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Problems of Drug Dependence 1986

Proceedings of the 48th Annual Scientific Meeting

The Committee on Problems of Drug Dependence, Inc.

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES • Public Health Service • Alcohol, Drug Abuse, and Mental Health Administration

Problems of Drug Dependence, 1986

Proceedings of the 48th Annual Scientific Meeting, The Committee on Problems of Drug Dependence, Inc.

Editor: Louis S. Harris, Ph.D.

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NIDA Research Monographs are prepared by the research divisions of the National Institute on Drug Abuse and published by its Office of Science. The primary objective of the series is to provide critical reviews of research problem areas and techniques, the content of state-of-the-art conferences, and integrative research reviews. Its dual publication emphasis is rapid and targeted dissemination to the scientific and professional community.

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Foreword

The National Institute on Drug Abuse (NIDA) is pleased to publish in its Research Monograph series the proceedings of the 48th Annual Scientific Meeting of the Committee on Problems of Drug Dependence, Inc. (CPDD). This meeting was held at Tahoe City, Nevada, in June 1986. The scientific community working in the drug abuse area was saddened by the untimely death of one of its very productive and active leaders: Joseph Cochin, M.D., Ph.D. Joe was a talented scientist who was greatly admired by his students and colleagues. For the past five years, Joe had served as the Executive Secretary of the CPDD. This monograph includes papers from a symposium on "Mechanisms of Opioid Tolerance and Dependence," dedicated to his memory. These papers were presented by many of his friends and colleagues, who took the opportunity to express their high esteem for Joe.

The CPDD is an independent organization of internationally recognized experts in a variety of disciplines related to drug addiction. NIDA and the CPDD share many interests and concerns in developing knowledge that will reduce the destructive effects of abused drugs on the individual and society. The CPDD is unique in bringing together annually at a single scientific meeting an outstanding group of basic and clinical investigators working in the field of drug dependence. This year, as usual, the monograph presents an excellent collection of papers. It also contains progress reports of the abuse liability testing program funded by NIDA and carried out in conjunction with the CPDD. This program continues to represent an example of a highly successful government/private sector cooperative effort.

I am sure that members of the scientific community and other interested readers will find this volume to be a valuable "state-of-the art" summary of the latest research into the biological, behavioral, and chemical bases of drug abuse.

Charles R. Schuster, Ph.D.
Director
National Institute on Drug Abuse

ACKNOWLEDGMENT

The Committee on Problems of Drug Dependence, Inc., an independent, nonprofit organization, conducts drug testing and evaluations for academic institutions, government, and industry. This monograph is based upon papers presented or read by title at the 48th Annual Scientific Meeting of the CPDD, held in Tahoe City, California, on June 16-18, 1986. In the interest of rapid dissemination, it is published in the NIDA Research Monograph series as reviewed and submitted by the CPDD. Dr. Louis S. Harris, the editor of the monograph, is chairman of the Department of Pharmacology, Medical College of Virginia, Richmond.

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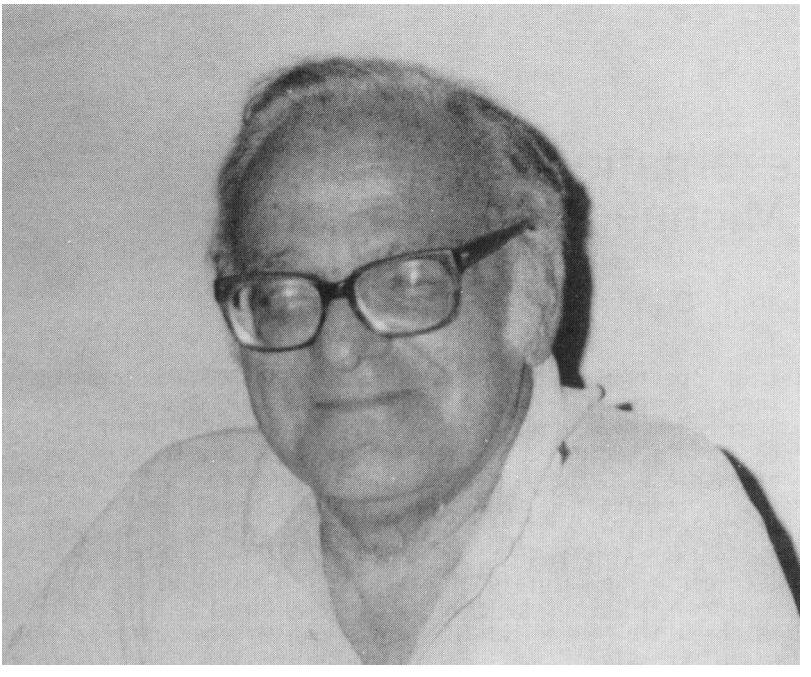

In Memoriam: Joseph Cochin, M.D., Ph.D. 1916-1985

This volume is dedicated to the memory of Dr. Joseph Cochin, whose death on October 19, 1985, saddened us all. Dr. Cochin had a long and distinguished career as a research scientist and teacher. His service to the Committee on Problems of Drug Dependence as a member and for the past five years as Executive Secretary was characterized by his patience and dedication. He is survived by his wife, Renee, and three sons, Joshua, Joel and Jesse.

His friends, colleagues, and family mourn the passing of this distinguished scholar and gentle man. We will all miss him.

Presentation of J. Michael Morrison Award

William L. Dewey

Ladies and gentlemen, I am pleased and honored to have the opportunity to present the J. Michael Morrison Award for 1986. Mike Morrison was a fine young man who worked diligently in science administration to the benefit of science and therefore, all of society. These qualities characterize this year's awardee, Dr. Edward C. Tocus. He earned his master's and Ph.D. degrees in Pharmacology at the University of Chicago. He worked at Lederle Laboratories for the first six years of his professional career. The majority of his time at Lederle was spent in endocrine research, but he also spent some time in toxicology. He has been in the Division of Neuropharmacological Drug Products at FDA since 1966. During that time, 50 different drug scheduling actions have gone through his office. These drug scheduling actions have been concerned with amphetamines, rescheduling barbiturates, scheduling of benzodiazepines, PCP and its analogs, cannabinoids and the narcotic agonist-antagonist, as well as many opiates. Equally important, during this time significant drugs have been evaluated which have been approved for the treatment of addiction. These include methadone and naltrexone. Other drugs with potential abuse potential which have been approved during his tenure include the agonist-antagonist analgesics, Nicorette, Delta-9-THC and Cismet for the treatment of nausea and vomiting associated with cancer chemotherapy.

Ed Tocus has also contributed to issues important to the Committee on Problems of Drug Dependence by serving on a number of government committees and study groups. He served for a number of years on the interagency committee on pain and discomfort. He worked with the SAODAP office in setting policy for the treatment of narcotic-dependent patients. Throughout his career he has served as a consultant to the World Health Organization and the Pan-American Health Organization on matters dealing with drug abuse treatment and control. He has served as an advisor to Congressional committees and to various state drug abuse authorities. His administrative expertise and his knowledge of drug abuse related issues have been recognized by the FDA by being chosen as their representative in the area of drug abuse at meetings of the American Medical Association, Drug

Enforcement Agency, National Institute on Drug Abuse, as well as nongovernmental scientific societies such as ACNP and CPDD. Dr. Tocus was honored by the FDA in 1981 when he was presented with their commendable service award for his contributions to the therapeutic treatment of dependency disorders and to the scheduling of abused drugs.

One of the most important aspects of the contributions of Ed Tocus is the manner in which he has carried out his duties. He has a judicious and fair way of protecting the subjects but yet allowing the evaluation of drugs to proceed.

Ed and his wife, Nora, who is earning her Ph.D. in psychology at this time, have two grown sons.

It is a pleasure for me to present this award to Dr. Tocus. The plaque is inscribed as follows:

In recognition of Outstanding Contributions in Science Administration, The J. Michael Morrison Award is presented to Edward C. Tocus at the 48th Annual Meeting of the Committee on Problems of Drug Dependence Tahoe City, California June 16, 1986.

AUTHOR

William L. Dewey, Ph.D., Medical College of Virginia, Virginia Commonwealth University, Box 613, Richmond, VA 23298

J. Michael Morrison Award Lecture, 1986

Edward C. Tocus

I am deeply honored to receive the third J. Michael Morrison Award and join the previous distinguished recipients, Robert C. Petersen now retired from the National Institute on Drug Abuse, and Kay Croker of the American Society for Pharmacology and Experimental Therapeutics. The award is for achievement as an administrator in the drug field. I have listened to recipients of various awards say how surprised they were and how they wondered what it was that led to their selection. I can say that I completely understand that reaction because it is precisely what I felt. This award represents the convergence of several phases of my professional career which I will share with you in the next several minutes.

The first phase was graduate training beginning in 1953. I like to brag that I am a direct descendent fourth generation pharmacologist; that is, starting with the father of modern experimental pharmacology, Oswald Schmiedeberg, whose student was John Jacob Abel (father of U.S. pharmacology), whose student was Eugene Maximillian Karl (EMK) Geiling, chairman of my Department at the University of Chicago. Dr. Geiling was a contemporary of Aldous Huxley, who wrote "Doors of Perception", an account of taking hallucinogenic drugs. Dr. Geiling was with Dr. Huxley *at* the time and took notes on the effects as they were experienced. Through several class lectures, I was introduced to the world of drugs of abuse including alcohol and narcotics. This exposure included an initiation into the need for careful observations and measurements in biological research. This characteristic of "staying with the data" of a carefully planned and executed study, in its broadest sense, has been a singular guiding principle in all facets of my career.

The second phase of my career leading to this recognition is the six years from 1960 to 1966 with a pharmaceutical company. Those were the anti-Vietnam War years. They were years of protests and marches against the government for social, economic, political and environmental reasons. It was a time of LSD. It was a time when I became disenchanted with both the

pharmaceutical industry and the Federal Government. I believed my future was destined in the international arena and, therefore, applied to several international health organizations for a job. After a number of months of waiting, it became apparent that you cannot get to an international health organization directly from an industry position. With the conviction that you cannot change government with marches and protests, but must join it and work from within, and with a plan to go from a federal to an international position, I applied to the FDA and was accepted to be a reviewing pharmacologist in the Division of Neuropharmacological Drugs.

That began in 1966, the third phase of my career, the bureaucratic years. Because of my interest in the pharmacology of the hallucinogenic substances, I requested and was assigned all of the drugs of abuse along with all of the narcotic analgesics. One of the first drugs which I evaluated and continued reviewing until marketing was naloxone. Of course, LSD, marijuana, CME (crude marijuana extract which later became delta-9-THC) were all assigned to me as a reviewer. Although pentazocine was available when I started, I had butorphanol and nalbuphine as my drugs. Then came methadone. In about 1968, I began attending the business and scientific meetings of the CPDD. I remember one particular business meeting at the National Academy of Sciences building in D.C. when Dr. Vincent Dole proposed establishing methadone treatment centers around the country for the heroin addicts who were without any treatment. That caused a flood of applications to the FDA, all of which were assigned to me. By 1972, it was obvious this treatment was no longer research and as a result, the methadone regulations were written to handle the several hundred programs going at that time. In 1970, the Controlled Substances Act was written and the FDA now had additional responsibilities in the drug abuse field. I was also promoted to the supervisory pharmacologist in the Division and all of the drug abuse work was too much, so we decided to establish a Drug Abuse Staff within the Division. This was done in 1971 with the agreement that I could continue to be responsible for my drug classes and the research in these classes. That began the era of LAAM and Naltrexone, the endorphins and peptide opiates, the cannabinols, and Nicorette chewing gum to name a few of the projects you might recognize. Every investigator in the country who studies a Schedule I substance in humans must have an IND with the Drug Abuse Staff of the FDA.

Recognition with the Morrison Award means that joining the government was the right choice, that my training, experience and objectives converged. Luck put me in a position to follow a class of drugs most pharmacologists rejected, drugs which my naive artist friends in New York who -protested against the war and nuclear bombs, and in support of civil rights and preservation of the environment were taking in spite of my advice. To end the story, I have been able to work with the World Health

and Pan American Health Organizations anyway, because of the experience at the FDA. Thank you.

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Introduction of Nathan B. Eddy Memorial Award Recipient—1986

Louis S. Harris

It gives me great personal pleasure to introduce this year's recipient of the Nathan B. Eddy Award. Dr. Harold Kalant is a native of Toronto, Canada. His formal education was at the University of Toronto where, as a member of the Royal Canadian Army Medical Corps., he earned an M.D. and B.Sc. in medicine. He later received a Ph.D. degree in Pathological Chemistry from the same institution. His post-graduate medical training was in Canada and Chile, an early indication of his broad international concern.

In the late 1950's he served as Biochemistry Section Head at the Defense Research Medical Laboratories in Toronto where he began his development of elegant analytical methods for the detection of small amounts of hormones and drugs in body fluids. In 1959, he began his association with the Department of Pharmacology of the University of Toronto and the Addiction Research Foundation of Ontario where he rose through the ranks to his present position as Professor of Pharmacology and Director of Biobehavioral Research. At the Addiction Research Foundation he began his pioneering studies on the pharmacology toxicology and mechanism of alcohol action, tolerance and dependence. It is fair to say that the work of Dr. Kalant and his colleagues over the past 25 years have provided much of the scientific basis for our understanding of alcohol intoxication. These studies were later extended to other drugs of abuse and form the scientific basis for his selection for the Eddy Award.

Dr. Kalant has also served the medical and scientific community by his service on numerous national and international commissions and boards. Of particular note are his services to the Committee on Problems of Drug Dependence, the Expert Panel on Drugs of Dependence of the World Health Organization and the National Institute of Alcoholism, and Alcohol Abuse, where he currently is Chairman of the Board of Scientific Counselors.

His distinguished career has earned him numerous honors. These include the Jellinek Memorial Award for Research on Alcoholism

(jointly with R.E. Popham), the Raleigh Hills Foundation International Gold Medal, and the Upjohn Award of the Pharmacological Society of Canada. In 1983, he was elected as a Fellow of the Royal Society of Canada.

Dr. Kalant is a brilliant but modest man whose career may be summed up by the following citation. "Harold Kalant distinguished scientist, gentle man. Your research on alcohol, alcoholism and other drugs of abuse has greatly benefited mankind."

The Committee on Problems of Drug Dependence is honored to name you the 1986 recipient of the Nathan B. Eddy Award.

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Nathan B. Eddy Memorial Award Lecture: Tolerance and its Significance for Drug and Alcohol Dependence

Harold Kalant

Tolerance has always been considered as one of the cardinal signs of addiction, whether to alcohol or other drugs, yet the reason for the importance attached to it has seldom been explained. Why, indeed, does tolerance really matter? The answer is not readily apparent from the research literature on this subject.

Early in the course of investigation of tolerance, a metabolic or pharmacokinetic variety was recognized, resulting from an increased rate of biotransformation of the administered drug (Pringsheim 1908). There are many examples of pharmacokinetic tolerance, affecting virtually all types of psychoactive drug. If tolerance is measured as a decrease in the duration of drug effect, pharmacokinetic factors may be of considerable importance, especially in relation to tests of long duration (e.g., duration of loss of righting reflex in rats or mice after administration of ethanol or other hypnotic-sedative drugs). However, for most experimental purposes it is much more common to express tolerance in terms of a reduction in the maximum intensity of response, or of increase in the dose required to achieve a given effect, such as increase in ED50 (Fernandes et al. 1977). Most test responses reach their maximum levels quite early after parenterial administration of drugs, or even in some cases after oral ingestion. Pharmacokinetic tolerance is of little importance under these circumstances, and one is dealing then with functional or pharmacodynamic tolerance (Kalant et al. 1971; Cicero 1980).

The traditional view has been that pharmacodynamic tolerance represents a physiological homeostatic adaptation to the direct pharmacological action of the drug, occurring at the molecular level (e.g., Collier 1965; Goldstein and Goldstein 1968). Such a homeostatic process is also generally presumed to underlie physical dependence: tolerance is shown by normal function in the presence of the drug, while withdrawal of the drug then unmasks the adaptive change which constitutes the basis of the withdrawal reaction. Since, in this view, tolerance is essentially a physiological adaptive process, it has been generally held to be specific for the type of drug used,

according to the mechanism of action of the drug. Thus, for example, tolerance to a drug such as morphine, which acts via stereospecific receptors, has been conceived as a different process from tolerance to ethanol, which acts by non-specific interaction with lipids and proteins of the cell membrane. According to this view, therefore, transfer of tolerance and of dependence between alcohol and opiates would be most improbable.

The relation of such postulated homeostatic change to continued drug-taking or drug dependence is seldom explained, except for the unproven assumption that the tolerant individual needs to increase the dose of the drug in order to achieve the euphoric or other desired effect of the drug, and for the more clearly proven observation that drug-taking may become, in part, a self-medication for the relief of withdrawal symptoms. Apart from these two possibilities, the question remains unanswered: why does tolerance really matter? Before attempting to answer it, one should examine carefully what tolerance really is, and how well or poorly the classical theories really explain it.

The traditional view of tolerance has been seriously challenged by a number of findings beginning over 20 years ago. Irwin (1963) observed that tolerance to phenothiazines developed more rapidly when the subjects were submitted to arousal stimuli during the course of drug action. Chen (1968) reported that animals which were obliged to perform a test daily under the influence of alcohol developed tolerance, whereas those which received the same dose of alcohol after the daily test performance did not become tolerant. Kayan et al. (1969) observed similarly that animals subjected repeatedly to tests of nociception under the influence of morphine developed a high degree of tolerance, while those receiving the same chronic dosage without the repeated tests developed less or none. From such observations, there arose the view that tolerance is essentially a learning process, rather than a physiological adaptation, and it has even been asserted that no tolerance occurs unless the drug-treated subject experiences test performance while in the drugged state (Wenger et al. 1981).

This view of tolerance as a learning process is consistent with a number of observations of formal resemblances between tolerance and learning. For example, when rats were exposed to repeated cycles of chronic alcohol exposure and withdrawal, they reacquired tolerance on the later cycles more rapidly than they had acquired it the first time (Kalant et al. 1978). The administration of cycloheximide, during the course of chronic ethanol administration, prevented the development of ethanol tolerance (LeBlanc et al. 1976) just as it had been previously observed to inhibit the acquisition of new learning. However, it is noteworthy that repeated cycles of alcohol exposure and withdrawal also enhanced the rate of development and intensity of expression of a withdrawal reaction (Branchey et al. 1971; Clemmesen and Hemmingsen 1984). The concept of tolerance as a learned compensation for drug effects does not offer any obvious explanation of such a facilitation of physical dependence.

Nevertheless, further support for the idea that tolerance involves learning of some type was provided by a variety of studies on both operant and classical (Pavlovian) conditioning. In studies of the effects of various drugs on operant behavior, it was observed repeatedly that drug effects which resulted in a loss of reinforcements resulted in more rapid development of tolerance. One of the earliest such observations was that by the Lexington group (Fraser 1957), that tolerance to barbiturates in human subjects was seen first on cash-rewarded tasks in which the drug effects caused a loss of earnings. Subsequently, the same phenomenon was observed in rats, in relation to the effects of amphetamine (Schuster et al. 1966) and of tetrahydrocannabinol (Elsmore 1976) on food-rewarded bar-pressing tasks. We were later able to confirm this with cocaine in a different type of operant procedure (H. Kalant and N. Woo, unpublished studies), and with ethanol and chlormethiazole (Kalant et al. 1986).

Siegel, in a thorough and systematic series of experiments beginning in the mid-1970's (Siegel 1975), has drawn attention to the role of Pavlovian conditioning of environmental cues in the development and expression of tolerance and physical dependence in rats treated with morphine. When animals were tested in the same environment in which they were treated with morphine daily, they developed increasingly rapid expression of tolerance to the analgesic and hypothermic effects, But when they were tested in a different environment, the manifestation of tolerance was markedly decreased or abolished. When they were treated with saline in place of morphine in the morphine-linked environment, they presented an overt withdrawal reaction. Similar findings have been obtained with respect to the hypothermic effect of ethanol (Lê et al. 1979; Crowell et al. 1981) and other drugs. The suggested explanation is that an innate (unconditional) adaptive response to the acute effect of the drug becomes linked conditionally to the specific environmental stimuli, so that subsequent exposure to those stimuli initiates the adaptive response as a conditioned response, beginning even before the administration of the drug. There have been numerous confirmations of the functioning of this mechanism, and its importance has been demonstrated in certain examples of cross-tolerance. For example, both ethanol and hydralazine produce hypothermia, but no cross-tolerance between ethanol and hydralazine effects on body temperature is seen (Rigter et al. 1980) unless the animals receive hydralazine in the same environment in which they have previously been made tolerant to ethanol (Lê et al. 1986c).

Further indirect support for the concept of learning as an integral part of tolerance is provided by observations of the effects of certain neuropeptides on ethanol tolerance. DeWied and his collaborators, in a long series of studies beginning in the 1960s, had observed that vasopressin, and the desglycinamide8 derivative of it (DGAVP) which is almost devoid of peripheral endocrine action, were able to maintain a learned avoidance behavior in the rat under conditions in which extinction would

otherwise have occurred (deWied and Bohus 1979). Similar doses of these hormones were found to maintain ethanol tolerance after the end of a period of chronic alcohol administration (Hoffman et al. 1978; Rigter et al. 1980; Lê et al. 1982). This effect of the peptide was demostrable only in the presence of an intact serotonergic pathway from the median raphe nucleus to the hippocampus (Lê et al. 1982; Speisky and Kalant 1985). It had previously been observed that lesioning of these pathways would, by itself, impair the acquisition of tolerance (Lê et al. 1981). While the specific involvement of a hippocampal pathway in the development and retention of tolerance can scarcely be considered proof of a learning process, it is at least compatible.

Despite all this evidence implicating a role of learning of various types in the development of alcohol and drug tolerance, it is nevertheless possible to produce tolerance in circumstances in which no opportunity for learning under drug influence is apparent. For example, continuous exposure of rats or mice to morphine, released from a subcutaneously implanted pellet, results in tolerance *to* the analgesic effects of morphine when the animals are tested for the first time after removal of the pellet. Similar results have been obtained with subcutaneous implantation of barbiturate pellets, or with continuous administration of various drugs from subcutaneously implanted osmotic minipumps. In analogous fashion, ethanol tolerance and dependence can be produced by continuous exposure to ethanol vapor in a closed chamber (Goldstein 1972), or by continuous administration of ethanol in a liquid diet modified from that of Lieber et al. (1965) (Khanna et al. 1979).

Does this mean that different types of tolerance exist, in learning and non-learning situations? At present, the answer can only be "not necessarily". There is no feature of the tolerance produced by these methods that distinguishes it from tolerance produced by the techniques which involve learning opportunities. In the studies referred to above, involving repeated cycles of alcohol exposure and withdrawal, a crossover between the first and second cycles from a learning to a non-learning model of tolerance does not interfere with the facilitation of tolerance seen in the second cycle (Kalant et al. 1978). On the other hand, tolerance to ethanol is accompanied essentially by a parallel shift of the dose-response curve, while tolerance produced by chronic high-dose treatment with morphine (Mucha and Kalant 1980) or of chlordiazepoxide (Lê et al. 1986a) gives rise to a flattening of the dose response curve rather than a parallel shift. Cross-tolerance between ethanol and chlordiazepoxide is therefore asymmetrical, involving only the "parallel shift" component (Lê et al. 1986c). Further, development of tolerance to ethanol is accompanied by cross-tolerance to barbital but not to pentobarbital, on a number of different tests (Gougos et al. 1986). These findings, which are suggestive of differences relating to the different molecular mechanisms of action of these drugs, leave open the possibility that there may in fact be more than one mechanism of tolerance.

