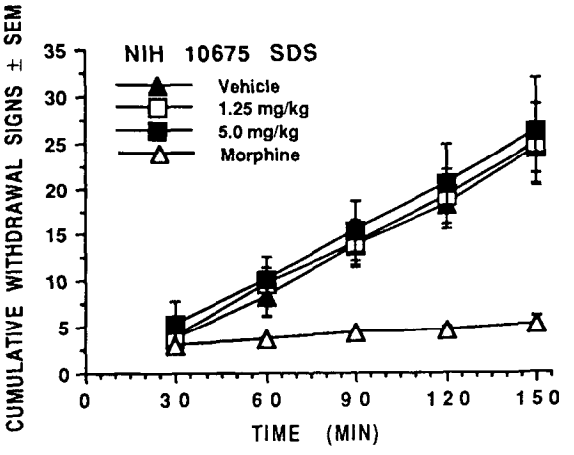
1) TF - 1.7 (1.1 - 2.7)[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.13 (0.02 - 0.99)[a]

[a]Vehicle - 25% propylene glycol and water.

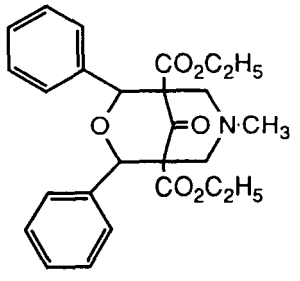
MONKEY DATA
(SDS)

At doses of 1.25 and 5.0 mg/kg, NIH 10675 neither substituted for morphine nor exacerbated withdrawal (see fig. NIH 10675 SDS). In the preliminary experiments, the monkey convulsed 30 m after receiving a total cumulative dose of 11.5 mg/kg within 1 h. Convulsions subsided after the monkey was given 2 i.p. doses of pentobarbital (approximately 40 mg).

NIH 10675 (-)-5,9α-Dimethyl-2-heptyl-2'-hydroxy-6,7-benzomorphan·HCl [α-(-)-N-Heptyl-N-normetazocine·HCl (cont.)



NIH 10676 1,5-Diethyl-7-methyl-9-oxo-2,4-diphenyl-3-oxo-7-azabicyclo-[3.3.1]nonane-1,5-dicarboxylate

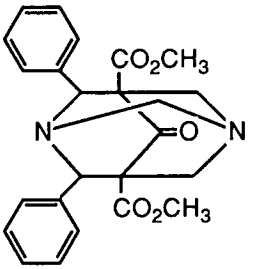


MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - Inactive at 1.0, 10.0 and 30.0[a]

[a]Vehicle - 45% DMSO, 45% propylene glycol and 10% ethyl alcohol.

NIH 10677 5,7-Dimethyl-6-oxo-8,9-diphenyl-1,3-diazaadamantane-5,7-dicarboxylate

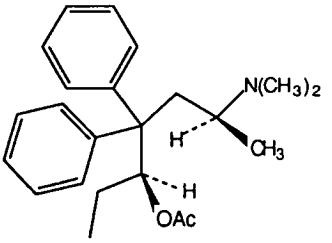


MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - Inactive at 1.0, 10.0 and 30.0[a]

[a]Vehicle - 45% DMSO, 50% propylene glycol and 5% ethanol.

NIH 10679 (-)-α-Acetylmethadol·HCl (LAAM)



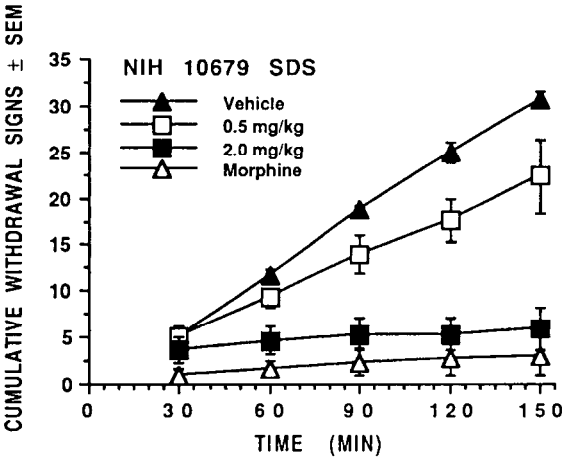
MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 7.2 (3.5 - 15.1)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.4 (0.2 - 0.8)

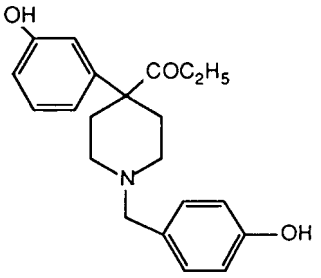
MONKEY DATA
(SDS)

As shown in the graph below, NIH 10679 produced a dose-related suppression of morphine withdrawal signs. Onset of action was a little slower than that of morphine (0.5 h) but duration was longer as judged by the fact that one monkey receiving the high dose did not require morphine until 6 p.m. Potency is estimated as approximately equal to that of morphine. Unfortunately, in the preliminary study, the monkey who received a cumulative dose of 14.5 mg/kg in 45 m died sometime during the night.

NIH 10679 (-)-α-Acetylmethadol·HCl (LAAM) (cont.)



NIH 10680 N-(4-Hydroxybenzyl)-N-norketobemidone·HBr



MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

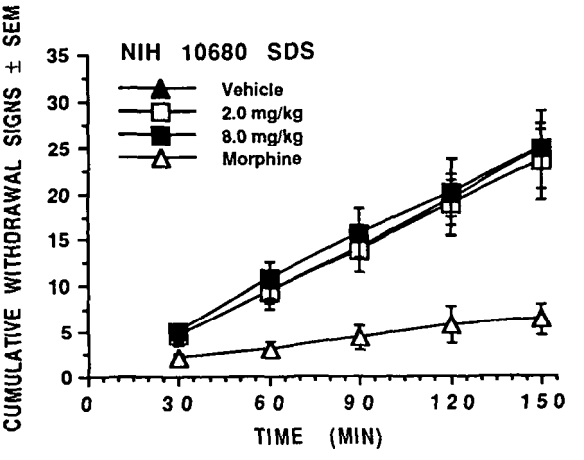
1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - Inactive at 1.0, 10.0 and 30.0[a]

[a]Vehicle - 40% propylene glycol, 10% ethyl alcohol and water.

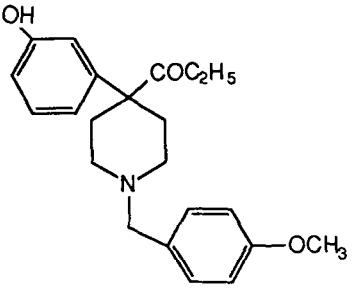
MONKEY DATA
(SDS)

As shown in the accompanying fig., NIH 10680 neither substituted for morphine nor exacerbated withdrawal. Vehicle consisted of 10% DMSO in water.

NIH 10680 N-(4-Hydroxybenzyl)-N-norketobemidone·HBr (cont.)



NIH 10681 N-(4-Methoxybenzyl)-N-norketobemidone·HBr



MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

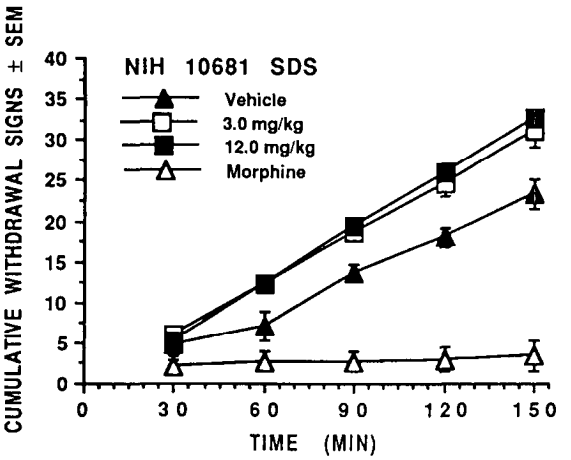
1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0 and 30.0[a]
3) PPQ - Inactive at 1.0 and 30.0[a]

[a]Vehicle - 10% DMSO in water.

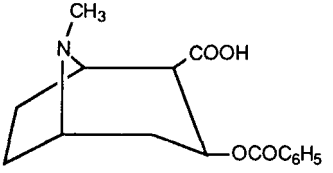
MONKEY DATA
(SDS)

NIH 10681 did not substitute for morphine but the drug produced a non-dose related exacerbation of withdrawal (see fig). The exacerbation may be attributable to an increase in wet-dog shakes and retching. Vehicle was 10% DMSO in aqueous solution.

NIH 10681 N-(4-Methoxybenzyl)-N-norketobemidone·HBr (cont.)



NIH 10682 Benzoylecgonine



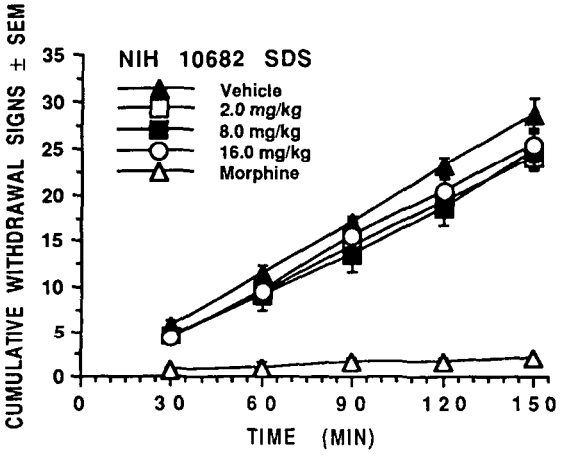
MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 18% at 1.0, 21% at 10.0 and Inactive at 30.0

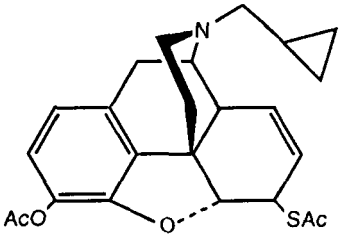
MONKEY DATA
(SDS)

Cocaine and its metabolite norcocaine attenuated withdrawal in withdrawn, morphine-dependent monkeys (reported 1990). Because attenuation was prolonged and not associated with half-life, it was felt that the metabolite benzoylecgonine (BEG) might be involved. However, BEG neither substituted for morphine nor exacerbated withdrawal at doses of 2.0, 8.0 and 16.0 mg/kg intravenously (see fig.).

NIH 10682 Benzoylecgonine (cont.)



NIH 10685 (-)-3-Acetyl-6-ß-(acetylthio)-N-(cyclopropylmethyl)normorphine



MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 2.8 (0.9-9.1)
2) TF vs. M - 28% of 1.0,; 18% and 24% at 10.0; 52% and 76% at 30; 71% at 60.0; and 51% at 80[a]
3) PPQ - 0.01 (0.002 - 0.04)
4) HP - 4.6 (1.4 - 15.4)

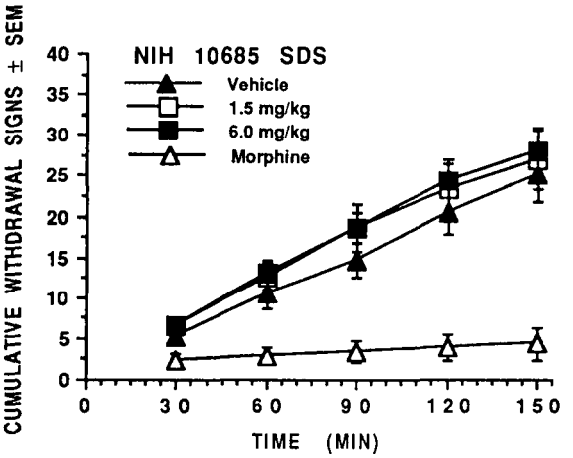
[a]Some of the doses were retested.

Special - Naloxone vs. ED80 of NIH 10685 in TF; AD50 = 1.2(0.3-4.1)

MONKEY DATA
(SDS)

NIH 10685 did not substitute for morphine. The drug appeared to exacerbate withdrawal (see fig. NIH 10685 SDS). However, this non-dose-related intensification was due primarily to an increase in the signs designated retching and vomiting. Since, only one score is allowed per sign per 0.5-h observation period, the intensity of the reaction is not apparent in the illustration. A number of other overt behavioral signs were observed. They included severe tremors, drowsiness, eyelid ptosis, jaw sag, salivation and slowing. At the high dose, one monkey seemed disoriented, it did not recognize its home cage.

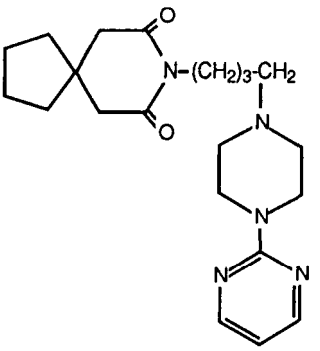
NIH 10685 (-)-3-Acetyl-6-ß-(acetylthio)-N-(cyclopropylmethyl)normorphine (cont.)



COMMENT

In the mouse, the drug had opioid agonist/antagonist properties. The agonist effects may have multiple opioid components because a high dose of naloxone was required to antagonize this action. In the monkey, NIH 10685 displayed mixed kappa (?) opioid and dopaminergic (?) actions.

NIH 10687 Buspirone



MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - 0% at 1.0 and 19% at 30.0
3) PPQ - 14.6 (4.6-46.1)[a]
4) HP - Inactive at 1.0, 10.0 and 30.0[a]

[a]ataxia

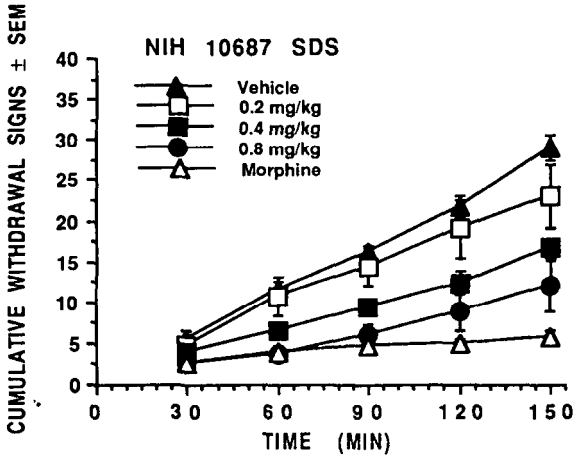
Special Test: Naloxone versus ED80 of Buspirone in PPQ - Inactive at 1.0 and 10.0 mg/kg.

NIH 10687 Buspirone (cont.)

MONKEY DATA

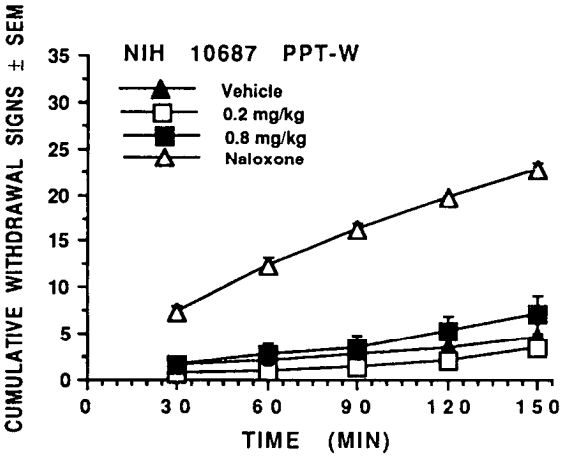
A. (SDS)

The clinically efficacious, non-benzodiazepine anxiolytic agent, buspirone, with a predominant action on the 5-HT1A receptor (Eison, Psychopath., 22, 1989) provided a unique opportunity to probe the serotonin-opioid interaction in morphine-dependent rhesus monkeys (M. mulatta). Buspirone dose-dependently reduced the number of withdrawal signs (see graph NIH 10687 SDS). Specifically, there was a reduction in the number of signs designated lying down, fighting, retching, restlessness, vocalization, vocalizes when abdomen palpated and rigid abdominal muscles. However, the sign wet-dog shakes may have increased slightly in frequency. Some other overt signs were seen, namely, jaw and body sag, ataxia, slowing, eyelid ptosis. In addition. 1 h after receiving buspirone some animals were more aggressive towards the handler (evidence of conflict reduction?). The results suggest that the serotonergic system may play a greater role in opioid withdrawal than was originally believed. Since this drug has also been reported to be relatively free of dependence liability in both preclinical (Balster, *et al.,* J. Clin. Psych., 43, 1982) and clinical studies (Griffiths, *et al.,* Am. J Med., 80, 1986), buspirone may be especially useful in the pharmacotherapy of opioid dependency.

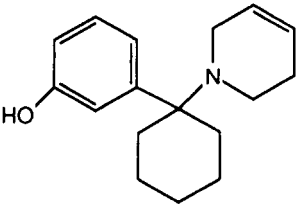


B. (PPT-W)

At doses of 0.2 and 0.8 mg/kg buspirone did not precipitate withdrawal (see graph). Some increased restlessness was noted. Other overt behavior signs noted were ataxia, body sag and immobility. In addition 1 h after receiving buspirone some of the animals were more aggressive towards the handler.



NIH 10700 1-[1-(3-Hydroxyphenyl)cyclohexyl]-3,4-dehydropiperidine·HCl

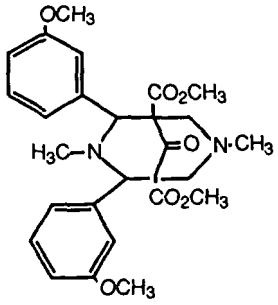


MOUSE DATA-ED50 OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 4.8 (1.7 - 13.4)[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.3 (0.1 - 0.7)
4) HP - 0% at 1; 88% at 3.0 and 100% at 10.0[b]

[a]Straub tail, ataxia, rapid breathing at 1.0. Clonic "popcorn" convulsions at 10.0.
[b]"Popcorn" convulsions at 1.0. At 3.0, 10.0 and 30.0. The hindlimbs were hyperextended so that bottom of feet could not touch the hot-plate surface. One mouse died at 30.0. Vocalization was also noted at 30.0.

NIH 10713 1,5-Dimethyl-2,6-di-(*m*-methoxyphenyl)-3,7-dimethyl-3,7-diazabiocyclo[3.3.1]nonan-9-one 1,5-dicarboxylic acid

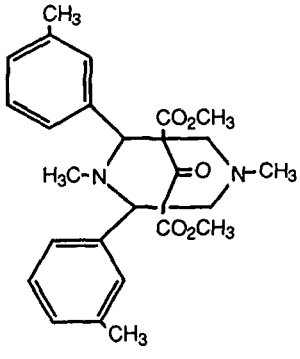


MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - Inactive at 1.0, 10.0 and 30.0[a]
4) HP - Inactive at 1.0, 10.0 and 30.0[a]

[a]Vehicle - 0.55% gum tragacanth suspension. Would not dissolve in dilute acid or DMSO.

NIH 10714 1,5-Dimethyl-2,6-di-(*m*-methylphenyl)-3,7-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one 1,5-dicarboxylic acid

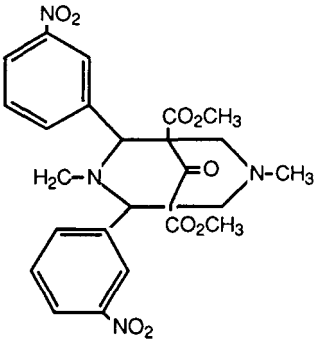


MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 30% at 1.0, 11% at 3.0, 41% at 10.0 and 46% at 30.0[a]
4) HP-Inactive at 1.0, 13% at 10.0 and 30.0[a]

[a]Vehicle - 0.5% gum tragacanth suspension. Would not dissolve in dilute acid or DMSO.

NIH 10715 1,5-Dimethyl-2,6-di-(*m*-nitrophenyl)-3,7-dimethyl-3,7-diazabicyclo[3.3.l]nonan-9-one 1,5-dicarboxylic acid



MOUSE DATA-ED50 OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0 and 10.0, 13% at 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 12% at 1.0, 9% at 10.0 and 18% at 30.0[a]
4) HP - 25% at 1.0, 13% at 10.0 and 25% at 30.0[a,b]

[a]Vehicle - 0.5% gum tragacanth suspension. Would not dissolve in dilute acid or DMSO.
[b]Note - Vehicle showed some activity - 13% effect in HP.

ACKNOWLEDGEMENTS

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Evaluation of New Compounds for Opioid Activity (1991)

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The evaluation of new compounds by the programs at the University of Michigan and the Medical College of Virginia is coordinated by Dr. Arthur E. Jacobson, Laboratory of Medicinal Chemistry, NIDDK, National Institutes of Health, Bethesda, MD. The drugs, which come originally from pharmaceutical companies, universities, government laboratories, and international organizations are submitted to Dr. Jacobson. The staff at NIDDK performs some of the MOUSE ANALGESIA tests. Values obtained in these tests for some representative opioid drugs are given in Table I.

At the UM and MCV laboratories, drug samples arrive from Dr. Jacobson with only the following information: (1) an identifying NIH number, (2) molecular weight, (3) solubility information and (4) a recommended starting dose. After the evaluation is complete and the report submitted to Dr. Jacobson, the submitter is requested to release the chemical structure to include with the evaluation data in the ANNUAL REPORT. The submitter has up to three years before release of the structure is required. When the structure is released all of the data on the compound are reported to the Committee.

DRUG DISCRIMINATION IN RHESUS MONKEYS

We currently use three groups of monkeys to test the discriminative stimulus effects of submitted drugs: one of these groups discriminates the administration of the κ agonist ethylketazocine (EKC); a second group discriminates the μ agonist alfentanil; a third group is treated daily with morphine and discriminates the opioid antagonist naltrexone.

The procedures used with the EKC-trained monkeys have been described by Bertalmio et al. (1982). The monkeys are removed from their home cages each day and seated in primate restraining chairs. These chairs are placed in isolation chambers quipped with two response levers, several stimulus lights and a cup to receive Noyes, banana-flavored pellets. These monkeys are required to make 100 consecutive responses on the correct one of the two levers and receive ten 300-mg food pellets. The right lever is correct if they were given a

subcutaneous injection of 0.0032 mg/kg EKC immediately prior to the start of the cycle. The left lever is designated correct if they were given a sham injection before the start of the cycle. Each cycle lasts 15-min and consists of an initial 10-min black out period followed by a period of as long as 5 min, during which a blue light is illuminated in the chamber and the monkey can respond for food. If the food pellets are delivered before the 5 min period is completed, the lights are extinguished for the remainder of this time. Typically, a daily session consists of several 15 min cycles. During a training session, if EKC is given, it is given on the penultimate cycle of that session. Responding on the drug-appropriate lever is reinforced during that cycle and on the subsequent, final cycle of the day. These last two cycles may be preceded by from zero to four sham cycles on a training day. A training session of six sham cycles is also scheduled from time to time.

With this type of multiple, discrete-cycle training, the animals can be tested with a cumulative dosing procedure. On a test session, the first cycle is preceded by an injection of saline, and prior to subsequent cycles, increasing, cumulative doses of the test drug are administered. One hundred consecutive responses on either lever are reinforced throughout the test session. The test drug is administered in increasing doses until the monkey either responds on the drug-appropriate lever, the response rate falls to less than half of the saline-control rate, or six cycles are given. In the latter situation, it is assumed that the selected dose range is too low, and the test is continued at higher doses on the next test session. Bach test session is preceded and followed by a training session. The criterion for satisfactory performance must be met on each training session that is followed by a test session. This criterion is that at least 90% of the responses during each cycle of a training session must be on the injection-appropriate lever, either sham or EKC.

The procedure for the alfentanil-trained monkeys is similar, but not identical. These animals are also trained and tested in a discrete, multiple-cycle procedure. The main difference between the alfentanil procedure and the EKC procedure is that the alfentanil monkeys are required to make 20 rather than 100 responses, and they receive a single pellet for correct responses. They can receive as many as 10 pellets during the 5-min, food-availability period of each cycle, but each pellet is delivered after 20 responses. Because in this procedure, monkeys can switch from one lever to another following the delivery of food, an additional criterion is added for satisfactory performance. In addition to making 90% or more of their responses on the correct lever, the monkeys must make fewer than 20 responses on the incorrect lever prior to delivery of the first food pellet of each cycle. Tests of the discriminative stimulus effects of submitted drugs in the alfentanil-trained monkeys are also done using a cumulative dosing procedure with dosing criteria identical to those used in the EKC-trained monkeys.

The procedure for studying discriminative stimulus effects in morphine-treated monkeys has been described previously (France and Woods, 1989). Daily sessions consist of between two and six discrete, 15-min cycles with each cycle

TABLE I

MOUSE ANALGESIA. Before submission to The University of Michigan, all compounds are evaluated for anlagesic activity by Dr. Arthur E. Jacobson. Shown below are comparative data (ED_{50} mg/kg/μmol/kg) (95% Confidence Interval) from Hot Plate[a-c] and Nilsen[d] assays

	HOT PLATE				NILSEN			
Compound	**(s.c./mg/kg)**		**(oral, mg/kg)**		**(s.c., mg/kg)**		**(oral, mg/kg)**	
NIH#	**(s.c., μmol/kg)**		**(oral, μmol/kg)**		**(s.c., μmol/kg)**		**(oral, μmol/kg)**	
Morphine Sulfate	0.98	(0.83-1.11)	6.3	(4.7-8.3)	1.3	(1.0-1.7)	8.3	(6.0-11.4)
NIH 0001, 9929	2.9	(2.5-3.3)	18.9	(14.1-24.9)	3.9	(3.0-5.1)	24.9	(18.0-34.1)
Codeine phosphate	6.8	(4.5-10.2)	13.5	(9.7-18.7)	7.4	(4.9-11.0)	14.7	(9.2-23.3)
NIH 0002	17.1	(11.3-25.7)	34.0	(24.4-47.1)	18.6	(12.3-27.7)	37.0	(23.2-58.7)
Levorphanol tartrate	0.2	(0.1-0.3)		----	0.2	(0.16-0.3)	2.5	(1.7-3.7)
NIH 4590	0.5	(0.2-0.7)		———	0.5	(0.4-0.7)	6.2	(4.9-9.1)
Meperidine·HCl	5.3	(4.0-7.1)		----		----		----
NIH 5221	18.7	(14.1-25.0)		----		----		----
(-)-Metazocine·HBr	0.6	(0.5-0.9)	10.6	(8.0-14.1)	0.5	(0.3-0.7)	26.0	(21.0-33.0)
NIH 7569	1.9	(1.4-2.8)	34.1	(25.7-45.3)	1.6	(1.0-2.3)	83.6	(67.5-106.1)

Table 1 continued

Dihydromorphinone·HCl	0.19	(0.15-0.25)	0.9	(0.7-1.2)	0.2	(0.15-0.3)	1.8	(1.5-2.1)
NIH 0123	0.6	(0.5-0.8)	2.8	(2.2-3.7)	0.6	(0.5-0.9)	5.6	(4.7-6.5)
Nalorphine	9.9	(5.7-17.1)		----	23.0	(16.2-32.7)		---
NIH 2105	28.4	(16.4-49.1)		----	66.1	(46.6-94.0		---
Cyclazocine	1.5	(1.1-2.1)		----	0.1	(0.07-0.16)		---
NIH 7981	5.5	(4.1-7.7)		----	0.4	(0.3-0.6)		---
Pentazocine	9.3	(6.7-12.8)		----	6.5	(4.4-8.8)		---
NIH 7958	32.6	(23.55-44.9)		----	22.8	(15.4-30.9)		---
Naltrexone·HCl NIH 8503					No dose response			
Naloxone·HCl NIH 7890					No dose response			

No antinociceptive activity in hot plate assay: Phenobarbital, amobarbital, diazepam, meprobamate, mescaline, oxazepam, flurazepam.

Chlorpromazine·HCl 1.1 (0.9-1.5)
3.2 (2.4-4.2)

a) Eddy and Leimbach (1953); b) Jacobson and May (1965); c) Atwell and Jacobson (1978); d) Perrine, Atwell, Tice, Jacobson and May (1972)

comprised of a 10-min time out during which lever presses have no programmed consequence and a 5-min response period during which green stimulus lights are illuminated and signal the activation of a schedule of stimulus-shock termination. Under these experimental conditions electric shock is scheduled to be delivered to the subject's feet every 15 seconds; monkeys can terminate the lights and postpone scheduled shocks for 30 seconds by pressing five times consecutively *(i.e.,* fixed-ratio 5) the lever appropriate for the solution administered during the first minute of the time out (left lever, saline; right lever, naltrexone). Monkeys receive an injection of saline (0.1 ml/kg) or drug (0.01 mg/kg naltrexone) during the first minute of each time out. On drug training days a single injection of nahrexone is administered during one time out and for that cycle and all subsequent cycles on that day only responding on the right lever postpones shocks. A variable number of saline cycles (0-5) precede the naltrexone cycle and on some days saline is administered during the time out of all cycles. Under these conditions monkeys switch their response choice from the saline lever to the naltrexone lever with complete generalization occurring in all three subjects at a dose of 0.01 mg/kg. Responding on the naltrexone lever is accompanied by other behavioral effects indicative of opioid withdrawal (*e.g.,* irritability, miosis, salivation). Moreover, when saline is substituted for the daily injection of 3.2 mg/kg of morphine monkeys respond predominantly on the naltrexone lever and show directly observable signs of withdrawal; the discriminative stimulus and other effects produced by morphine abstinence are reversed by some opioid agonists (*e.g.,* alfentanil; France and Woods, 1989; France et al., 1990).

For test sessions increasing doses of drug are administered during the first minute of consecutive time outs and five consecutive responses on either lever postpone shocks. In monkeys that receive 3.2 mg/kg of morphine 3 hours earlier, increasing doses of a test compound are administered up to doses that produce an average of at least 80% responding on the naltrexone lever or to doses that disrupt responding and result in the delivery of electric shock. Drugs that do not substitute for naltrexone *(i.e.,* precipitate withdrawal) are also studied for their ability to reverse responding on the naltrexone lever in morphine-abstinent *(i.e.,* withdrawn) subjects. Test compounds are studied using a cumulative-dosing procedure in morphine-abstinent monkeys up to doses that reverse completely responding on the naltrexone lever (< 20%) or to doses that disrupt responding. Some compounds that substitute for naltrexone also are studied for their capacity to prevent the effects of cumulative doses of opioid agonists. Monkeys that receive saline three hours earlier, rather than the daily injection of morphine, receive saline (control) or a single injection of test compound during the first cycle and increasing doses of agonist (alfentanil or morphine) during subsequent cycles. Agonists are administered up to doses that produce a switch from the naltrexone lever to the saline lever or to doses that disrupt responding and result in the delivery of electric shock.

DEPENDENCE EVALUATION IN RHESUS MONKEYS

The single-dose suppression (SDS) test determines the ability of a drug to

suppress the signs of withdrawal in monkeys which have been made dependent by the chronic administration of morphine (3 mg/kg every six hours). Compounds suspected of having morphine- antagonist properties are tested for their ability to precipitate the withdrawal syndrome in nonwithdrawn (NW) morphine-dependent monkeys. Nondependent monkeys (Normals) are used to determine whether the acute effects of the test drug are reversible by nalorphine or naloxone. In a primary dependence (PDS) study, non-dependent monkeys receive the test drug every six hours for *30* days to determine whether withdrawal signs will appear when the animals are challenged with an antagonist or when drug administration is discontinued.

Details of these techniques have been presented in the ANNUAL REPORT to the Committee in 1963 (Minutes of the 25th Meeting) by Deneau and Seevers (1963) and by Villarreal (1973).

ANALGESIA IN RHESUS MONKEYS

The tail withdrawal procedure used to study analgesic effects of test compounds in rhesus monkeys has been described previously (Dykstra and Woods, 1986). Monkeys are restrained loosely at the neck and arms while seated in Plexiglas primate chairs. For tests of tail withdrawal latency, the lower 10-12 cm of the shaved tail is immersed in a thermos containing water at 40°, 50°, or 55° C and the latency until the tail is withdrawn from the thermos is recorded for each monkey at each temperature. When the tail is not withdrawn within 20 seconds (cut-off latency) the experimenter removes the thermos and a latency of 20 seconds is recorded. Experimental sessions begin with several exposures to 40° C water. Four or five monkeys are tested consecutively and the time between tail immersions for individual monkeys is 5 minutes. Generally, 40° C water does not produce tail withdrawal in rhesus monkeys (Dykstra and Woods, 1986); however, if a monkey fails to keep its tail in 40° C water for 20 seconds on at least 3 of 4 immersions, that animal is not tested further for that particular session. In a subsequent pre-test component, tails are immersed in 40°, 50°, and 55° C water. The order in which the three temperatures are presented is varied among subjects. If the latencies for tail withdrawal in the pre-test component are at or near 20 seconds for 40° C water and less than 5 seconds for 55° C water, monkeys receive the test compound. The test is identical to the pre-test, except that monkeys receive s.c. injections of drug 10 minutes prior to tail immersion. The time between immersions for individual subjects is 5 minutes and the order in which temperatures are presented varies among subjects and across cycles. The interinjection interval typically is 30 minutes and between four and six doses are studied in a single experiment using the cumulative dosing procedure. For some studies a single dose of an opioid antagonist is administered prior to the test compound and for other studies a single dose of test compound is administered prior to increasing doses of a μ (*e.g.,* alfentanil) or κ (*e.g.,* U-50,488) opioid agonist.

RESPIRATORY FUNCTION IN RHESUS MONKEYS

The effects of test compounds on ventilatory function are studied in rhesus monkeys breathing air or 5% CO_2 in air (France and Woods, 1990; Howell et al., 1988). Monkeys are restrained at the neck and waist while seated in a Plexiglas primate chair. Normal air or 5% CO_2 in air is delivered at a rate of 10 1/min into a sealed helmet placed over the subject's head. Changes in pressure within the helmet are measured and recorded by a transducer and a microprocessor, and are transformed according to known standards to frequency of respiration (f) in breaths/minute and to tidal volume (V_T) in ml/inspiration. Data are recorded continuously during 23-minute exposures to air alternating with 7-minute exposures to CO_2. The last 3 minutes of exposure to CO_2 are used for data analyses and are compared to the last 3 minutes of exposure to air only. Increasing doses of drug are administered during the first minute of consecutive time outs so that the interinjection interval is 30 minutes. For some studies a single injection of an opioid antagonist is administered prior to increasing doses of a test compound and for other studies a single injection of test compound is administered prior to cumulative doses of a standard compound (*e.g.,* alfentanil).

SELF-ADMINISTRATION BY MONKEYS

Tests of self-administration determine the ability of the drug to maintain responding in monkeys trained to self-inject codeine. Each of at least three monkeys is studied with saline as a negative control and a number of doses of the test compound until a maximum rate of responding was obtained or until, in the absence of evidence of a reinforcing effect, observable changes in behavior are produced by the compound.

The schedule of intravenous drug delivery is a fixed-ratio 30; when a light above a lever is illuminated, the 30th response produce a five-set intravenous drug injection accompanied by another light that is illuminated during drug delivery. After each injection, a ten-min timeout condition is in effect, during which responses have no scheduled consequence and neither light is illuminated. Each of the two daily sessions consist of 13 injections or 130 min, whichever occurs first. Other details of the procedure and initial findings with a variety of narcotics are given in previous reports (*e.g.,* Woods, 1977; 1980).

Doses of the drugs are typically described in terms of mg/kg/ injection (inj). Duplicate observations of codeine (0.32 mg/kg/inj) and of saline are obtained for each monkey. A saline substitution is conducted before and after the series of observations on a test drug; the control rates of codeine- reinforced responding are obtained by a random sampling of two sessions interpolated between the drug-substitution sessions. These data are represented in the following graphs with individual symbols for each of the monkeys; each symbol is the mean of duplicate observations for a given dose in each monkey. The closed circles indicate the averaged data for observations on the subset of monkeys used to study each drug under each of the experimental conditions. In all cases, the

rates of responding given are those calculated during only the fixed-ratio portion of each session.

DISPLACEMENT OF RADIOLABELED LIGAND BINDING

Details of the binding assay based on the displacement of ^{3}H-etorphine in rat brain membranes have been described previously (Medzihradsky et al, 1984). Briefly, aliquots of a membrane preparation from rat cerebrum are incubated with ^{3}H-etorphine in the presence of 150 mM NaCl, and in the presence of different concentrations of the drug under investigation. Specific, *i.e.,* opioid-receptor-related interaction of ^{3}H-etorphine is determined as the difference in binding obtained in the absence and presence of an appropriate excess of unlabeled etorphine. The potency of the drugs in displacing the specific binding of ^{3}H-etorphine is determined from log-probit plots of the data. See Table II for representative results with different opioids.

To enhance the characterization of novel opioids, we are also investigating their selectivity in binding to μ-, δ-, and κ-opioid receptors in membranes from monkey brain cortex. Thus, we are now providing EC_{50} values of the tested compounds in displacing the following radiolabeled opioid ligands:

etorphine (nonselective, reflects opioid character),
sufentanil or Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (DAMGO);
(μ selective),
[D-Pen^2-D-Pen^5]enkephalin (DPDPE;δ selective),
U-69,593 (κ selective).

Using the receptor-specific assays, we have described the selectivity of various established opioids in brain membranes of different species (Clark *et al.,* 1988). The selection of ***monkey brain*** as the tissue for the selective binding assays strengthens the correlation between this *in vitro* assessment and the behavioral evaluation of the tested compounds. In the **ANNUAL REPORT,** the results of the selective binding assays are listed under "Binding in monkey brain cortex". See Table III for representative results with different opioids in rat and monkey brain.

Within our goal to enhance the molecular characterization of novel opioids (Medzihradsky, 1987) we have established functional assays for assessing receptor-effector interactions, reflecting receptor coupling to regulatory G protein and adenylate cyclase, respectively. The methods are based on the stimulation of brain GTPase and inhibition of adenylate cyclase by opioid agonists, processes blocked by antagonists (Clark and Medzihradsky, 1987; Carter and Medzihradsky, 1991). We are presently evaluating the quantitative responses of partial and irreversible agonists in these assays.

TABLE II

ECSO's of representative opioids for displacement of 0.5 nM ^{3}H-etorphine from rat brain membrane, and inhibition of the twitch of the mouse vas deferens preparation.

Compound	BINDING* EG_{50} (nM)	MVD
DPDPE	---	5.52
U50,488	---	6.29
Fentanyl	36.2	37.1
DAMGO	23.9	81.3
Etorphine	0.37	0.0068
(-)Cyclazocine	0.53	11.9
Naltrexone	0.63	---
Bremazocine	1.42	0.29
UM 1071R**	1.55	---
Sufentanil	1.60	4.43
(-)SKF 10047	3.93	---
Ethylketazocine	6.60	11.6
Ketazocine	14.1	1.18
Morphine	23.6	395
DSLET	43.0	1.71
Dextrorphan	<6000	1010

* In the presence of 150 mM NaCl.

**1R-5R-9R-2"R-5,9-dimethyl-2'-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan hydrochloride

TABLE III

Inhibition of radiolabeled sufentanil, DPDPE and U69,593 binding in rat and monkey brain. In membranes from rat cerebrum and monkey brain cortex, the inhibition of specific equilibrium binding of 0.5 nM [^{3}H]sufentanil, 1.5 nM [^{3}H]DPDPE and 1.5 nM [^{3}H]U69,593 by five different concentrations of the listed compounds was investigated in the presence of 150 mM NaCl (modified from Clark et al., 1988).

Compound	EG_{50} (nM) [^{3}H]Sufentanil	[^{3}H]DPDPE	[^{3}H]U69,593
Rat cerebrum			
DAMGO	13.2	690	
Sufentanil	1.25	45.0	
Morphine	31.4	422	
ß-FNA	6.99	43.9	
ß-CNA	1.29	7.48	
Naloxone	6.37	14.3	
Etorphine	0.60	1.13	
Buprenorphine	1.07	1.12	
Bremazocine	1.79	1.12	
Superfit	576	16.5	
DSLET*	121	1.05	
ICI-174,864	58900	59.0	
DPDPE	7720	6.44	
U50,488	7230	13100	
U69,593	38000	13400	
Monkey cortex			
Sufentanil	1.18	81.1	> 10000
DPDPE	18900	4.21	> 10000
U69,593	10700	17000	8.41

*(D-Ser^2,Leu^5)-enkephalin-Thr^6

ISOLATED, ELECTRICALLY-STIMULATED MOUSE *VAS DEFERENS* PREPARATION

The development of new, highly selective antagonists such as the reversible, noncompetitive κ receptor antagonist norbinaltorphimine (Smith *et al.,* 1989) and the competitive δ receptor antagonist ICI-174864 have made possible the evaluation of selectivity of opioid agonists and antagonists by use of the mouse *vas deferens* preparation. Male, albino ICR mice, weighing between 25 and 30 g, are used. The mice are decapitated, the *vas deferentia* removed, and 1.5 cm segments are suspended in organ baths which contain 30 ml of a modified Kreb's physiological buffer. The buffer contains the following (mM): NaCl, 118; KCl, 4.75; $CaCl_2$, 2.54; $MgSO_4$, 1.19; KH_2PO_4, 1.19; glucose, 11; $NaHCO_3$, 25; pargyline HCl, 0.3; and disodium edetate, 0.03. The buffer is saturated with 95% O_2 - 5% CO_2 and kept at 37° C. The segments are attached to strain gauge transducers and suspended between two platinum electrodes. After a 30-min equilibration period, the segments are stimulated once every 10 sec with pairs of pulses of 2 msec duration, 1 msec apart and at supramaximal voltage. See Table II for potencies of representative agonists.

The following antagonists are studied: naltrexone HCl, ICI- 174864 [N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH] and norbinaltorphimine. The antagonists are added to the organ baths 15 minutes before the determination of cumulative concentration-effect relationships for the various agonists. See Table IV for the potencies of different competitive antagonists studied in relation to prototypic agonists. EC_{50}'s are calculated by probit analysis, and pA_2 values are determined to assess relative potencies of antagonists.