However, the most economical hypothesis to account for the differences in tolerance produced by the same drug on the same test, under different behavioral or environmental circumstances, is that the stimulus to the development of tolerance is not the presence of the drug *per se*, or its direct molecular interaction with a specific receptor or other cell component, but rather, that the stimulus is the functional impairment produced by the drug by whatever means through which it acts. Clearly, the type and dose of drug will have an important bearing on the functional disturbance, but the latter can also be enhanced by environmental or behavioral circumstances. For example, ethanol impairs thermoregulation, and thus can give rise to hypothermia at low ambient temperatures or to hyperthermia at high temperatures. When rats are treated with ethanol repeatedly at an ambient temperature (T_a) of 4°C, which results in marked hypothermia, they develop a high degree of tolerance to the hypothermic effect of ethanol tested at normal room temperature. In contrast, rats of the same strain, receiving the same dosage of ethanol at T_a of 36°, do not develop tolerance to the hypothermic effect at room temperature (Lê *et al.* 1986b). When rats of different strains, differing in initial sensitivity to the hypothermic effect of ethanol, are treated chronically with ethanol, the strains which were most sensitive initially show the greatest acquisition of tolerance, so that they may ultimately reach the same level of response as the more resistant strains (Riley and Lochry 1977; Khanna *et al.* 1985). The cross-tolerance between ethanol and morphine, which has been observed *in vivo* and *in vitro* (Khanna *et al.* 1979; Mayer *et al.* 1980) is most easily explained the basis of tolerance to the same functional disturbances that are produced by both drugs. The same principle can be observed to apply even to relatively simple response systems. Tolerance to the effect of ethanol on post-tetanic potentiation in the abdominal ganglion of aplysia is observed only if the afferent fibres to the ganglion are stimulated repeatedly during the exposure to alcohol (Traynor *et al.* 1977). Tolerance to the effect of ethanol on a spinal reflex in the spinally transected rat is seen only when the reflex is elicited repeatedly during alcohol exposure (Jørgensen and Hole 1984). One may conjecture that neurons or synapses, in an altered state associated with functional activity, are more sensitive to the effects of ethanol and other drugs and therefore experience a greater stimulus to adapt to these effects.

This view of tolerance carries several important implications. The first is that tolerance is not a global state of resistance to the drug in question, but a delimited state that applies under certain circumstances and not others. In experimental terms, this implies that tolerance may be a test-specific phenomenon, especially at low doses of drug which do not necessarily affect all functions of the organism. Thus one sees, for example, that rats subjected to daily gavage with ethanol in a dose of 2 g/kg show substantial tolerance to the hypothermic effect of ethanol, and to its impairment of operant responding for food reward, but no tolerance on a test of motor impairment (Lê *et al.* 1984). At higher doses, which impair many basic physiologial-functions, one

is more likely to see generalized tolerance simply because almost all of the animal's normal activities will have been affected even in the absence of specific tests, and therefore an adequate stimulus to tolerance development will have arisen in most neuronal pathways. Another implication is that any proposed molecular basis for tolerance must be able to account for the interaction of the drug, the behavioral state and the environmental context in which the drug is presented. Any theory of tolerance based exclusively on molecular mechanisms such as receptor change, altered membrane composition or function, is likely to be overly simple unless it includes some postulated means by which the level of CNS arousal or the impact of incoming neuronal stimuli can modify the drug effect at a particular locus.

Another implication of this concept of tolerance is that the relationship of tolerance to physical dependence is not necessarily constant. In most purely pharmacological studies, in which large doses of morphine, alcohol, barbiturate or benzodiazepine, for example, are given regularly by intubation or by intraperitoneal injection, and a global tolerance is produced on the basis described above, physical dependence does usually develop in parallel with tolerance and is manifested as a spontaneous or evoked withdrawal reaction when the drug is stopped. However, when tolerance is produced by lower doses of drug, in a model involving a substantial role of environmental conditioning, this does not necessarily apply. For example, Siegel (1975) found no withdrawal symptoms in morphine-tolerant rats until they were exposed to the same environmental stimuli after injection with saline instead of drug. One may well ask whether such a subject is indeed dependent. Cochin and Kornetsky (1964) described very long-lasting tolerance in rats to which no morphine had been given for many months, but which still showed reduced antinociceptive effect of morphine when tested in the same test environment as before. This is clearly suggestive of a conditioned tolerance that has persisted because no extinction trials have been carried out. Is this possibly related to what has been described as "protracted abstinence"? Does this have implications for treatment of addiction in humans? Perhaps the withdrawal-like symptoms and craving experienced by addicts on returning to the environment of their habitual drug use is in fact the same phenomenon, as suggested many years ago (Wikler 1948; Ludwig and Wikler 1974). Should treatment normally include deliberate extinction trials in order to eliminate this potentially important cause of relapse?

A final question concerns the relationship of this complex and multi-faceted pattern of tolerance to reinforcement of drug intake. An individual who is exposed for the first time to the action of a drug is likely to experience a variety of effects, some pleasurable or reinforcing and others unpleasant or aversive. The relative balance between the aversive and reinforcing effects presumably determines whether or not drug consumption is likely to be repeated on other occasions. The aversive effects include most

of those which are used as common laboratory measures of drug action, such as hypothermia or impaired intestinal motility with morphine, dizziness or motor incoordination with ethanol, or appetite suppression by amphetamine or cocaine. Even the antinociceptive effect of morphine, which is a therapeutically desirable effect in patients experiencing pain, is linked to aversive effects in the healthy volunteer subject. The reinforcing effects, designated in humans by subjective terms such as euphoria and "high", must be inferred in experimental animals from objectively measured behaviors such as drug self-administration, or conditioned taste or place preferences.

Tolerance can be shown to develop readily to all of the aversive effects. In contrast, there is little or no evidence that tolerance develops to the reinforcing effects. In animal experiments involving intravenous self-administration of morphine or cocaine, for example, prolonged experience with a fixed drug dosage does not result in extinction of responding, as would be expected if the animal failed to experience the reinforcing effect as a result of tolerance. Many investigators have suggested that the reinforcing effects of drugs such as morphine, amphetamine, or low doses of sedative drugs are linked to their stimulant effects, including increased arousal, exploratory activity and elevated body temperature. Though there are a few reports of the development of tolerance to morphine-induced hyperthermia, for example, most investigators have failed to find clear-cut tolerance to these excitatory or stimulant effects. This may be considered perhaps as indirect evidence supporting the view that little or no tolerance occurs to reinforcement by drugs. In that case, the development of tolerance would mean essentially tolerance to the aversive effects, so that the preponderance of reinforcement over aversive consequences of the drug would be increased (Cappell and LeBlanc 1981). This should substantially increase the strength of the behavioral dependence. One may wonder whether an intuitive grasp of this relationship may have been responsible for the importance which has been traditionally attached to tolerance as a component of drug addiction. If this picture is correct, it raises an important goal for new therapeutic efforts in the treatment of drug dependence. This objective would be to develop methods of diminishing the relative strength of reinforcement, either by reversing the tolerance to the aversive effects, or devising some strategy for producing tolerance to the reinforcing properties. How such an objective might be attained is a subject for future recipients of the Eddy Award to discuss.

The history of the study of tolerance and drug dependence is full of examples of important observations which have been made, forgotten, and rediscovered years later. A few examples are sufficient to illustrate the point. Pringsheim (1908) reported clearly the development of pharmacokinetic tolerance to ethanol in rats subjected to daily intubation with large doses; this finding was reported nearly 60 years later by our own group (Hawkins *et al*. 1966), who performed experiments which we would not have had

to do if we had known of Pringsheim's work in time. Fraser et al. (1957) described clearly the pattern of "learned tolerance" which was rediscovered independently by Chen in 1968 and again by Wenger et al. (1981) and others later. Himmelsbach (1943) described the increased rate of development of tolerance on repeated cycles of exposure to meperidine, which our own group observed over 30 years later in relation to ethanol. Many other examples can easily be found in the present-day literature.

Purposeful repetition of earlier work is often necessary or desirable, because earlier methods are later shown to be unreliable, or because the work was not given a theoretical context which a modern repetition can provide. In many cases, however, the people who rediscover the phenomenon are not even aware after the fact that it had already been described years earlier by other investigators. This loss of continuity of knowledge is probably due to at least two factors. The first is the excessive and increasing tendency toward compartmentalization, so that what one investigator discovers with an opiate is not read by another investigator working with ethanol or benzodiazepines. The second is excessive preoccupation with what is most recent, leading many investigators to carry their reviews of the literature no farther back than five or at most 10 years. The argument is often advanced that anything older than that has been done with antiquated techniques, and is therefore not worthy of further consideration. I should like to close with a plea for a more general awareness of the need, in this as in other fields of research, for scholarship and not only for technology. Knowing what has been done in the past, and formulating the right questions on the basis of that knowledge, are at least as important as applying the most modern and sophisticated methods.

FOOTNOTE

[1]This paper is dedicated to the memory of the late Dr. Joseph Cochin, a pioneer in research on drug tolerance.

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Early Studies of Tolerance to Morphine

Conan Kornetsky

I believe it is most appropriate that the Committee on Problems of Drug Dependence have this special symposium in honor of Joseph Cochin. As all of you know, Joe invested a great deal of his energy in this organization. And the topic of this special symposium, Tolerance and Dependence is one that was the subject of his scientific career. Although Joe's death was a sad occasion for us all, we are not here to mourn his death but we are here to applaud his life.

Joseph Cochin was born the 21st of July 1916 in Winnipeg, Canada; however, his family moved to Detroit where he attended secondary school and Wayne University, receiving his B.S. in chemistry in 1937. He worked as a chemist from 1937 to 1939 at an oil refining company. However, in 1940 he received a teaching certificate from the state of Michigan and taught in the Detroit public schools from 1940 to 1942. He served in the U.S. Army from 1942-1946 with considerable duty in the South Pacific. After leaving the Army he became a graduate student in the Department of Pharmacology as well as a medical student at the University of Michigan at Ann, Arbor. He received his M.D. degree in June 1953 and his Ph.D. the following year. Joe's first choice was biochemistry, but due to an error in interpreting his undergraduate point average he was not admitted to the program in biochemistry. However, the Pharmacology Department at Michigan accurately interpreted his point average and admitted him. Biochemistry's loss was pharmacology's gain.

As many of you know, Joe was very loyal to the University of Michigan. He took almost as much pleasure with every win of its football team as with every publication from the Pharmacology Department. He would use the metaphor that Michigan was the "Harvard of the West" - I pointed out to him that he was somewhat incorrect, the metaphor really was that Harvard was the Michigan of the East.

Joe had a strong attachment to the University of Michigan. In 1958 the ASPET meeting was at Ann Arbor. It was the year that the

Pharmacology Department moved to new quarters. Although the building was empty he dragged me to it so that I would see the house built by John Jacob Abel, and he, like many other former Michigan pharmacology students, picked up a piece of the building as a memento. I believe he took a thermostat off of the wall.

Joe's Ph.D. dissertation was on the chemical determination of morphine and its biological fate. His doctoral committee was comprised of Associate Professor Lauren A. Woods as chairman, Instructor Theodore M. Brody, Associate Professor Joseph P. Chandler, Assistant Professor Edward F. Domino and Professor Maurice H. Seevers. The first paragraph of the abstract of his dissertation outlined the aims of the research and it was Joe's first but not last study designed to determine the variables accounting for tolerance and physical dependence.

> The metabolic fate of morphine and its possible interrelationship with the problems of tolerance and physical dependence have long been a matter of great interest. It is the aim of this study to attempt to elucidate the fate of the alkaloid in the animal organism and to investigate the changes, if any, which take place in the excretory pattern of morphine in the dog during chronic administration of the drug. (Cochin 1954, p. 1 Abstract).

This work was published in May 1954 in the Journal of Pharmacology and Experimental Therapeutics. (Woods et al. 1954; Cochin et al. 1954).

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PLASMA LEVELS, URINARY AND FECAL EXCRETION OF MORPHINE IN NON-TOLERANT AND TOLERANT DOGS[1-3]

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FIGURE 1
Title page of Joe's published dissertation. Copyright 1954, Williams and Wilkins Company.

Figures 1 and 2 are reproductions of the first page and a summary table, respectively from Cochin et al. (1954). Figure 2 clearly shows that there was not a significant difference in the metabolic fate between the morphine tolerant and nontolerant animal. As the authors pointed out the slight but not significant difference between the two groups are of "little importance in explaining the marked tolerance developed to morphine . . ." Joe, as always, was very conservative in his interpretation for he added, "in the dog," to the statement.

TABLE 6

Parallel urinary and fecal excretion of free, bound and total morphine (expressed as per cent of administered dose) in non-tolerant and tolerant dogs after subcutaneous administration of 30 mgm./kgm. of morphine

	DOG NO.	FECAL PLUS URINARY MORPHINE			
		Free	Bound	Total	Mean Total ± S.D
Non-tolerant	M-11	23	57	80	84 ± 6*
	M-14	18	60	78	
	M-15	20	66	86	
	M-16	25	66	91	
Tolerant	M-6	24	72	96	90 ± 10*
	M-10†	36	54	90	
	M-12†	31	47	78	
	M-13	31	66	97	

* $P < 0.2$ for the difference between the mean total excretion of the non-tolerant and tolerant dogs; therefore the difference is not satistically significant.

† The urine values used for these calculations were the average of the last five consecutive daily excretion values (see table 3).

FIGURE 2

Summary table of the results from the dissertation publication (Cochin et al. 1954). Copyright 1954, Williams and Wilkins Company.

A minor historical note is that in the footnote of this paper the word statistically is spelled incorrectly. I am not sure Joe ever noticed the error but it clearly points out to me a flaw in a Michigan education.

This paper, I believe, put to rest the hypothesis that alteration in the pattern of morphine excretion accounts for the development of tolerance and physical dependence. In order for Joe to make that simple statement it was first necessary for him to develop a sensitive, reliable and practical method for the quantitative determination of morphine in plasma and urine, not an easy task.

Joe left Michigan in the Fall of 1954 where he became a member of the Laboratory of Nathan Eddy in the National Institute of Arthritis and Metabolic Diseases in Bethesda. Here Joe did clinical studies on the efficacy of various new drugs in the relief of pain. It was in Bethesda that I met Joe. We collaborated on two series of experiments. The first of these was not directly related to the phenomenon of tolerance. The second series of experiments developed from Joe's interest in tolerance and dependence. We set out to study the time course for the development and disappearance of tolerance to morphine in the rat using both the "hot plate" and swimming speed, a simple test of motor effects.

This seemed like a fairly simple thing to do. We measured the response of the same animals to the hot plate" and speed of swimming in a 15 foot long water trough. Figure 3, taken from that paper (Cochin and Kornetsky 1964) clearly shows the rapid

development of tolerance to a 20 mg/kg test dose. Daily dose started at 20 mg/kg twice daily increasing to 20 mg/kg twice daily so that by the 5th day the total daily dose was 200 mg/kg. They

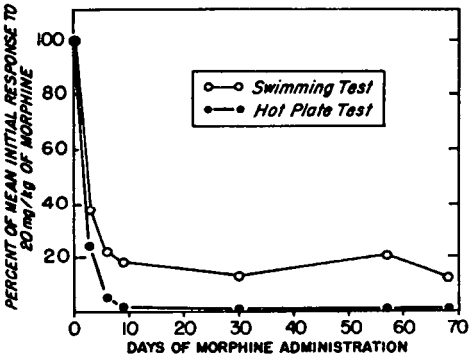

Figure 3
The development of tolerance in the same animals to 20 mg/kg of morphine in the rat on the "hot plate" and a swimming test (Cochin and Kornetsky 1964). Copyright 1964, Williams and Wilkins Company.

were then maintained on this dose until the end of the chronic drug period. We then abruptly withdrew the animals and hoped to see a rapid loss tolerance. To our surprise no rapid recovery was forthcoming.

Figure 4 summarizes the results from this experiment. There were two groups of animals, those that received the daily injections of morphine and a control group that received a single injection at the same time that the experimental animals were administered their first dose and then they were not given a second dose until 68 days later. Each animal was tested on both the "hot plate" procedure as well as speed of swimming. As can be seen there were

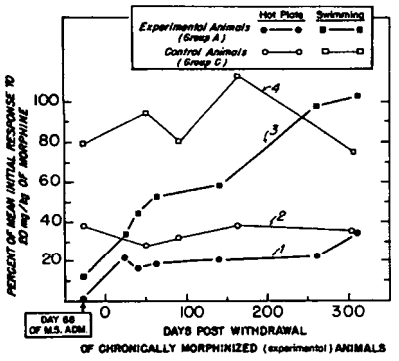

Figure 4
A comparison of recovering of sensitivity to 72 days of daily morphine administration in the rat on the "hot plate" and the swimming test. Group 1 & 2 depict the performance of the experimental group on the "hot plate" and swimming test, respectively. Groups 2 & 3 depict the performance of the control group. Copyright 1964, Williams and Wilkins Company.

clear differences in loss of tolerance in the animals on the two procedures. Striking was the marked attenuation of morphine effect in the control animal's response on the "hot plate". Their response was only 40% of their original response to morphine. The

experimental animals only reached the level of the control animals on day 300 of withdrawal where both groups still showed a response that was less than 40% of their original response. This phenomenon was not observed in the swimming procedure, a simple test of motor performance. However, even here recovery of sensitivity to the level of the control animals did not occur until somewhere between 150 to 250 days post-withdrawal. One problem that we immediately became aware of was that in order to test for tolerance we were in fact priming the tolerance.

In order to determine whether priming played a role we designed an experiment where animals would be tested for tolerance only once. In order to determine the time necessary to lose tolerance to morphine different groups of animals were used, each to be tested only once at a specified time period. The results of this experiment are illustrated in figure 5. What was striking in this experiment was that the control animals that only received morphine once at the start of the experiment, once 2 months later, and then approximately once every two months (groups C1), seemed to develop what appears to be tolerance. Group A1, the experimental group who received daily doses of morphine for the first 60 days also showed

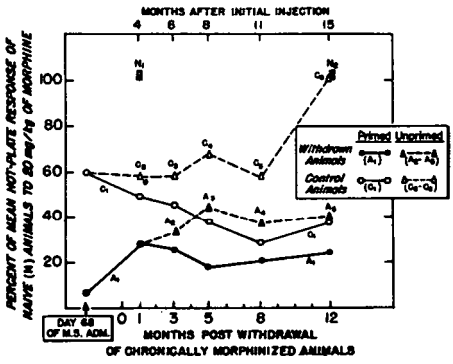

Figure 5
A comparison of "primed" and "unprimed" animals recovery of sensitivity to morphine after chronic administration doses (groups A1-5) or after an initial acute dose 68 days prior (groups C1-6). Points N1 & N2 are the response of two groups of naive animals to the test dose of morphine response is expressed as a percent of the initial response to the morphine of the experimental and control groups. Copyright 1964, Williams and Wilkins Company.

little evidence of recovery of sensitivity to morphine. The C2-6 and A2-5 animals were only tested once after the second month after the first injection of morphine. The results using the "hot plate," shown here, were clearly different from that of the swimming animals (not shown). The latter once again showed recovery of sensitivity and it was the same for primed and unprimed animals. What is most interesting in this figure is the control animal curves (C1, etc) suggesting that a single dose of morphine might have profound lasting effects.

Subsequently we (Kornetsky and Bain 1967) looked at the effect of a second dose of morphine on a foot shock titration analgesic

procedure. The results of this experiment are shown in figure 6. In this study all animals were tested only on the designated test day with half of them receiving saline on the 1st day. The indicated control level is the response of the saline animals to their first dose of morphine on the designated day. The response of the other animals is the effect of their 2nd dose but first

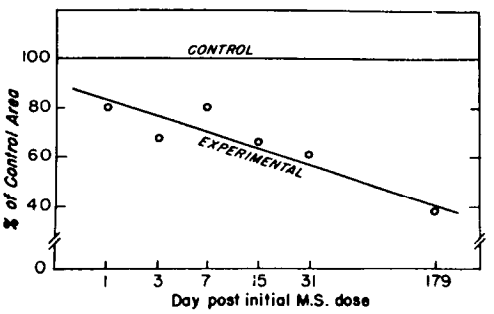

Figure 6
The effect of a single injection of 10 mg/kg of morphine in the rat on the effects of a subsequent 5 mg/kg test dose on a foot-shock attention procedure. Scores are expressed as a percent of the effect of morphine in a control group of animals who originally received saline rather than morphine (From Cochin and Kornetsky 1968 based on Kornetsky and Bain 1967). Copyright 1968, Williams and Wilkins Company.

time tested with morphine. This experiment is not dissimilar from one reported in 1953 by Nathan Eddy who showed that in mice the effects of a second dose of morphine given 24 hours following the first dose will not be influenced by the first dose, but if 72 or 92 hours intervenes the effect of the second injection is significantly altered.

The interesting thing that this experiment shows is that when only one day intervened between doses of morphine tolerance was slight. It is as if there must be some sort of consolidation, much like what is seen for short term memory. Only here we have the time period spread out and that a given dose immediately after the first, one day in this case, does not give the cell sufficient time for the consolidation of the receptor to the opiate molecule. Feinberg and Cochin (1972) reported that cycloheximide, a potent inhibitor of protein synthesis, which will prevent consolidation of short term memory, will also block the development of tolerance if given one hour prior to the morphine administration in the mouse.

In the 1964 paper (Cochin and Kornetsky) in which we demonstrated single dose tolerance, we suggested that "one of the possible explanations may be the induction of some sort of immune mechanism by the administration of morphine." This concept had been previously considered by others, Gioffredi in 1897 and 1898 (as reported by Krueger et al. 1941) reported that injection of serum

from tolerant animals into naive animals protected the naive recipients from the effects of an otherwise lethal dose of morphine. Later investigators were not able to replicate these findings and it was generally concluded that the evidence is against the presence of any specific antibody as a mechanism for tolerance. These earlier investigators mainly studied the lethal effects of morphine and did not determine the effects of passive transfer on sub-lethal doses.

The first experiment in which we attempted to passively transfer tolerance was done with the dog as the donor animal (Kornetsky and Kiplinger 1963). Dogs were given gradually increasing daily doses of morphine for two weeks. The drug was abruptly withdrawn and 48 hrs later blood was taken and serum prepared from these animals as well as comparable controls. Serum from the morphine-tolerant donors and from control donor animals was injected into two separate groups of rats and the effects of a test dose of morphine on the time needed to swim a straight alley was measured 48 hours after the injection of either control serum or serum from tolerant animals. To our surprise we observed a potentiation of the morphine effect that was subsequently replicated by Kiplinger in another laboratory using the "hot plate as a measure of effect (Kiplinger and Clift 1964).