All drugs which are submitted for evaluation are studied in the following manner: 1) the submitted drug is tested on the *vas deferens* preparation in the absence and in the presence of a concentration of naltrexone sufficient to block μ, κ and δ receptors. 2) If the submitted drug inhibits the twitch and its actions are blocked by naltrexone, it is evaluated further in the absence and presence of ICI-174864 and norbinaltorphimine used in concentrations at which these antagonists are selective for δ and κ receptors, respectively. 3) If the submitted drug is a partial agonist or devoid of agonistic activity at opioid receptors, it is evaluated further as an antagonist against the following agonists: sufentanil (μ selective), DSLET (δ selective) and U50,488 (κ selective). If the submitted drug has antagonistic activity against any or all of the receptor-selective agonists or upon any of the other preparations used in the Drug Evaluation Unit, the type of antagonism (competitive, noncompetitive, irreversible) is determined. For further details of the procedure and for a description of experiments in which β-funaltrexamine was used see Smith (1986). Drugs studied in the preparation prior to 1987 were evaluated with the protocol reported in the 1985 Annual Report.

TABLE IV

Potencies of antagonists assessed in the mouse vas deferens

	pA_2, values* determined with three agonists		
	Sufentanil (μ)	U50,488(κ))	DSLET (δ)
Antagonist			
Naltrexone	8.76	7.74	7.41
Naloxone	7.99	6.90	7.35
Cyprodime**	7.41	6.15	5.98
Nalbuphine	7.23	6.31	5.76
Naltrindole	7.71	7.38	9.44
ICI-174,864	<5.00	<5.00	7.90

*The pA_2 value is the negative logarithm of the molar concentration of antagonist necessary to shift the agonist concentration-effect curve to the right by a factor of 2-fold.

**(-)-N-cyclopropylmethyl)-4,14-dimethoxymorphinan-6-one

SUMMARY OF TESTS PERFORMED

The compounds which were evaluated at the University of Michigan during the past year, and the individual tests which were performed are shown in Table V. Also shown are dates of Reports to the Biological Coordinator, Dr. A.E. Jacobson, in which results are reported.

TABLE V

SUMMARY OF TESTS PERFORMED

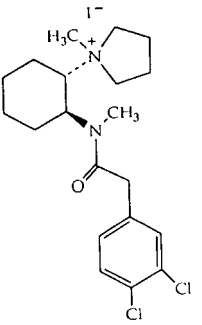
NIH	CHEMICAL CLASS OR GENERIC NAME	SA	MVD	BIND	DD	ANAL	RSP	REPORT
10577	Benzeneacetamide	-	+	+	-	-	-	10/17/88
10578	Benzeneacetamide	-	+	+	-	-	-	10/17/88
10585	Ethyloxymorphone	-	+	+	-	-	-	12/06/89
10593	Buprenorphine	-	Insol.	Insol.	-	-	-	06/06/90
10629	Morphinan	-	Insol.	+	-	-	-	01/03/89
10637	Phenylpiperidine	-	+	+	-	-	-	12/06/89
10639	Phenylpiperidine	-	+	+	-	-	-	11/20/90
10645	Naloxone	-	+	+	-	-	-	12/06/89
10646	Nalozone	-	+	+	-	-	-	12/06/89
10647	Phenylpiperidine	-	+	+	-	-	-	12/06/90
10656	Naloxone	-	+	+	+	+	+	12/08/89 05/07/91
10657	Dipyridine piperidone	-	+	+	-	-	-	01/03/89
10658	Dipyridine piperidone	-	+	MBC	-	-	-	901/03/89 11/20/90
10663	Nalmefene	-	+	+	-	-	-	02/12/90
10665	Etonitazene	-	+	+	-	-	-	06/06/90
10666	6,7-Benzomorphan	-	+	+ MBC	-	-	-	06/06/90
10667	6,7-Benzomorphan	-	+	+	-	-	-	06/06/90
10668	Haloperidol	-	+	+	+	-	-	06/20/90
10669	See NIH 10647	-	+	+	-	-	-	06/06/90
10672	Benzeneacetamide	+	+	+	+	+	+	06/06/90 05/07/91
10673	6,7-Benzomorphan	-	+	+ MBC	-	-	-	06/20/90 11/07/90
10674	6,7-Benzomorphan	-	+	+ MBC	-	-	-	06/20/90 11/07/90

Table IV (continued)

NIH	CHEMICAL CLASS OR GENERIC NAME	SA	MVD	BIND	DD	ANAL	RSP	REPORT*
10675	6,7-Benzomorphan	-	+	+ MBC	-	-	-	11/20/90 11/07/90
10676	Diphenyl nonane	-	+	+	-	-	-	11/20/90
10677	Diphenyl adamantane	-	+	+	-	-	-	11/20/90
10679	Acetylmethadol	-	+	+ MBC	-	-	-	05/07/91
10681	Norketobemidone	-	+	+	-	-	-	11/20/90
10685	Morphine	-	+	+	+	+	+	05/07/91
10686	6,7-Benzomorphan	-	+	+	-	-	-	02/08/91
10691	6,7-Benzomorphan	-	+	+	-	-	-	01/02/91
10694	6,7-Benzomorphan	-	+	+	-	-	-	02/08/91

*Date report was submitted to CPDD Biological Coordinator.
MBC = Monkey Brain Cortex

NIH 10577 1*S*,2S-*trans*-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl]benzeneacetamide methiodide [1S,2S-U50,488 methiodide]



MOUSE ANALGESIA
Hot Plate: Inactive (5 mg/kg or 20 mg/kg)

DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

6.3% inhibition at 6 uM in the presence of 150 mM NaCl.

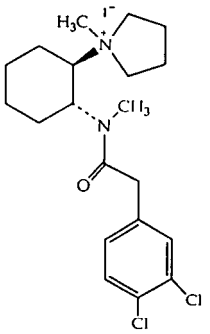
MOUSE *VAS DEFERENS* PREPARATION

NIH 10577 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-8}, M to 10^{-4} M. Concentrations between 10^{-6} M and 10^{-4} M caused an inhibition of the twitch. Because of limitations in the supply of this drug higher concentrations could not be studied, and EC50's could not be determined. At a concentration of 10^{-4} M, NIH 10577 produced a 71.9 $\pm$ 3.4% inhibition of the twitch. In the presence of naltrexone, 10^{-7} M, the response at a concentration of 10^{-4} M was a 49.8 $\pm$ 13.4% inhibition of the twitch. In the presence of ICI-174,864 (an antagonist selective for δ receptors), 10^{-7} M, the response at 10^{-4} M was a 61.5 $\pm$ 20.4% inhibition of the twitch (n=3).

SUMMARY

NIH 10577 was an opioid agonist of extremely low potency.

NIH 10578 1*R*,2R-*trans*-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methiodide [1R,2R-U50,488 methiodide]



MOUSE ANALGESIA
Hot Plate: Inactive (5mg/kg or 20 mg/kg)

DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

13.2% inhibition at 6 uM in the presence of 150 mM NaCl.

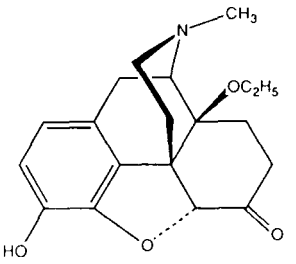
MOUSE *VAS DEFERENS* PREPARATION

NIH 10578 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-8} M to 3 x 10^{-5} M. Concentrations between 10^{-6} M and 3 x 10^{-5} M caused an inhibition of the twitch. Because of limitations in the supply of this drug higher concentrations could not be studied, and EC_{50}'s could not be determined. At a concentration of 3 x 10^{-5} M, NIH 10578 produced a 32.9 $\pm$ 7.1% inhibition of the twitch. In the presence of naltrexone, 10^{-7} M, the response at a concentration of 3 x 10^{-5} M was a 12.1 $\pm$ 2.5% inhibition of the twitch. In the presence of ICI-174,864 (an antagonist selective for δ receptors), 10^{-7} M, the response at 3 x 10^{-5} M was a 35.9 $\pm$ 10.7% inhibition of the twitch (n=3). At a concentration of 10 M this drug did not block the inhibitory actions of sufentanil, DSLET or U50,488H.

SUMMARY

NIH 10578 had very low potency in both preparations. It may have had slight opioid agonist actions in the *vas deferens*

NIH 10585 14-O-Ethyloxymorphone



DISPLACEMENT OF SPECIFIC [^{3}HIETORPHINE BINDING

EC_{50} of 2.32 nM in the presence of 150 mM NaCl.

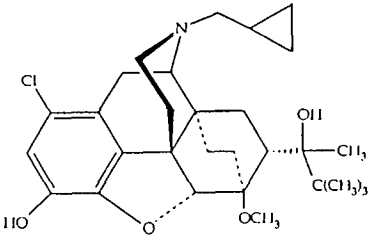
MOUSE *VAS DEFERENS* PREPARATION

	Inhibitory EC_{50} (nM)	***Maximum Response***
Drug alone	18.7	100%
After naltrexone	546.0	100%
After β-funaltrexamine	78.6	90.9%
After ICI-174,864	46.2	100%

SUMMARY

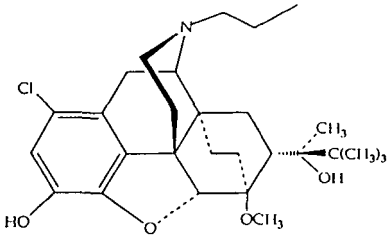
NIH 10585 was a potent compound in both preparations with its agonist actions predominantly mediated through the u receptor in the vas deferens preparation.

NIH 10593 1-Chloro-17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-6,14-ethenomorphinan-7-methanol [1-Chlorobuprenorphine]



NIH 10593 was insoluble and could not, therefore, be evaluated in either the binding or smooth muscle assays.

NIH 10629 1-Chloro-17-(*n*-propyl)-α-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-6,14-ethenomorphinan-7-methanol



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

NIH 10629 was insoluble and, therefore, EC_{50},s could not be determined.

MOUSE *VAS DEFERENS* PREPARATION

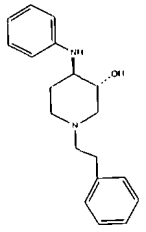
NIH 10629 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-10} M to 3 x 10^{-5} M. No concentration of this drug inhibited the contractions of the vas deferens and it was evaluated as an antagonist. This drug, starting at a concentration of 0.1 nM, caused a noncompetetive antagonism of the actions of U-50,488. pA_2, values against the following agonists were:

Agonist	*pA_2 ± S.D.*	*Slope*
Sufentanil	8.49 ± 0.63	1.74
DSLET	5.59 ± 0.29	0.87

SUMMARY

NIH 10629 is a potent, noncompetitive antagonist (0.1 nM) at κ receptors and equivalent to naltrexone in its potency at u and δ receptors.

NIH 10637 trans-3-Hydroxy-4-anilino-1-(2-phenyllethyl)piperidine



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (8.2% inhibition at 6 uM) in the presence of 150 mM NaCl.

NIH 10637 *trans*-3-Hydroxy-4-anilino-1-(2-phenylethyl)piperidine

continued...

MOUSE ***VAS DEFERENS*** PREPARATION

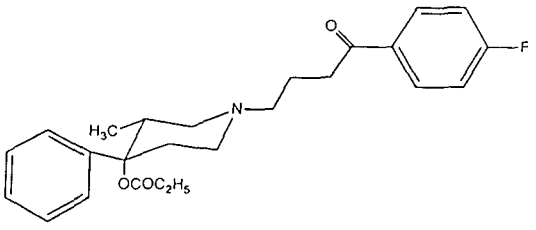
	Inhibitory EC_{50} (nM)	***Maximum Response***
Drug alone	51.7	50.5%
After naltrexone	31.0	37.8%
After norbinaltorphimine	8.93	49.4%
After ICI-174,864	66.4	46.4%

NIH 10637 was also a weak antagonist at u and κ receptors, but quantities were insufficient for determination of pA_2, values.

SUMMARY

NIH 10637 had very little opioid activity in either preparation. There was a suggestion of antagonist activity in the *vas deferens* preparation at high concentrations.

NIH 10639 3*R*,4*S*-(+)-N-3-(p-Fluorobenzoyl)propyl-3-methyll-4-phenyl-4-pro-pionyloxypiperidine. fumarate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 4 1.3 nM in the presence of NaCl.

NIH 10639 3*R*,4*S* -(+)-N-3-(p-Fluorobenzoyll-3-methyl-4-propionyloxypiperidine. fumarate

continued...

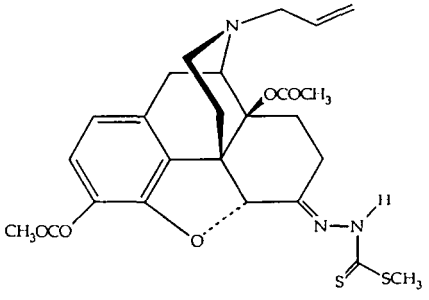
MOUSE *VAS DEFERENS* PREPARATION

	Inhibitory EC_{50}, (nM)	***Maximum Response***
Drug alone	56.1	100%
After naltrexone	2325.8	100%
After norbinaltorphimine	27.8	100%
After ICI-174,864	70.1	100%

SUMMARY

NIH 10639 was a potent displacer of etorphine, and it was a u agonist in the mouse *vas deferens.*

NIH 10645 Naloxone methyldithiocarbohydrazone 3,14-diacetate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 18.3 nM in presence of 150 mM NaCl.

NIH 10645 Naloxone methyldithiocarbohydrazone 3,14-diacetate

continued...

MOUSE *VAS DEFERENS* PREPARATION

NIH 10645 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations that ranged from 10^{-9} M to 3 x 10^{-5} M. This drug acted as an antagonist with some nonopioid agonistic activity.

	Inhibitory EC_{50} ***(nM)***	***Maximum Response***
Drug alone	532	100%
After naltrexone	720	100%

pA_2 values against the following agonists were:

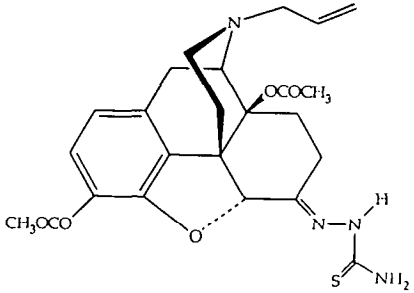
Agonist	$pA_2 \pm$ ***S.D.***	***Slope***
Sufentanil	7.69 $\pm$ 0.51	1.41
DSLET	7.88 $\pm$ 0.49	1.16

The U50,488 concentration-effect curve was shifted to the right at the 3 x 10^{-6} M concentration of U50,488, and the maximum response was reduced to 55.5 % at the 10^{-5} M concentration.

SUMMARY

NIH 10645 had significant affinity for the etorphine binding site, and it displayed opioid antagonist actions with respect to each agonist. It was less potent than naltrexone in both preparations.

NIH 10646 3.14-Naloxone diacetate thiosemicarbazone



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 17.4 nM in the presence of 150 mM NaCl.

NIH 10646 3,14-Naloxone diacetate thiosemicarbazone

continued...

MOUSE *VAS DEFERENS* PREPARATION

NIH 10646 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. No concentration of this drug inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2, values against the following agonists were:

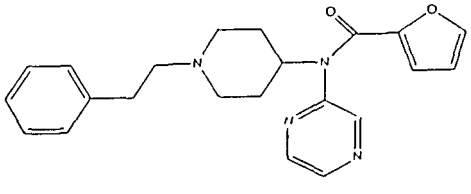
Agonist	*pA_2 + S.D.*	*Slope*
Sufentanil	7.88 + 0.41	1.21
DSLET	6.86 + 0.43	1.24

pA_2 values were not calculated for its antagonism of U50,488 because that antagonism was either irreversible or noncompetitive, but significant antagonism occurred at a concentration of 10^{-8} M.

SUMMARY

NIH 10646 was a potent compound in both assays; antagonist action in the vas *deferens* was found with each of the prototypic agonists. There was TLC evidence that the compound was impure.

NIH 10647 l-(2-Phenylethyl)-4-(N-(2-pyrazyl)-2-furoylamido)piperidine.HC1
(Also NIH 10669; information repeated for comparison from the 1990 Annual Report)



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 91.0 nM in the presence of 150 mM NaCl

MOUSE *VAS DEFERENS* PREPARATION

NIH 10647 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. No concentration of this drug inhibited the contractions of the vas deferens and

NIH 10647 1-(2-Phenylethyl)-4-(N-(2-pyrazyl)-2-furoylamido)piperidine.HC1

continued...

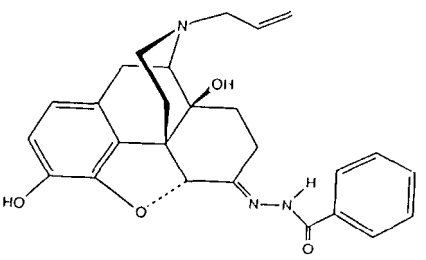
it was evaluated as an antagonist. pA_2, values against the following agonists were:

Agonist	***$pA_2 \pm$ S.D.***	***Slope***
Sufentanil	6.84 ± 0.42	1.24
U50,488	5.80 ± 0.41	1.21
DSLET	6.45 ± 0.35	1.05

SUMMARY

In the mouse *vas deferens,* NIH 10647 was a relatively nonselective, competitive opioid antagonist.

NIH 10656 Naloxone benzoylhydrazone



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 6.08 nM in presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

NIH 10656 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. No concentration of this drug inhibited the contractions of the vas deferens and

NIH 10656 Naloxone benzoylhydrazone

continued...

it was evaluated as an antagonist. pA_2, values against the following agonists were:

Agonist	*pA_2 ± S.D.*	*Slope*
Sufentanil	8.30 ± 0.36	1.07
U50,488	7.44 ± 0.28	0.83
DSLET	7.53 ± 0.49	1.43

DRUG DISCRIMINATION

NIH 10656 substituted for naltrexone in morphine-dependent rhesus monkeys. It was similar in potency to naltrexone. The apparent affinity of NIH 10656 was estimated following the procedure described previously (France *et al.,* 1990); the pA_2 was 7.80.

ANALGESIA STUDIES

NIH 10656 was without effect at 50° or 55° C. up to 0.1 mg/kg. At 0.3 and 1.0 mg/kg, NIH 10656 produced a 20% effect at 50° C. It antagonized the effects of alfentanil, ethylketazocine, and U50,488; it was more potent as an antagonist of alfentanil. A dose of 0.1 mg/kg NIH 10656 shifted alfentanil potency by 3-fold. 1.0 mg/kg NIH 10656 was required to shift U50,488's potency by 3-fold.

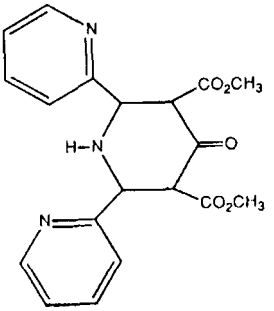
RESPIRATORY FUNCTION

NIH 10656 had no reliable effects up to 3.2 mg/kg. NIH 10656 at 0.1 mg/kg shifted the effects of alfentanil 3-fold to the right in monkeys breathing air or CO_2.

SUMMARY

NIH 10656 was quite potent in both *in vitro* assays. It was less potent than naltrexone in the binding assay, but comparable to naltrexone in the *vas deferens.* In the *in vivo* assays, it was without agonist effect, and was an effective antagonist against μ and κ agonists; it was more potent as a μ antagonist in the behavioral assays.

NIH 10657 Dimethyl 2,6-di(2-pyridine)-4-piperidone-3,5-dicarboxylate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (4.4% inhibition at 6 μM) in presence of 150 mM NaCl.

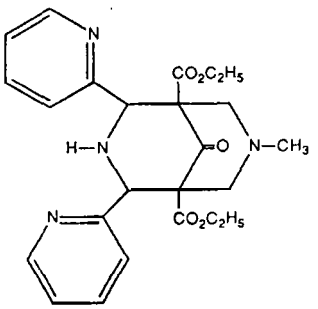
MOUSE *VAS DEFERENS* PREPARATION

NIH 10657 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-10} M to 3 x 10^{-5} M. This drug did not inhibit the twitch at any concentration. NIH 10657 (10^{-8} M) did not antagonize responses to sufentanil, DLSET or U50,488.

SUMMARY

NIH 10657 failed to have significant opioid activity in either preparation.

NIH 10658 Diethyl 2,4-di(2-pyridine)-3,7-diazebicyclo[3.3.1]nonane-9-one 1,5-dicarboxylate



BINDING IN MONKEY BRAIN CORTEX

This finding was obtained in displacing the specific equilibrium binding of (a) 0.5 nM [^{3}H]DAGO (μ-selective assay), (b) 1.5 nM [^{3}H]DPDPE (b-selective assay), and 1.5 nM [^{3}H]U69,593 (K-selective assay) in membranes from monkey brain cortex suspended in 50 mM Tris.HCl buffer (pH 7.4) containing 150 mM NaCl. EC_{50} values for the assays are:

(a)	μ-receptor	358 nM
(b)	δ-receptor	1.3 % inhibition at 6 μ M
(c)	κ-receptor	984 nM

NIH 10658 Diethyl 2,4-di(2-pyridine)-3,7-diazebicyclo[3.3.1]nonane-9-one 1,5-dicarboxylate

continued...