Joe and I decided that we would pursue this work first using the rabbit as the donor animal and the mouse as the test animal (Cochin and Kornetsky 1968). Figure 7 shows the results of one of these experiments. Serum was collected one week after withdrawal and the effects of a test dose of 10 mg/kg in the mouse, given at

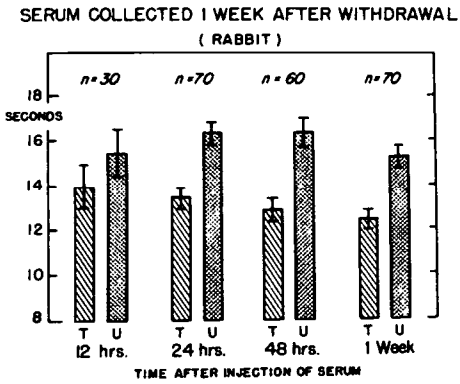

Figure 7
Effect (x ± SEM) of serum obtained from morphine-tolerant (T) and nontolerant (V) donor rabbits on the "hot plate" response of mice to 10 mg/kg of morphine. Copyright 1968, Williams and Wilkins Company.

various time periods after the injection of the serum, were tested using the "hot plate." The ordinate is the latency of response to the 10 mg/kg dose. As can be seen the animals receiving serum from the morphine tolerant animals showed significant reduction in effect at 24 and 48 hours and one week after the serum administration. We had similar results when the rat was the donor animal and the mouse the test animal (Cochin and Kornetsky 1968).

We continued this line of work attempting to see if we could replicate the Kiplinger and Clift findings and our previously mentioned findings unfortunately none of the results were as robust as previously reported. However, there was a trend in that either we found no differences or differences in the direction previously reported, that is, serum from dogs chronically treated potentiated the effects of morphine on the test procedure whereas those results in which the donor animal was the rabbit the trend was in the direction of an attenuated response in the test animals.

Friedler, in her dissertation under the direction of Joe Cochin (1968), tested the hypothesis of a possible immune factor in tolerance by removing the thymus within 24 hours after birth and then subsequently testing the mouse on the "hot plate" procedure. Although no difference was found between these two groups, she did make an important observation in that offspring of mothers treated with morphine prior to mating at 4-5 weeks of age showed a depression in the "hot plate" response to an initial dose of morphine, suggesting in some way that tolerance was passed from the mother to the offspring (Friedler and Cochin 1972). It should be noted that if the offspring were exposed to morphine in utero it could only be to trace amounts since the mothers were not bred for at least 4 or 5 days after drug withdrawal. This has led to a series of experiments by Friedler demonstrating that not only can pregestational treatment of the dam with morphine affect the offspring but the even more surprising finding, that the pregestational treatment of the sire will also affect the offspring (Friedler 1985).

Although these experiments did not lead to a clear demonstration of a immunological mechanism they did indicate that morphine and possibly other drugs may have profound effects months after the administration of morphine and further that these effects may be passed on to the offspring.

Joe added much to our understanding of the phencmenon of tolerance and physical dependence. I have described, for the most part, only those experiments that he and I worked on together or specifically related experiments. The legacy that Joe left to us is not only the answers but also the questions that he asked concerning the phenomenon of tolerance and physical dependence. We will miss him.

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Acute and Chronic Tolerance: Relevance for Clinical Practice

Carl Rosow

INTRODUCTION

Joe Cochin devoted much of his scientific career to the characterization of opioid tolerance and physical dependence in various animal models. He never forgot his clinical "roots", and we discussed the potential clinical relevance of his work on a number of occasions. He pointed out that a great deal of the human data on tolerance and physical dependence was obtained in drug abusers, particularly those at the Addiction Research Center in Lexington. It was never clear to him just how much of this information was applicable to patients receiving opioids for pain.

There is no general consensus on the importance of opioid tolerance in "normal" patient populations. In 1982 Abbott et al. published a study of opioid tolerance using an animal model of chronic pain. They concluded that"... the degree of tolerance seen is consistent with clinical reports that tolerance is not an important consideration in the management of prolonged pain." Twycross (1974) has stated that tolerance is not much of a practical problem in 85 - 90% of patients receiving heroin for pain of terminal cancer. Isbell et al. (1947) were able to use methadone in a surgical population 3 to 5 weeks without substantial dose escalation. These clinical anecdotes are certainly at odds with the preclinical literature which suggests that profound opioid tolerance can be produced in a very short period of time.

Why worry about tolerance? As long as we give the appropriate dose of medication, is the tolerant patient compromised in any way? As a clinician, I might worry about tolerance for several reasons:

1. Opioid tolerance frequently manifests initially as a decrease in duration of analgesic effect. This exacerbates the "pain cycle" with increasing pain and anticipation of pain

before each round of medication. Increasing the frequency of injections to compensate means more discomfort and inconvenience for the patient and a greater burden on the nursing staff.

2. Tolerance develops most rapidly to depressant opioid effects like analgesia, so stimulant effects like constipation become proportionately more of a problem.

3. Very high levels of tolerance may actually lead to receptor desensitization and flattening of the dose-response curve. (This was discussed earlier by Dr. Kalant). Some patients may not get sufficient relief of pain with <u>any</u> dose of opioid.

Although I am unaware of any prospective trials to assess the impact of tolerance in ordinary clinical practice, there are clues to be found in the medical literature: First, acute and chronic tolerance have both been demonstrated in "normal" patient populations. Second, the clinical use of opioids has undergone some radical changes during the last five to ten years. Opioids are now being administered in ways which appear to maximize the chances for tolerance to develop--if one believes the animal data. Finally, profound opioid tolerance has been described repeatedly in man, and the frequency of clinical reports suggests that it will become more of a problem in the future. In the remainder of this brief presentation I would like to elaborate on these three points.

ACUTE TOLERANCE

Let us consider first two phenomena described in the experimental literature, "acute" tolerance and "single-shot" tolerance. In animals, sufficient doses of opioids will produce the rapid onset of tolerance (usually within hours) to a second dose. This has been demonstrated with respect to morphine-induced hypothermia, hypotension and analgesia as well as levorphanol-induced lenticular opacification. This "acute" form of tolerance may require a high degree of receptor occupancy by agonist, while so-called "chronic" tolerance ma occur after relatively low doses. Cochin and Kornetsky (1964) demonstrated that long-term tolerance to hot-plate analgesia can be produced by one large injection of morphine. This phonomenon of "single-shot" tolerance develops over time and is easily demonstrable months after the initial injection.

I need look no further than my own specialty of anesthesiology to find a human model for acute tolerance. In 1969, Lowenstein described the use of high doses of morphine for anesthesia in open-heart surgery. It has now become common clinical practice to administer very high doses of opioid (usually fentanyl or one of its new congeners) to produce hypnosis and profound analgesia during cardiac surgery. Some representative drugs and doses are listed in Table I.

TABLE 1: Opioid doses of cardiac surgery

Drug	Analgesic Potency (Morphine = 1)	Dose (mcg/kg)
Fentanyl	100	50-150
Sufentanil	1000	10-30
Alfentanil	30	100-200

Based upon its relative analgesic potency, 30 mcg/kg of sufentanil is comparable to 30 mg/kg of morphine given as a single dose (2.1 grams/70 kg). Patients given these enormous doses of sufentanil usually require ventilation for 18-24 hours. Doses of fentanyl and alfentanil are comparably high.

Bovill et al. (1983) have demonstrated the development of acute tolerance to the hypnotic effect of alfentanil when it is used in this manner. They showed a strong positive correlation (r = 0.94) between the total dose of alfentanil administered during surgery and the plasma level of alfentanil measured at the point the patient regained conciousness. Acute tolerance to thiobarbiturates has been demonstrated in man and animals using a similar approach (Maynert and Klingman (1960). I am not aware of any descriptions of acute tolerance to the analgesic or respiratory depressant effects of alfentanil, although tolerance could well be overlooked in this clinical setting.

It is entirely speculative whether single-dose tolerance could be demonstrated in man. The cardiac surgical patients I described actually received larger absolute doses of opioid than those used by Cochin and Kornetsky in their animal experiments. No one knows how these cardiac patients respond to opioids six months after operation. If any differences have been noted, they have not been sufficiently striking to be reported.

CHRONIC TOLERANCE

The long-term or chronic tolerance which occurs upon repeated administration of opioids has much more obvious parallels in clinical practice. Cochin and others have demonstrated some rather distinctive features of chronic tolerance in animals (Cochin 1970). It can occur after low doses, requires time to develop, and the rate of tolerance development is influenced by the pattern of dosing. Mushlin et al. (1976) showed that the degree of tolerance to a second dose of morphine is approximately the same whether the doses are spaced 1 day apart or three weeks apart. In contrast, continuous parenteral administration (e.g. by subcutaneous implantation of a morphine pellet) was found to produce a very high degree of tolerance within one to three days (Way et al., (1969).

It therefore appears that continuous low plasma levels produced by frequent small doses or infusions may be more effective in

initiating tolerance than intermittent high doses. This is an important point, since maintenance of constant plasma opioid levels by frequent injections seems to be a very good way to control clinical pain (Stapleton et al., 1979). Many clinicians have lamented the inadequacy of our traditional methods of pain control (Angell 1982). Large doses of opioid prescribed "prn" tend to produce cycles of pain followed by oversedation. If the analgesic is given at regular intervals by the clock, patients are kept more comfortable since the drug is given in anticipation of pain rather than in response to pain. In these cases blood levels may fluctuate, but they tend to be maintained at or above therapeutic levels.

An even more effective treatment modality is the PCA or Patient Controlled Analgesia device which allows the patient to push a button for a small intravenous bolus of opioid (Bennett and Griffen 1983). Patients generally establish very stable blood levels of analgesic (approximating a continuous infusion) and reportedly achieve very good pain relief using relatively low total doses. Patient satisfaction is very high with this technique, and the incidence of oversedation and respiratory embarrassment is reported to be quite low.

Continuous intravenous infusion of opioid is becoming increasingly popular, especially in the intensive care unit. The most common indication is a need for sedation and suppression of airway reflexes in a patient who is artifically ventilated. Continuous infusion is also utilized in pain of terminal malignancy when intramuscular injections are not feasible or not effective.

The newest form of opioid analgesia involves direct instillation into the central nervous system. Opioids may be given intermittently or continuously through lumbar epidural or intrathecal catheters. This produces a dense segmental analgesia without other motor or sensory deficits. Morphine may also be administered by intracerebroventricular injection in selected cases. The profound and long-lasting analgesia produced this way is due to the immense concentration of opioid at a central nervous system receptor sites. This would be difficult--if not impossible--to achieve by intravenous injection.

One would predict that these trends in clinical analgesia might produce high levels of tolerance, and this does seem to be the case for some patients. Consider the following examples:

---Woods and Cohen (1982) described a patient with hip pain secondary to metastatic cervical cancer who required daily doses of methadone (90 mg p.o.) to supplement an i.v. infusion of morphine (280 mg per 24 hours). She was changed to epidural morphine, and she initially obtained adequate analgesia from 2.5 mg by this route. Within ten days she required an epidural infusion of 10 mg per hour. This heroic dose of morphine

produced no sedation or respiratory depression and barely adequate analgesia.

---Greenberg et al. (1982) describe a similar patient who required a ten-fold increase in her dose of intrathecal morphine over a one week period. Increasing tolerance and progression of her tumor led to large increases in morphine dose over the next three months. Shortly before her death the patient was receiving up to 150 mg of intrathecal morphine per day supplemented by 20-30 mg of oral levorphanol. She experienced no sedation or respiratory depression at these doses.

---Coombs et al. (1985) report a patient with metastatic adenocarcinoma of the rectum who developed complete tolerance to morphine. She experienced no sedation, no respiratory depression, and no analgesia after the injection of 17.5 mg of morphine directly into the lateral cerebral ventricle (!)

How can we account for such profound changes in some individuals? Why don't more patients get into trouble like this? Which environmental or physiological factors might identify those patients at risk? Unfortunately, these case reports show that the issue is of more than theoretical importance. The patient who has been made refractory to opiates finds himself with a sadly diminished set of therapeutic options.

Joe Cochin would have found more than a touch of irony in all of this. He spent more than thirty years lecturing medical students on the evils of underprescribing opiates for pain. I am sure he would agree that the development of tolerance is a modest risk associated with the compassionate use of opiate analgesics--but it is a risk that should not be taken lightly.

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Recent Studies on Heterogeneity and Isolation of Opioid Receptors

Eric J. Simon

I am very honored to be invited to be a speaker at this fine symposium in honor of the memory of Joe Cochin. I am sure that my being here has more to do with my friendship and love for Joe than with the relevance of my topic to this symposium, which is, of course, as it should be. I should add that Joe had a very keen interest in the biochemical pharmacology of opioid receptors and their endogenous ligands.

CPDD will not be the same without Joe, but then many things will not be the same. His impact was felt in so many areas. I would like to mention some that have not been mentioned by the other speakers. I had the privilege of serving on the NIDA Study Section with Joe. I do not remember anyone who was a more thorough and fairer reviewer of research grants than he. It was a pleasure to work with him and to spend our leisure hours together. I also had much contact with him in his role as Field Editor for JPET and, as Eve Killam will tell you, he was an exceptional one. He handled 70-80 reviews a year, twice as many as the other Field Editors, yet he managed to call every reviewer on the telephone before sending him or her the paper. As has been said many times, Joe was an outstanding pharmacologist, but, above all, he was a wonderful, warm human being with a great sense of humor and an astonishing range of interests and knowledge. This included the ability to find the best restaurants no matter where we were, Rockville, MD or Milan, Italy. We had many unforgettable dinners together and several of the participants today were often with us. Sometimes our wives were with us, including Renee, who due to the pressures of her own work was not able to travel with Joe as often as she would have liked. I am very happy that she is able to be here today to see first hand how we feel about Joe. We will miss him enormously. It was a privilege to have been his friend.

I plan to summarize for you some recent results from my laboratory concerning our efforts to understand the molecular basis of opioid receptor heterogeneity. I shall list only the most pertinent references to the work to be discussed. For more complete literature citations please see references listed at the end of this paper.

Before beginning, a word is in order concerning the relation of my topic to tolerance and dependence. The discovery of opioid receptors and their putative natural ligands, the endogenous opioid peptides, have resulted in enormous research activity and given rise to many useful and important findings. The one disappointment has been that the expected resolution of the biochemical mechanism of the long term effects of opiates has not yet occurred. However, many of the physiological effects in which the endogenous opioidergic system seems implicated bear a close resemblance to the acute pharmacological effects of opiate narcotic analgesics. It is therefore expected by most workers in this field that this system will prove to be involved in tolerance and dependence and that a thorough understanding of it will lead to the elucidation of the molecular basis of these phenomena. It would indeed be surprising if the CNS had an opioidergic system and it had nothing at all to do with the chronic action of opiates.

This wishful thinking is supported by several types of evidence. I will just mention two. One is the finding that tolerance can develop to pharmacological quantities of opioid peptides. The other is the *recent* finding that it is possible to produce selective tolerance to the separate types of opioid receptors.

The existence of multiple types of opioid receptors seems firmly established now. The major types are mu, kappa and sigma, first postulated by Martin, and the delta receptor, which has a preference for enkephalins, first suggested by Kosterlitz. There may be many other receptor types as well as subtypes, and a considerable number has already been postulated in the literature, but the evidence for these is currently less compelling. The question to which the answer is not yet known is what is the molecular basis of this receptor heterogeneity. To put it simply: are the various receptor types separate molecular entities or are they different conformations of a single receptor molecule? Our studies are aimed towards an answer to this question. Many experiments have been done in a number

of laboratories, including our own, using membrane binding and pharmacology to establish the existence and characteristics of the various types of opioid binding sites, but these studies, while extremely important, cannot shed light on their molecular basis. This is best approached by attempting physical separation of receptors and their subunits. Ultimately, it will be necessary to purify each receptor type and elucidate its structure. My summary will address these approaches.

In our laboratory Dr. Y. Itzhak was able to separate kappa sites from mu and delta sites. This was done by using a technique called sucrose density centrifugation on extracts of guinea pig brain, which retained their receptor binding activity. Such physical separation, also achieved by Dr. R.S. Zukin using a column separation, strongly suggests that the kappa site is on a separate molecule from the others. All attempts to achieve a similar separation of mu from delta sites were unsuccessful.

We therefore resorted to a different approach. These experiments were carried out by several people in my laboratory, most notably, my graduate student Andrew Howard. Iodine-labeled beta-endorphin, able to bind to opioid receptors with high affinity, had just become available. Using crosslinking reagents (double-headed reagents available commercially) it was possible to link this ligand covalently to cell membrane preparations from different brain regions and from neuroblastoma x glioma hybrid cell cultures (NG108-15). Beta-endorphin has high affinity for both mu and delta sites but very low affinity for kappa sites. This therefore provides a method for linking beta-endorphin irreversibly to these two types of receptors in the same membranes and to determine labeling patterns in tissues with widely varying ratios of mu to delta. The covalently labeled binding sites were extracted with the detergent SDS and run on SDS-polyacrylamide gel electrophoresis (SDS-PAGE). This technique separates different protein subunits by size and permits one to see whether the subunits of mu and delta sites are the same or different. The bands on the gel are visualized by autoradiography, a technique which allows the radioactive particles to expose an x-ray film. The results of this research gave strong support to the idea that the major binding proteins of mu and delta receptors are of different molecular size. The first evidence was that a protein of MW 65000 occurred in tissues enriched in mu sites but not in NC-108-15 cultures known to contain only delta receptors.

A protein of MW 53000 was observed in the cell cultures and in tissues rich in delta sites but not in tissues containing mainly mu sites, such as the rat thalamus. To prove more conclusively the nature of these binding proteins, the binding and crosslinking of ^{125}-I-beta-endorphin to brain and cultured cell membranes was run in the presence of the highly selective mu ligand, DAla^{2}-MePhe^{4}-Gly-ol^{5}-enkephalin (DAGO) and the equally selective delta ligand, Pen^{2}-Pen^{5}-enkephalin (DPDPE). It is noteworthy that both are peptides related to enkephalin but the modifications made have resulted in markedly different receptor selectivity for these two peptides. It was found that the labeling of the 65000 MW protein was selectively suppressed by DAGO, while the 53000 MW protein was no longer labeled when DPDPE was present during beta-endorphin binding and crosslinking to membranes. These results, taken together, provide strong evidence that mu and delta receptors have binding subunits that differ from each other in size and suggest that these two receptors are likely to be separate molecular species.

As stated earlier, the ultimate goal is to purify to homogeneity all separable types of opioid receptors and to obtain their complete amino acid sequence, carbohydrate and lipid composition and tertiary structure. Progress towards purification of receptors has been made in several laboratories, notably those of Abood, Barnard, Klee, Loh, and Zukin, as well as in our own. Only our own work will be summarized but references to the other studies will be found at the end of this paper.

The solubilization of opioid receptors in active form from mammalian brain membranes was accomplished by Richard Howells as part of his graduate research. This method, which involves the use of digitonin in the presence of a high concentration of sodium chloride, is still the preferred method used in our laboratory and in several others. For purification, opioid receptors were solubilized from bovine striatum. The method of purification to be described was developed by A. Howard, T.L. Gioannini and J.M. Hiller in my laboratory. The soluble receptor is purified in two major steps. The first involves affinity chromatography. A derivative of naltrexone is covalently bound to Sepharose beads. The receptor is selectively retained on columns of this material due to its affinity for the opioid ligand, while other proteins pass right through. The receptor can be eluted by high concentrations of an opioid such as naloxone. Purification of 3000-5000-fold was achieved in this

single step. The second purification step involves a column of wheat germ agglutinin (WGA) bound to agarose beads. This is a lectin which retains glycoproteins that contain the sugar, N-acetylglucosamine. Since we found that the opioid receptor is such a glycoprotein it can be purified by retention on WGA-agarose and elution by N-acetylglucosamine. This results in another 15-20-fold purification. The final product has a specific binding activity of ca. 13000 pmol/mg protein, close to the theoretical value for a pure protein of MW 65000. It gives a single band of that MW on SDS-PAGE. Considerable evidence has been accumulated that the protein purified from cow striatum is the binding subunit of the mu receptor. We are currently purifying large amounts (several hundred picomoles) of this receptor in order to obtain a partial amino acid sequence. This task has been made significantly more difficult because the amino terminal of the protein is not free. Free amino terminals must therefore be generated by fragmentation and purification of the resulting peptides before sequencing is possible. Once a suitable sequence is obtained it will be used to synthesize oligonucleotide probes. Such probes will be used to isolate the cDNA corresponding to receptor mRNA from a brain cDNA library. This cDNA will then be cloned to obtain large quantities for total sequencing. The nucleotide sequence will provide the amino acid sequence of the entire protein. This seemingly involved method is far more rapid than the direct sequencing of a large protein. This research is being done in collaboration with Dr., S. Udenfriend at Roche Institute, in whose laboratory two of my former students, R. Howells and A. Howard, are doing post-doctoral training.

A final word is in order about the significance of all this work. In the forseeable future the full structures of the major types of opioid receptors will be known. This will furnish the final proof as to which types of receptors are different molecules. We will also know whether differences in receptor type reside in the protein chain or in other parts of the molecule, such as the carbohydrate or lipid portion. The latter would mean that the same gene codes for them and that the variation is due to post-translational modification. The former would suggest different genes. The presence of considerable homology between reeceptor genes would suggest that they originated from a common ancestor gene. It will then also become possible to probe receptor function on the molecular level. This is done with a variety of techniques, e.g. reconstitution into membranes and recoupling of binding sites to other proteins such as the G-proteins and the enzyme

adenylate cyclase. Site-directed mutations can be produced and their effect on function studied. It will also become easy to make highly specific antibodies to various portions of the receptor molecules. These can be used for fine mapping of receptor distribution and they will facilitate isolation. The availability of cDNA probes will make it much easier to test for subtle differences in receptor level or turnover during the development of tolerance and dependence.

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Role of the Spinal Cord in the Development of Opiate Tolerance and Dependence

A. E. Takemori, Gary E. DeLander, and P. S. Portoghese

INTRODUCTION

Wikler and Frank (1948) were among the first to suggest the importance of the spinal cord in tolerance and dependence by demonstrating in chronic spinal dogs that supraspinal influences were not necessary for the development of these adaptive phenomena. These findings have been confirmed and extended in spinal dogs (Martin and Eades, 1964) and in spinal rats (Berge and Hole, 1981). Yaksh and coworkers (1977; 1981) showed that tolerance and physical dependence could be induced in rats and other species by repeated injections of morphine via an indwelling spinal catheter. Participation of the spinal cord in analgesia induced by systemically administered opiates has also been demonstrated (Yeung and Rudy, 1980a,b). Thus it was of interest to study the role of spinal sites and opioid receptor type which are responsible for the development of tolerance and dependence to systemically administered opiates.

In this study, we have taken advantage of the use of two of our newly developed affinity labels for opioid receptors, ß-chlornaltrexamine (ß-CNA, Portoghese et al., 1978) and ß-funaltrexamine (ß-FNA, Portoghese et al., 1980). ß-CNA is the nitrogen mustard derivative of naltrexone and is a highly selective, irreversible antagonist for opioid receptors in general. ß-FNA is the fumaramate methyl ester derivative of naltrexone and is an irreversible opioid antagonist but in contrast to ß-CNA, it is highly selective for opioid receptors of the mu type. Opioid receptors of the mu type have already been shown to be the main type in the central nervous system that are responsible for the development of physical dependence both in rats (Aceto et al., 1986) and monkeys (Gmerek and Woods, 1985; Aceto et al., 1986).

METHODS

Rats were given saline or the irreversible antagonists, ß-CNA or ß-FNA through indwelling spinal catheters 24 hr before inducing tolerance and dependence by implanting s.c. 3 morphine (50 mg base) pellets. Tolerance was assessed 72 hr later by determining the ED50 values for morphine using the tail flick and hot plate analgesic assays. Physical dependence was assessed by noting several characteristic signs after withdrawal was precipitated by injections of various doses of naloxone.

RESULTS AND DISCUSSION

ß-CNA and ß-FNA not only inhibited slightly morphine-induced analgesia 72 hr after i.t. administration but they appeared to block completely the development of analgesic tolerance as seen by the lack of change in the ED50 of morphine 72 hr after implantation of morphine pellets. Lack of change in the ED50 value does not necessarily mean that tolerance did not develop. It has been shown previously that pretreatment of animals with moderate doses of morphine several hours before analgesic testing does not change the ED50 of morphine but these animals had much higher levels of morphine in their brain indicating that some degree of central tolerance had occurred (Contreras and Takemori, 1981). This may merely mean that the usual analgesic assays are not sensitive enough to detect the development of low degrees of tolerance. The activities of the alkylating antagonists were presumed to be due solely to spinal effects because neither drug affected the analgesia induced by morphine given i.c.v. Although ß-CNA and ß-FNA were localized in the lumbar-sacral region of the spinal cord, the tolerance observed by testing morphine given i.c.v. was also inhibited suggesting a supra-spinal interaction.

Rats pretreated with either ß-CNA or ß-FNA were significantly less dependent than control rats when the withdrawn rats were observed for mastication, ear blanching, abnormal posturing, diarrhea, wet dog shakes, jumping and hypothermia. The amount of naloxone required to elicit the withdrawal signs was significantly greater in the ß-CNA- or ß-FNA-treated animals than in the control dependent rats.

CONCLUSIONS

We can conclude with the use of ß-CNA that spinal opioid receptors play a prominant role in the development of analgesic tolerance and physical dependence to systemically administered morphine. The use of ß-FNA allows us to conclude that the mu type opioid receptors in particular are important in these adaptive changes. The results also indicate that the development or expression of tolerance depends on the interaction of spinal and supraspinal sites

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Neural Substrates of Opioid Tolerance and Dependence

George F. Koob

CONCEPTUAL ISSUES

Opiate dependence has been a subject of significant interest not only because it has served as a prototype for chemical dependency in general, but also because it presumably reflects an important biological plasticity, a large extent of which is hypothesized to be located in the central nervous system. For the present, our approach is a "top-down" approach where we operationally define behavioral phenomenon and then seek to identify brain sites and neurochemical substrates for these phenomenon (Koob and Bloom, 1983). Here, opioid agonists or antagonists are administered to animals and underlying substrates are identified by alterations in behavior that reflect physical dependence or alterations in drug self-administration. Clearly integration of this information with evidence produced by the opposite approach of developing evidence at the molecular level for those cells likely to release and respond to opiates ("bottoms up") will ultimately be necessary for a multidisciplinary understanding of opiate dependence,

An important influence on my thinking in this area was a 1970 review paper of Dr. Joe Cochin (Cochin, 1970). While the subject of this paper was largely, or at least ostensibly, opiate tolerance, the principles outlined apply almost equally well to the phenomenon of dependence. One of the objectives of this paper was an attempt to describe the different hypotheses that had been used to explain opiate tolerance and dependence. The hypotheses that were discussed included altered metabolic disposition, prevention of access of drug to the site of action, occupation and saturation of receptor sites, and cellular adaptation. Dr. Cochin elaborated on the fourth hypothesis which was the cellular adaptation hypothesis. Here a biochemical transformation or an immune-like reaction was hypothesized to be the substrate for tolerance and dependence to opiate compounds.

Dr. Cochin went even further to describe the current cellular adaptation theories that had been described at that time. There

was a dual-action theory by Seevers and Woods (1953), a depression theory by Shuster (1961), and a receptor induction theory of Collier (1965) that was already alluded to in this symposium. But my favorite part of Dr. Cochin's paper was the following quote: "One of the common features of the two theories is that there is a neurohumor, C, that is affected by chronic opiate administration."

It is this premise upon which a number of us have continued to work. What is the neurohumor, and what is the neural substrate in the brain for opiate dependence? Rephrasing the question, what are the neurobiological sites that are most important for opiate dependence?

NEURAL SUBSTRATES OF PHYSICAL DEPENDENCE

Physical dependence is classically defined as "intense physical disturbances (that result) when the administration of a drug is suspended" (Eddy et al., 1965). In an early study on neural substrates of precipitated withdrawal, crystalline naloxone was injected into different loci in an attempt to precipitate a withdrawal syndrome in dependent rats (Wei et al., a 1973). The withdrawal syndrome was characterized by escape behavior and wet dog shakes. Two major areas that elicited naloxone-induced precipitated withdrawal were the medial thalamus and an area which was described as the mesencephalic-diencephalic junction. This study suggested a site-specific brain location for opiate withdrawal, and by extension, opiate dependence. More recent work, however, suggests that the conclusion that these sites in the medial thalamus and the mesencephalic-diencephalic junction may be important substrates for an "abstinence syndrome" may have been overstated. For example, large lesions of these substrates in the rat do not abolish the "abstinence syndrome" produced by injection of systemic naloxone in dependent rats (Adler et al., 1978).

A more recent study took a conceptually similar but experimentally quite different approach (Bozarth and Wise, 1984). Here, dependence was induced by the infusion of small amounts of morphine directly into different regions of the brain using a minipump. Dependence again was measured by escapes from a small container, precipitated by a systemic injection of naloxone. Perfusion of morphine into the periventricular gray region, the area around the central gray, produced increased escape when those animals received systemic injections of naloxone. The ventral tegmental area, an area at the base of the midbrain which has been implicated in opiate, stimulant and reinforcing actions and which is the source of the mesolimbic dopaminergic cell bodies, did not produce this manifestation of physical dependence. Indeed, the authors found instead that non-dependent animals readily self-administered morphine into the ventral tegmental area but not the periventricular gray (Bozarth and Wise, 1981; Bozarth and Wise, 1984).

NEURAL SUBSTRATES OF PSYCHOLOGICAL DEPENDENCE

"Psychic" dependence, as it was classically defined, is a condition in which a drug produces "a feeling of satisfaction and psychic drive that requires periodic continuous administration of the drug to produce pleasure or to prevent or avoid discomfort" (Eddy et al., 1965). Our recent work has been directed at identifying the neurosubstrates that might be involved in opiate dependence from the psychic dependence point of view. The animal model used in these studies involves intravenous self-administration of heroin in non-physically dependent rats. Rats in these studies are allowed three hours access a day to heroin, and no more. By all measures that are classifically used to define physical dependence in the rat, these rats are not physically dependent. They do not show precipitated withdrawal whether they are injected with naloxone before the session or even immediately after the session (Table I). The rats inject 0.06 mg/kg of heroin per injection intravenously on a continuous reinforcement schedule. Responding is very stable from day to day, and when an animal has established a baseline, the animals vary less than 10% from day to day. The inter-injection interval becomes very regular and very stereotyped, and in this animal model of "chipping," the rats do not show tolerance in that they do not increase the amount of heroin taken over time. Injections increase during the first two weeks and then level off to a stable number self-administered injections a day and stay at that level (Figure 1).

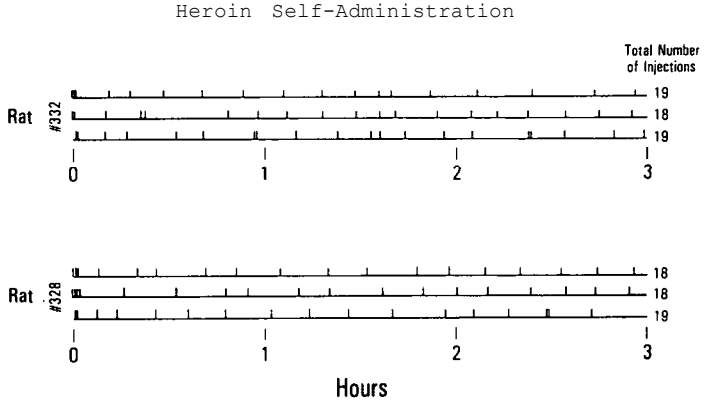

FIGURE 1. Representative response records for 2 rats self-administering heroin. Test sessions were 3 hours in duration. Each mark represents a response/infusion of intravenous drug.

However, if the rats are allowed continuous 24 hour access to, heroin for 30 days, then they will continually increase over the 30 day period (responses/24 hour were 62.6 ± 8.9 for days 1-5 and 135.5 ± 25.0 for days 26-30 for 4 rats). Injections of naloxone at this point will produce precipitated withdrawal (Table I).

TABLE I Naloxone precipitated withdrawal in dependent and non-dependent rats

	Daily 3 Hour Access	23 Hour Access
	Values represent mean ± S.E.M.	
Rearing	9.8 ± 2.5	12.8 ± 3.7
Teeth chattering	6.5 ± 1.2	13.0* ± 2.2
Weight loss (3 hours)	5.3 gms ± 1.8	17.0 gms* ± 2.9
	Values represent number of rats showing the presence of a response over the total rats/group	
Ptosis	1/4	3/4
Diarrhea	0/4	4/4*

**Significantly different from 3 hour group, information statistic or Students t-test.*

Measures of naloxone-precipitated withdrawal signs for 10 minutes in the home cage of rats with limited access to heroin (n = 4) and in rats allowed continuous access 23 hours/day (n = 4). Rats were tested in their self-administration cage after 3 hours access to heroin on a CRF schedule. Four mg/kg naloxone was injected S.C.

In the non-dependent, restricted access studies, there is an inverse dose effect function. Increasing the dose decreases the number of self-injections and increases the inter-injection interval; decreasing the dose increases the number of self-injections and decreases the inter-injection interval (Ettenberg et al., 1982; Koob et al., 1986). Thus, pharmacological antagonism is reflected as a shift to the right of the dose-effect function, and what one would predict at a dose in the middle of the function is an increase in the self-administration of heroin (Koob et al., 1986). Indeed, systemically administered naloxone increases the number of injections taken during a session. Similar effects have been observed by injecting methylnaloxonium, a quaternary derivative of naloxone that does not readily cross the blood-brain barrier, directly into the central nervous system in an attempt to block the effects of heroin self-administration. Intraventricular injection of methylnaloxonium hydrochloride produced a dose-dependent increase in the amount of heroin the animals would self-administer in a three-hour session (Vaccarino et al., 1985a). The threshold here

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was approximately 1 μg of methylnaloxonium injected intraventricularly.

Injections of methylnaloxonium into the ventral tegmental area produced basically the same dose effect function observed after intraventricular injection. However, injections of methylnaloxonium in small quantities into the area of the nucleus accumbens produced at least an 8 to 10-fold shift to the left of the dose-effect curve of methylnaloxonium compared to intraventricular injections of methylnaloxonium (Vaccarino et al., 1985b).

Thus, methylnaloxonium was much more effective when injected directly into the nucleus accumbens than into the lateral ventricle. Based on that study and other observations examining the neural substrates for stimulant-type effects of opiates in non-dependent rats (Amalric and Koob, 1985; Vaccarino et al., 1986), these data suggest the region of the nucleus accumbens might be an important substrate for opiate reinforcement. The nucleus accumbens receives significant information from limbic structures and as a part of the ventral striatum is situated in an important position to influence motivated behavior (Kelley and Stinus, 1984). There are important inputs from the amygdala, the hippocampus, the prefrontal cortex and of course, the dopaminergic system which profoundly innervates this area, and the present thesis is that this might be an important substrate for the reinforcing actions of opiates.

SUMMARY AND CONCLUSIONS

Opiate dependence is not represented by a single neuronal substrate but probably reflects actions at multiple functional sites in the central nervous system. Classical measures of opiate dependence such as escape reactions and wet dog shakes may be elicited by neural substrates in the medial thalamus and periaqueductal gray. Our work and others suggests that the region in the nucleus accumbens and its afferent and efferent connections may be an important substrate to reinforcing properties of opiates. The nucleus accumbens is also an important substrate for the reinforcing properties of stimulant drugs. These data suggest that opiate dependence may represent functional changes not only in neural substrates responsible for physical dependence, but also in brain sites responsible for the reinforcing actions of opiates.

It should be noted that there was inability in the early days when the endorphins first came to the forefront, to show correlations between changes in opiate binding and endorphin levels associated with dependence. This may be due to a failure to identify functionally significant substrates that may be implicated from what one would call the top-down approach. Perhaps the "neurohwnor" that Dr. Cochin was thinking about is an opioid peptide located in brain regions high in opiate receptors and functionally sensitive to opiate antagonists. New work

isolating particular loci for opiate actions may point to potential sites and neurochemical substrates for opiate dependence.

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Tolerance and Dependence: Implications for the Pharmacological Treatment of Addiction

Mary Jeanne Kreek

Dr. Joseph Cochin was a highly respected scientist, whose research papers teach the important fundamentals about the actions of exogeneous opiates and therefore by inference also about the endogenous opioids. I was first introduced to the works of Dr. Joseph Cochin by one of my early mentors, Professor Vincent P. Dole, who, in 1964, first introduced me to these teachings. I first met Dr. Cochin when he was a very kind and compassionate, though rigorous, site visitor on my first grant on a narcotics related topic. We then become very good friends as fellow scientists, working in areas related to narcotic pharmacology and the biology of the addictive diseases. Later I also had the priviledge of becoming a close working colleague with Dr. Cochin on this Committee on Problems of Drug Dependence.

Dr. Cochin's own teachings concerning the development of tolerance to narcotics and also the long-term persistence of these narcotic effects, made a very significant impact upon the concept of the development of a pharmacological treatment of narcotic addiction using an orally effective, long-acting narcotic, as presented by Dr. Vincent Dole and Dr. Marie Nyswander whom I had the priviledge of joining at The Rockefeller University at the initiation of their research work on the pharmacological treatment of narcotic addiction in 1964. Dr. Cochin's teachings also has had an important impact on the development of our concept of narcotic addiction as a metabolic disease.

Dr. Marie Nyswander, who was also a close friend of Joe Cochin, died two months ago on April 20, 1986. Therefore, my comments are presented in memory of both Joe and Marie, two great leaders in this field.

The problem of narcotic addiction remains an enormous one and is growing. In the United States it is estimated that there are at least 500,000 "hard core" narcotic addicts ("hard core" defined as multiple opioid injections each day with opiate dependency). It has also been estimated that there may be four

times this number of intermittent use narcotic abusers. These numbers are devastating in societal terms, in health care terms, and also now with the epidemic of AIDS growing especially important with respect to that new medical problem.

The stages of narcotic addiction can be briefly summarized: acute exposure to narcotics may lead to chronic narcotic use which,in turn, will lead to development of tolerance and development of physical dependence (which means, by definition, abstinence syndrome on drug withdrawal); protracted abstinence which may be detected much longer than acute withdrawal symptoms; and persistent drug hunger, with resultant return to narcotic use; or alternatively, use of other drugs or alcohol on the way to return to narcotic use. The acute effects of short or long acting narcotics, in man, are well known: analgesia, somnolence, and euphoria, sometimes preceded by dysphoria, as well as respiratory depression, pupillary constriction, urinary retention, constipation and a variety of alterations in endocrine function. The well known important desired effects are analgesia or euphoria; the other effects are not usually the desired effects. Tolerance develops during chronic use of narcotics in humans. Tolerance develops to most, but not all, of the acute narcotic effects, whether caused by short-acting or long-acting narcotics, although tolerance develops at varying rates for the different effects.[4] These profound differences in the rates of development of tolerance to the specific narcotic effects are of interest because they can be observed to occur in humans during constant dose therapy with the same pharmacokinetically long-acting narcotic drug, with the person exposed to the same environment on a daily basis. Thus, here we are able to observe and consider the biochemical and physiological factors which may contribute to the development of tolerance and which are different from some of the factors of conditioning or learning which were discussed in preceeding presentations. Multiple opiate receptor subtypes, with specific subtype(s) responsible for any given effect, may be in part the explanation, with varying affinities of receptors for different ligands at specific sites of action. possibly, according to recent intriguing data, different transducers and/or second messenger systems, my be operative in response to different ligands, or at different receptor subtypes, or within different tissues or organs. Also, diverse neural networks, and different varieties of neurotrasmitter/hormone modulators at different sites of action, in addition to differences in local concentrations of drugs, may also contribute to the varying rates for development of tolerance to different narcotic effects.

Because of the development of tolerance, the amount of narcotics which must be used, either administered or self-administered, to achieve the desired effect, either analgesia or euphoria, usually have to be increased. The doses of narcotic also usually have to be increased to prevent sighs and symptoms of narcotic abstinence in an individual who is physically dependent. Drug-seeking behavior may result, in part, from real or perceived symptoms of relative opiate withdrawal, and in part, as a

continuing response to some unidentified alteration in normal physiology which, as reported by Cochin and Kornetsky in 1964, may persist long after even a single narcotic administration, or could be permanent cessation of chronic regular exposure to narcotics.

Hypotheses, which we have formulated as a basis for some our work are: (1) A narcotic drug must be available to specific receptors, (i.e., opiate receptors) exerting biological activity for a finite and definable period of time for tolerance and physical dependence to develop. (2) Drug-seeking behavior may follow as a natural consequence when the signs and symptoms which result when drug (narcotic) is withdrawn from the tolerant and physically dependent individual are identified as related to drug withdrawal, and are relieved by re-administration of drugs. (3) Constant availability of drugs to specific receptors is essential for steady state maintenance of the tolerant and dependent state, and it is this constant availability of the drug to specific receptors, resulting in a steady state, which has been essential for the pharmacological, physiological and thus clinical efficacy of methadone maintenance treatment. (4) Finally, availability of drug to specific receptor sites may be affected by host response and other exogenous factors, that is, any factors which may affect overall distribution, metabolism, and excretion of the drug.

Many of the pharmacological as well as physiological effects of heroin when used on a chronic basis may be explained by its pharmacokinetic properties. The heroin addict must use muiltiple heroin injections each day, first to achieve euphoria or "high", and, with the development of tolerance, simply to prevent the onset of narcotic withdrawal, or "sick" state. Readministration of drug prevents withdrawal. Because of the short-acting pharmacokinetic properties of heroin, (or morphine), several daily injections are necessary to either get the desired effect of euphoria (or analgesia), or simply to prevent narcotic withdrawal symptoms. Methadone is a synthetic opioid drug, which we are know acts primarily at mu opioid receptors but possibly also in part at other receptor subtypes. When given in high dosages to narcotic tolerant individuals, the effects of heroin and methadone, are very different from each other. Heroin, to be fully effective must be administered intravenously, because of extensive "first pass" hepatic biotransformation after oral administration, whereas methadone can be administered orally, since its absorption from the gastointestinal tract is essentially complete, its initial hepatic uptake extensive (around 90%) but release from hepatic binding (storage) sites also extensive and biotransformation gradual.[15] The onset of action of heroin when given intravenously is immediate, whereas methadone has a 30 minute onset action time after oral administration.[6] Duration of action of heroin is 3 to 6 hours, whereas the duration of action of a moderate to high dose (30 to 120 mg./day) of methadone is 24 to 36 hours.[5,12,13,20,24] Euphoria will be experienced during the first 1 to 2 hours after

heroin administration if a large enough dose is self-administered to prevent narcotic withdrawal symptoms for six hours, whereas, if the proper dose of methadone is administered, with strategy that the dose given should be less than to which a person has become tolerant, then no euphoria, and no narcotic affect of any kind can be perceived by the patient, or observed or measured by the physician or scientist will occur, and withdrawal symptoms will not appear until 24 hours or more, unless there is some factor affecting overall drug dispostion.[10,12,22] Therefore, when moderate to high daily doses of methadone, (30-120mg/d), are; given, as single oral daily dose, one can achieve a stabilized state, a functional state, which is described as "straight", or normal, with no euphoria and no abstinence symptoms. Because of the very high degree of tolerance which is developed when moderate or large doses of methadone are administered daily, (80 mg/d studied extensively), if a dose of heroin is superimposed, administered in a research setting on a double binded basis, or self-administered on the street, then no euphoria and no subjective effects of a superimposed narcotic may be perceived by the patient nor observed or measured by the physician or scientist because of narcotic cross-tolerance. When we developed specific and sensitive analytical methods for measurements of methadone, and its metabolites in blood and other body fluids, we were able to show that this functional state was closely paralleled by the plasma levels and,' therefore, it is assumed, receptor levels of drug, with very small peak levels, (barely a doubling of the nadir or sustained plasma level at the end of a 24 hour dosing interval), along with a sustained steady-state plasma level of drug over a 24 hour dosing interval.[5,8,9,13,20] Using a variety of techniques primarily stable isotope trace, technology, and chemical ionization mass spectometry with selected ion monitoring, we now know that the apparent terminal half-life of the racemic methadone, is around 24 hours, whereas that of the active levo (-R)- enantiomer is 48 hours.[5,13,20] We also have learned that methadone is essentially completely absorbed after oral administration, and is secreted into bile and excreted in feces almost exclusively as metabolites, but in urine partially as unchanged methadone and that steady state levels of methadone in cerebralspinal fluid are achieved by daily oral dosing.[8,9,14] Methadone maintenance treatment for narcotic addiction has now been carried out in over 150,000 heroin addicts; approximately 100,000 are currently in treatment. Voluntary retention in treatment for more than 2 years is greater than 50%, ranging from 55% to 80%, depending upon the clinic arid the methadone treatment modality, with the highest retention rate apparently in those treatment programs which combine pharmacological treatment with the best features of a health maintenance organization and of a drug-free environment (counseling, general health care, mental health care along with various kinds of special groups, rehabilitation and education services being offered as well). Illicit narcotic (heroin) abuse during treatment is less than 10%. Recidivism after cessation of methadone treatment, however, is essentially the same as recidivism after exit from incarceration or from residential

drug-free treatment, or discharge from drug-free or "detoxification" programs employing short-term methadone treatment or other pharmacolcgical agents. Only 20 to 30 percent of former "hardcore" heroin addicts are found to stay narcotic free for three years or more. Seventy to eighty per cent of drug-free former, rehabilitated methadone-maintained patients will return to narcotics use following voluntary or involuntary discharge from treatment, whereas less than 10% will use illicit narcotics during treatment, and most will prefer to stay in methadone maintained treatment, if that is allowed. The actions of chronic methadone treatment, are to prevent withdrawal symptoms, prevent so-called "drug hunger", whatever the basis of that is chronic methadone treatment also "blocks", by cross-tolerance, the euphoric effects of other short acting narcotics. The mechanism of action of methadone is that this long-acting narcotic provides steady levels of opioid at opiate receptor sites thus allowing normalization of most, if not all, physiological processes which are disrupted and become abnormal during chronic heroinuse.

There has been one very intriguing yet unexplained observation with respect to chronic methadone maintenance treatment. Discussed earlier in this symposium was the problem of increasing tolerance which develops in chronic pain patients when the analgesia is the desired effect. It has been of great interest to us that, as we have now been able to observe and follow prospectively patients in methadone maintainence treatment for over 20 years, with a constant single daily dose of methadone administered orally, there is no tolerance developed to the narcotic-withdrawal prevention effect of that dose, and no tolerance to "drug-hunger" prevention by that dose. Yet when, on a double-binded basis, one significantly reduces that methadone dose abruptly, the full spectrum of narcotic withdrawal signs and symptoms along with neuroendocrine changes, which will be discussed briefly, may be observed and documented. Therefore, by steady perfusion of opiate receptors, possibly both mu and other sub-types, we are not seeing the development of increasing tolerance to the abstinence-preventing effect or drug-hunger preventing effect when this long-acting synthetic narcotic methadone is used.