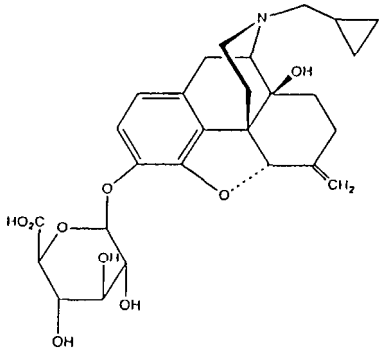
MOUSE *VAS DEFERENS* PREPARATION

	Inhibitory EC_{50} (nM)	***Maximum Response***
Drug alone	429	100%
After naltrexone	976	89.9%
After norbinaltorphimine	1050	70.0%
After ICI-174,864	131	100%

SUMMARY

NlH 10658 had low affinity for both μ and κ binding sites and displayed agonist activity in the *vas deferens* that was antagonized by NBNI

NIH 10663 Nalmefene-3β-D-glucuronide



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 1624 nM in presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

NIH 10663 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. This drug did not inhibit the twitch at any concentration. NIH 10663 caused parallel shifts to the right in the concentration-effect curve for sufentanil. The pA_2, values for antagonism of sufentanil was 5.44 $\pm$ 0.32 (λ = 1.19). No concentration of NIH 10663 antagonized responses to either DSLET or to U50,488.

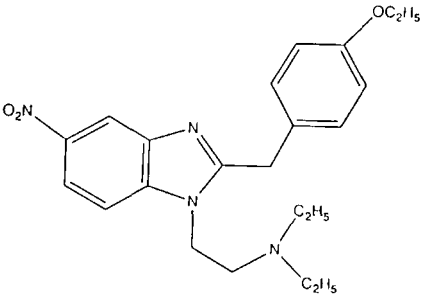
NIH 10663 Nalmefene-3β-D-glucuronide

continued...

SUMMARY

NIH 10663 had low potency in both preparations. It was a μ-selective antagonist in the *vas deferens* preparation.

NIH 10665 Etonitazene methanesulfonate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 0.506 nM in presence of 150 mM NaCl.

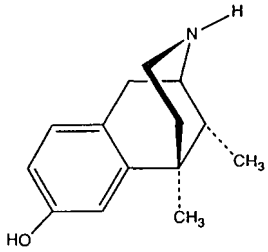
MOUSE *VAS DEFERENS* PREPARATION

	Inhibitory EC_{50} (nM)	***Maximum Response***
Drug alone	0.567	86.3%
After naltrexone	33.8	76.6%
After norbinaltorphimine	3.21	56.2%
After ICI-174,864	4.32	49.0%

SUMMARY

NIH 10665 had interesting properties; high affinity for etorphine binding sites and similar affinity but partial agonist activity the mouse *vas deferens*. It was relatively selective for μ receptors in the mouse vas deferens.

NIH 10666 (±) -5,9α-Dimethyl-2'-hydroxy-6,7-benzomorphan [α-(±)-N-normetazocine]



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 664 nM in the presence of NaCl.

BINDING IN MONKEY BRAIN CORTEX

This finding was obtained in displacing the specific equilibrium binding of (a) 0.5 nM [^{3}H]DAG0 (μ-selective assay), (b) 1.5 nM [^{3}H]DPDPE (δ-selective assay), and 1.5 nM [^{3}H]U69,593 (K-selective assay) in membranes from monkey brain cortex suspended in 50 mM Tris.HCl buffer (pH 7.4) containing 150 mM NaCl. EC_{50} values for the assays are:

(a) μ-receptor 435
(b) δ-receptor 1412
(c) κ-receptor 433

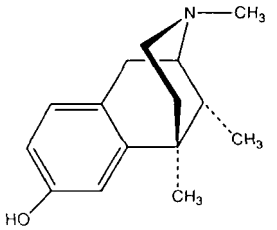
MOUSE *VAS DEFERENS* PREPARATION

	Inhibitory EC_{50} (μM)	*Maximum Response*
Drug alone	6.81	100%
After naltrexone	56.8	96.6%
After norbinaltorphimine	32.4	100%
After ICI-174,864	30.0	100%

SUMMARY

NIH 10666 appeared to act as an agonist of low potency on opioid receptors in the *vas deferens* with some selectivity for δ receptors.

NIH 10667 (±)-2'-Hydroxy-2,5,9α-trimethyl-6,7-benzomorphan.HCl
[α-(+)-Metazocine.HCl



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 119 nM in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

NIH 10667 studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-8} M to 3 x 10^{-4} M. This drug acted as a mixed agonist-antagonist.

	Inhibitory $EC_{50}(\mu M)$	***Maximum Response***
Drug alone	1.31	100%
After naltrexone	26.0	64.5%
After norbinaltorphimine	27.4	57.6%
After ICI-174,864	17.6	69.5%

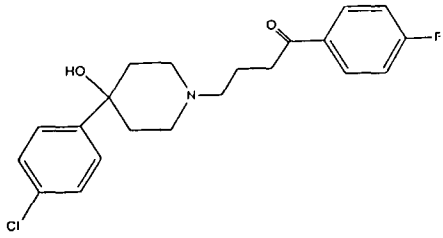
pA_2 values against the following agonists were:

Agonist	***pA_2 ± S.D.***	***Slope***
Sufentanil	6.31 ± 0.20	0.56
U50,488	5.90 ± 0.30	0.91
DSLET	5.62 ± 1.30	0.97

SUMMARY

NIH 10667 had significant activity in both assays. Its potency was comparable in both. In the *vas deferens* preparation, it was a weak, mixed agonist-antagonist.

NIH 10668 Haloperidol



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (39% inhibition at 6 μM) in presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

	Inhibitory EC_{50} (nM)	*Maximum Response*
Drug alone	46.6%	61.2%
After naltrexone	26.4%	64.6%

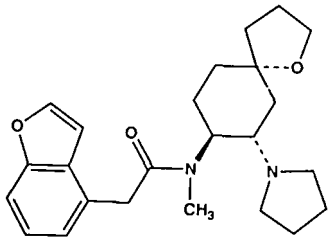
DRUG DISCRIMINATION

NIH 10668 (also NIH 10625) was studied in three morphine-dependent rhesus monkeys discriminating naltrexone. Up to a dose that eliminated responding (0.1 mg/kg), NIH 10668 failed to substitute for naltrexone. When saline was substituted for morphine, NIH 10668 failed to reverse responding on the naltrexone lever up to doses that suppressed responding (0.1 mg/kg).

SUMMARY

NIH 10668 failed to display significant opioid activity in the three preparations in which it was assessed.

NIH 10672 (-)-[5*R*-(5α,7α.,8β))]-N-Methyl-N-[7-(l-pyrrolidinyl)-l-oxa-spiro-[4.5[dec-8-yl]-4-benzofuranacetamide.HCl



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 1182 nM in presence of 150 mM NaCl.

NIH 10672 (-)-[5*R*-(5α,7α,8β)]-N-Methyl-N-[7-(1-pyrrolidinyl)-l-oxa-spiro-[4.5[dec-8-yl]-4-benzofuranacetamide.HCl

continued...

MOUSE *VAS DEFERENS* PREPARATION

	Inhibitory EC_{50} (nM)	***Maximum Response***
Drug alone	1.44	96.5%
After naltrexone	10.1	92.8%
After binaltorphimine	48.4	100%
After ICI-174,864	0.92	97.2%

DRUG DISCRIMINATION

NIH 10672 substituted for ethylketazocine at a dose of 0.00032 mg/kg. It was 100 times more potent than ethylketazocine. NIH 10627 failed to reverse naltrexone lever responding in morphine-abstinent monkeys up to doses that suppressed responding. NIH 10672 did substitute for naltrexone in two of three subjects.

ANALGESIA STUDIES

NIH 10672 increased in a dose-related manner the latency with which monkeys removed their tails from 50° or 55° C. water. A maximum effect was obtained at 0.018 mg/kg. The analgesic effects were antagonized by quadazocine in a comparable manner to κ agonists.

RESPIRATORY FUNCTION

NIH 10672 was studied in three rhesus monkeys up to a maximum dose of 0.032 mg/kg. NIH 10672 decreased f and V_T, to averages of 65% and 64% of control, respectively, in air, and to averages of 66% and 75% of control, respectively, in CO_2.

SELF-ADMINISTRATION

NIH 10672 was evaluated in three rhesus monkeys trained to self-administer alfentanil. It was evaluated over a large dose range; 0.00001-0.001 mg/kg/inj in half-log increments. NIH 10672 failed to maintain self-injection responding at any dose.

SUMMARY

NIH 10672 was more potent in the *vas deferens* than in the binding assay and appeared to be a potent opioid agonist selective for κ receptors in the former.

NIH 10672 (-)-[5R-(5α,7α,8β)]-N-Methyl-N-[7-(1-pyrrolidinyl)-1-oxa-spiro-[4.5[dec-8-yl]-4-benzofuranacetamide.HCl

continued...

It is interesting to note that, unlike for other standard κ agonists, the antagonism produced by norbinaltorphimine was surmountable. *In* vivo studies suggest that NIH 10672 is a very potent κ agonist in the rhesus monkey

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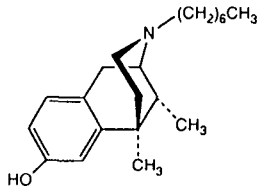
NIH 10673 (±)-5,9α-Dimethyl-2-heptyl-2'-hydroxy-6,7-benzomomorphan.HCl [α~(±)-N-Heptyl-N-normetazocine.HCl]



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 128 nM in presence of 150 mM NaCl.

BINDING IN MONKEY BRAIN CORTEX

This finding was obtained in displacing the specific equilibrium binding of (a) 0.5 nM [^{3}H]DAG0 (μ-selective assay), (b) 1.5 nM [^{3}H]DPDPE (&selective assay), and 1.5 nM [^{3}H]U69,593 (K-selective assay) in membranes from monkey brain cortex suspended in 50 mM Tris.HCl buffer (pH 7.4) containing 150 mM NaCl. EC_{50} values for the assays are:

(a)	μ~receptor	191
(b)	δ~receptor	144
(c)	κ~receptor	430

MOUSE *VAS DEFERENS* PREPARATION

	Inhibitory EC_{50} (μM)	***Maximum Response***
Drug alone	0.443	96.1%
After naltrexone	5.52	86.8%
After norbinaltorphimine	0.271	80.4%
After ICI-174,864	0.547	80.5%

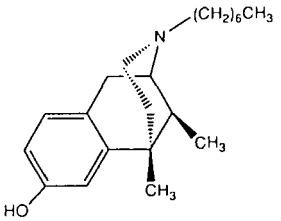
NIH 10673 (±)-5,9α-Dimethyl-2-heptyl-2'-hydroxy-6,7-benzomorphan.HCl

continued...

SUMMARY

NIH 10673 exerted μ agonist actions in the *vas deferens* preparation. It also displaced etorphine with a comparable potency to that shown in the *vas deferens.*

NIH 10674 (+)-5,9α-Dimethyl-2-heptyl-2'-hydroxy-6,7-benzomorphan.HCl [α-(+)-N-Heptyl-N-normetazocine.HCl]



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 4722 nM in presence of 150 mM NaCl.

BINDING IN MONKEY BRAIN CORTEX

This finding was obtained in displacing the specific equilibrium binding of (a) 0.5 nM [^{3}H]DAGO (p-selective assay), (b) 1.5 nM [^{3}H]DPDPE (b-selective assay), and 1.5 nM [^{3}H]U69,593 (K-selective assay) in membranes from monkey brain cortex suspended in 50 mM Tris.HCl buffer (pH 7.4) containing 150 mM NaCl. EC_{50} values for the assays are:

(a)	μ-receptor	619
(b)	δ-receptor	44% at 6 μM
(c)	κ-receptor	1914

MOUSE *VAS DEFERENS* PREPARATION

	Inhibitory EC_{50} (nM)	***Maximum Response***
Drug alone	11.1	31.1%
After naltrexone	28.6	21.1%

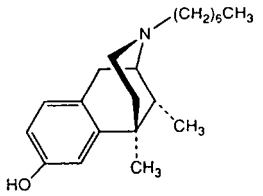
NIH 10674 (+)-5,9α--Dimethyl-2-heptyl-2'-hydroxy-6,7-benzomorphan.HCl

continued...

SUMMARY

NIH 10674 had very low potency in the binding assay, and low efficacy in suppressing the twitch of the *vas deferens.* Its actions on the mouse *vas deferens* are probably not mediated by opioid receptors.

NIH 10675 (-)-5,9α~Dimethyl-2-heptyl-2'-hydroxy-6,7-benzomorphan.HCl [α-(-)-N-Heptyl-N-normetazocine.HCl]



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

$EC_{,,}$ of 89.1 nM in presence of 150 mM NaCl.

BINDING IN MONKEY BRAIN CORTEX

This finding was obtained in displacing the specific equilibrium binding of (a) 0.5 nM [^{3}H]DAGO (μ-selective assay), (b) 1.5 nM [^{3}H]DPDPE (b-selective assay), and 1.5 nM [^{3}H]U69,593 (κ~selective assay) in membranes from monkey brain cortex suspended in 50 mM Tris.HCl buffer (pH 7.4) containing 150 mM NaCl. EC_{50} values for the assays are:

(a)	μ-receptor	71.6
(b)	δ·-receptor	82.0
(c)	κ·-receptor	216

MOUSE *VAS DEFERENS* PREPARATION

	Inhibitory EC_{50} (μM)	*Maximum Response*
Drug alone	0.353	100%
After naltrexone	352.0	100%
After norbinaltorphimine	3.14	100%
After ICI-174,864	1.04	100%

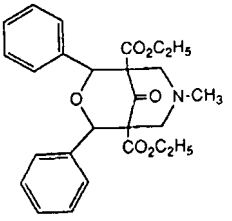
NIH 10675 (-)-5,9α-Dimethyl-2-hepty1-2'-hydroxy-6,7-benzomorphan.HCl

confinued...

SUMMARY

NIH 10675 was potent in the binding assay. In the mouse *vas deferens* it was a relatively weak opioid agonist with actions primarily at μ receptors.

NIH 10676 1,5-Diethyl-7-methyl-9-oxo-2,4-diphenyl-3-oxa-7-azabicyclo-3.3.1] nonane-1,5-dicarboxylate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (19% inhibition at 6 μM) in presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

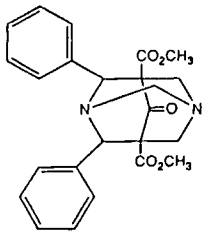
	Inhibitory EC_{50} (nM)	***Maximum Response***
Drug alone	452	94.9%
After norbinaltorphimine	896	77.0%
After ICI-174,864	389	94.7%

The maximum response in the presence of naltrexone was negligible and an EC_{50} could not be determined.

SUMMARY

NIH 10676 was without significant effect in the binding assay. It was an unusual, weak agonist in the *vas deferens* preparation, with activity at both μ and κ opioid receptors.

NIH 10677 5,7-Dimethyl-6-oxo-8,9-diphenyl-1,3-diaza-adamantane-5,7-dicarboxylate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (29% inhibition at 6 μM) in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10677 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations that ranged from 10^{-8} M to 3 x 10^{-6} M. This drug caused a partial inhibition of the twitch.

	Inhibitory EC_{50} (μM)	***Maximum Response***
Drug alone	2.91	28.3%
After naltrexone	0.143	14.6%

A concentration of 3 x 10^{-5} M NIH 10677 caused a 3.8-fold shift to the right in the U50,488 concentration-effect curve, and the maximum response was reduced to a 78.8% inhibition of the twitch.

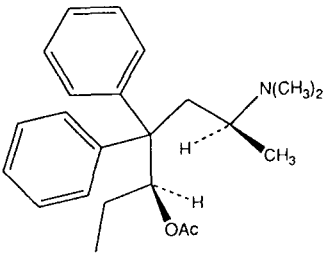
pA_2, values against the following agonists were:

Agonist	***pA_2 ± S.D.***	***Slope***
Sufentanil	4.92 ± 0.53	1.48
DSLET	5.46 ± 0.49	1.43

SUMMARY

NIH 10677 was not potent in either preparation. It had unusual opioid agonist-antagonist actions in the *vas deferens* preparation at high concentrations.

NIH 10679 (-)-α-Acetylmethadol.HCl (LAAM)



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

$EC_{,,}$ of 388 nM in presence of 150 mM NaCl.

BINDING IN MONKEY BRAIN CORTEX

This finding was obtained in displacing the specific equilibrium binding of (a) 0.5 nM [^{3}H]DAGO (μ-selective assay), (b) 1.5 nM [^{3}H]DPDPE (δ-selective assay),= and 1.5 nM [^{3}H]U69,593 (K-selective assay) in membranes from monkey brain cortex suspended in 50 mM Tris.HCl buffer (pH 7.4) containing 150 mM NaCl. EC_{50} values for the assays are:

(a)	μ-receptor	34.1 nM
(b)	δ–receptor	2259 nM
(c)	κ–receptor	3564 nM

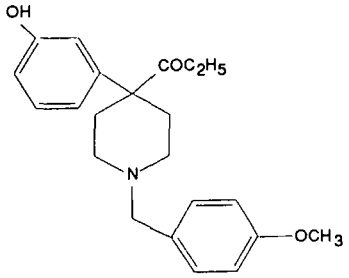
MOUSE *VAS DEFERENS* PREPARATION

	Inhibitory EC_{50} (nM)	*Maximum Response*
Drug alone	974.3	82.7%
After naltrexone	Not measurable	0%
After norbinaltorphimine	582.0	74.8%
After ICI-174,864	568.7	63.2%

SUMMARY

NIH 10679 selectively displaced the μ receptor ligand in monkey brain. It had a biphasic concentration-effect curve in the *vas deferens.* Naltrexone abolished the effect of NIH 10679 in the *vas deferens;* the other antagonists had little effect. Thus, the compound was a selective compound at μ sites with an unusual profile in the *vas deferens.*

NIH 10681 N-(4-Methyoxybenzyl)-N-norketobemidone.HBr



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 625 nM in presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

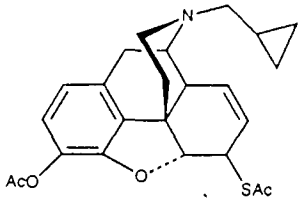
No concentration of NIH 10681 inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	*pA_2*	*Slope ± S.D.*
Sufentanil	5.95	1.62 ± 0.03
U50,488	5.75	1.66 ± 0.56
DSLET	6.85	1.06 ± 0.11

SUMMARY

NIH 10681 had no significant activity in the binding assay, but was a moderately selective b-receptor antagonist in the mouse *vas deferens* preparations.

NIH 10685 (-)-3-Acetyl-6-ß-(acetylthio)-N-(cyclopropylmethyl)normorphine



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 7.12 nM in the presence of 150 mM NaCl.

BINDING IN MONKEY BRAIN CORTEX

This finding was obtained in displacing the specific equilibrium binding of (a) 0.5 nM [^{3}H]DAGO (p-selective assay), (b) 1.5 nM [^{3}H]DPDPE δ-selective

NIH 10685 (-)-3-Acetyl-6-ß-(acetylthio)-N-(cyclopropylmethyl)normorphine

continued...

brain cortex suspended in 50 mM Tris.HCl buffer (pH 7.4) containing 150 mM NaCl. EC_{50} values for the assays are:

(a) μ-receptor: 1.09 nM
(b) δ--receptor: 13.3 nM
(c) κ--receptor: 0.781 nM

MOUSE *VAS DEFERENS* PREPARATION

	Inhibitory EC_{50} (nM)	***Maximum Response***
Drug alone	20.4	34.5 %
After naltrexone	84.8	53.2%

Agonist	***pA_2***	***Slope ± S.D.***
Sufentanil	7.93	1.34 ± 0.37
U50,488	8.36	0.93 ± 0.09
DSLET	8.09	0.58 ± 0.17

DRUG DISCRIMINATION

Up to doses that eliminated responding (0.03 mg/kg) NIH 10685 failed to substitute for alfentanil in rhesus monkeys. It did substitute for ethylketazocine at 0.01 mg/kg. It also substituted for naltrexone at a dose of 0.032 mg/kg; it was equipotent with naltrexone in this regard. NIH 10685 antagonized alfentanil's ability to reverse withdrawal in these monkeys, as well.

ANALGESIA STUDIES

NIH 10685 produced analgesia in the tail withdrawal assay in rhesus monkeys; full effect (50° C.) and near full effect at 55" C. was obtained with 0.32 mg/kg. Quadazocine (1.0 mg/kg) antagonized this effect.

RESPIRATORY FUNCTION

NIH 10685 (0.1 mg/kg) antagonized the effect of alfentanil upon respiratory function. Slightly higher doses of NIH 10685 had a modest respiratory depressant effect (decreased both frequency and tidal volume). This effect was not clearly attenuated by 1.0 mg/kg quadazocine.

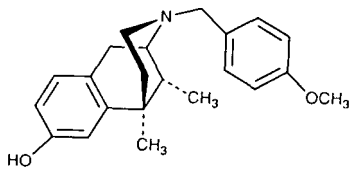
NIH 10685 (-)-3-Acetyld-6-ß-(acetylthio)-N-(cyclopropylmethyl)normorphine

continued...

SUMMARY

NIH 10685 had high affinity for opioid binding sites with a slightly higher affinity for μ and κ sites relative to the δ site. In the *vas deferens,* it had antagonist actions against each of the prototypic agonists. When slopes of pA_2 regressions were constrained, there were no significant differences among the antagonist affinities. On the *vas deferens* it was also an agonist of low efficacy but similar in potency to its antagonist actions. In the rhesus monkey, NIH 10685 clearly had both agonist and antagonist actions. It was an antagonist of alfentanil in the respiratory function assay and in the morphine-dependent monkey naltrexone discrimination procedure. It also had κ agonist effects in the ethylketazocine drug discrimination assay.