Metabolic bases for addiction has been proposed by us and by many others. Those metabolic bases for addiction may include factors associated with genetics, pharmacology, but also pharmacology intersecting with physiology which was an early teaching of Cochin. Variable outcome following drug exposure may be due in part to host response differences, due to coexistent disease or altered physiological states, or drug interactions which play of course an enormous role in this treatment of the user.[7,14,12] Short-acting narcotics have "on-off" effects at specific receptor sites of action, simply because of their pharmacokinetic properties. With heroin, 3-4 cycles a day of receptor action can be observed as the heroin is administered 3-4 times a day with the peak plasma level followed by a nadir level, a peak and a

sharp decline, giving this "on-off" effect at receptor sites. Although tolerance develops to the acute opioid effects and physical dependence also develops, the physiological effects are the net result of intermittent opiate effect, followed by relative opioid withdrawal, two or three times a day. Contrasted with this are the effects of a long-acting narcotic such as methadone or levo-alpha-acetylmathadol. When methadone is administered orally, there is a sustained action at specific receptor sites, again simply because of the pharmacokinetic properties of the drug, with one cycle a day, one dose administration a day.[2] A high degree of tolerance develops to all of the various acute opioid effects and this degree of tolerance is sustained; the physiological effects are the result of a constantly steady state opioid effect, without any intermittent opioid withdrawal.

Acutely, short-acting or long-acting opioids have a variety of effects on endocrine functions. Most of these effects persist during chronic administration of short acting narcotics with resultant chronic alterations in neuroendocrine function. However, during chronic treatment with the long-acting narcotic methadone, these abnormalities slowly disappear with resultant normalization of endocrine or neuroendocrine function.[4,11,16,18,19,23] They include, in man, inhibition of release of FSH, LH, and suppression of LHRM with resultant changes in testosterone production and in ovulation, inhibition of release of ACTH and beta-endorphin which comes from the same parent peptide precursor,proopiomelanocortin, along with suppression of adrenal cortical production of glucocorticoids (cortisol in man). Abnormalities in the circadian rhythm of release of these peptides and steroid hormones also results from such acute narcotic administration. Increase in release of antidiuretic hormone, and increase release of prolactin are also observed. We have had the opportunity to follow patients maintained on a single dose of methadone administered orally, and have shown that prior to the development of full tolerance to narcotics one can observe all of the abnonmlities, described above, and in addition an abnormal metyrapone response, which is a test of hypothalamic reserve to stress, the failure of the hypothalamus. and pituitary to respond to chemically induced stress, is observed during the first 2 to 3 months of chronic methadone treatment.[4,6,11] However, after 12 months or more of steady dose methadone treatment normalization of most induced neuroendocrine functions occur.[6,16,19] However, we have also shown that minimal tolerance develops to the prolactin releasing effect on a peak level of opioid. When methadone is administered orally, the peak level occurs at around 4 hours after dose administration; and peak level of prolactin, although not elevated above normal levels, occurs at about that same time, rather than in the early morning hours. Since prolactin is usually under tonic inhibitory control by dopamine, these findings suggest that there is no full tolerance developed to the opioid effect on prolactin releases even when increased amounts of opioid are present on the sustained basis at receptor sites.

The roles of the endogenous opioids ("endorphins") in addiction remains unknown. However there have been three very different hypotheses proposed which have been that 1) narcotic addiction could be a disease of endorphin deficiency; 2) a disease of endorphin excess with failure to respond to endorphins; or 3) a disease in which there may be an altered feedback control of synthesis, release or degradation of one or more of the classes of endogenous opiates, or altered receptor response. The latter seems most likely.

We have developed and modified various techniques for qualitative and quantitative analyses of sane endogenous opioids.[16, 18, 19, 25] We have now been able to look at one simple question of: a) are there any age related changes in plasma levels of beta-endorphin in man, using a highly sensitive and specific radioimmunoasay for measurement in beta-endorphin levels. Levels were all measured in the early morning level; morning, when the levels have been shown to be the highest, following the circadian rhythm of ACTH release. One rather intriguing observation which we have made, is that be appears to be an increase in the morning resting level of beta-endorphin with age.[25]

Also, using this technology as wall as other technologies for peptide analyses we have been able to address more directly than in our earlier studies some other questions. Acute and chronic use of short acting narcotics in man with three or four doses administered each day cause chronic alterations in neuroendocrine functions, including chronic suppression of levels of release of ACTH, cortisol, and probably beta-endorphin. Although tolerance develops to many narcotic effects, tolerance, or at least adaptation, does not develop to the intermittently high and low levels of narcotic. During chronic use of methadone which is a long-acting opioid we propose that we would see normalization of most aspects of neuroendocrine function which could occur because of the long acting properties of the drug giving steady state perfusion of opioid at receptor sites. Normally one of the most important stress response mechanisms in man is that of release of ACTH, from the anterior pituitary which in turn is followed by the release from the adrenal cortex of cortisol. The hypothalamus plays an important role by releasing corticotropin releasing factor, which in turn causes release of peptides from the porrpiomelano cortin presursor peptide from the antuary pituitary. There is a negative feedback control mechanism effected by cortisol released by its adrenal cortex in response to increased circulating levels of ACTH which may be operative both at hypothalamic sites and directly at anterior pituitary sites. When plasma cortisol levels go up there is a decrease in release of the control peptide hormones, including adrenal ACTH and also beta-endorphin release. In the chronic heroin addict and in the methadone maintained patient who is in the first one or two months of treatment, at a time when doses of methadone are being increased and tolerance has not been developed, abnormal plasma levels and patterns of release of beta-endorphin ACTH and abnormalities in cortisol are observed.[4,6,11] However in a

chronic methadone maintained patient there are normal plasma levels of beta-endorphin, with a normal circadian rhythm (highest levels in the morning, lowest in the evening) normal levels of ACTH, arid nod levels of cortisol.[16,18,19] When metyrapone, a compound which blocks the 11 beta-hydroxylation of the precursor of cortisol, thus preventing cortisol production, by the adrenal cortex, which therefore interrupts the normal negative feedback signal for control at hypothalamic and pituitary sites of tropic peptide hormone release, an abnormal response is seen in the heroin addict which is an inability to release increased amounts of ACTH and an inability to release increased amounts of beta-endorphin in response to this chemically induced stress. A similar abnormal response is observed in patients during the first me to three months of methadone maintainence treatment, during which time the doses of methadone are being increased and stabilized, and full tolerance is developing.[4,6] In the chronic methadone maintained patient who has been in treatment for one year or more, after cortisol levels are dropped abruptly by metyrapone administration, an absolutely normal ability to increase release of tropic peptides hormones, as reflected by increases in plasma levels of ACTH and beta-endorphin. Thus the normalization of the hypothalamic-pituitary-adrenal axis function as reflected by normal levels and circadian rhythm of release of hormones, along with normalization of responses to chemically induced stress is achieved through this long term, steady state methadone maintenance treatment of heroin addicts. It is of interest that this normalization comes around the time when there is normalization of functional status and behaviors. Other scientific groups are studying the possible relationships between these and other stress hormones in the brain and periphery, along with patterns of release of stress hormones, with abnormal behavior.

We are also now studying the neuroendocrine status of the drug-free former heroin addict and the drug-free former methadone maintained patient. Some of our early studies lead us to believe that for a very long period of time, following restoration of a drug-free state, several neuroendocrine responses are abnormal, and that these abnormalities may indeed contribute to the behavior of drug seeking.

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Mechanisms of Opioid Tolerance and Dependence: Symposium Summary

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Because this symposium is dedicated to Joe Cochin's memory, I would like to say a word about the relationship that I had with him, aside from our close friendship. Joe got me into this field over 15 years ago when he urged me to switch from studying the effects of brain damage in animals on brain excitability to looking at the effects of brain damage on narcotic dependence and determining what brain sites might be involved in producing dependence. At the time, I was (as the euphemism goes) between grants, and it seemed like a very good idea. Because I was a young investigator at that time, I find it particularly fitting that the award being established by the CPDD in Joe Cochin's memory is for a young investigator. With those few comments, I would like to turn to the symposium.

We heard an excellent overview from Dr. Harold Kalant about the field of tolerance, with particular emphasis on the role of conditioning and learning. He discussed some of the factors, including pharmacokinetics, environment, and very importantly, the test being used, that enter into the development of tolerance with a number of drugs of abuse. It should be remembered that although we usually focus on alcohol or on opioids when we talk about tolerance and dependence, we are really dealing with a wide variety of substances, each of which has very specific pharmacodynamic properties and very specific pharmacokinetic properties associated with its use. The characteristics of dependence and tolerance and the sites of action probably vary with these different compounds although there may be some commonality, an issue I will address again at the end. Dr. Kalant pointed out that tolerance is not global, it can be test-specific, and the quantitative relationship between tolerance and dependence is not constant, especially at low doses. A particularly important

corollary to this point is that without a complete dose-response curve when looking at phenomena like tolerance and dependence, one can be led down the garden path very easily. One of the most critical questions raised by Dr. Kalant was why is tolerance important. There are many answers, or at least partial answers, as we have heard during the course of this symposium. Dr. Kalant pointed out that tolerance develops earlier to the aversive effects than to the stimulatory effects; since stimulatory effects may include the reinforcing effects, there could be a shift towards the so-called desired effects with chronic use of a substance. Despite the fact that most textbooks state that tolerance does not develop to the stimulatory effects, it is now known that it does. We and others, for example, have demonstrated that tolerance develops to the pupillary effects of opioids (Adler et al., 1980; Adler *et al.*, 1981; Higgins *et al.*, 1985; Tress *et al.*, 1978).

Dr. Conan Kornetsky spoke about the duration of tolerance that even single doses of a drug can produce and I think that this is particularly important not only in human but also in animal studies. If one gives a dose of a drug and then waits a period of time, several days or even a week or two in some studies, we make the assumption (probably incorrectly in many cases) that we are not seeing any long-lasting effect since the drug itself has been eliminated from the body. One of the problems that those of us doing animal studies encounter is that in order to do them properly we either have to resort to sophisticated cross-over designs or we have to use large numbers of naive animals in order to avoid the possibility of confounding the interpretation of results with long-lasting effects such as those discussed by Dr. Kornetsky.

Dr. Carl Rosow talked about some of the reasons why tolerance is important clinically. I might sum up part of his talk by saying that he demonstrated that as the duration of the effect diminishes, one must give injections more frequently. As shown in a number of animal studies, tolerance develops to different effects at different rates and physical dependence can be produced by regimens of frequent drug administration. Profound tolerance leads to a lessening of the maximal analgesia produced by the drug. A particularly important point that he made is that we may be doing exactly the wrong thing with the current procedures for administering drugs to patients for chronic pain or to relieve acute episodes of pain. If we can extrapolate our findings in animals to human therapeutics, then giving patients the drugs almost continuously might be

expected to result in the more rapid development of tolerance and dependence. Dr. Rosow mentioned the possibility of trying different regimens. I think that we may eventually find that the most preferable regimens are those that lie between a continuous infusion and an intermittent administration - something akin to the drug holidays that were used with psychotropic agents - in order to minimize the development of tolerance and dependence.

The next speaker was Dr. Eric Simon. Although he said that his work with the opiate receptor might not appear to pertain directly to tolerance and dependence, I believe that it does. If we do not understand the interplay between receptors and their endogenous ligands, we are not going to understand very much about the processes involved in these phenomena. Dr. Simon's work on the chemical characterization of *mu* and *kappa* receptors has important implications for understanding many of the effects of opioids, including tolerance and dependence.

Dr. Akira Takemori focused on the spinal cord in terms of the development of tolerance to the analgesic effects of morphine. He drew our attention to the ability of two long-lasting antagonists that he and his colleagues developed, ß-CNA and ß-FNA, to block the development of tolerance. In addition, he showed that tolerance depends on the interaction of spinal and supraspinal sites. That is an important concept, not only for analgesia, but for a number of effects of opioids. It appears more and more likely that we are going to see the implications of the interactions at different levels since we know that the opioid receptors are located both peripherally and centrally.

Dr. George Koob talked about dependence and its neural basis, one of the crucial areas of study in the field of centrally acting drugs. He asked the question of what are the neural biological sites that are most important for opiate dependence and said that we can raise the question of whether there is a single locus in the brain responsible for dependence and the abstinence syndrome. I do not believe that a single site is involved. There are so many different aspects to tolerance and dependence and to the expression of abstinence that it appears highly unlikely that there is one primary site. More probable is that the site depends on the particular sign that we are observing. Eddie Wei and his colleagues, using implantation of naloxone crystals into different brain areas, came to that conclusion a number of years ago (Wei *et al.*, 1972). On the basis of brain lesion experiments (Adler *et al.*, 1978), we also concluded that there was not one

site; rather, specific sites were associated with specific signs. Dr. Koob found that, for self-administration, the medial thalamus and the periaqueductal grey may be of great importance, while the nucleus accumbens may be critical for reinforcement. His studies are vital for an understanding of reinforcement and the reason that people continually seek to use drugs. Identifying the sites of action with some of the techniques that Dr. Koob discussed will certainly play a role in our understanding of the use of abused drugs, and potentially in treatment methods for patients in the future.

Finally, the studies reported by Dr. Mary Jeanne Kreek brought together information about pharmacodynamic and pharmacokinetic factors for treating addicts with methadone. She also showed that tolerance to some of the neuroendocrine effects of methadone developed rapidly while tolerance developed more slowly to some other endocrine effects. Furthermore, she pointed out that normalization of the hypothalamo-pituitary-adrenal axis functions may be important. We seem to be dealing here with some of the compensatory mechanisms that occur and that are important in terms of the signs associated with chronic use of a number of drugs, including methadone.

I would now like to indicate a few of my own thoughts on tolerance and dependence. I can do that by focusing on one of the effects that Joe Cochin studied and in which he was very interested - the effects of opioids on body temperature. I believe that the evidence is accumulating that mu and kappa receptors play a vital role in thermoregulation itself. Dr. Cochin and his colleagues (Rosow *et al.,* 1982) found that 10 mg/kg of morphine given ip to mice at 20 or 25°C ambient results in a small drop in body temperature. Upon chronic administration of that dose, the effect gradually reverses and one sees an increase in body temperature. This reversal becomes even more apparent as the dose of morphine is increased. These studies, as well as others in which ambient temperatures were altered led to the conclusion that there was marked tolerance to some of the temperature effects of opioids but not to others. In fact, there was an enhancement of some effects. To bring in drugs other than the opioids, one merely has to look to cocaine and amphetamine to see not only tolerance to some effects, but what has been called "reverse tolerance" or an actual increase in sensitivity to others. This phenomenon has been seen with opioids, as well, but has not been recognized until fairly recently. The study of tolerance mechanisms is not restricted to just those methods that

have been discussed at this symposium. For example, studies by Klee *et al.* (1984), Law *et al.* (1984) and others used neuroblastoma clonal cell lines to show that receptor desensitization, receptor downregulation, and an increase in adenyl cyclase occurred during chronic opiate treatments. Their work indicates that tolerance and dependence are separate cellular adaptation processes, something that a number of investigators have concluded using *in vivo* approaches in addition to in vitro ones. Work by Cochin and Kornetsky (1964) on the idea that there may be some involvement with the immune system is pertinent to what we are finding today. They suggested that aspects of tolerance and dependence might be due to the induction of an immune mechanism. That idea is particularly intriguing because we now know that opioids can affect some parts of the immune system, as will be discussed at a later session of this meeting by Dr. Arthur Falek and his group. Even more intriguing are the findings that opioid receptors exist in several components of the immune system.

Although we do not yet know the basic mechanisms that underlie tolerance and dependence, a number of discoveries in the past few years have obvious implications for our understanding of these states. These findings include the discovery of the opioid receptor, the realization that multiple opioid receptors are present *in vivo,* the discovery of three families of endogenous opioid peptides, the anatomical localization of the receptors and the peptides, and the gradual elucidation of the functional roles of the receptors and the peptides. The relevance of our findings with opioids bears more than a tenuous relationship to other drugs of abuse. Not only does there appear to be some commonality among the pathways and the factors that influence reinforcement, but the interactions of the various drugs of abuse with neuropeptides might have some similarities. This is an area of research that is just beginning. On the other hand, it still seems that there are specific characteristics associated with the different drug classes. The advances in the opioid field have played a significant role in opening the neuropeptide field in general; thus, the study of opioids, although not yet providing an answer to the question of the basic mechanisms involved in tolerance and dependence, have succeeded in opening new vistas in understanding the interplay among numerous chemicals and receptors in the brain and elsewhere. In the long run, the lasting legacy of research on tolerance and dependence may be in our overall understanding of brain mechanisms.

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Morphine Antinociception in the Chronic Spinal Rat

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Spinal opiate administration produces potent, selective and long-lasting analgesia in a variety of acute and chronic pain states. Recent evidence, however, indicates that the efficacy of spinal morphine does not decline during chronic exposure, suggesting that the spinal cord does not exhibit tolerance. To examine directly the spinal contribution to opiate analgesia and tolerance we studied opiate antinociception in spinally transected rats.

Spinal transections (T6-T9) and catheterizations were performed under ether anesthesia on male albino rats (250-400g). At various intervals after transection the latency of tail withdrawal (flick) to a high intensity thermal stimulus was determined. Individual groups of intact and spinal rats were then injected with morphine sulfate, either, subcutaneously (sc, 0.75 mg/kg - 6.0 mg/kg) or intrathecally (ith, 0 ug - -15 ug) and retested 40 m. later.

Within one day after transection the dose response curve to sc morphine was shifted to the right of the curve obtained in unoperated control rats. At intervals of 3, 10 and 20-30 days, this shift was more pronounced. The ED50 of chronic spinal rats increased progressively during the first three weeks after transection.

To determine whether the decrease in opiate sensitivity was a direct function of changes within the spinal cord the effect of ith morphine was determined in intact and spinal rats. In contrast to the results of SC administration, the ith dose response curve of acute, 24 h. spinal rats was not different from that of intact rats. However, the intact and spinal rats differed in the distribution of their antinociceptive scores. Intact rats showed a graded increase in reaction as a function of increasing dose. Spinal rats showed an all-or-nothing response to ith morphine; either no change in latency, or a maximum response at each dose.

Additional experiments investigated the reason for this difference between intact and spinal rats. It was found that the dose-response curve of intact rats to ith morphine could be modified by experience with the nociceptive test: Their response to a second ith injection was significantly reduced by the interpolation of additional tail flick tests, but not by the passage of time alone. This result suggested that the effect of spinal morphine could be modified by nonpharmacological processes, such as arousal and habituation to the experimental context.

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Intrapartum Resuscitation of Infants Born to Drug Dependent Women

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A study was undertaken to investigate the effects of maternal drug use on neonatal resuscitation. Infants of drug dependent women (n=56) were compared to a drug-free control group (n=63). Drugs of abuse for the drug dependent women included both opiates and non-opiates (57%) or non-opiates only (43%). Socioeconomic status, gestational age and parity were similar in both groups. A high incidence of low birth weight was seen in the infants born to drug dependent women: 13 infants versus 7 infants in the control group. Resuscitation was divided into four levels with increasing degrees of complexity: Level I-suction bulb and/or suction catheter only: Level II-oxygen inhalation and/or positive pressure inhalation in addition to suction: Level III-intubation and visualization of the cords in addition to suction and oxygen administration: Level IV-external cardiac massage. Infants born to drug dependent women had a higher incidence of Level III and Level IV resuscitation. This was found despite the overwhelming number of black infants in the comparison group (black infants are thought to require more intervention possibly due to lower birthweights and a negative response to perinatal stress). Levels I and II were similarly administered to the two groups of infants. The type of analgesia or anesthesia received by the mothers during the intrapartum course was not related to the level of resuscitation required by the infants. The results of these data suggest that the risk of requiring increased levels of resuscitation at birth is greater for infants born to drug dependent women. In the application of this data to current practice, medical facilities which provide care for pregnant drug dependent women should anticipate problems in the delivery room, and establish appropriate emergency procedures in order to provide optimal care for this high-risk population.

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Adinazolam: Comparative Evaluation of Acute Behavioral and Subjective Effects

George E. Bigelow, Roland R. Griffiths, Frank Funderburk, and Ira A. Liebson

Adinazolam is a new triazolobenzodiazepine compound showing anti-depressant as well as anxiolytic activity. This paper reports results of a clinical laboratory evaluation of the acute behavioral and subjective effects of adinazolam in comparison to the standard benzodiazepine anxiolytic lorazepam. The purpose of the study was to assess the relative abuse liability and performance impairing effects of the compounds, and to assess the utility of the test procedures for differentiating among benzodiazepines. Twenty-four volunteers with histories of "recreational" sedative use were randomized to receive each of four dose levels of either adinazolam (0, 15, 30, and 50 mg) or lorazepam (0, 1.5, 3, and 5 mg) in a mixed order and under double-blind procedures. Throughout each 8-hr session repeated measures were made of subjective and behavioral indices, including: subject and observer ratings of drug effect and drug liking, Addiction Research Center Inventory, Profile of Mood States, digit symbol substitution performance, eye-hand coordination, short-term memory, choice reaction time, and tracking performance. Results showed both similarities among and differences between the two study drugs. Both adinazolam and lorazepam showed qualitatively similar effects indicating significant dose-related sedation on both subjective and performance indices. However, significant between-drug differences and/or drug x time interactions on measures of eye-hand coordination, tracking performance, and digit symbol substitution performance indicated that adinazolam tended to produce a lesser magnitude and/or a lesser duration of psychomotor performance impairment than did lorazepam. These data indicate that the test procedures can differentiate among benzodiazepines, suggest that subjective effects and performance-impairing effects of benzodiazepines may be dissociated, and suggest that adinazolam may present a reduced risk of behavioral impairment.

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Water Soluble Tetrahydrocannabinoids: Pharmacological and Radioligand Binding Studies

David R. Compton, Patrick J. Little, and Billy R. Martin

The mechanism of action of the cannabinoids is believed to to be mediated either by general membrane perturbation or through an interaction with a specific receptor. Binding site assays with cannabinoids are hindered by the extreme lipophilicity of these compounds. Therefore, attempts have been made to evaluate water soluble THC analogs [morpholinobutyric acid ester (MB) and trimethylammonium (TMA) derivatives of Δ^8-THC] in ligand binding and pharmacological assays. The radioligand binding studies using either 10 or 100 nM ^{3}H-MB-Δ^{8}-THC (37°C, 1 mg prot/ 5 ml TES-NH_4OH buffer, pH 7.0) failed to find any evidence of a specific, saturable binding site for the cannabinoid class of drugs. These data are similar to those previously reported for ^{3}H-Δ^{8}-THC binding to brain tissue (Harris et al., 1978). The results from radioligand binding studies using 75 pM ^{3}H-TMA-Δ^8-THC are not similar to data published previously (Nye et al., 1985). Binding studies with fresh tissue indicate a large degree of unsaturable binding, plus a small amount of saturable binding (K_D = 26 nM, Bmax = 33 pmol/ mg prot). However, ^{3}H-TMA-Δ^{8}-THC also bound to heat denatured (95°C, 1 hr) tissue in a saturable manner (K_D = 771 nM, Bmax = 3.02 nmol/ mg prot). The pharmacological effects of MB-Δ^{8}-THC on several parameters (hypoactivity, hypothermia, analgesia) suggests that it is less potent (0.3-0.1) than Δ^{9}-THC. Values obtained with Δ^{9}-THC on these parameters are similar to those previously reported; also the potency range for the effects of MB-Δ^8-THC are similar to those obtained for Δ^{8}-THC (Martin et al., 1984). The partition coefficient obtained for Δ^{9}-THC (12,898 ± 550) is more like the 6,000 value obtained by Gill and Jones (1972) than the 60,000 value obtained by Roth and Williams (1979). The extremely low coefficient obtained for TMA-Δ^{8}-THC (13 ± 1) indicates it is very water soluble. Zitko et al. (1972) reported MB-Δ^8-THC to be freely soluble in water (at approximately mM levels), yet at the µM levels being used in these binding studies it was necessary to prepare solutions in ethanol in silanized glassware, which suggests limited hydrophilicity. The coefficient obtained for MB-Δ^8-THC (526 ± 27) supports this contention, These data suggest that the ^{3}H-TMA-Δ^8-THC site is probably not responsible for the behavioral effects of the cannabinoids. Secondly, although MB-Δ^{8}-TI-IC is active behaviorally, this cannabinoid did not exhibit saturable binding, which may be due to the fact that MB-Δ^{8}-THC is only partially water soluble, suggesting other water soluble analogs should be investigated.

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Carbon Monoxide for Assessing and Treating Tobacco Dependence in COPD

Thomas J. Crowley, A. E. Andrews, J. Cheney, G. Zerbe, and T. L. Petty

Cigarette-induced chronic obstructive pulmonary disease (COPD) kills about 60,000 Americans each year. Continued smoking by severely handicapped COPD patients testifies to the strength of their tobacco addiction. Tobacco abstinence slows COPD's progression, but clinicians often doubt the veracity of patients' self-reported abstinence. Among 182 COPD patients attending the Chest Clinic of the Denver V.A. Hospital, we measured breath carbon monoxide (CO) with the Minico Monitor to assess smoking prevalence, seeking correlations with self-reported tobacco abstinence. Of these patients, 72% received home oxygen therapy, and the mean FEV_1 for the group averaged 40% of predicted normal. When confronted by the monitor, 37% of patients admitted recent smoking. Of those who admitted smoking, 93% exceeded 8 parts per million (PPM) CO in breath; of those who denied smoking, 92% had breath CO at or below 8 PPM. Those who admitted smoking had a mean CO of 23.1 PPM; those who denied smoking had a mean CO of 5 PPM.

We assessed decay rates of breath CO among twelve patients who smoked, monitoring them hourly from immediately after a last cigarette through 8 hours of abstinence. The group mean CO declined from 32.2 PPM to 17.2 PPM over 8 hours; this is 55.9% of the possible fall, assuming an asymptote of 5 PPM. Exponential fits to the data of each of the 12 patients produced half-life estimates ranging among patients from 2.7 to 12.7 hours (mean 6.5 ± 0.81 s.e.m. hours). Finally, we have attempted to shape reduced smoking by reinforcing reduced CO levels among COPD patients. Breath CO samples were assessed at subjects' homes 3 times per week for month. In the last 2 weeks, subjects were paid Colorado lottery tickets (on a sliding scale) for reductions in breath CO below baseline levels. Three patients have now completed this protocol, and the final measured values are approximately 1/3 lower than initial values.

CO measurement appears valuable for providing reasonably valid, inexpensive, and instantaneous information on smoking behavior to pulmonary patients and their clinicians. Reinforcement of reduced CO levels may be a practical way of encouraging abstinence in these severely addicted, tobacco-dependent patients.

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Effects of MDA and MDMA on Neurotensin and Substance P Systems in Rat Basal Ganglia

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Considerable public attention has been focused recently on the actions of amphetamine-like designer drugs such as 3,4mthylenedioxyanphetmine (MDA) and its congener, 3,4 methylenedioxymethamphetamine (MDMA, ecstasy). We (Stone, D. et al., FASEB abstracts, 1986) and Ricaurte et al. (Science 229, 1985) have found that these compounds exert profound biochemical changes in CNS serotonergic pathways while apparently having lesser impact on CNS dopaminergic projections. However, prior to the present report, there have been no studies which have examined the influence of MDA and MDMA on neuropeptide pathways.

Rats were given 5 subcutaneous injections (6-h intervals) of saline MDA (10 mg/kg/dose), MDMA (15 mg/kg/dose) or METH (15 mg/kg/dose) and sacrificed 18 h following the final injection. In some experiments the dopamine D-2 antagonist, sulpiride (80mg/kg/dose), or the 5HT-2 antagonist, ritanserin (1 mg/kg/dose), were given either separately or concurrently with the designer drugs or METH. Neurotensin-like (NTLI) and substance P-like (SPLI) immunoreactivity were determined by RIA in the striatum and substantia nigra employing highly specific antiserum.

We found that concentrations of striatal NIL1 were similarly elevated by treatment with MDA, MDMA or METH (200% of control). Increases of striatal NTLI also were seen following sulpiride administration, an effect which was additive with that of the designer drugs and METH However, while each of these stimulants also increased nigral NTLI concentrations (200% of control), D-2 blockade, by itself, did not have an effect and in combination it attenuated the METH effect in this tissue, but did not alter the changes induced by MDA and MDMA. In contrast, 5HT-2 blockade had no effect on NTLI concentrations either alone or in combination in striatal or nigral tissues.

The substance P systems responded somewhat differently in that SPLI concentrations were elevated in the striatum following treatment with both MDA and MDMA, but nigral levels of SPLI were only elevated by MDA treatment.

These findings demonstrate that the designer drugs MDA and MDMA, can have profound effects on the neurotens in and substance P systems associated with the basal ganglia. Some of these effects appear to be related to dopaminergic, but hot serotonergic activity, although other mechanism are definitely involved. (Supported by USPHS grants DA 00869 and MH 40175)

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Human Coffee Drinking: Reinforcing and Physical Dependence-Producing Effects of Caffeine

Roland R. Griffiths, George E. Bigelow, and Ira A. Liebson

In a residential research ward, coffee drinking was studied in 9 volunteer human subjects with histories of heavy coffee drinking. The presence or absence of caffeine in the coffee was manipulated under double-blind conditions by using caffeinated (CAF) or decaffeinated (DECAF) coffee. When subjects were alternately switched for 10 or more consecutive days between CAF and DECAF, the daily number of cups consumed tended to be relatively stable. In a different experiment, preference for CAF vs. DECAF was assessed. After experimenter-scheduled exposures, subjects were given choices between CAF and DECAF. When subjects were presumably caffeine tolerant/dependent, CAF was better liked than DECAF and was reliably preferred to DECAF in choice tests. When subjects were not caffeine tolerant/dependent, CAF was not reliably preferred to DECAF, nor were there pronounced differences in liking. Under these conditions, some subjects preferred DECAF to CAF, citing adverse symptoms (suggesting caffeine toxicity) as reasons for avoiding CAF. The effects of caffeine withdrawal were studied by abruptly substituting DECAF for CAF for 10 or more days. This resulted in an orderly withdrawal syndrome, having an onset latency of 19 hours, peaking on days 1 or 2, and progressively decreasing over the next 5 or 6 days. The withdrawal syndrome, which was detected on subject-rated, staff-rated, and objective behavioral measures, was characterized by increased headache, sleepiness and laziness, and decreased alertness and activeness. The present study provides the first unequivocal demonstration of the reinforcing effects of caffeine in humans and also documents the severity of the caffeine withdrawal syndrome. It is concluded that caffeine has the cardinal features of a prototypic drug of abuse.

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Subjective Effect of Diazepam Withdrawal: Severity Depends on Chronic Diazepam Dose

M. Emmett-Oglesby, D. Mathis, S. Idemudia, H. Lal, and C.Harris

The subjective effect of withdrawal from diazepam has been measured in an animal model for anxiety, the pentylenetetrazol (PTZ) discrimination paradigm (La1 and Emmett-Oglesby, Neuropharmacology 22:1422, 1983). In this paradigm rats are trained (with food reinforcement) to press one lever after injections of the anxiogenic drug PTZ, and to press an alternate lever after saline. In previous studies, the stimulus effect of Ro 15-1788 (given to precipitate withdrawal) substituted in a dose-dependent manner for the PTZ stimulus. The present study was conducted to determine the relationship between dose of diazepam (given chronically) and degree of PTZ-lever selection precipitated by Ro 15-1788. Prior to chronic diazepam$_I$ rats selected the PTZ-appropriate lever after PTZ, and the PTZ stimulus was blocked by diazepam, indicating reliable control of behavior by drug stimuli. Rats were then injected intraperitoneally, at 8-hour intervals, with diazepam, 20, 40, or 80 mg/kg. After 6 days of chronic diazepam all rats were given an injection of diazepam, 20 mg/kg, followed 15 min later by Ro 15-1788, 40 mg/kg. They were tested for lever selection 15 min after the second injection. The percent of rats selecting the PTZ-lever increased from 20 to 86% over the dose range from 20 to 80 mg/kg, tid. (7-12 rats per group). These data indicate that, as with overt withdrawal signs, the severity of the subjective effect of withdrawal is also directly related to the dose of chronic diazepam. These results further characterize PTZ discrimination as a sensitive assay for a subjective effect of diazepam withdrawal.

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Kappa and Mu Opiate Effects on Information Processing

Ronald I. Herning, Wallace B. Pickworth, and Karen Kumor

In drug abusers morphine, a mu agonist, and ketocyclazocine, a kappa agonist, produced different profiles of subjective, physiologic, and discriminative effects. Subjects reported euphoria after morphine and dysphoria and LSD-like effects after ketocyclazocine. Event related potentials (ERPs) provide a means of assessing the effects of drugs on sensory and cognitive function in humans. We used an ERP measure to distinguish mu and kappa opiate effects on human cognition.

Twelve male volunteers with drug experience participated in an inpatient study. They received: placebo, naloxone (210 mg), morphine (30 mg) or ketocyclazocine (1.2 mg) in a double-blind randomized experiment. The subject performed the oddball ERP task before, 60 and 300 minutes after drug administration by listening to rare and frequent tones and counting the rare tones. The EEG was recorded from five scalp locations. The peaks and latencies of the N100 and P300 components of the average ERP were determined for the rare and frequent tones. The N100 amplitude to the rare tone was reduced only by ketocyclazocine ($p < .05$). Morphine, ketocyclazocine and naloxone reduced P300 amplitude to the rare tone ($p < .05$); only morphine increased P300 latency ($p < .05$).

The decrease in N100 amplitude with ketocyclazocine indicates a deficit in selective attention and is consistent with a previous report of perceptual alterations in humans. During morphine-induced euphoria and ketocyclazocine-induced dysphoria the P300 amplitude is reduced. The effect is not specific to opiates since P300 amplitude is also reduced by alcohol and cocaine. The reduction in P300 amplitude reflects an inability to update working memory during the psychoactive effects of these drugs. This inability may be a direct drug effect or a consequence of the euphoria or dysphoria produced by abused drugs. The increase in P300 latency produced by morphine suggests a delay in processing the target stimuli. This delay was not observed with ketocyclazocine. Thus, mu and kappa agonists, cause differential effects on sensory information processing in drug abusers.

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The Effects of MDMA (Ecstasy) on Brain-Stimulation Reward and Detection Thresholds

Michael P. Bird, Carol B. Hubner, Stefanie Rassnick, and Conan Kornetsky

Methylendioxymethamphetsminne (MDMA) is a psychoactive compound having structural similarities to both amphetamine related sympathomimetics and hallucinogens like mescaline. It is reported to produce profound pleasurable effects including acute euphoria and longer lasting positive changes in attitude and self-confidence, and recent reports indicate extensive recreational use.

In this study the abuse potential and behavioral effects of rscemic 3.4-MDMA were assessed by brain-stimulation reward and detection, respectively. Increased sensitivity (a lowering of threshold) for rewarding brain stimulation has been used as an animal model of drug-induced euphoria and is thought to be predictive of abuse liability in man. A raising in the detection threshold suggests a disruption in attentional and perceptual capacities. In both procedures, a bipolar electrode was stereotaxically implanted in the medial forebrain bundle-lateral hypothalamic (MFB-LH) area of male CDF rats. Reward and detection thresholds were determined using a modification of the psychophysical method of limits. Subjects were trained to turn a wheel manipulandum to obtain electrical stimulation to the MFB-LH. In the reward procedure the animal learns to respond only to stimuli which are rewarding, while in the detection procedure the animal is motivated to respond to the lowest intensity cue it can detect.

MDMA significantly lowered the reward threshold at doses (1.O-2.0 mg/kg. sc) which did not alter the detection threshold. Higher doses (4.0-8.0 mg/kg. sc), however, did not lower the reward threshold but did significantly raise the detection threshold and increased nonreinforced responding. These findings suggest that MDMA has euphorigenic properties that can be dissociated from its attentional and motor effects. The close correspondence between high doses which fail to lower the reward threshold and the minimally effective dose that raises the detection threshold illustrates the influence of attentional and perceptual effects of MDMA in disrupting the animal's ability to perform in the reward procedure.

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Contribution of Personality Variables to the Subjective Effects Produced by Methaqualone and D-Amphetamine

Martin Ionescu-Pioggia, George S. Welsh, and Jonathan O. Cole

Welsh *(Creativity & Intelligence,* Chapel Hill, NC: IRSS, 1975) proposed a personality model employing two independent dimensions: **origence (OR)** and **intellectence (IN).** OR lies on the vertical dimension and IN on the horizontal. Continuous scores can be obtained on both dimensions which are orthogonal and also yield four distinct personality types. Low OR subjects prefer structure, while high scorers favor unstructured and unconventional experiences. Low IN subjects prefer concrete experiences, while high scorers are more comfortable with the abstract. If a relationship between personality and substance use exists, then acute drug-induced subjective effects may vary as a function of subjects' scores on OR and IN.

MMPI-derived OR **(M-OR)** and IN **(M-IN)** scores were obtained from 23 males and 20 females who met provisional criteria for recreational substance use: 1) use of $\geq$ psychoactive substance (excluding etoh or marijuana at least 4 times); 2) does not meet DSM-III criteria for a substance abuse disorder; 3) use of a substance primarily for relaxation or experiencing novel mood states; and, 4) MMPI profile T scores generally <70.

Twenty-two subjects received 200 and 400 mg of methaqualone, and 21 subjects 15 and 30 mg of d-amphetamine under placebo-controlled double-blind conditions. Each experiment consisted of three sessions during which subjective states were measured pretreatment, and at 4 one-hour intervals posttreatment, using an abbreviated version of the ARCI.

ARC1 scale scores were predicted from M-OR and M-IN using multiple linear regression. For methaqualone, high M-IN scores were associated with high scores on the Sedation scale (R^2= .31, p.< 05). For amphetamine, high M-OR scores were associated with high scores on the Amphetamine (R^2=.43, p<.05) and Euphoria scales (R^2= .16, p<.O5); in one case, a low M-IN score was associated with a low score on the Euphoria scale (R^2=.31,p<.05).

M-OR and M-IN scores were used to classify recreational substance users into one of Welsh's four personality types. A significant proportion of subjects (28/42) fell within the type characterized by high scores on both OR and IN. In addition, a significant proportion of subjects (32/43) also had peak MMPI profiles involving some combination of scales 4/Pd and/or 9/Ma (p < .01).

Results suggest that personality variables can be used as preditors of acute drug-induced subjective states. While substances may have a uniform effect, its perception, and hence the estimate of abuse potential, is partially mediated by individual differences. This subgroup of substance users was characterized by high scores on both OR and IN, and by MMPIs on scales 4/Pd and/or 9/Ma.

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Opioid Profile of BW942C in Man

Rolley E. Johnson and Donald R. Jasinski

BW942C is a chemically novel enkephalin-like pentapeptide under evaluation fox the treatment of acute non-specific diarrhea. BW942C exhibits naloxone reversible opioid activity in the guinea pig ileum and animal antitussive model. Parenterally, BW942C is three to six times more potent than morphine in producing analgesia in rats and mice. The oral dose required to produce antidiarrheal effects is reported to be thirty to sixty-two hundred times less than the oral dose required to produce analgesia. This wide separation between apparent central nervous system and peripheral activity provides a rationale for the efficacious use of BW942C without abuse liability.

The high water solubility allowing for possible parenteral administration and reports of euphoria, and naloxone reversible opioid effects led us to assess the drug for morphine-like subjective effects, miosis and abuse liability in nine opioid abusers using a double-blind, randomized, crossover design. Comparisons of physiological and subjective effects were made between intramuscularly administered morphine (7.5, 15, and 30 mg), BW942C (0.5, 1.0, and 2.0 MG) and placebo. Although both drugs constricted pupils, only morphine constricted pupils in a dose dependent manner. Neither drug significantly affected respiration, blood pressure, or temperature. Subjective effects were measured using the Single-Dose Opiate Questionnaire (Feel Drug, Drug Identification, Opiate Symptoms and Liking scales) and a short form of the Addiction Research Center Inventory (MBG, PCAG, and LSD scales). Both drugs were psychoactive as measured by the Feel Drug scale; however, responses were monotonic for BW942C and dose dependent for morphine. BW942C did not increase Liking scores while morphine increased Liking scores in a dose responsive manner. Only morphine at the high dose significantly ($p < 0.05$) increased the MBG (euphoria) scale score. Only BW942C at the high dose significantly ($p < 0.05$) increased the PCAG (apathetic sedation) and LSD (dysphoria) scale scores; effects which are consistant with nalorphine-like agonists. A dose responsive increase in "dope" identification was observed with morphine but not BW942C.

From this data, it is concluded that BW942C is not a typical morphine-like drug and is not likely to be abused.

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A Calorimetric Analysis of Body Temperature Changes Produced in Rats by Morphine, Methadone, and U50,488H

Thomas J. Lynch, Rebecca P. Martinez, Michael B. Furman, Ellen B. Geller, and Martin W. Adler

Continuous chart traces of body temperature (Tb), O_2 consumption (VO_2) and heat loss (Q) from Sprague-Dawley rats were obtained before and after s.c. injection of morphine, methadone or the selective kappa agonist, U50-488H (Upjohn). A gradient-layer calorimeter with semiconductor, heat flux sensors permitted measurement of whole-body Q, while VO_2 was calculated from the pO_2 deficit measured in the calorimeter. Calorimeter temperature was 20°C.

Control and saline-injected rats showed only slight changes in T_b, Q and VO_2 and these were in parallel. However, increasing T_b, was consistently correlated with a decreasing Q/VO_2, ratio (calories per ml O_2) and vice versa.

A low dose of morphine sulfate (4 mg/kg) caused a small increase in both Q and VO_2, accompanied by hyperthermia of 0.5-1.0°C lasting about min. The hyperthermia conincided with a Q/VO_2 decrease of 0.2-0.4 cal/ml. Injection of 8 mg/kg caused slight hypothermia in some cases and greater hyperthermia in others. The relationship between changes in T_b and Q/VO_2, with 8 mg/kg was similar to that with 4 mg/kg only more clear.

A high dose of morphine (64 mg/kg) caused hypothermia of 4-5°C within 4 hr. The drop in T_b was initiated, typically, by a 0.7 cal/ml increase in Q/VO_2 within 1 hr. After 1 hr, Q/VO_2 dropped continuously until T_b stabilized at a low level between 2 and 4 hr post-injection. The start of recovery was signalled by a sharp increase in VO_2 while Q remained steady or dropped even further. Within minutes T_b began an exponential recovery with a time constant of 30-60 min. With the kappa agonist, U50-488H (40 mg/kg) changes in T_b, VO_2 were similar during the onset of hypothermia, but very different during recovery.

Since Q and VO_2 both decrease during the onset of hypothermia induced by all three drugs, the apparent cause of hypothermia is decreased metabolism rather than an absolute increase in heat loss. We don't know if the drop in Q/VO_2, suggesting peripheral thermoregulation, would occur faster in native rats during a similar decrease.

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Interactions Between Naloxone and T-Lymphocytes: Do They Indicate the Presence of Specific Opiate Binding Sites?

J. J. Madden, R. M. Donahoe, J. Zwemer-Collins, and A. Falek

Opiates have been reported to modulate various biological functions of T lymphocytes when administered either in vivo or in vitro. Lymphocytes from opiate addicts, for example, exhibit diminished capacity for DNA repair, increased cytogenetic damage, and decreased E-rosette forming ability and mitogen-stimulated blastogenesis. However, the mechanism(s) by which opiates modulate these lymphocyte functions has not yet been determined although the hypothesis has been advanced that T lymphocytes have specific opiate binding sites.

Naloxone, the opiate antagonist, binds specifically to highly enriched, unstimulated peripheral T lymphocytes that are relatively platelet (<20%) and monocyte free (<2%). Platelets and monocytes interfere with the measurement of specific binding for lymphocytes because they take up large quantities of labeled compound yielding high non-specific binding values. The specific binding of naloxone to the lymphocytes is media dependent. It can be demonstrated in a minimal buffer like Hanks Balanced Salt Solution (GIBCO) but not a highly enriched medium like RPMI 1640 (GIBCO) with or without fetal bovine serum. The inhibition of specific binding by RPMI 1640 is not a function of ionic strength, pH, the presence of reducing agents (glutathione), or of adenine-containing compounds (NAD^+ and nicatimamide). Specific binding can also be demonstrated in sonicates of lymphocytes particularly if the soluble material is first removed from the active membrane particulates by high speed centrifugation. The supernatant apparently contains a factor(s) which inhibits specific opiate binding. The specific binding to whole lymphocytes is saturable at approximately 100nM, although this varies between individuals.

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Discriminative Properties of Flupirtine, a New Centrally Acting Analgesic, in Rats

Bernd Nickel, Suzanne E. Schell, Harlan Shannon, and Michael Swedberg

Flupirtine (FPT), ethyl-N-[2-amino-6-(4-fluor-phenylmethyl-amino)-pyridin-3-yl]carbamate, a new analgesic has been shown to be as effective clinically as pentazocine (PENT) and codeine in relief of severe pain. No significant difference was observed in overall analgesic effect assessed by a verbal rating system yet a fairly pronounced sedative-type effect was found with FPT. Preclinical pharmacological studies confirm these findings but little is known about the possible mechanisms by which FPT may exert this activity or about its subjective effects.

In view of this, studies were initiated in an animalmodel to identify discriminative effects of drugs (Shannon, JPET 216(3):543-551, 1981). Male F344 rats were trained in a two-choice, FR5 shock termination task. Studies were conducted to determine if animals could be trained to discriminate between FPT (10mg/kg i.p., 30 minutes pre-test) and vehicle. Following some 64 sessions in which FPT was administered 30 minutes before the start of the training session, the level of drug appropriate responding did not reach the pre-established criterion for acquisition. When the pretreatment time was decreased to 10 minutes, the training criteria were met. However, performance in individual animals was not always reliable. In addition doses of FPT (0.3to 10 mg/kg) were evaluated in rats trained to discriminate between vehicle and 10 mg/kg of PENT. PENT alone produced dose related increases in responding on the PENT lever (nearly 100%). In animals tested with FPT responses on the PENT lever remained at or near zero at all dosage levels. FPT was also compared with tramadol, another analgesic widely used in Europe. As in other test procedures, FPT showed behavioral effects clearly different from those exhibited by tramadol in these studies. That is, tramadol substituted for PENT whereas FPT did not. Since sedative-like properties had been seen with FPT, the compound was also tested in a separate group of rats trained to discriminate between lorazepam (LZP) (0.3 mg/kg) and vehicle. Rats trained on and tested with LZP responded on the drug appropriate lever nearly 100% of the time. In contrast, when FPT was the test drug, responding on the LZP appropriate lever remained at or near zero.