NIH 10686 (-)-5,9α-Dimethyl-2'-hydroxy-2-(4-methoxybenzyl)-6,7-benzomorphan.HBr



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 7014 nM in the presence of 150 mM NaCl.

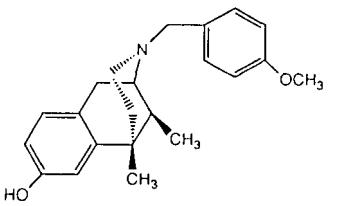
MOUSE *VAS DEFERENS* PREPARATION

NIH 10686 was devoid of agonist activity at concentrations up to 1 μM. At a concentration of 3 μM the magnitude of the twitch increased. This drug is a very weak antagonist. Although pA_2 values were not determined, the following shifts occurred at a concentation of 10 μM: against sufentanil, 3.1 l-fold; against DSLET, 2.01-fold; and against U50,488, 12.05-fold. Thus, this drug would seem to have some selectivity against the κ agonist.

SUMMARY

NIH 10686 was active in both assays only at very high concentrations.

NIH 10691 (+)-5,9 α-Dimethyl-2'-hydroxy-2-(4-methoxybenzyl-6,7-benzomorphan.HBr



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 6109 nM in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

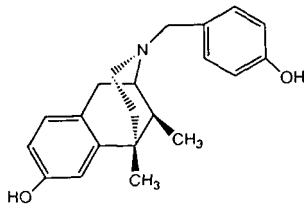
No concentration of NIH 10691 inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2, values against the following agonists were:

Agonist	***pA₂***	***Slope*** ± S.D.
Sufentanil	5.60	1.30 ± 0.24
DSLET	5.33	1.65 ± 0.33

SUMMARY

NIH 10691 was a nonselective receptor antagonist in the mouse *vas deferens* with very low affinity for the etorphine binding site in rat brain membranes. It was an insurmountable antagonist at κ receptors in the *vas deferens*. The slopes of the Schild plot suggested that the antagonism at μ and δ receptors was not simply competitive.

NIH 10694 (+)-5,9α-Dimethyl-2'-hydroxy-2-(4-hydroxybenzyl)-6,7-benzomorphan.hemioxalate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (23% at 6 μM) in presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

No concentration of NIH 10694 inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	*p A 2*	*Slope ± S.D.*
Sufentanil	5.51	1.06 ± 0.06
U50,488	5.72	1.17 ± 0.27
DSLET	<5.00	---

SUMMARY

NIH 10694 was active in the mouse vas deferens only at very high concentrations. At these concentrations, it was an antagonist. It had slight non-opioid actions (10% inhibition). NIH 10694 had no significant activity in the binding assay.

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Progress Report from the Testing Program for Stimulant and Depressant Drugs (1990)

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The research group involved in the evaluation of stimulant and depressant compounds has been in existence for approximately eight years. This group includes laboratories at The University of Chicago (Nader, Woolverton), The University of Michigan (Winger, Woods), The Medical College of Virginia (Patrick, Harris) and NM (Jacobson). The group is part of the Drug Evaluation Committee, chaired by Ted Cicero, of the Committee on Problems of Drug Dependence. The purpose of the group is to evaluate new compounds generally classified as stimulants or depressants for their abuse liability and dependence potential. Compounds are received, coded and distributed by Jacobson for blind testing in the various laboratories. They are evaluated for discriminative stimulus effects (UC), reinforcing effects (UM) and capacity to produce physical dependence (MCV). This report includes some of the results of the evaluation of the following compounds: CPDD 0025 (brotizolam, cf. 1989 Report), 0028 (chlordiazepoxide), 0030 (alprazolam), 0031 (triazolam) and 0032 (flunitrazepam).

METHODS

Drug Discrimination in Rhesus Monkeys

The subjects were one female and five male rhesus monkeys (*Macaca mulatta*) that weighed between 6.5 and 12.1 kg. All monkeys had extensive experience with the present drug discrimination procedure before starting this experiment. They were housed individually in stainless-steel cages in which water was available continuously. They were fed 150 to 200 g of monkey chow after each session and were given a chewable vitamin tablet 3 days/week. During experimental sessions the monkeys were seated in a Plas-Lab restraining chair and placed in a wooden cubicle (175 cm high X 85 cm wide X 65 cm deep) containing two response levers mounted 100 cm above the floor. A 34 w white house light was mounted on the ceiling. The monkey's feet were placed into shoes, the bottoms of which were fitted with brass plates which could deliver electric shocks. All programming and recording of experimental events were accomplished by an Aim 65 microprocessor located in an adjacent room.

The monkeys had been trained previously to discriminate d-amphetamine (AMPH; 7737, 7739, 8515) or pentobarbital (PB; 8106, 8236, 7976) from saline in a two-lever, discrete-trial shock-avoidance procedure similar to the one described by Holtzman (1982). One hour after an intragastric (via n.g. tube) infusion of the training drug (0.56-1.0 mg/kg AMPH or 10 mg/kg PB) or saline,

the houselights and lever lights were illuminated (trial) and responding on one lever (the correct lever) avoided electric shock and extinguished the lights. Responding on the incorrect lever started a 2-second change-over delay during which correct lever responding had no consequence. If a correct response was not made within 5 seconds of onset of the lights, shock (250 msec duration, 5.0 mA intensity) was delivered every 2 seconds until a correct response was made. After a correct response, there was a 55-second intertrial interval, after which a new trial began. The session lasted for 30 trials or 40 min, whichever came first. The correct lever was determined by the infusion that was administered before the session. For three monkeys the right lever was correct after drug infusions and the left lever was correct after saline infusions. This condition was reversed for the other three monkeys.

Monkeys were considered to be stable in the discrimination when more than 90% of the trials were completed on the correct lever for six consecutive sessions. At this point testing was begun with the training drugs and the test drugs. Two 5-day sequences alternated drug, vehicle and test sessions so that the first test session was preceded by two training sessions, one with saline and one with drug pretreatment and the second test session of the sequence was preceded by either vehicle or drug pretreatment. In the event that the criterion for stimulus control was not met during the training sessions, the training sequence was continued. During test sessions, both levers were operational, i.e., shock could be avoided by responding on either lever.

Saline, at least three doses of the training drug, and three doses of each test drug were evaluated under the test conditions for each monkey. The percentage of trials that were completed on the drug lever is presented for each test session. In addition, the average time between the onset of a trial and a lever press (average latency) was calculated for each test session. Because these test compounds were evaluated blind without any dose-response information, initial test doses were done in an ascending order from 0.1 mg/kg to doses that either significantly increased latency or resulted in at least 90% drug-appropriate responding. If response generalization occurred, that dose and doses higher and lower were tested again, in a random order. The drug vehicle was also tested.

PB and the test drugs were prepared immediately before testing, while a stock solution of AMPH was prepared each week. PB (40 mg/ml) and AMPH (5 mg/ml) were dissolved in saline, chlordiazepoxide was soluble in sterile water, while the other benzodiazepines were administered as suspensions in saline or alpha-cyclodextrin vehicle.

Self-Administration by Rhesus Monkeys

The reinforcing effects of test compounds were evaluated in a substitution self-administration procedure with methohexital serving as the baseline drug. Rhesus monkeys were surgically prepared with indwelling silicone catheters using 10 mg/kg i.m. ketamine and 15 mg/kg i.v. PB as anesthetic. Catheters were implanted in jugular (internal or external), femoral or brachial veins, as necessary. The catheter passes subcutaneously from the site of the incision to the mid-scapular region, where it exited the monkey and continued, through a hollow restraining arm, to the outside rear of the cage.

The restraint and catheter protection device has been described in detail by Deneau et al. (1969). Monkeys were individually housed in stainless steel cages,

measuring 83.3 X 76.2 X 91.4 cm deep. Each monkey wore a tubular stainless steel harness that protected the exit site of the catheter and allowed relatively unrestricted movements within the chamber. A Teflon cloth jacket (Alice King Chatham Medical Arts, Los Angeles, CA) provided further protection for animals who tended to locate and pull their catheters. The harness was connected to a tubular, jointed arm that carried the catheter to the back of the cage where it joined tubing passing through a roller infusion pump (Watson and Marlow Co., Model MHRK 55; Falmouth. UK).

A 15.4 cm square stimulus panel was located on the side of each cage, approximately 10 cm from the front and 19 cm from the bottom of the cage. Across the top of the stimulus panel, 2.5 cm apart, were three circles, 2.5 cm in diameter, covered with translucent plastic and capable of being illuminated from behind by 5 W colored bulbs. The two side lights could be illuminated red and the center light could be illuminated green. Below each of the two red stimulus lights was a response lever (Model 121-07; BRS-LVE, Beltsville, MD), capable of being operated by 10-15 gms of force. Experimental control was provided by individual IBM PCjr computers located in an adjoining room.

Monkeys were adapted to restraining arms for a week or more; then an intravenous catheter was implanted and they were given the opportunity to respond and receive drug. At the beginning of each session, a red light was illuminated over one of two levers in each monkey's cage and 10 responses (fixed-ratio 10: FR 10) on that lever resulted in a 5-second infusion of 0.1 mg/kg sodium methohexital, followed by a lo-second timeout during which all stimulus lights were extinguished and responding had no programmed consequence. During an infusion the red lever light was extinguished and the overhead green houselight was illuminated for the duration of the infusion. Experimental sessions were limited to 210 min or until a maximum of 200 injections were delivered. No monkey ever received 200 injections of methohexital. Two sessions were scheduled each day, separated by at least four hours. Occasionally, saline was made available and when there was a clear difference in the number of injections of saline and methohexital, a dose of the test compound was substituted for one session. All conditions were similar to training sessions except the maximum number of injections of the test compound was 150/session. If there was no visible trend in responding, compounds were tested on Tuesday and Fridays. Each dose was tested at least twice in each monkey.

Physical Dependence (Substitution) in Rats and Potency Estimation in Mice

Male Sprague-Dawley rats (Dominion Labs, Dublin, VA) initially weighing 175-200 g were individually housed in stainless steel cages with food and water available ad lib. Male CF-1 mice (Dominion Labs, Dublin, VA) weighing 25-30 g were housed in plastic cages with food and water ad lib. All animals were acclimated to the animal facility for several days prior to use in any study.

Rats were surgically prepared with an intraperitoneal cannula (PE9O) while under methoxyflurane anesthesia. All rats were allowed several days to recover from surgery prior to being placed into an infusion harness. Acclimation to the infusion system occurred for 3 days during which the rats were continuously infused with 0.9% saline. This was followed by the continuous infusion of either saline (control) or pentobarbital sodium for 12 consecutive days using an escalating drug dosage schedule (Yutrzenka, et al., 1985). At the end of the

infusion period most rats were receiving pentobarbital at a dose of 900 mg/kg/24 hour. Body weight was monitored daily during the drug infusion period.

Following the final day of pentobarbital infusion a 24 hour substitution period commenced during which pentobarbital-dependent rats were infused with either saline, vehicle, or test drug. This was followed by a 24 -hour drug withdrawal period during which all rats received saline.

Every 2 hours for the first 12 hours and again, at 24 hours of each period, rats were assigned a withdrawal score based on the degree of expression of several behavioral responses and signs. Scores were assigned by two observers who were blind to the drug treatment. In addition, body weight was determined at 0,8 and 24 hours of each period. Investigators were blind to the identity of the compounds until all data was collected and analyzed (Yutrzenka et al., 1989).

Preliminary studies to ascertain potency of these benzodiazepine compounds, relative to pentobarbital, were conducted in mice. Drug-treated mice were assayed using the inverted screen test (Coughenour et al., 1977) and alteration of spontaneous locomotor activity. At least three doses of each drug, with at least six mice per dose, were used to determine dose-response curves. Vehicle-treated mice served as controls and were assayed concurrently with drug-treated mice.

The inverted screen test was conducted at 20, 30, 60 and 120 minutes following drug administration. The ED_{50} dose, which was determined to be the dose at which one-half of the treated mice failed to right themselves within the 60 second time period, was computed for each time period. Spontaneous locomotor activity was determined using a single beam photocell which bisected a plastic cage containing 2 mice. Movement of the mice disrupted the beam and a "count" of activity was recorded. Following drug administration, activity was recorded at the following time intervals; 5-15 minutes, 35-50 minutes, 65-95 minutes, and 125-185 minutes. The ED_{50} dose was determined to be that dose which reduced spontaneous locomotor activity to one-half that recorded for concurrently tested vehicle-treated control mice. Potency ratios of each test drug relative to pentobarbital were determined at time of peak of activity and when, in addition, the vehicle effect was no longer evident.

Pentobarbital sodium was dissolved in distilled water made isotonic with sodium chloride. Brotizolam was dissolved in a water-Emulphor EL-620-ethanol (68:20:12 by volume) vehicle; chlordiazepoxide was dissolved in distilled water; alprazolam was dissolved in a vehicle of distilled water-propylene glycol-ethanol (80:16:4 by volume); and flunitrazepam was dissolved in distilled water-propylene glycol-ethanol (50:40:10 by volume) at pH 2.3. Eight ml of each solution was infused over a 24 hour period. For this study, the benzodiazepines were provided in coded vials by the Committee on Problems of Drug Dependence (CPDD). Investigators were blind to the identity of the compounds until after the completion of the study.

Withdrawal scores for each treatment group were compared to the control by use of the Mann-Whitney U-test. Alteration in body weight was tested for significance by use of f-test. ED_{50} values and 95% confidence intervals in the inverted screen test and locomotor activity measure were also determined (Litchfield and Wilcoxon (1949).

RESULTS

Compound #0025! (Brotizolam : cf 1989 report)

Brotizolam was quite potent in both the inverted screen test (see Table 1) and in reducing spontaneous locomotor activity (Table 2), being approximately 100 times as potent as pentobarbital in both measures. When substituted for pentobarbital in dependent rats, brotizolam suppressed overt signs of withdrawal (Figure la). Following discontinuation of the brotizolam substitution, overt signs of withdrawal increased in rats which received the lower dose (5 mg/kg/day) but not in those which received the higher dose (Figure lb). Both doses of brotizolam significantly suppressed the loss of body weight associated with barbiturate withdrawal, and weight loss typical of abstinence occurred when brotizolam was discontinued (see Figure 2).

Compound #0028 (Chlordiazepoxide)

Chlordiazepoxide was active in the inverted screen test (Table 1) and in reducing locomotor activity (Table 2), with potency slightly greater than pentobarbital in both assays.

When chlordiazepoxide was made available to monkeys who were experienced with i.v. self-administration of sodium methohexital, all three animals did self-administer chlordiazepoxide as well (see Figure 3). The number of injections self-administered increased with dose per injection between 0.01 and 0.10 mg/kg/injection. Peak rates of self-administration occurred at doses of 0.10 or 0.30 mg/kg/injection. One monkey that exhibited maximal responding at 0.30 mg/kg/injection was not exposed to a higher dose, while the other monkey who responded maximally at that dose showed a marked decrease in responding at 1.0 mg/kg/injection.

In drug discrimination studies in rhesus monkeys, chlordiazepoxide produced a dose-related increase in drug-appropriate responding in animals trained to discriminate pentobarbital (10 mg/kg) from saline (see Figure 4). Complete generalization (100% drug-appropriate responding) occurred at doses of 10 to 17 mg/kg. These doses also caused a modest increase in response latency. When chlordiazepoxide was given to monkeys trained to discriminate amphetamine (0.56 or 1.0 m/kg) from saline, 2 of the 3 subjects responded on the saline-appropriate lever. For the one monkey that exhibited drug-appropriate responding the magnitude of response was not dose-related.

When chlordiazepoxide was infused into pentobarbital-dependent rats in a substitution study for dependence liability, the higher dose administered (900 mg/kg/day) effectively suppressed overt behavioral signs of withdrawal while the lower dose (450 mg/kg/day) did not (see Figure 5a). When the drug infusion was discontinued, mild signs of abstinence were observed in the rats receiving the lower doses (Figure 5b). Both doses of chlordiazepoxide prevented the loss of body weight associated with barbiturate abstinence, but there was relatively little effect on body weight when chlordiazepoxide itself was withdrawn (see Figure 6).

(Figure 10b). Substitution of flunitrazepam also prevented the loss of body weight associated with abstinence. However, weight loss did occur when the drug was withdrawn (see Figure 1l), indicating its dependence liability.

DISCUSSION

Compound #0025 (Brotizolam)

Brotizolam, in agreement with findings reported last year, produced dose-related effects typical of CNS depressant drugs in the inverted screen test and in reducing spontaneous locomotor activity. It substituted for pentobarbital in suppressing signs of abstinence in dependent rats, and signs of abstinence did emerge when the substitution was halted. These results support earlier findings in drug discrimination and self-administration studies, suggesting that brotizolam possesses barbiturate-like subjective effects and abuse and dependence liability.

Compound #0028 (Chlordiazepoxide)

Chlordiazepoxide produced depressant effects on motor coordination and locomotor activity, with potency similar to pentobarbital. It was self-administered by monkeys at a rate slightly less than methohexital, and it produced drug-lever responding in pentobarbital-trained monkeys. It substituted partially for pentobarbital in dependent rats, but withdrawal from it was quite mild. These data suggest that chlordiazepoxide produces subjective effects similar to barbiturates, and that it has abuse potential and dependence liability which are qualitatively similar to pentobarbital, but lesser in degree.

Compound #0030 (Alprazolam)

Alprazolam produced dose-related effects typical of CNS depressant drugs, with potency somewhat greater than diazepam. It produced discriminative stimulus effects resembling pentobarbital in monkeys, and slightly increased response latency. It substituted for pentobarbital in dependent rats, and mild signs of withdrawal appeared following cessation of administration. Alprazolam appears to have subjective effects and dependence liability resembling the barbiturates.

Compound #0031 (Triazolam)

Triazolam produced drug-lever responding when administered to pentobarbital-trained monkeys in drug discrimination studies, and this effect was observed at doses that did not significantly increase response latency. Triazolam would be predicted to have pentobarbital-like subjective effects in humans.

Compound #0032 (Flunitrazepam)

Flunitrazepam was very potent in producing typical depressant effects on motor coordination and locomotor activity. It produced discriminative stimulus effects similar to pentobarbital. Upon substitution in pentobarbital-dependent rats it suppressed signs of abstinence, but such signs did appear upon cessation of administration. Flunitrazepam would be predicted to have pentobarbital-like subjective effects and dependence liability in humans.

Compound #0030 (Alprazolam)

Given acutely to mice, alprazolam was quite potent (approximately 50 to 100 times as potent as pentobarbital) in both the inverted screen test (Table 1) and the suppression of locomotor activity (Table 2).

In rhesus monkeys trained to discriminate pentobarbital from saline, administration of alprazolam, suspended in an alpha-cyclodextrin vehicle, via nasogastric tube occasioned drug-appropriate responding. One monkey exhibited 100% drug-lever responding at a dose of 0.03 mg/kg, while the other two exhibited a similar effect at doses of 0.3 to 0.56 mg/kg. Response latency was increased to the same degree as occurred with the training dose of pentobarbital. Monkeys trained to discriminate amphetamine from saline responded on the saline-appropriate lever when treated with alprazolam.

When alprazolam was substituted for pentobarbital in dependent rats, it effectively suppressed, behavioral signs of abstinence (Figure 7a) at doses of 10 and 20 mg/kg/day. Following withdrawal of alprazolam, there were few overt signs of abstinence (Figure 7b). The loss in body weight associated with barbiturate abstinence was suppressed completely by the higher dose (20 mg/kg/day) of alprazolam and partially by the lower dose (see Figure 8). Withdrawal from the alprazolam substitution led to a loss of about 5% of body weight, suggestive of a mild abstinence syndrome (Figure 8).

Compound #0031 (Triazolam)

Triazolam, suspended in an alpha cyclodextrin vehicle, was administered via nasogastric tube to 2 rhesus monkeys trained to discriminate pentobarbital (10 mg/kg) from saline. At doses of 0.1 to 0.3 mg/kg triazolam produced 100% drug-appropriate responding, indicative of pentobarbital-like subjective effects. These doses of triazolam did not affect response latency relative to saline controls, in contrast to the increased latency seen with the training dose of pentobarbital.

Compound #0032 (Flunitrazepam)

Flunitrazepam was extremely potent in the inverted screen test (Table 1) and in decreasing spontaneous locomotor activity (Table 2). These effects were dramatically reduced by 60 to 120 minutes after acute injection.

A suspension of flunitrazepam in saline was administered via nasogastric tube to 2 groups of rhesus monkeys (3 per group) trained to discriminate either pentobarbital or amphetamine from saline. In pentobarbital-trained monkeys, flunitrazepam occasioned 100% drug-appropriate responding at doses of 0.3 to 1.0 mg/kg (see Figure 9). This effect was accompanied by a dose-related increase in response latency with the latency being greater than that produced by the training dose of pentobarbital in most subjects (Figure 9). Administration of flunitrazepam to amphetamine-trained monkeys resulted in only saline-lever responding in 2 of 3 subjects, while the 1.0 mg/kg dose disrupted avoidance responding in all 3 monkeys.

Substitution of flunitrazepam at doses of 5 and 10 mg/kg/day in pentobarbital-dependent rats effectively suppressed overt signs of withdrawal compared with vehicle substitution (see Figure 10a). When infusion of flunitrazepam was discontinued, rats exhibited increased signs of abstinence over the next 24 hours

TABLE 1

Effects of CPDD Compounds in the Inverted Screen Test in Mice

	Time of Test After Treatment			
Drug Treatment	20	30	60	120
Pentobarbital	17.8(14.1-22.4)[1]	24.5(20.7-29.0)		
Brotizolam	0.194(0.094-0.399)	0.241(0.100-0.586)	1.74(0.40-7.60)	
Chlordiazepoxide	18.8(11.5-30.8)	14.4(7.15-29.1)	30.5(24.8-37.6)	34.7(29.9-41)
Alprazolam		0.321(0.200-0.516)	0.78(0.46-1.33)	4.18(0.938-18.6)
Flunitrazepam	0.092(0.047-0.178)	0.097(0.051--0.184)	0.53(0.12-2.37)	0.79(0.20-3.15)

[1]Values in table indicate ED-50 in mg/kg and 95% confidence limits.

TABLE 2

Sedative Effects of CPDD Compounds on Locomotor Activity in Mice

	Time of Test After Treatment (min)	
Drug Treatment	5-15	35-50
PentobarbitaI	24.7(20.6-29.7)[1]	
Brotizolam	0.090)0.31-0.264)	0.035(0.0074-0.162)
Chlordiazepoxide	24.7(17.7-24.5)	9.1(4.7-17.5)
Alprazolam	0.562(0.208-1.52)	0.111(0.030-0.417)
Flunitrazepam	0.040(0.0074-0.22)	0.036(0.0028-0.44)

[1]Values in table indicate ED-50 in mg/kg and 95% confidence limits.

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Figure 1a: Withdrawal Scores on Substitution of Vehicle(V) and Brotizolam(BTZ)

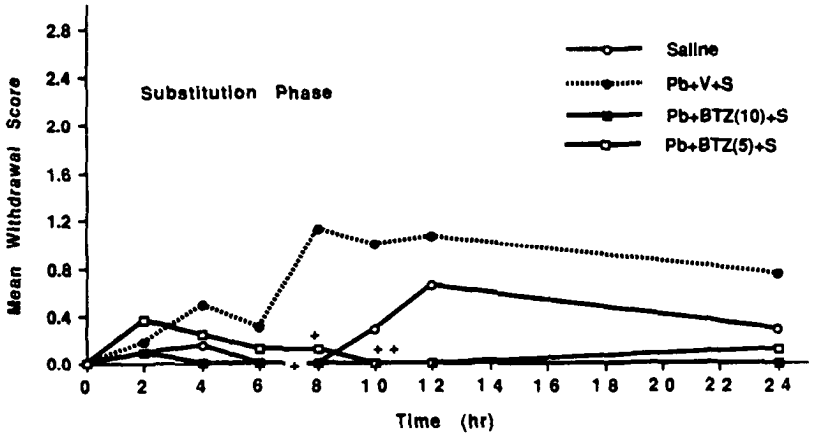


Figure 1b: Withdrawal Scores on Substitution of Vehicle(V) and Brotizolam(BTZ)

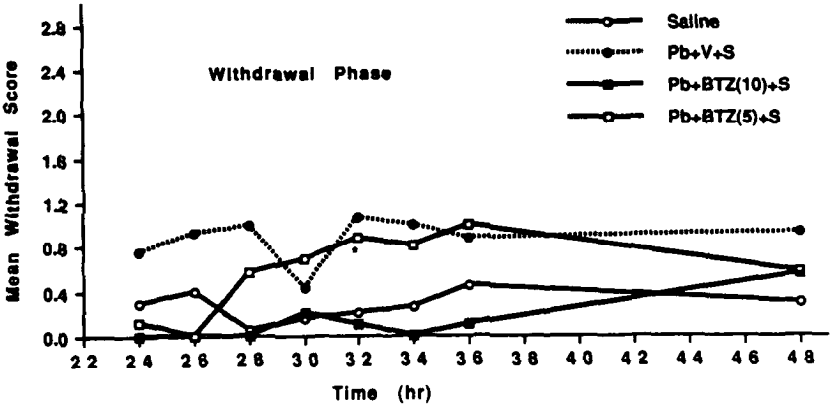


FIG. 1 Mean withdrawal scores of control rats and pentobarbital-dependent rats during (A) brotizolam or vehicle substitution (Day 13) or (B) saline substitution (Day 14). Brotizolam infused in doses of 5 or 10 mg/kg/24 hr. Each point represents the mean withdrawal score of 3 to 6 rats.

Figure 2: Changes in Body Weight with Substitution of Vehicle(V) and Brotizolam(BTZ)

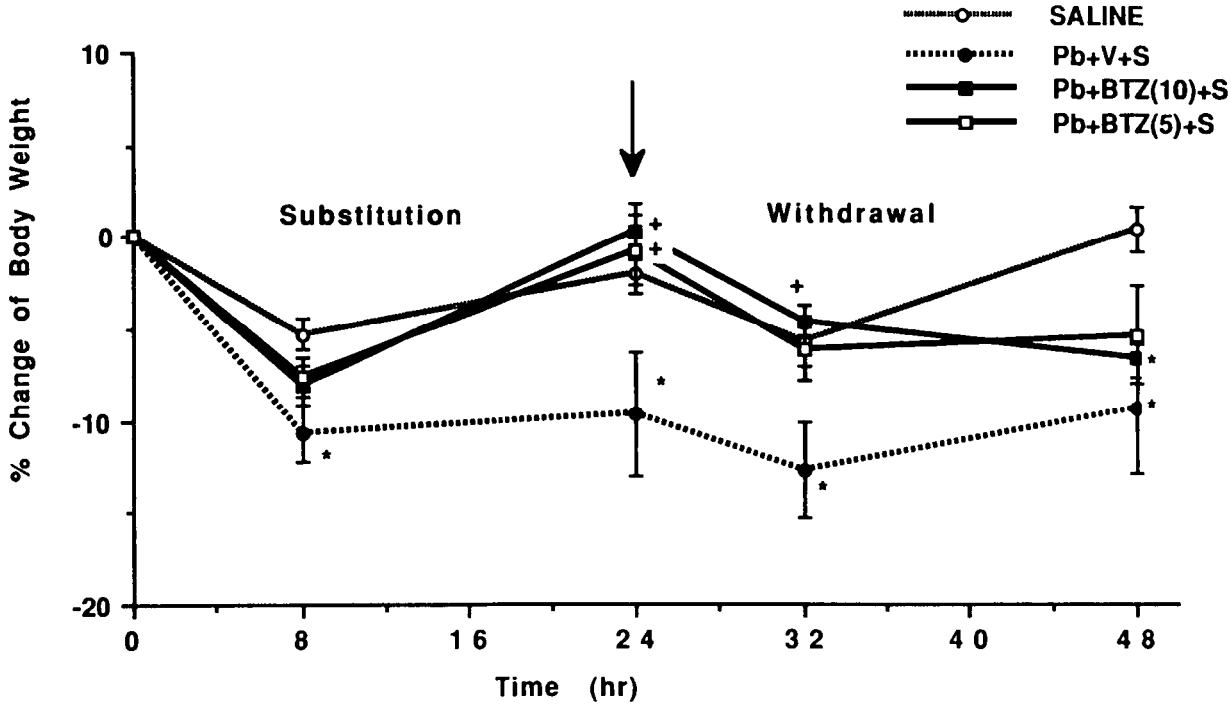


FIG. 2. Percent change in body weight of control rats and pentobarbital-dependent rats during both the drug substitution period (Day 13) and subsequent saline substitution period (Day 14). Pentobarbital-dependent rats infused with either vehicle, or brotizolam in doses of 5 or 10 mg/kg/24 hrs (n=3-6).

Figure 3

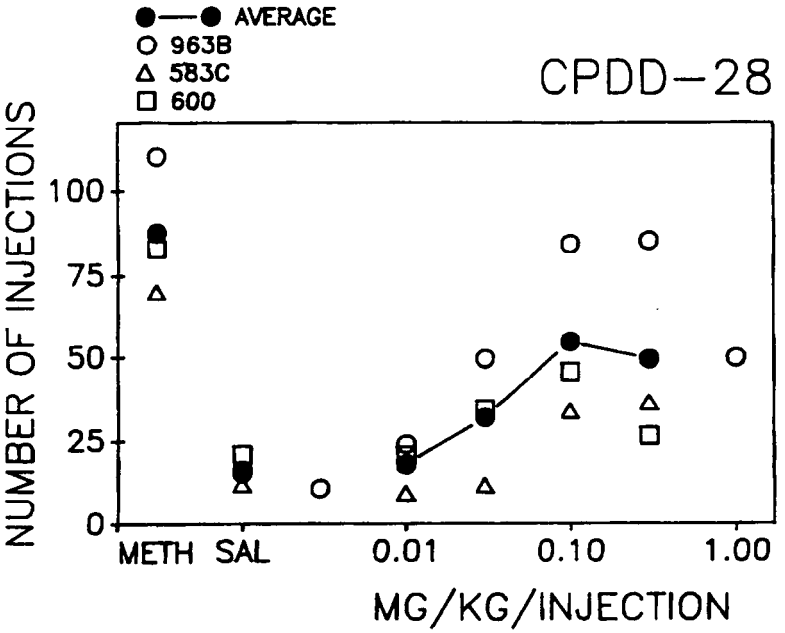


Figure Caption: Effects of response-contingent CPDD-28 on responding in rhesus monkeys.

The points shown at METH are the average number of injections taken of 0.1 mg/kg/inj sodium methohexital on the sessions just preceding each substitution of CPDD-28. These averages are shown for the individual monkeys (open symbols) and for the group (closed circle). Similar data are shown for saline availability over the point indicated by SAL. Data shown for CPDD-28 are averaged across two or three observations in each monkey. The closed, connected circles are the average of the individual monkey data shown in the open circles.

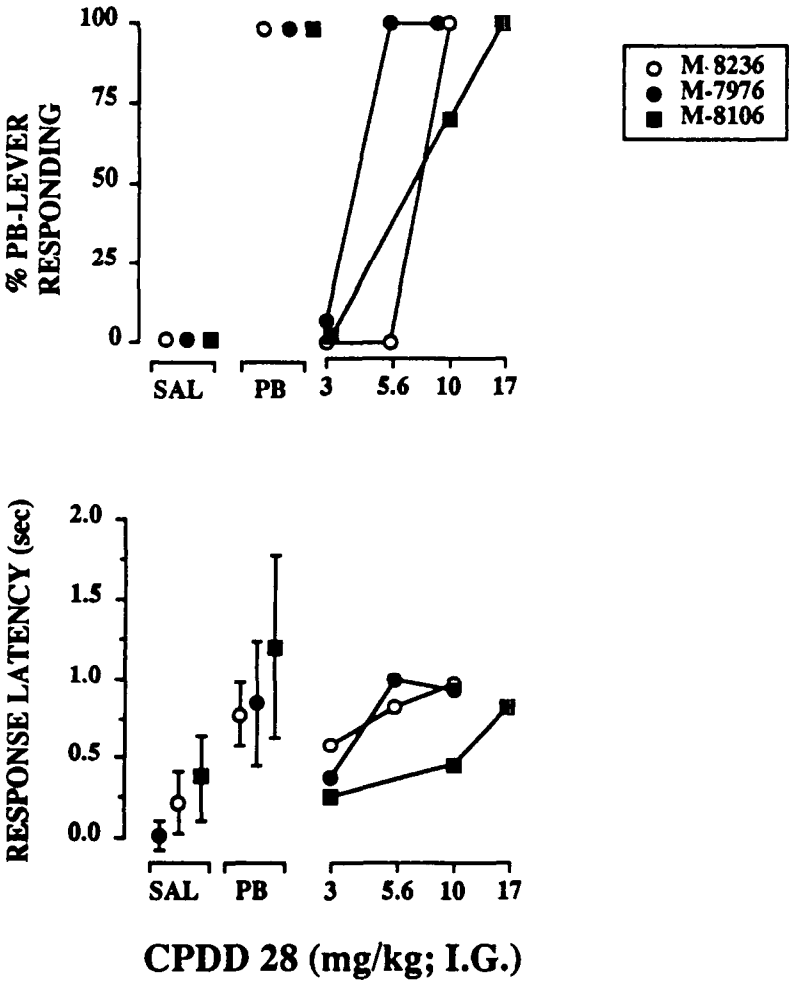


FIG. 4 Effects of CPDD 28 in rhesus monkeys trained to discriminate 10 mg/kg pentobarbital from saline. The top panel represents the percentage of total responses during test sessions in which pentobarbital-lever responding occurred, while the lower panel depicts the average response latency per trial. The unconnected symbols at the left represent the average of Thursday control sessions.

FIGURE 5a: Withdrawal Scores on Substitution of Saline(S) and CPDD#0028

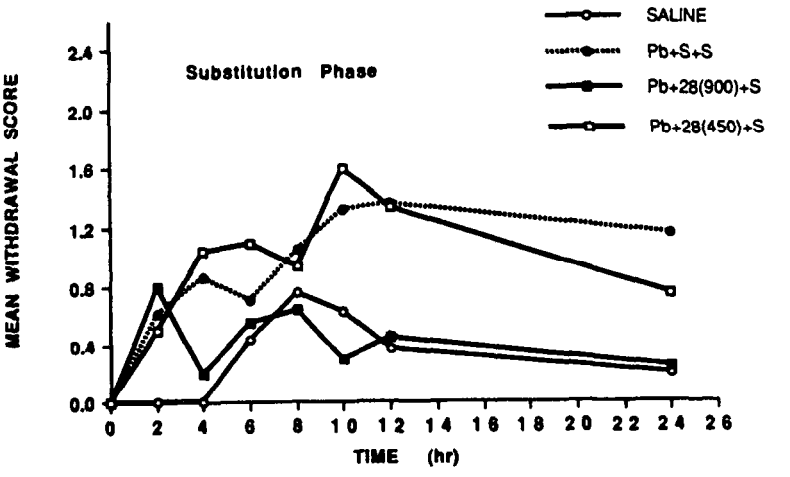


Figure 5b: Withdrawal Scores on Substitution of Saline(S) and CPDD#0028

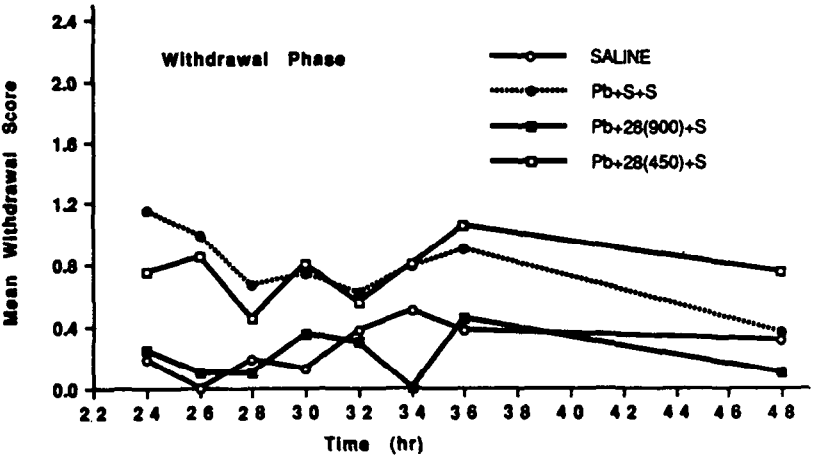


FIG. 5 Mean withdrawal scores of control rats and pentobarbital-dependent rats during (A) chlordiazepoxide or vehicle substitution (Day 13) or (B) saline substitution (Day 14). Chlordiazepoxide infused in doses of 450 to 900 mg/kg/24 hr. Each point represents the mean withdrawal score of 3 to 6 rats.

Figure 6: Changes In Body Weight with Substitution of Saline(S) and CPDD#0028

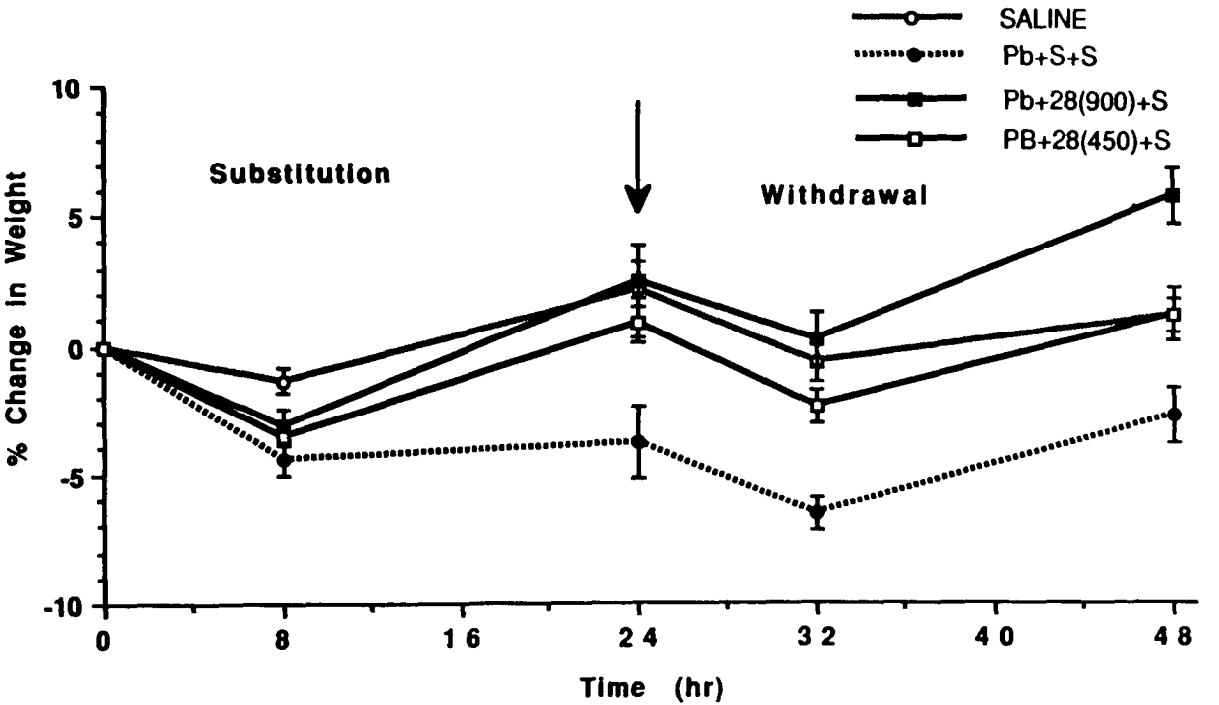


FIG. 6. Percent change in body weight of control rats and pentobarbital-dependent rats during both the drug substitution period (Day 13) and subsequent saline substitution period (Day 14). Pentobarbital-dependent rats infused with either vehicle, or chlordiazepoxide in doses of 450 or 900 mg/kg/24 hrs (n=3-6).

Figure 7a: Withdrawal Scores on Substitution of Vehicle(V) and CPDD#0030

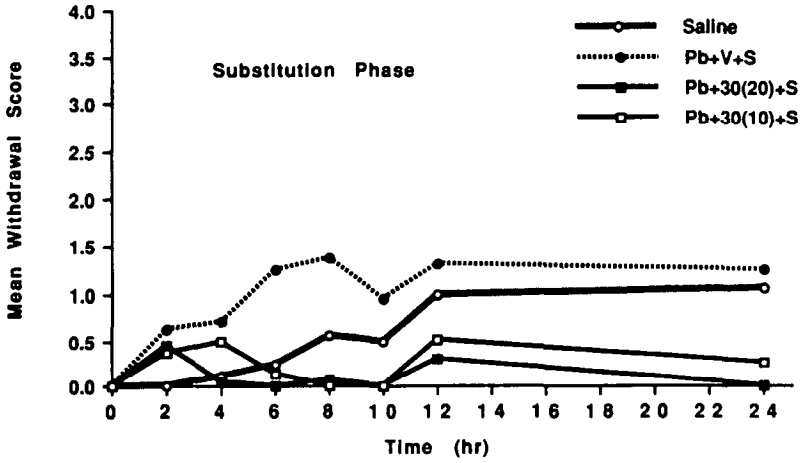


Figure 7b: Withdrawal Scores on Substitution of Vehicle(V) and CPDD#0030

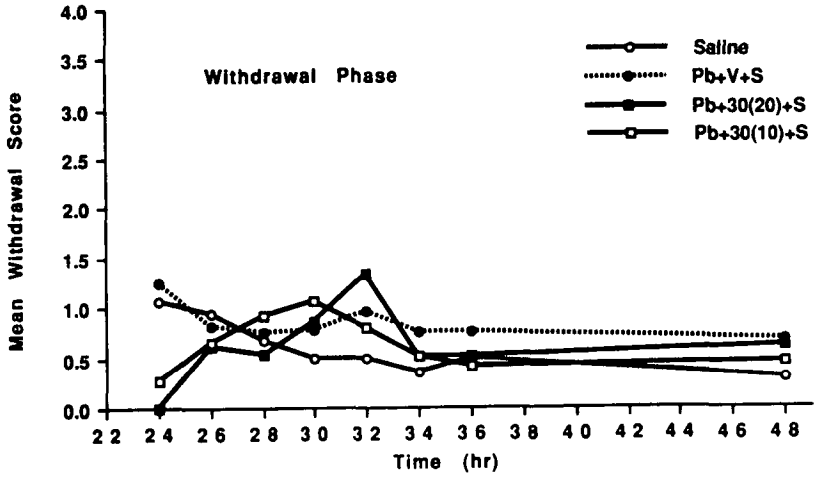


FIG. 7 Mean withdrawal scores of control rats and pentobarbital-dependent rats during (A) alprazolam or vehicle substitution (Day 13) or (B) saline substitution (Day 14). Alprazolam infused in doses of 10 to 20 mg/kg/24 hr. Each point represents the mean withdrawal score of 3 to 6 rats.