Thus, the profile of behavioral activity FPT exhibits appears to be somewhat unique compared to that shown by other strong centrally acting analgesics in these testing procedures. Additionalwork is ongoing to further describe its discriminative properties.

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Flupirtine, a New Analgesic With a Novel Profile of Activity

Bernd Nickel, Karen L. McCullough, and Bruce Vaupel

Early results with flupirtine (FPT) strongly suggested a novel profile of pharmacological activity. FPT demonstrated relatively high potency as an analgesic but did not show signs in common with opiates or other potent analgesics. Studies of tolerance, physical dependence and self-adninistration as wel1 as binding to opiate receptors ware negative.

To further examine the profile of activity FPT demonstrates, the chronic spinal dog preparation, previously shown to provide valuable insight into the comparative spectra of activity of a large number of centrally acting agents, was used. Studies were conducted comparing three (0.31, 1.25, and 5.0 mg/kg) doses of pentazocine (PENT) and four doses (0.31, 1.25, 5.0, and 10.0 mg/kg) of FPT. I.v. administered PENT and FPT were evaluated for effects on two nociceptive reflexes, the flexor (spinal) and the skin twitch (supraspinal), as well as on respiration, heart rate, pupillary diameter, nictitating membrane width, rectal temperature, and behavior over a 150 minute period (Vaupel et al., Drug Alc. Dependence 2:45-63, 1977).

PENT was clearly more effective than FPT in depressing the amplitude of the flexor reflex. Both drugs produced increases in the latency of the skin twitch reflex with pentazocine being apparently more efficacious. PENT and FPT-induced antinociception determined with the skin Witch reflex peaked within the first 10 minutes of administration; however, the effect of PENT was sustained over 150 minutes. FPT and PENT produced comparable miotic and hypothermic effects. The profile of FPT is characterized by hypothermic antinociception, miosis, slight hypothermia, and decreased behavioral arousal. Notably dogs which appeared to be sleeping were easily awakened. PENT produced a profile characterized by supraspinal and spinal antinociceptive properties, miosis, and slight hypothermia.

In summary, these studies are in agreement with earlier results suggesting that FPT may exhibit a profile of activity distinctly different from classical centrally acting analgesic and other centrally acting compounds previously characterized in the spinal dog preparation. More work is underway to elaborate on the mechanisms which may underlie the compound's activity.

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The Effect of Chronic Maternal Drug Addiction on Placental Drug (Xenobiotic) Metabolism

Enrique M. Ostrea, Jr., Thomas Porter, and James N. Wardell

The human placenta (plac.) is normally incapable of xenobiotic biotransformation, although there are circumstances such as in maternal cigarette smoking, where specific placental xenobiotic monoxygenase activity has been induced. The aim of this study was to establish if chronic drug addiction during pregnancy would induce placental metabolism of drugs, as well. Microsomes (105,000 g pellet) were prepared from tissue homogenates of placentas obtained from 4 control and 5 drug dependent (DD) women and from the liver of 3 non-drug dependent fetuses (22-24 weeks). The microsomal mixed function oxidase reactions (hydroxylation and dealkylation) and conjugation were assayed as follows: **A.** hydroxylation of aniline (assayed as Drug p-aminophenol formed/mg protein). **B.** demethylation of aminopyrine (assayed as nmol HCHO formed/mgprotein) and **C.** conjugation of bilirubin (assayed as µg conjugated bilirubin formed/mg protein). The liver from an adult Sprague-Dawley rat was used during each assay to control for enzyme activity. The concentration of products formed were plotted against time and from the slope of the regression line, the enzyme activity was calculated as products formed/mg protein 160 minutes.

RESULTS (enzyme activity)

	Control Plac.	DD Plac.	Liver (fetus)	Liver (fetus)
A.	0.93	0.28	3.12	44.76
B.	2.05	2.97	6.21	30.49
c.	0.18	0.25	0.14	1.39

CONCLUSION

Despite chronic addiction, the placenta of the DD mother shows no enhancement of its xenobiotic biotransformation activity and therefore does not offer the fetus who also has limited hepatic drug biotransforming capability, any protection from drugs.

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Infants with Slit-Like Ventricles: Incidence, Perinatal Factors, Birth Weight, and Neonatal Abstinence

Matthew E. Pasto, Saundra Ehrlich, and Loretta P. Finnegan

Infants exposed to narcotic agents in-utero show a higher incidence of slit-like ventricles (SLV) than infants born to drug-free control mothers. Seventy-three infants were studied at birth, one month, and six months (43 drug-exposed and 30 control infants). As in previous studies, a persistently high percentage of SLV were seen in the drug-exposed group; 93% at birth, 72% at one month and 26% at six months in comparison to the control group which had 37%, 17%, and 3% at similar time intervals. Grouping both the drug exposed and control infants together, SLV were more likely to occur in white infants (87%) than in black infants (62%). SLV were also more likely to occur and persist in infants with lower birth weights (2992.5 gms. vs. 3130.2 gms.) and in those who required treatment for neonatal abstinence (p=<.05). Although the frequency of SLV in infants decreased between birth and six months of age, an increasing percentage of treated infants continued to show slit-like ventricles - 65% at birth, 68% at 1 mo. and 72% at 6 months. Though these infants had severe symptoms of abstinence (on assessment with the Finnegan Neonatal Abstinence Score) , these findings do not exclude the possibility that the pharmacotherapy required for abstinence influenced the persistence of SLV. For drug-exposed infants, there were no significant differences between infants with slit-like and normal ventricles in number of days to control and treat abstinence or maternal methadone dose during pregnancy. For infants with SLV, no differences were found in age of mother, gestational age, or infant head circumference. In conclusion, factors which may possibly play a role in the occurrence and incidence of SLV are: 1) race, 2) low birth weight, 3) drug exposure in-utero, 4) neonatal abstinence, and possibly 5) pharmacotherapy for abstinence.

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THC Plasma Distribution in Rhesus Monkey Mother and Fetus Near Term

Merle G. Paule, John R. Bailey, and W. Slikker, Jr.

In order to quantify the placental transfer of delta-9-tetrahydrocannabinol (THC, the major psychoactive component of marijuana) to the fetus, three late term (146-151 day! rhesus monkeys (Macaca mulatta) were given 0.3 mg/kg THC iv (maternal radial vein). With the use of an intraplacental cannulation technique, simultaneous blood samples were obtained from a maternal uterine vein and an intraplacental artery at 0, 1, 3, 6, 15, 30, 60, 120 and 180 min after dosing. Plasma samples were analyzed by radioimmunoassay for THC and a major metabolite 11-NOR-9-carboxy-THC (11-NOR). Peak plasma THC values were obtained 15 min after dosing in the fetus and demonstrate that THC rapidly crossed the placenta into the fetal circulation. The concentration difference between maternal and fetal plasma THC, which was striking over the first 15. min, decreased rapidly and after 3 hrs, maternal and fetal levels were equal (37 ng/ml), indicating that fetal THC exposure after maternal administration is substantial. Maternal concentrations of THC were greater than maternal ll-NOR concentrations at all time points examined. 11-NOR was virtually undetectable in fetal plasma which suggests 1) this metabolite that is formed by the mother does not readily cross the placenta and 2) the fetus does not readily metabolize THC to 11-NOR. In another group of animals it was determined that plasma THC areas under-9 the concentration versus time curves (AUCs, as ng-min/ml x 10-2 15-300 min after injection of 1.0 mg/kg THC iv, did not differ significantly between pregnant (n=6, AUC=7041 and nonpregnant (n=3, AUC=876) females indicating that pregnancy did not appear to alter maternal disposition of parent compound after THC administration.

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Local Cerebral Glucose Utilization Following Intravenous Cocaine Administration

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Behavioral studies have shown cocaine to be a potent reinforcing stimulus. As with other psychomotor stimulants cocaine administration also increases locomotor activity. Both the locomotor and reinforcing effects of cocaine are thought to be mediated by its actions at dopaminergic synapses, particularly those of the mesolimbocortical system. We have used the quantitative autoradiographic 2[^{14}C]deoxylglucose (2-DG) method to determine the anatomic distribution of alterations in metabolic activity in this system as well as other portions of the CNS that are produced by cocaine in rats. Rates of local cerebral glucose utilization (LCGU) were measured according to the standard protocol in four groups of male adult Sprague-Dawley rats to which 0.5, 1.0 and 5.0 mg/kg cocaine or vehicle alone was administered i.v. 5 minutes prior to the initiation of the experimental procedure. Locomotor activity was measured concurrently with the 2-DG procedure. Cocaine administration resulted in discrete changes in LCGU at all doses. Significant dose-dependent increases were observed in the medial prefrontal cortex, nucleus accumbens and lateral septum. At the lower doses, those which rats will self-administer, LCGU alterations were mainly restricted to these portions of the mesolimbocortical system. At the highest dose widespread increases were also found in structures of the extrapyramidal motor system including the caudate, globus, subthalamic nucleus and the substantia nigra, and a significant decrease was observed in the lateral habenula. Locomotor activity positively correlated with LCGU in the caudate, globus pallidus, substantia nigra and inversely in the lateral habenula. The rate of glucose metabolism in the nucleus accumbens, a structure thought to have a role in stimulant-produced locomotor activity, did not correlate with locomotor activity in the present study. These data are consistent with the view that portions of the mesocorticolimbic system, in particular the medial prefrontal cortex and the nucleus accumbens are a part of the neural circuitry subserving the behavioral response to cocaine. The locomotor stimulatory effects of cocaine, however, appear to be mediated by the extrapyramidal motor system.

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Induction of Midazolam Dependence in the Rat

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Midazolam (MID) is a new benzodiazepine currently marketed in the U.S. for preoperative sedation and induction of anesthesia. MID is the most fat soluble, rapidly acting and short acting benzodiazepine. Among a group of benzodiazepines Studied (self-administration), MID had the highest abuse liability. Despite this, a withdrawal syndroma has not been demonstrated. Accordingly, experimantal investigations of physical dependence to MID in the rat were performad under different schedules of administration. Acute dose response and time action for CNS depression (overt intoxication) were evaluated by gross neurological testing to guide design of treatments (Tr). Signs of withdrawal (WD) were monitored by 2-3 Tr blind, independent raters. Tr-I, 120 mg/kg, p.o., g.i.d., which produced continuous CNS depression, was stopped at 72 hrs. and testing for WD began. WD emerged after 1.5 days, peaked at 3.8 days with recovery by day 5. The long latency for WD suggested saturation of body and/or metabolism. Tr-II, 120 mg/kg, p.o., b.i.d. x 3 weeks, produced a 28% more severe WD than Tr-I. Onset was more abrupt, less than one day, and peaked. at 2.2 days with recovery by day 9. Tr-III, MID 120 mg/kg, p.o. chronically escalating to 180 mg/kg, b.i.d. x 5 weeks, produced the most severe WD (19% more than Tr-II) and was quickest to peak (1.8 days). Treatments I-III produced tolerance to gross intoxication. In Tr-IV, (a single 120 mg/kg, p.o.) MID showed no evidenceof spontaneous withdrawal. These results provide evidence for the intrinsic potential of midazolam to induce chronic dependence; acute dependence seems to be absent perhaps because of MID's extremaly short duration of action. Further study is needed to rigorously compare MID to other benzodiazepines for relative dependence potential. Nevertheless, MID withdrawal was no more severe than for the long acting historical standard of maximal chlordinzepoxide dependence (JPET 226: 100, 1983).

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Genetic Differences in the Development of Physical Dependence on Pentobarbital in Four Inbred Strains of Rats

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The purpose of the present study was to systematically investigate physical dependence on pentobarbital (PB) in four inbred strains of female rats, Fischer 344 (F344), Lewis (LEW), Spontaneously Hypertensive (SHR) and Wistar Kyoto (WKY), and to investigate the possible relationship between degree of motor incoordination during PB treatment and severity of withdrawal.

Rats were chronically fed food containing PB on an escalating drug dosage schedule (1 to 16 mg/kg of food) over a period of 47 days. Motor incoordination was elevated by a rotarod performance test. Withdrawal was conducted by substituting normal food for PB-admi!xed food. Withdrawal signs were observed for 48 hrs after termination of drug treatment.

During treatment, the growth curve of the LEW, SHR and WKY rats was suppressed as compared with respective controls. The ranking of the motor incoordination was as follows: WKY > LEW > SHR > F344. After withdrawal, various signs of PB withdrawal occurred. The withdrawal signs from PB in F344, LEW and SHR rats were mild as compared with those in WKY rats. The order of the severity of withdrawal signs in the four inbred strains was parallel to that for motor incoordination.

The results of this study emphasize the important role of genetic factors as determinants of both response to chronic drug treatment and degree of withdrawal severity. These results suggest that the differences between strains in withdrawal can be attributed to differences in the degree of chronic CNS depression.

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Tripelennamine Potentiation of Opioid Reinforcement (T's and Blues)—Mediation by Central Histamine?

Ellen M. Unterwald and Conan Kornetsky

In recent years, the abuse of pentazocine combined with tripelennanine (street name "T's and Blues") has become widespread. This combination is frequently substituted for heroin and apparently gives the user a more heroin-like effect than is experienced with pentazccine alone. The mechanism by which tripellenanine enhances the euphoric properties of pentazocine is poorly understood. We have previously reported that pentazccine lowers the threshold for rewarding brain stimulation and that tripelennanine potentiates this lo&ring effect (Unterwald and Kornetsky, Pharmacol. Biochem. Behav. 21:961-964, 1984). A lowering of reward threshold appears to reflect drug-induced euphoria and hence is predictive of abuse liability (Kornetsky et al., Arch. Gen. Psycriat. 38:289-292, 1979). Tripelennanine has several pharmacological actions including antihistrminic and local anesthetic effects. The present study was undertaken to determine which of these actions is responsible for tripelennanine's ability to enhance euphoria.

Bipolar stimumlating electrodes were implanted in the medial forebrain bundle-lateral hypothalmus of male rats. A rate-independent procedure was used to determine the reward threshold and involved varying the current intensity of the stimulation according to a modification of the psychophysical method of limits. The effects of diphenhydranine, an antihistamine, and procaine, a local anesthetic, on the threshold for reinforcing brain stimulation were determined. In addition, their ability to potentiate the effect of pentazocine was also tested.

Results indicate that acute administration of diphenhydranine produced a significant although modest, lowering of reward threshold. When a low, ineffective dose of diphenhydranine was co-administered with an ineffective dose of pentazocine, a significant lowering of reward threshold was seen. Procaine, over a wide range of doses, produced no significant effect alone or in combination with pentazocine. Therefore, it appears that diphenhydranine, but not procaine, has weak reinforcing activity. These results suggest that antihistamines can increase the euphoric quality of opioids and that the reinforcing property of tripelennamine is mediated at least in part through its effect on central histanine systems.

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Effect of Cigarette Rod Length on Smoking Topography

Phillip P. Woodson and Roland R. Griffiths

Nine habitual smokers smoked their preferred brand cigarette under three conditions. In the "Full-Length Condition" (FLC), one puff was taken from each of eight full length cigarettes while in the "Butt-Length Condition" (BLC), one puff was taken from each of eight butt length cigarettes prepared by clipping off all but 5 mm of burnable tobacco rod distal to the filter. In the third "Whole-Length Condition" (WLC), one cigarette was smoked (eight puffs) progressively down the burning rod to within 5 mm of the filter. Carbon monoxide (CO) boost after each condition was used as an indice of smoke exposure.

The BLC produced a smaller CO boost than did the FLC and WLC. The lower draw-resistance level of the BLC may partially account for this in that it generated a more intensive puff (i.e., shorter latency to maximum flow and higher maximum flow). This should make the combustion process more efficient; resulting in less CO production. The more intensive puff profile of the BLC may also explain the finding that even though BLC puff durations were shorter than those of the FLC, BLC puff volumes were only marginally less (p .1) than those of the FLC. The CO findings may also be a function of the smaller smoke inhalation volume (i.e., shallower inhaling) and slower smoke inhalation rate of the BLC. BLC smoke was rated as hotter than the other two conditions, suggesting that the less intense inhalation pattern may be a defensive mechanism.

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An 11-Year Study of Mortalities in Methadone Patients: The Growing Impact of AIDS

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Many heroin addicts upon entering methadone treatment show good therapeutic gains, discontinue drug use, and generally lead a productive and useful life. Unfortunately, a certain number of methadone patients do not make therapeutic improvement, but instead continue their drug use and continue to engage in the concominant lifestyle. Consequently, it is not surprising that methadone patients have increased rates of mortality resulting from drug related accidents and violence, overdoses, and a variety of medical problems stemming from substance abuse.

A study was done in order to characterize the mortalities that occurred in a large methadone program serving 2400 patients in the Bronx borrough of New York City during the period of 1975 to 1985. Medical records and death reports were reviewed in order to obtain the exact cause of death. Mortalities were classified as follows: drug overdose, AIDS, medical, alcohol related, violent and accidental, suicide and unknown.

A total of 263 persons died over an 11 year period while enrolled in the methadone program. The overall death rate has risen from 8.35/1000 in 1975 to 19.34/1000 in 1985. Although the numbers have been increasing, the contribution of the various causes to the overall mortality have been changing. There is evidence that over time the impact of overdoses, violent and accidental deaths are waneing while the impact of medically related deaths and AIDS are coming to the fore. In 1982, AIDS accounted for 4.6% of all mortalities; by 1985 AIDS was responsible for 38.6% of the mortalities. Clearly, AIDS is becoming the most significant factor in the mortalities encountered in methadone treatment; if the present trend continues, in 1986 AIDS will be the single largest cause of death in our program.

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Effects of Diazepam on Human Laboratory Aggression: Correlations with Alcohol Effects and Hostility Measures

D. R. Cherek, J. L. Steinberg, and T. H. Kelly

Clinical studies have typically reported that diazepam (Valium) reduced aggressive behavior of patients (e.g., Cherek and Steinberg, 1986). However, diazepam resulted in "paradoxical" increased aggressive behavior in some patients (Hall and Zizook, 1981), and increased reports of hostility in subjects residing on a research ward (Griffiths, et. al., 1983). The following experiment was undertaken to determine the effects of acute diazepam administration on aggressive responding of normal males in a laboratory setting.

METHOD

Subjects

Seven males have participated after giving their informed consent. Volunteers were recruited by advertisements soliciting participation in behavioral research projects. The advertisements and consent forms did not mention aggressive behavior, since we did not want to imply that the research subjects must respond aggressively to participate in the experiment or to earn monetary payments.

All subjects were given a complete physical exam and structured psychiatric exam prior to drug administration. To avoid problems associated with drug usage, daily breath alcohol measures were taken and urine samples were obtained for complete drug screen analysis.

PROCEDURE

Subjects were told that they would be randomly paired with other people participating in the research project at the same time, but in a different location. The situation was described as one in which they could influence the amount of money earned by these other individuals by subtracting money from them. Subjects were told that people with whom they

were paired could choose to subtract money from them at any time during the experimental sessions.

All subjects came to the medical center for daily fifty (50) minute sessions five days per week (Mon. thru Fri.). Subjects were required to swallow two #00 gelatin capsules thirty (30) minutes prior to the session. These capsules contained either placebo or 2.5, 5 or 10 mg of diazepam per 70 kg of body weight. Successive drug doses were separated by at least 96 hours, and were administered if preceding placebo session responding was within variability ranges established prior to drug administration.

The response console contained two response manipulanda, response button A and button B. Pressing button A was maintained by a fixed ratio (FR) 100 schedule of point presentation. Each point delivery was Indicated by incrementation of a counter mounted directly adjacent to button A and was equivalent to ten cents. Pressing button B ostensibly delivered an aversive stimulus to another person and was defined as aggressive. The completion of each fixed ratlo (FR) 10 on button B resulted in the ostensible subtraction of one point, i.e., ten cents from the other person.

Aggresslve responding was elicited by subtracting money from the subjects, which was attributed to the other participants. Point subtractions (provocations) were scheduled to occur at random points throughout the session. In the absence of aggressive responding, subjects were scheduled to receive 40 provocations (point subtractions) per session. In addition to ostensibly subtracting a point from the other person, ten responses on button B also Initiated a provocation-free Interval (PFI) during which point subtractions were not presented. PFI durations were either 125 or 500 seconds. Subjects were able to initiate a PFI only following at least one point subtraction, i.e., an escape contingency. When the PFI elapsed, point subtractions were again presented randomly.

Following the completion of the diazepam dose-response curve, subjects participated in additional sessions and were administered placebo drinks or a single alcohol drink containing 0.5 g/kg of alcohol thirty (30) minutes prior to these additional sessions.

Subjects completed the Profile of Mood States (POMS) questionnaire and were evaluated for clinical signs of intoxication after each session. Subjects also completed the Buss-Durkee Hostility Questionnaire at the end of the study. Subjects were not actually paired with another person during the experiment, and they were debriefed and informed of this at the end of the experiment.

RESULTS

Aggressive responding usually occurred immediately following provocations i.e., point subtractions. The effects of placebo and the three doses of diazepam on the number of aggressive responses per session are shown in Figure 1. Dose-response curves at PFI values of 500 seconds are shown in the top half of the figures, and those at PFI values of 125 seconds are shown in the bottom half. The dose-response curves are expressed as percent changes from placebo baseline set at zero, in order to compare effects upon different frequencies of aggressive responding. The frequency of provocation was relatively low (5-10 per session) at a PFI of 500 seconds (top half of figure). and higher (16-25 per session) at a PFI of 125 seconds (bottom half of figure). Aggressive responding was maintained by escape from scheduled provocations as previously discussed.

One subject (S-173) increased aggressive responding following the administration of 10 mg of diazepam per 70 kg. The effect was first observed at a high frequency of provocation (lower left curve), and then replicated at a low frequency of provocation (upper left hand curve). Increased aggressive responding in this subject typically represented aggressive responding in the absence of provocation which did not occur under placebo conditions. No other subjects evidenced this effect.

Diazepam had no effect upon the aggressive responding of subject S-178. All other subjects had reduced aggressive responding following the administration of the highest diazepam dose. Three subjects had decreased aggressive responding following the administration of the 5 mg per 70 kg diazepam dose.

Figure 2 shows the effects of placebo and the three diazepam doses on non-aggressive monetary reinforced responses. Dose-response curves are again expressed as percent of placebo controls. Diazepam produced slight and dose-dependent decreases in non-aggressive responding in most subjects. Some subjects evidenced slight increases in non-aggressive responding.