Figure 8: Changes in Body Weight with Substitution of Vehicle(V) and CPDD #0030

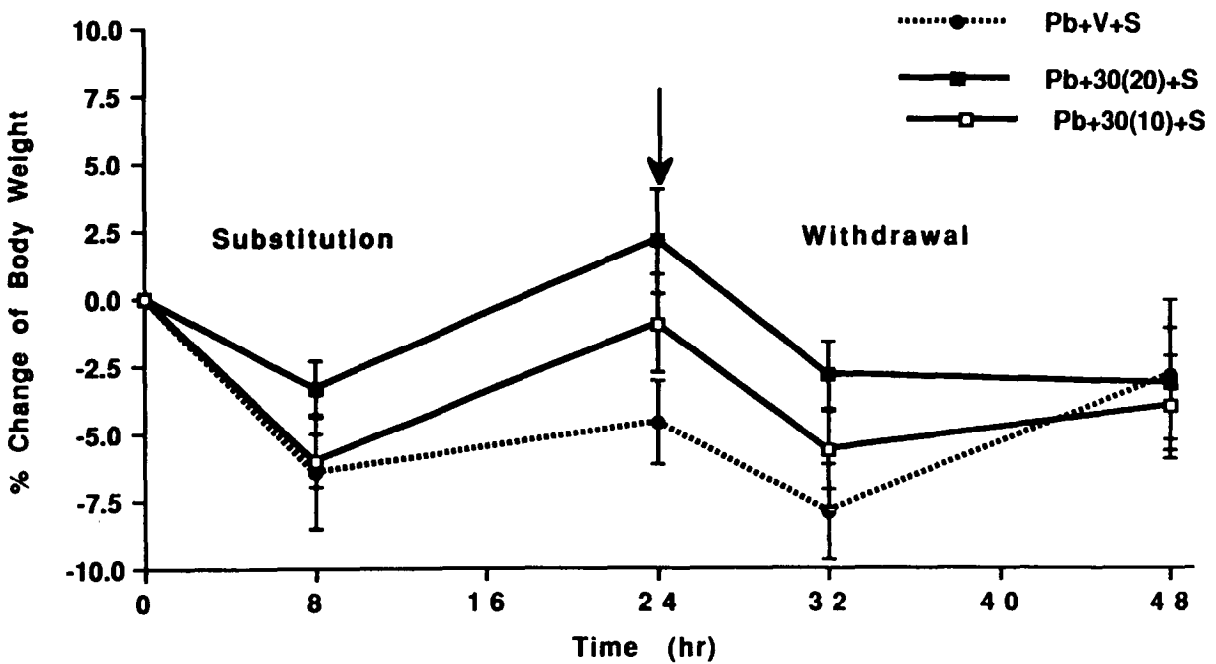


FIG. 8. Percent change in body weight of control rats and pentobarbital-dependent rats during both the drug substitution period (Day 13) and subsequent saline substitution period (Day 14). (A) Pentobarbital-dependent rats infused with either vehicle, or alprazolam in doses of 10 or 20 mg/kg/24 hr (n=3-6).

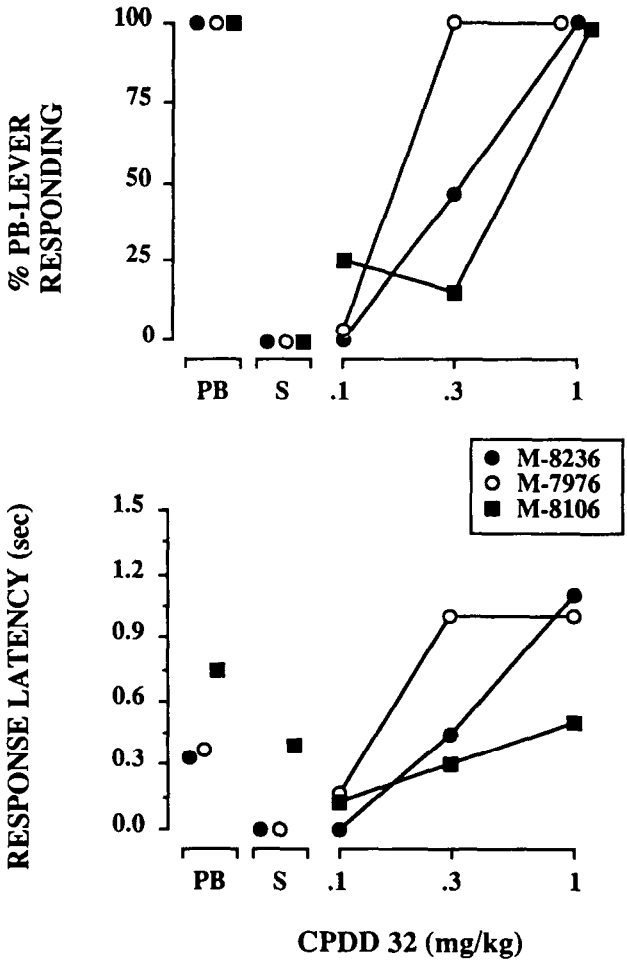


FIG. 9 Effects of CPDD 32 in rhesus monkeys trained to discriminate 10 mg/kg pentobarbital from saline. The top panel represents the percentage of total responses during test session in which pentobarbital-lever responding occurred, while the lower panel depicts the average response latency per trial. The unconnected symbols at the left represent the average of Thursday control sessions.

Figure 10a: Withdrawal Scores on Substitution of Vehicle (V) and CPDD#0032

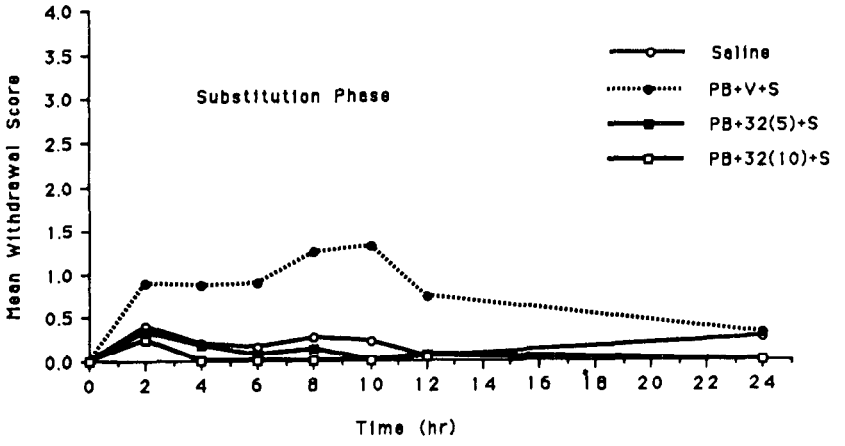


Figure 10b: Withdrawal Scores on Substitution of Vehicle (V) and CPDD#0032

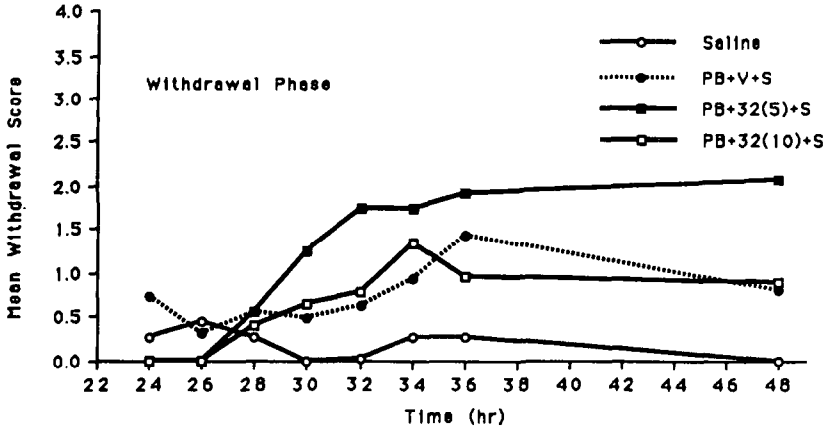


FIG. 10 Mean withdrawal score of control rats and pentobarbital-dependent rats during (A) Flunitrazepam or vehicle substitution (Day 13) or (B) during saline substitution (Day 14). Flunitrazepam was infused in doses of 5 or 10 mg/kg/24 hr. Each point represents the mean withdrawal score of 3 to 6 rats.

Figure 11: Changes in Body Weight With Substitution on of Vehicle(V) and CPDD#0032

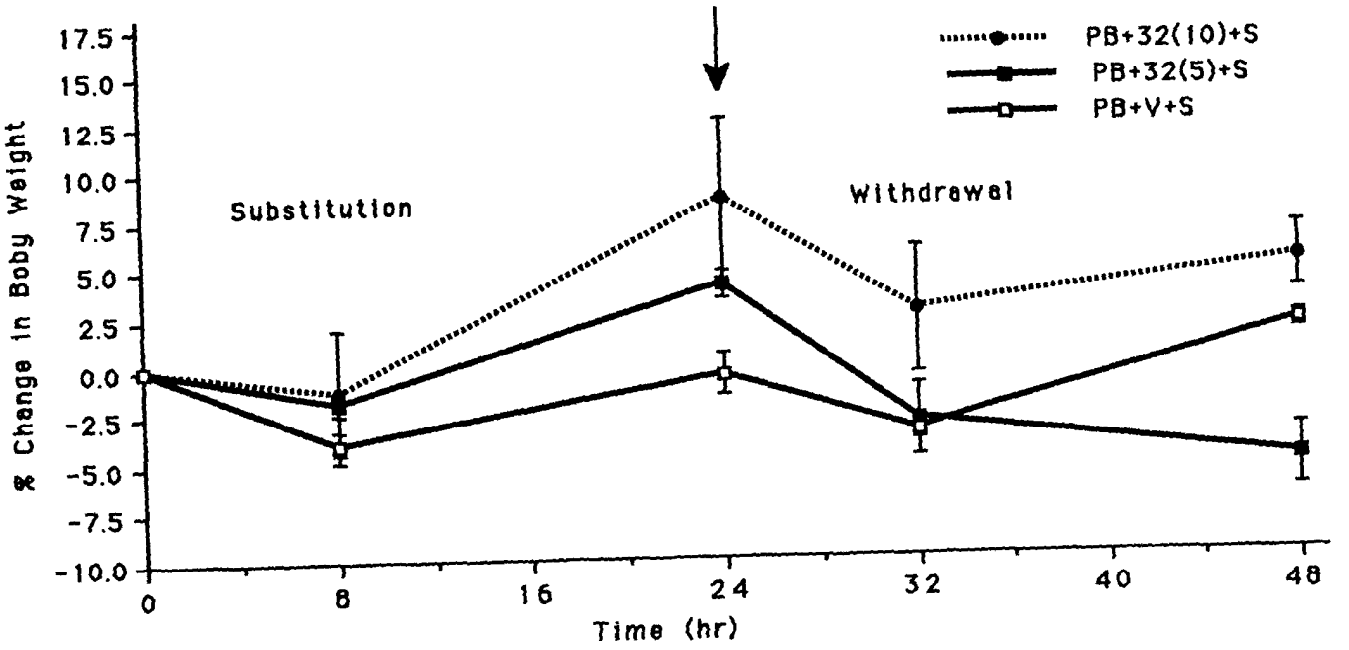


FIG. 11. Percent change in body weight of control rats and pentobarbital-dependent rats during both the drug substitution period (Day 13) and subsequent saline substitution period (Day 14). Pentobarbital-dependent rats infused with either vehicle, or flunitrazepam in doses of 5 or 10 mg/kg/24 hr (n=3-6).

Progress Report from the Testing Program for Stimulant and Depressant Drugs (1991)

G. Winger, J.H. Woods, G.A. Patrick; L.J. Powell, L.S. Harris, M.A. Nader and W.L. Woolverton

The research group involved in the evaluation of stimulant and depressant compounds has been in existence for approximately nine years. The groups includes laboratories at the University of Chicago (Nader, Woolverton), The University of Michigan (Winger, Woods), Virginia Commonwealth University (Patrick, Powell, Harris), and NIH (Jacobson). The group is part of the Drug Evaluation Committee, chaired by Ted Cicero, of the Committee on Problems of Drug Dependence. The purpose of the group is to evaluate new compounds generally classified as either stimulants or depressants for their abuse liability and dependence potential. Compounds are received, coded and distributed by Dr. Jacobson for blind testing in the various laboratories. They are evaluated for discriminative stimulus effects (UC), reinforcing effects (UM), and capacity to produce physiological dependence (VCU). This report includes some of the results of the evaluation of the following compounds: CPDD 0020, 0022, 0023, 0029 (nitrazepam), 0032 (flunitrazepam, cf. 1990 report), and 0033 (U78,875).

METHODS

Physical Dependence (Substitution) in Rats and Potency Estimation in Mice

Male Sprague-Dawley rats (Dominion Labs, Dublin, VA) initially weighing 200-225 g were individually housed in stainless steel cages with food and water available ad lib. They were used in the infusion and substitution experiments. CF-1 mice (Dominion Labs, Dublin VA) weighing 25-30 g were housed in plastic cages with food and water *ad lib.* The mice were used in initial studies for potency estimation. All animals were acclimated to the animal facility for several days prior to use in any study.

Rats were surgically prepared with an intraperitoneal cannula (PE90) while under methoxyflurane anesthesia. All rats were allowed several days to recover from surgery prior to being placed into an infusion harness. Acclimation to the infusion system occurred for three days during which the rats were continuously

infused with 0.9% saline. This was followed by the continuous infusion of either saline (control) or pentobarbital sodium for 12 consecutive days using an escalating dosing schedule (Yutrzenka, 1985). At the end of the infusion period most rats were receiving pentobarbital at a dose of 900-1000 mg/kg/24 hours. Body weight was monitored daily during the drug infusion period.

Following the final day of pentobarbital infusion, a 24-hour substitution period commenced during which pentobarbital-dependent rats were infused with either saline, vehicle, or test drug. This was followed by a 24-hour drug withdrawal period during which all rats received saline.

Every two hours for the first 12 hours and again at 24 hours of each period, rats were assigned a withdrawal score based on the degree of expression of several behavioral responses and signs. In addition, body weight was determined at 0, 8, and 24 hours of each period. Scores were assigned by two observers who were blind to the drug treatment. Investigators were blind to the identity of the compounds until all data were collected and analyzed (Yutrzenka et al, 1989).

Preliminary studies to ascertain potency of the test compounds, relative to pentobarbital were conducted in mice. Drug-treated mice were assayed using the inverted screen test (Coughenour *et al*., 1977) and alteration of spontaneous locomotor activity. At least three doses of each drug, with at least six mice per dose, were used to determine dose-response curves, Vehicle-treated mice served as controls and were assayed concurrently with drug-treated mice.

The inverted screen test was conducted at 20, 30, 60, and 120 minutes following drug administration. The ED,, dose, which was determined to be the dose at which one-half of the treated mice failed to right themselves within the 60 second time period, was computed for each time period. Spontaneous locomotor activity was determined using a single beam photocell which bisected a plastic cage containing two mice. Movement of the mice disrupted the beam and a "count" of activity was recorded. Following drug administration, activity was recorded at 5-15 min, 35-50 min, 65-95 min, and 125-185 min. The ED_{50}, dose was determined to be that dose which reduced spontaneous locomotor activity to one-half that recorded for concurrently tested vehicle-treated control mice. Potency ratios of each test drug relative to pentobarbital were determined at time of peak activity and when, in addition, the vehicle effect was no longer evident.

Pentobarbital sodium was dissolved in distilled water made isotonic with sodium chloride. CPDD 20 was prepared in a 20% propylene glycol, distilled water solution with pH adjusted with NaOH. Eight ml of each solution was infused over a 24 hour period. For this study, the test drugs were provided in coded vials by the Committee on Problems of Drug Dependence. Investigators were blind to the identity of the drugs until after completion of the study.

Withdrawal scores for each treatment group were compared to the control by use of the Mann-Whitney U-test. Alterations in body weight was tested for significance by use of t-tests. ED^{50} values and 95% confidence intervals in the

inverted screen test and locomotor activity measure were also determined (Litchfield, Wilcoxon, 1949).

Drug Discrimination in Rhesus Monkeys

The subjects were two female and five male rhesus monkeys *(Maccaca mulatta)* that weighed between 6.5 and 12.1 kg. All monkeys had extensive experience with the present drug discrimination procedure. They were housed individually in stainless steel cages in which water was continuously available. They were fed 100 to 150 g of monkey chow after each session and were given a chewable vitamin tablet 3 days/week. During experimental sessions the monkeys were seated in a Plas-Lab restraining chair and placed in a wooden cubicle (175 cm high x 85 cm wide x 65 cm deep) containing two response levers mounted 100 cm above the floor. A 40 w white house light was mounted on the ceiling. The monkey's feet were placed into shoes, the bottoms of which were fitted with brass plates which could deliver electric shocks. Programming and recording of experimental events were accomplished by an Aim 65 microprocessor located in an adjacent room.

The monkeys had been trained previously to discriminate d-amphetamine (AMPH; 7737, 7739, 8515) or pentobarbital (PB; 8106, 8236, 7976, 8814) from saline in a two-lever, discrete-trial shock-avoidance procedure similar to the one described by Holtzman (1982). One hour after an intragastric infusion (naso-gastric tube) of the training drug (0.56-1.0 mg/kg AMPH or 10 mg/kg PB) or saline, the houselights and lever lights were illuminated (trial) and responding on one lever (designated the correct lever) avoided electric shock and extinguished the lights. Responding on the incorrect lever started a 2 second change-over delay during which correct lever responding had no consequence. If a correct lever response was not made within 5 seconds of onset of the lights, an electric shock (250 msec duration, 7 mA intensity) was delivered; if a correct response was made (escape) within 2 sec of the first shock, the trial was terminated, otherwise, a second shock ended a trial. Trials were separated by a 30-s timeout. The session lasted for 30 trials or 20 min, whichever came first. The correct lever was determined by the infusion that was administered before the session. For three monkeys, the right lever was correct after drug infusions and the left lever was correct after saline infusions. This condition was reversed for the other four monkeys.

Monkeys were considered to be stable in the discrimination when more than 90% of the trials were completed on the correct lever on at least seven out of eight consecutive sessions. At this point, testing was begun with the training drugs and the test drugs. Two 5-day sequences alternated drug, vehicle and test sessions so that the first test session was preceded by two training sessions, one with saline and one with drug pretreatment and the second test session of the sequence was preceded by either vehicle or drug pretreatment. In the event that the criterion for stimulus control was not met during the training sessions, the training sequence was continued. During test sessions, both levers were operational, i.e., shock could be avoided by responding on either lever.

Saline, at least three doses of the training drug, and three doses of each test drug, in addition to the test drug vehicle, were evaluated under the test conditions for each monkey. The percentage of trials that were completed on the drug lever is presented for each test session. In addition, the average time between the onset of a trial and a lever press (average latency) was calculated for each test session. Because these test compounds were evaluated blind without any dose-response information, initial test doses were done in an ascending order from 0.1 mg/kg to doses that either significantly increased latency to respond or resulted in at least 90% drug-appropriate responding. Doses greater than 30 mg/kg were not tested. If response generalization occurred, that dose and doses higher and lower were tested again, in a random order.

PB and the test drugs were prepared immediately before testing, while a stock solution of AMPH was prepared each week. PB (40 mg/ml and AMPH (5 mg/ml) were dissolved in saline.

Self-Administration by Rhesus Monkeys

The reinforcing effects of test compounds were evaluated in a substitution self-administration procedure with methohexital serving as the baseline drug. Rhesus monkeys were surgically prepared with indwelling silicone rubber catheters using 10 mg/kg i.m ketamine and 2 mg/kg i.m. xylazine as anesthetics. Catheters were implanted in jugular (internal or external), femoral or brachial veins as necessary. The catheter passed subcutaneously from the site of the incision to the mid-scapular region, where it exited the monkey and continued, through a hollow restraining arm, to the outside rear of the cage.

The restraint and catheter protection device has been described in detail by Deneau *et al*. (1969). Monkeys were individually housed in stainless steel cages, measuring 83.3 X 76.2 X 91.4 cm deep. Each monkey wore a tubular stainless steel harness that protected the exit site of the catheter and allowed relatively unrestricted movements within the cage. A Teflon cloth jacket (Alice Ring Chatham Medical Arts, Los Angeles, CA) provided further protection for animals who tended to locate and pull their catheters. The harness was connected to a flexible spring arm that carried the catheter to the back of the cage where it joined tubing passing through a roller infusion pump (Watson and Marlow Co., Model MHRK 55; Falmouth, UK).

A 15.4 cm square stimulus panel was located on the side of each cage, approximately 10 cm from the front and 19 cm from the bottom of the cage. Across the top of the stimulus panel, 2.5 cm apart, were three circles, 2.5 cm in diameter, covered with translucent plastic and capable of being illuminated from behind by 5 W colored bulbs. The two side lights could be illuminated red and the center light could be illuminated green. Below each of the two red stimulus lights was a response lever (Model 121-07; BRS-LVE, Beltsville, MD) capable of being operated by 10-15 gms of force. Experimental control was provided by an IBM PS/2 computer programmed with Med-PC (Med-Associates,

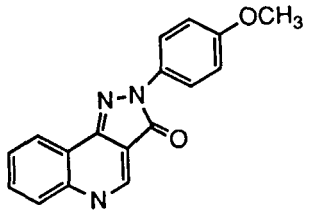
Fairfield, VT) software and located in an adjoining room.

Monkeys were adapted to restraining arms for a week or more, then an intravenous catheter was implanted and the animals were given the opportunity to respond and receive drug. Evaluation of drugs with depressant properties was carried out in monkeys trained to self-administered sodium methohexital. For these monkeys, at the beginning of each session, a red light was illuminated over one of two levers in each monkey's cage and 10 responses (fixed-ratio 10; FR 10) on that lever resulted in a 5-second infusion of 0.1 mg/kg/sodium methohexital, followed by a lo-second timeout during which all stimulus lights were extinguished and responding had no programmed consequence. During an infusion, the red lever light was extinguished and the center green light was illuminated for the duration of the infusion. Experimental sessions were limited to 210 min or until a maximum of 200 injections were delivered. No monkey ever received 200 injections of methohexital. Two sessions were scheduled each day, separated by at least four hours. On approximately half the baseline sessions, the monkeys were exposed to response-contingent saline. When there was a clear and consistent differential response between saline and methohexital, a dose of the test compound was substituted for one session. All conditions were similar to training sessions except the maximum number of injections of the test compound was limited to 150/session. Each dose was tested twice in each monkey.

Monkeys scheduled to evaluate the potential reinforcing effects of stimulant drugs were trained to self administer cocaine under a schedule much like that described by Winger *et al.* (1989). In this paradigm, the onset of the red stimulus light signalled the availability of one of four doses of intravenously delivered cocaine. Drug delivery was contingent on 30 responses on the response lever (FR30) and was followed by a 45 sec time out period (TO45). As many as 20 infusions of a given dose was permitted, or 25 min of access to each dose, whichever came first. Once one of these limits had been reached, a 10 min time out period occurred with all stimulus lights extinguished, and responses without programmed consequences. When the red stimulus light was again illuminated, another dose of cocaine was delivered following completion of the FR30 TO45 response requirement. Over a 130 min session, four doses of cocaine were made available. These doses were selected to produce a monotonically increasing function between rate of response and dose per injection of cocaine.

RESULTS

Compound #0020



Potency estimation in mice

Compound #0020 showed both stimulant and depressant effects on locomotor activity in mice. These effects were mild, erratic and unrelated to dose with the exception of the smallest dose tested (10 mg/kg) which showed a mild stimulant effect that decreased steadily over time.

Compound #0020 had only a 20% effect on the inverted screen test in mice at a dose of 300 mg/kg. This was the largest dose tested and caused death in one of six mice. No further testing was done because of the lack of effects in the initial testing as well as solubility problems and toxicity associated with this compound.

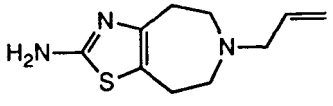
Drug discrimination studies in rhesus monkeys

Compound #0020 did not occasion pentobarbital-lever responding in any monkey tested up to doses of 30 mg/kg. There were no systematic effects on response latency at these doses.

Self-administration studies in rhesus monkeys

Because of solubility problems, Compound #0020 was not tested for intravenous self-administration in monkeys.

Compound #0022



Potency estimation in mice

Compound #0022 had mild stimulant and then mild depressant effects on locomotor activity in mice. These effects were erratic and unrelated to dose. The effects of compound #0022 on the inverted screen test were similarly erratic, generally modest, and not dose-related.

Compound #0022

continued...

Physical dependence (substitution) studies in rats.

No dose of compound #0022 was found to be equivalent to pentobarbital. When substituted for pentobarbital in pentobarbital-dependent rats, compound #0022 did not suppress signs of barbiturate abstinence and may have actually enhanced them.

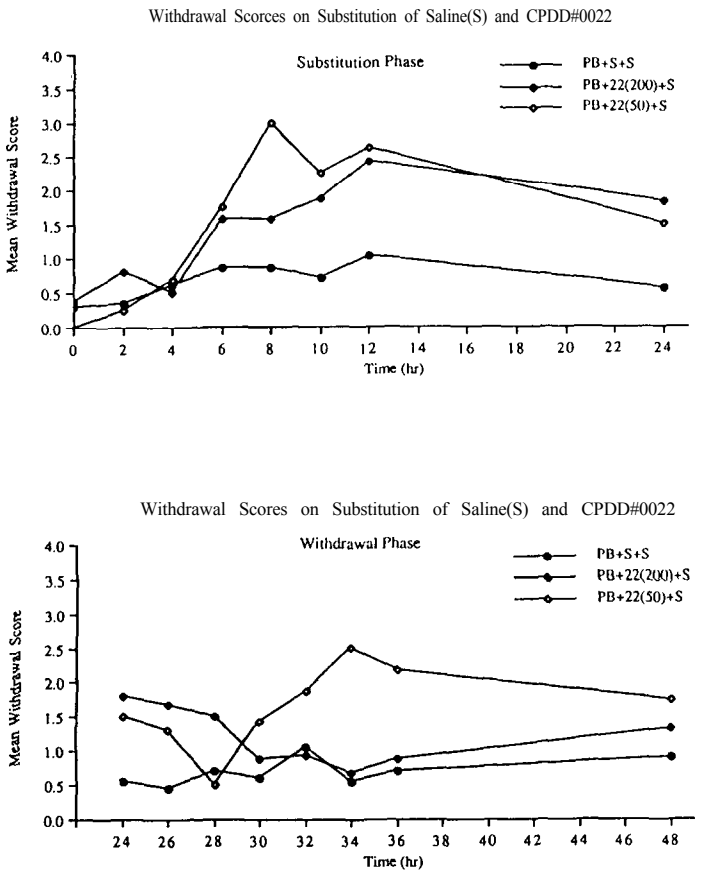


Figure 1. Mean withdrawal scores of control rata and pentobarbital-dependent rats during [a] Compound #0022 or saline substitution (Day 13) or [b] saline substitution (Day 14). CPDD 22 was infused at doses of 50 or 200 mg/kg/24 hr. Each point represents the mean withdrawal score of 3-6 rats.

Compound #0022

continued...

There was a greater loss of body weight in the compound #0022-substituted rats than in the saline-substituted rats. When the administration of compound #0022 was discontinued, the withdrawal signs did not differ markedly from those of the saline group.

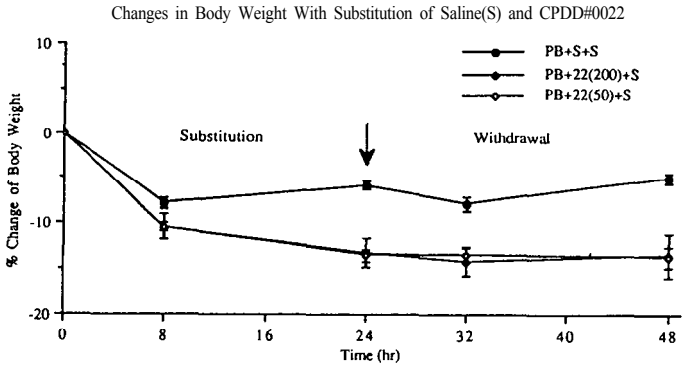


Figure 2. Percent changes in body weight of pentobarbital-dependent rats during both the drug substitution phase (Day 13) and subsequent saline substitution period (Day 14). Pentobarbital-dependent rats were infused with either vehicle or Compound #0022 in doses of 50 or 200 mg/kg/24 hrs.

Drug discrimination studies in rhesus monkeys

This drug did not occasion pentobarbital- or amphetamine-lever responding in any monkey tested up to doses of 0.30 mg/kg. At the highest doses tested, response latency was increased above saline control levels.

Self-administration studies in rhesus monkeys

Neither methohexital (Fig. 3) nor cocaine-maintained monkeys (data not shown) self-administered compound #0022. Two of the three cocaine-maintained monkeys showed a depressed response rate in the cocaine session immediately following access to compound #0022.

Compound #0022

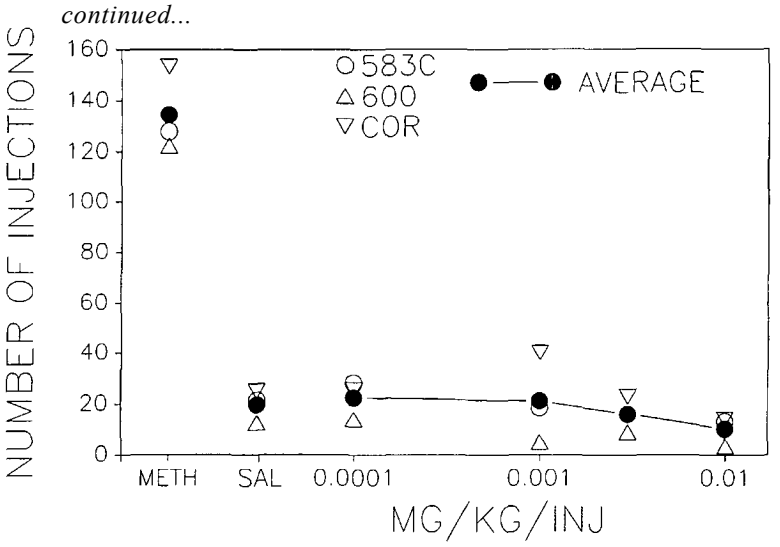
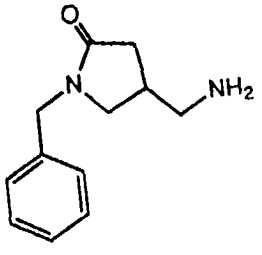


Figure 3. Effects of response contingent Compound #0022 on responding by rhesus monkeys. The points above METH are the average number of injections taken of 0.1 mg/kg/inj sodium methohexital on the sessions just preceeding each substitution of Compound #0022. These averages are shown for each individual monkey (open symbols) and for the group average (•). Similar data are shown for saline availability over the point indicated by SAL. Data for Compound #0022 are averaged across 2 observations in each monkeys. The closed, connected circles are the average of the individual monkey data.

Compound #0023

Potency estimation in mice and physical dependence (substitution) studies in rats



In mice, compound #0023 produced short-lived central nervous system depressant effects. When it was substituted in pentobarbital-dependent rats, there was no alteration in the overt signs of barbiturate withdrawal. Neither was their any indication of dependence to compound #0023 itself. Equisedative doses of this drug were not evaluated due to limited solubility of the compound.

Compound #0023

continued...

Drug discrimination studies in rhesus monkeys

In rhesus monkeys trained to discriminate the stimulus effects of oral amphetamine, compound #0023 did not mimic the amphetamine stimulus, even at a dose of 30 mg/kg. No change in response latency was observed at this dose,
suggesting a general lack of potency. Similarly, in monkeys trained to discriminate pentobarbital from saline, Compound #0023 occasioned only saline-lever responding.

Self-administration studies in rhesus monkeys

When compound #0023 was made available to rhesus monkeys experienced with self-administration of sodium methohexital, no reinforcing effects were observed (Fig. 4). This drug was also evaluated in monkeys experienced with self-administration of cocaine hydrochloride. Compound #0023 showed no reinforcing effects in these monkeys.

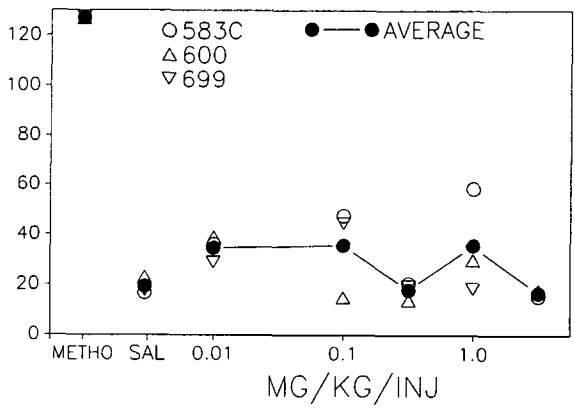
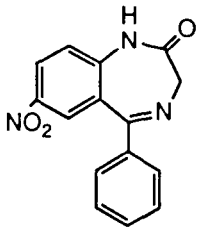


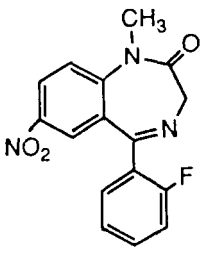
Figure 4. Effects of response contingent Compound #0023 on responding in rhesus monkeys. Details are as in Fig. 3.

Compound #0029 (Nitrazepam)



Due to difficulties putting nitrazepam into solution, no *in vivo* evaluation could be made of this drug.

Compound #0032 (Flunitrazepam)



Physical dependence (substitution) studies in rats.

In test results reported in 1990, flunitrazepam was reported to be very potent in producing typical depressant effects in locomotor activity and motor coordination. In pentobarbital-dependent rats, it suppressed signs of abstinence and, when its regular administration was stopped, barbiturate-like withdrawal signs were observed.

Withdrawal Scores on Substitution of Vehicle (V) and CPDD#0032

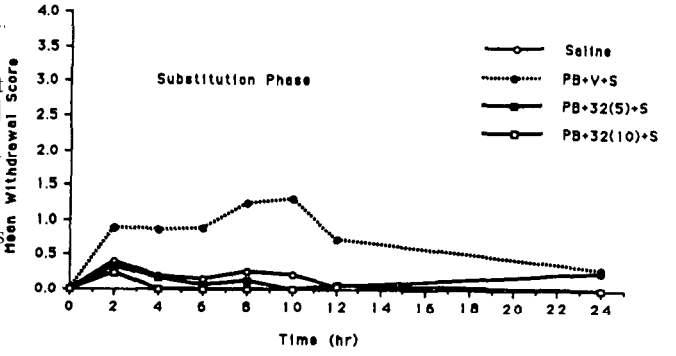
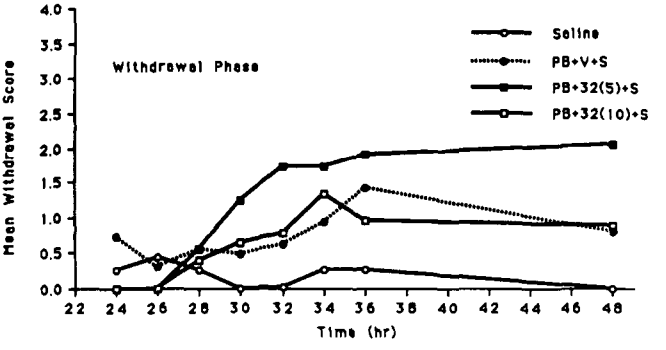


Fig. 5. Mean withdrawal score of control rats and: pentobarbital-dependent rats during [a] Compound #0032 or vehicle substitution (Day 13) or vehicle substitution (Day 14). Compound #0032 was infused in doses of 5 or 10 mg/kg/24hr. Each point represents the mean withdrawal scores of 3 to 6 rats.

Withdrawal Scores on Substitution of Vehicle (V) and CPDD#0032



Compound #0032

continued...

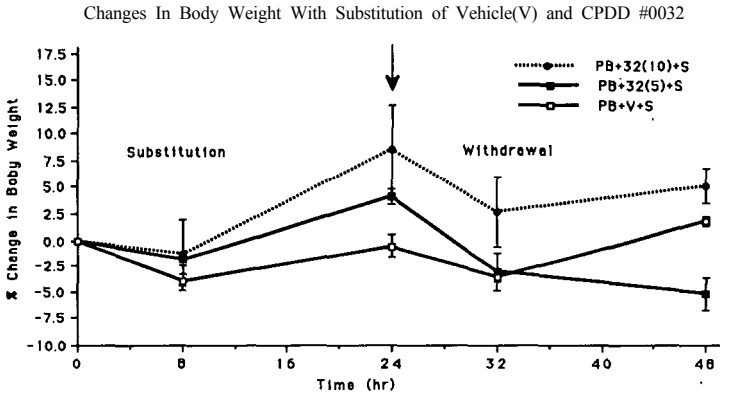
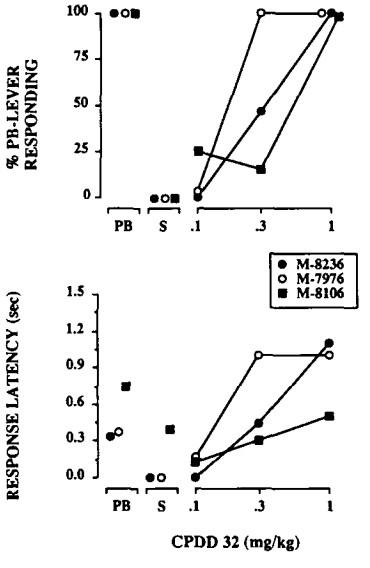


Figure 6. Percent change in body weight of pentobarbital-dependent rats during both the drug substitution period (Day 13) and subsequent saline substitution period (Day 14). Pentobarbital-dependent rats were infused with either vehicle or Compound #0032 in doses of 5 or 10 mg/kg/24 hrs.

Drug discrimination studies in rhesus monkeys



In each of three rhesus monkeys trained to discriminate the effects of pentobarbital, flunitrazepam produced pentobarbital-like discriminative stimulus effects, One monkey trained to discriminate amphetamine also generalized to flunitrazepam .

Figure 7. Effects of flunitrazepam in rhesus monkeys trained to discrimination 10 mg/kg pentobarbital from saline. The top panel represents the percentage of total responses during test sessions in which pentobarbital-lever responding occurred, while the lower panel depicts the average response latency per trial. The unconnected symbols at the left represent the average of Thursday control sessions.

Self-administration studies in rhesus monkeys

In self-administration studies, all of the three monkeys tested took more injections of flunitrazepam than they took of saline, but only one of three monkeys took as

many injections of flunitrazepam as it took of methohexital self-administration

Compound #0032

continued...

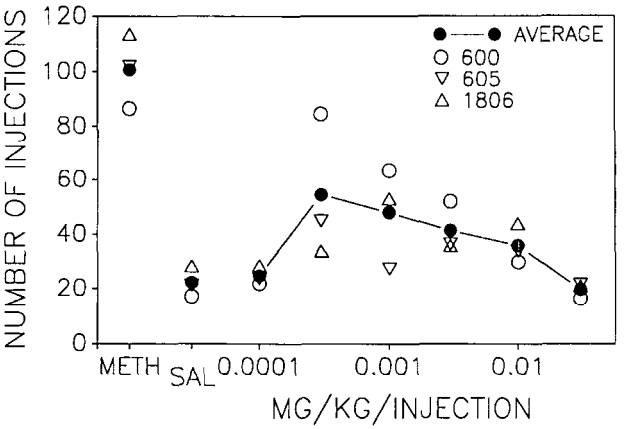
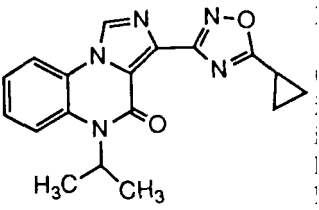


Figure 8. Effects of response-contingent flunitrazepam on responding in rhesus monkeys. Details are as in Fig. 3.

(Fig. 8). Thus, flunitrazepam appears to have a modest capacity to maintain self-injection behavior.

Compound #0033 (U78,875)



Potency estimation in mice

Compound #0033 had either no effect or an inconsistent, not dose-related effect in the mouse inverted screen assay. In the spontaneous locomotor activity assay in mice, U78,875 was less potent than pentobarbital but with a longer duration of action.

Drug discrimination studies in rhesus monkeys

Up to doses of 10 mg/kg, Compound #0033 did not substitute for either pentobarbital (n=4) or d-amphetamine in discriminative stimulus tests in rhesus monkeys.

Self-administration in rhesus monkeys

Compound #0033 was not self-administered by rhesus monkeys (Fig. 9).

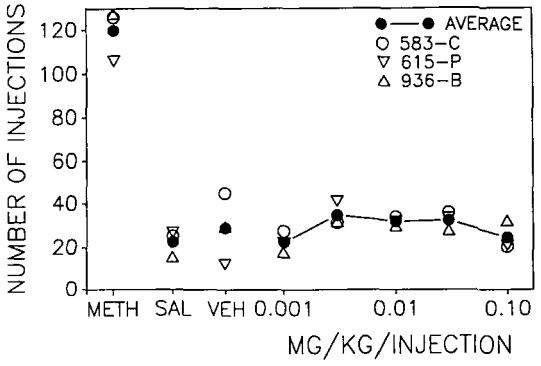


Figure 9. Effects of response-contingent Compound #0032 on responding in rhesus monkeys. Details are as in Fig. 3.

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