The large increased aggressive responding observed in subject S-173, cannot be attributed to a non-specific or generalized stimulant effect since non-aggressive responses are not affected or slightly decreased. The decreased aggressive responding observed in most of the subjects appears to be selective. Both aggressive and non-aggressive responding were decreased, but decreases in non-aggressive responding were less than ten percent, while decreases in aggressive responding were 25 to 50 percent. Diazepam effects on aggressive and non-aggressive responding were poorly

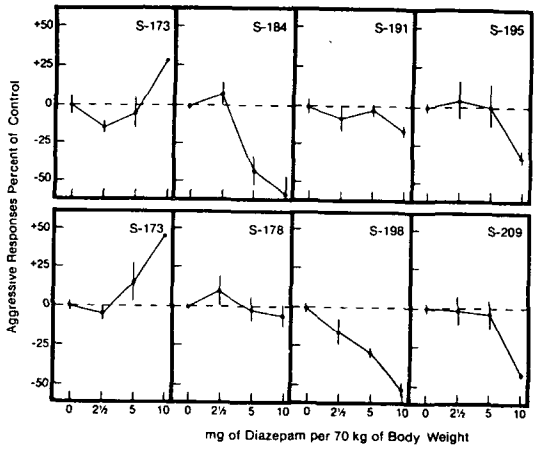

Figure 1. The effect of placebo (0) and three doses of diazepam 2.5, 5 and 10 mg per 70 kg on aggressive responses. Data points are expressed as percent changes from the mean placebo session values set at zero. Drug data points represent the mean of three different sessions. Vertical lines at all data points represent 1 SEM. Subjects assigned to PFI durations of 500 seconds are shown in the top half of the figure, and those assigned to PFI durations of 125 seconds are shown in the bottom half of the figure.

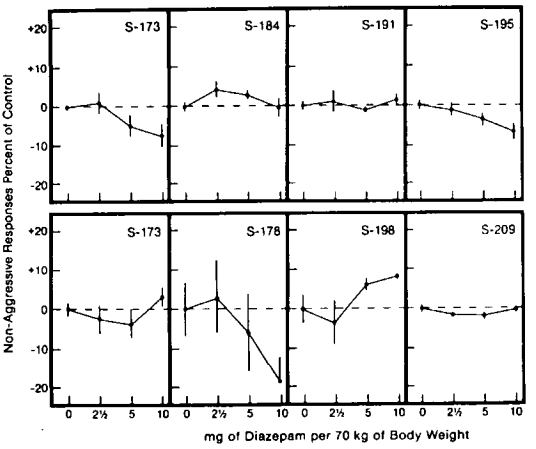

Figure 2. The effect of placebo (0) and three doses of diazepam 2.5, 5 and 10 mg per 70 kg on non-aggressive monetary reinforced responses. Data points are expressed as percent changes from the mean placebo session values set at zero. Drug data points represent the mean of three different sessions. Vertical lines at all data points represent 1 SEM. Subjects assigned to PFI durations of 500 seconds are shown in the top half of the figure, and those assigned to PFI durations of 125 seconds are shown in the bottom half of the figure.

correlated (r = -0.09). Subjects S-178 had substantial decreases in non-aggressive responding while aggressive responding was unchanged; Subject S-198 had slightly increased non-aggressive responding while aggressive responding was markedly reduced.

The Profile of Moods State (POMS) questionnaire was completed by all subjects at the end of each session. Scores from the POMS were combined into six categories: tension, depression, confusion, anger and fatigue. The anger scores as well as all other categories of the POMS were essentially unchanged following diazepam administration.

Subjects completed the Buss-Durkee Hostility Questionnaire at the end of the study. This questionnaire consisted of 75 true-false items. The scale contains both objective and subjective items regarding aggression and hostility. The categories of this scale are: assault, indirect hostility, irritability, negativism, resentment, suspicion, verbal hostility and guilt. The total hostility score is the sum of the scores of all of these categories. Subject S-173 who had marked increases in aggressive responding following the administration of the highest diazepam dose, had higher scores on the assault (a measure of physical violence against others) and verbal hostility scale. Subject S-173 overall hostility score was 46 and was much higher than scores of any of the other subjects (17-25).

Discussion

Our preliminary findings indicate that “paradoxicaltt increases in aggressive responding were observed in a single subject in a laboratory. Reports of paradoxical increases in aggressive behavior have been observed in patients administered certain benzodiazepines in a therapeutic context. The present experiment indicates that increased aggressive behavior can occur in normal males following the administration of diazepam at a dose of 10 mg per 70 kg. However, the acute administration of diazepam resulted in a relatively selective suppression of aggressive behavior in all other subjects, validating the “aggressive” effects of diazepam that have been reported clinically and in laboratory studies with non-humans.

The correlation between the effects of diazepam and the effects of alcohol on aggressive responding was highly significant ($r=0.918$, $p<.001$). In addition, the total hostility scores on the Buss-Durkee Hostility Scale and the effects of diazepam on aggressive responding were also significantly correlated ($r=0.823$, $p<.025$). Our preliminary data indicate that high scores of self-reported hostility and increased aggressive responding following alcohol administration may predict paradoxical increases in

aggressive behavior following diazepam administration. However, the typical response to diazepam administration is a rather selective decrease in aggressive responding and little or no effect on non-aggressive responding.

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The Role of Temptation in Reactivity to Alcohol Stimuli

Eric Corty, Charles P. O'Brien, and Stephan Mann

Relapse may be the major problem in the treatment of addictive behaviors. While treatment can detoxify patients from alcohol and/or drugs, one of the diagnostic hallmarks of this class of disorders is the resumption of substance use in the absence of physiological withdrawal. Wikler (1965) noted that some addicts, after months or years of abstinence, returned to drug use when they returned to an environment where they had previously used drugs. This relapse appeared to be motivated by the reappearance of withdrawal symptoms. Wikler postulated that classical conditioning of a specific environment and withdrawal symptoms occurred via repeated associations. The instrumental activity of drug seeking and drug use was then reinforced by the alleviation of withdrawal symptoms when the drug was used. Finally, upon return to the environment withdrawal symptoms ware re-elicited and subsequent drug use became more probable.

There is some support for this model in the area of alcohol dependence. Kaplan et al. (1983) found that cues associated with alcohol led to physiological and cognitive responses that correlated with an increased probability of drug seeking behavior. Others have also shown in humans that alcohol cues affect the physiological effects of alcohol (Dafters and Anderson, 1982) and affect craving, physiology, and behavior (Ludwig et al., 1974). Pomerleau et al. (1983) measured cognitive and physiological responses in a group of alcoholics and a group of control subjects who sniffed cedar chips and alcohol. Alcoholics significantly differed by reporting more craving for alcohol and by swallowing more frequently when sniffing alcohol. Pomerleau et al. considered swallowing to be a conditioned response and suggested that such conditioned responses may be an effective way to assess desire to drink and to predict relapse.

The present studies replicated and extended the Pomerleau et al. study. The first study was a replication with several methodological changes. Notably, salivation rather than swallowing was the dependent variable, the order of the stimuli

was counterbalanced, and a caloric control stimulus was used. In the second study, a series of case reports, the role of cognitive set in conditioned reactions to drug cues was explored.

STUDY I: Exposure to Alcohol Cues

Subjects. Ten subjects who had completed detoxification at the Philadelphia VA Medical Center and ten nonalcoholic control subjects (recruited via signs posted at the VA) were used. Informed consent was obtained and subjects were reimbursed $5 for their participation. The alcoholic subjects ware 90% black, had a mean age of 40.7 (s.d. = 11.7), had their first drink at a mean age of 14.2 years (s.d. = 4.6), had been heavy drinkers for a mean of 14.0 years (s.d. = 12.0), had a mean score on the MAST (Selzer, 1971) of 35 (s.d. = 12.2), had been in treatment for alcoholism a mean of 2.8 times (s.d. = 2.2) and drank a mean of 8480 ml. of ethanol (s.d. = 8600) over a mean of 17.9 (s.d. = 9.9) of the last 30 days. In contrast, the nonalcoholic subjects ware 50% black, had a mean age of 35.7 (s.d. = 10.5), had their first drink at the mean age of 19.3 (s.d. = 7.5), had a mean score of 1.0 (s.d. = 1.2) on the MAST, and drank a mean of 90 ml of ethanol (s.d. = 100) over a mean of 2.7 (s.d. = 2.1) of the last 30 days.

Procedure. The experimental session always began at the same time of day. Each session began with a ten minute baseline period. The subject then placed three dental rolls in his mouth to collect saliva. After the dental rolls had been placed, the subject opened the door to a cupboard, removed the container that had been placed there, and began to sniff the contents of the container. After five minutes the subject was told to remove the dental rolls and to replace the container. Fifteen minutes later the procedure was repeated and subject found a different stimulus to be sniffed in the cupboard. Fifteen minutes after this second trial the procedure was repeated a third time with yet a third substance to be sniffed. Ten minutes later the session was terminated.

The order of the substances found in the cupboard varied. The first substance that all subjects sniffed was cedar chips. The second substance was either a cup containing 60 ml of an orange flavored drink or the smallest available bottle of their preferred alcoholic beverage. The orange beverage and the alcoholic beverage were sniffed in a predetermined random order. Subjects were told that they could not consume any of the substances.

Finger temperature, skin resistance level, and heart rate were measured continuously. The other dependent variables, salivation, blood pressure, and self-reported urge to drink alcohol, were measured at each of the stimulus periods.

Results. Repeated measures ANOVAs ware used to examine all dependent variables. The design was group (alcoholic vs. nonalcoholic) by order (cedar, alcohol, orange vs. cedar, orange, alcohol) by time (baseline vs. stimulus I vs. stimulus II). A significant group x order x time interaction would show that alcoholics and nonalcoholics reacted differentially to the

different stimuli. However, no such significant interactions ware found. For most of the variables, the only significant effect found related to time. Both heart rate and finger temperature decreased significantly over the experimental session ($F(2,32) = 20.23$, $p < .001$ and $F(2,32) = 12.11$, $p < .001$, respectively). Both measures of blood pressure evidenced time x group interactions ($F(2,28) = 3.9$, $p < .05$ and $F(2,28) = 5.93$, $p < .01$, for systolic and diastolic respectively). Salivation showed a significant group x order interaction ($F(1,16) = 5.06$, $p < .05$) with the alcoholics salivating less overall when the stimuli they sniffed were in the order of cedar chips, then orange beverage, then alcohol. For the other two dependent variables, skin resistance and reported urge for a drink of alcohol, no significant effects were found.

Discussion. Group data from this study do not support the conditioning model of alcoholism. That is, Study I failed to show that alcoholics and nonalcoholics react differentially to alcohol stimuli. Though this may be because the conditioning model of alcoholism is invalid, the failure to reject the null hypothesis may also be due to procedural factors. If the procedure is at fault then it is likely that the phenomenon of conditioned responses to alcohol stimuli is not robust. That is, the phenomenon might only be elicitable under certain, specified conditions and only be measurable by certain, specified variables.

Two procedural differences seem most important. Hodgson et al. (1979) found that severely dependent subjects exhibited a greater degree of craving than did moderately dependent subjects. Though the alcoholic subjects in the present study all had diagnoses of alcohol dependence, none of them had a SADQ score high enough to be placed in the severely dependent range (Stockwell et al., 1983). Thus, it is possible that evidence supporting the conditioning model of alcoholism would be found with more severely dependent subjects.

In the present study, subjects were told that though they would be asked to sniff various substances they would not be asked to and would not be allowed to consume any of them. Pomerleau (1984) reported that he kept instructions to subjects deliberately vague as to the possibility of consumption. Different settings, environmental and/or cognitive, may be more or less conducive to the elicitation of conditioned responses or urges. That is, being faced with a beverage that one knows one will not be allowed to consume may be phenomenologically different from confronting a drink that one could actually consume.

Evidence supporting this notion can be found in Kaplan et al., (1984). Alcoholics were asked to sniff a malt beverage that they knew may or may not contain alcohol before consuming it. After consumption the subject was asked whether he thought the beverage contained alcohol. Subjects who reported believing the beverage to be alcoholic showed a significantly greater skin conductance level change to the presentation of the beverage. Thus, cognitions appear to play a role in what could be considered a conditioned

reaction to alcohol related stimuli.

STUDY II: Exposure to Alcohol Cues with Permission to Drink

Study II was designed to explore the role that expectations (or cognitions, or setting) nay play in eliciting conditioned responses to alcohol relevant stimuli, In addition to changing the setting, we also changed the salience of the stimulus, the order of presentation, and selected as subjects extremely dependent alcoholics. Study II was designed as a series of case studies.

Subjects. As this study was going to involve the ingestion of alcohol by alcoholics, the inclusion/exclusion criteria ware stringent. over a six month period only 11 subjects volunteered for this study and only three of these met the following screening criteria. In order to participate, volunteers had to be between age 25 and 50 and had to have a DSM III diagnosis of alcohol dependence. They had to report at least a five year history of heavy drinking; during the past 30 days they had to report drinking at least 250 ml. of ethanol on at least 20 days. Subjects had to have an SADQ score of greater than 30. Subjects had to agree to enter into treatment for alcoholism at the conclusion of the study. subjects were eligible to earn $50 if they completed five days of alcoholism treatment at the conclusion of the study. (All three subjects earned the $50.) And, of course, subjects had to sign statements of informed consent.

Subjects were excluded if they had any systemic condition that was caused or worsened by alcohol. In addition they were excluded for the following psychiatric reasons: any psychotic condition, major affective disorder, sociopathy, organic mental syndrome or dementia, and retardation. Subjects had to be free of any other major substance abuse in the past six months. And finally, subjects could not be taking any medication with the ability to alter the psychophysical dependent variables.

Subject 1 (S1) was a 30 year old black male with a seven year history of heavy drinking and an SADQ score of 36. Prior to admission for detoxification he reported drinking 30 days per month, consuming a mean of 310 ml. of ethanol per drinking day. Subject 2 (S2) was a 26 year old black male with a seven year history of heavy drinking and an SADQ score of 50. S2 reported drinking 29 out of 30 days, consuming an average of 440 ml. of ethanol per drinking day. Subject 3 (S3) was a 32 year old black male with a 15 year history of heavy drinking and an SADQ score of 45. He reported drinking 30 days in the month prior to admission, consuming 496 ml. of ethanol per day.

Procedure. The procedure was aimed at getting subjects to believe that when the researcher offered them a drink of alcohol and told them that they could drink it, that they actually would be allowed to consume it. This was deemed vital, for we believed that the stimulus would elicit a conditioned reaction only if the subject was tempted by it. To this end, we designed the study to take several days. The first two days set the stage for day 3, the

test day.

Subjects were admitted into the hospital on a Friday and began the study on Monday. At the completion of the study the subject stayed in the hospital for two more days in order to make sure that the alcohol withdrawal syndrome had not been re-instituted. After this the subject was discharged and began alcohol rehabilitation treatment.

Day 1 and Day 2. On each of these days the subject was seated in the experimental chamber and had electrodes attached. After a 20 minute baseline period the subject drank either alcohol (0.6 ml/kg of body weight) mixed one part to five with tonic water or a similar quantity of water mixed one part to five with tonic water. The alcohol was calculated to raise blood alcohol level to 0.05%. subjects were told that on different days they would consuming different amounts of alcohol. Both subjects and experimenters were blind as to the amount of alcohol received on each day. Sixty minutes after the ingestion of the alcohol the session concluded.

Day 3. was the test day and consisted of two sessions Day separated by a 45 minute break. Each session had a 20 minute baseline period during which the first measures of salivation and physiological responses were taken. After baseline the subject retrieved a glass containing the stimulus beverage and an opened manufacturer's bottle of the beverage from the cupboard. The subject sniffed this for five minutes. During this time physiological recordings were made and the subject was asked if he had any urge for a drink of alcohol. Also during this time the subject watched an audio-video tape that consisted of stimulus relevant material. After the five minutes were up, the subject was told that he could consume the beverage if he wanted. All of these directions were contained in the audio-video tape in order to minimize experimenter influence. The session continued for another 25 minutes.

Session I on day 3 always consisted of the non-alcoholic stimulus. The beverage was a cola and the audio-video tape showed pictures of soft drinks and soft drink dispensers with a soundtrack from advertisements. Session II always consisted of the alcoholic stimulus. The beverage was the subjects' favorite alcoholic beverage in the form that the subject preferred (e.g., straight or mixed). The audio-video tape showed pictures of alcoholic beverages and containers and had footage of a person buying alcohol in a liquor store and drinking in a bar. Again, there was a soundtrack from relevant advertisements.

Results and Discussion. The only physiological variable presented will be salivation as this variable has been found to be relevant in other studies. We would also like to stress that these results are based on three case studies.

For both stimuli, the cola and the alcohol, the subjects ingested the stimulus drink. Thus it seems that subjects believed that the

beverages could be consumed and found the beverages attractive.

Subjects' reported urges for a drink of alcohol did not vary much with the stimulus presented. Subject 1 reported no urge for alcohol in either situation and Subject 3 reported a slight urge in both situations. only for Subject 2 was there an increase from a moderate urge for a drink of alcohol when confronted with the cola stimulus to a considerable urge when confronted with the alcohol stimulus. On several grounds self-report of urge does not seem to be adequate in assessing urge. First, it does not show the variability across situations that one would expect and second a subject who reports no urge for a drink still consumed a drink.

We calculated a change score for salivation. A change score of 100% indicates no increase in salivation to the stimulus over the baseline period. Salivation data are shown in Table 1.

Table 1. Individual subjects' salivation and change scores during cola and during alcohol stimulus periods.

	Baseline Salivation		Change Score	
	Cola	Alcohol	Cola	Alcohol
S1:	1.96	2.61	162%	163%
s2:	1.22	.74	134%	240%
s3:	1.78	1.43	136%	233%

Note: Baseline salivation measured in grams.
Change score = salivation to stimulus as a % of baseline.

All subjects in all conditions evidenced a change score greater than 100%. Thus it seems that the presentation of a stimulus beverage with the presentation of ancillary stimulus material and under a condition where the subject knows that he can consume the beverage elicits increased salivation.

In two subjects, the change score for the alcohol condition was considerably greater than it was for the cola condition. Thus, it seems that for these conditions and for these subjects the presentation of the alcohol stimulus elicited a greater increase in salivation.

Overall Discussion. Study I failed to show that conditioned reactions to alcohol stimuli can be elicited in alcoholics. Study II suggested that such reactions can be found under certain conditions and with certain subjects. Thus, at present it seems safe to conclude that such conditioned reactions do exist but that their prevalence is unknown and that the parameters under which they can be elicited are not well known.

Our impression is that the most important parameter for the elicitation of such a conditioned reaction is a setting in which the temptation is real. The stimulus must be available and the

subject must truly believe that it may be consumed. Both the cognitive and the physical settings are important. This impression is based on the differences in results between the two studies. Many parameters beside temptation were varied from one study to the next and the second study is based on only a small number of subjects. Nonetheless, we hold that without a tempting stimulus (one that is available and that the subject believes could be consumed) the second study would have shown no conditioned reactions to alcohol related stimuli.

This need for temptation may limit the utility in treatment of the conditioned reactions to alcohol related stimuli. Initially it was hoped that this methodology could be used to assess craving and/or relapse potential during treatment (Pomerleau et al., 1983) or that it could be used as an active component of treatment for heroin or cocaine dependence (Childress et al., 1985). Unfortunately, when actual temptation with the possibility of consumption is present, the presentiment that assessment or treatment may contribute to relapse occurs. Although data do not support the notion that one drink leads to a drunk (e.g., Paredes et al., 1973), the tenor of the times (e.g., Pendery et al., 1982) may make it difficult to support the research that would determine whether the integration of a temptation extinction component into a comprehensive treatment program would decrease the rate of relapse. We feel, given the relatively low rates of success in treatment, that research on conditioning factors in alcoholism is necessary and should be undertaken.

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Alcohol Effects on Human Aggressive Behavior: Influence of Concurrent Fixed-Ratio Reinforcement Contingencies

Thomas H. Kelly, Don R. Cherek, and Joel L. Steinberg

When humans are provoked in a laboratory setting, they respond in a manner that presents noxious stimuli, such as electric shock, loud noise, or monetary point subtractions, to other persons. This behavior has been investigated because of its functional similarity to human aggression. Several research groups have reported that alcohol increases the probability of these aggressive responses. Recent reviews of these findings have stressed the importance situational factors in determining the effects of alcohol on aggressive responding (Taylor and Leonard 1983: Cherek and Steinberg 1986). Clinical evidence also confirms the situation-dependent nature of alcohol effects on aggression. This paper presents evidence suggesting that concurrent reinforcement contingencies influence the effects of alcohol on human aggressive behavior.

Several studies using various procedures have demonstrated the role of concurrent reinforcement contingencies in controlling probabilities of aggressive responding. For example. aggressive behavior increases in probability during extinction of previously reinforced behavior (e.g., Azrin et al., 1966; Thompson and Bloom 1966: Kelly and Hake 1970; Harrell 1972). The probability of aggressive responding is also functionally related to presentation of other reinforcers (e.g., Hutchinson et al., 1968; Flory 1969). Drugs such as delta-9 tetrahydrocannibinol, produce selective effects on aggressive responding that is maintained by concurrent reinforcement schedules (Cherek and Thompson 1973: Cherek et al., 1980). Neither the effects of concurrent reinforcement schedules on human aggressive behavior or drug effects on these behaviors have been investigated.

METHOD

Subjects: Four subjects between the ages of 18 and 40, in good physical health and devoid of any major psychiatric condition completed the study. They were recruited with newspaper advertisement for part-time employment. No mention of drugs or aggression was included in advertisements to avoid bias in the

responding population. All gave informed consent prior to participation. Subjects were asked to refrain from caffeine and nicotine consumption for one hoar prior to each session and illicit drug use throughout the study. Urine samples were collected at the first session and periodically throughout the study to monitor drug usage, and breath alcohol levels were measured daily. Repeated alcohol detection prior to sessions and any incident of illicit drug consumption were exclusion criteria.

Apparatus: Before sessions, subjects were seated in front of a human response console equipped with two illuminating response buttons, an add/subtract counter, pseudo-thermistor and speaker. The thermistor was taped to the middle finger of the left hand prior to each session, and subjects were informed that body temperature, pulse rate and pulse pressure were being monitored. These instructions were used to minimize the importance of aggressive responding as a primary dependent variable. No mention of aggressive responding was offered during initial instructions.

Procedure: Subjects were exposed to a three component reinforcement schedule. A and B buttons were illuminated during components. Responses on the A button intermittently increased point totals on the counter. Point increases produced an audible click, and a 0.5 second flash of a green light occurred. A and B buttons were dark during this time. Each point was exchanged for ten cents following sessions. During separate components, 50, 200 or 500 responses produced a point. The three components were presented in a random order twice per session. Components changed following the first point delivery nine minutes after the start of a component, or after eleven minutes (i.e., 2 minute limited hold). A 5 second timeout, during which A and B buttons were dark, separated components. Session time averaged 56 minutes.

Human response rate during fixed ratio reinforcement contingencies is typically greater than 4.5 responses per second and independent of response requirement. Responses occurring less than seconds following a previous response (IRT <.2 seconds) were counted but had no effect on response requirements. Approximately 20% of responses were emitted with IRT's less than .2 seconds. The added IRT contingency maintained stable reinforcement density when response rates changed.

Periodically throughout sessions, points were subtracted from subjects. Point subtractions produced an audible click, and a 0.5 second red light flash occurred. Subjects were told that they were paired with another participant selected at random from a group of participants and that this participant would control point subtractions. In reality, points were subtracted according to a variable-time schedule throughout the first nine minutes of each component. No points were subtracted daring the 2 minute limited hold.

Subjects were informed that they could also subtract points from

the other participant throughout each session. 10 responses on the second, or B button darkened the A and B buttons for 0.5 seconds and ostensibly subtracted a point from the other participant. A 5 second change-over-delay reduced contiguity between B button responses and point delivery following A button responses.

Subjects were initially exposed to a minimum of one session with no provocations. Next, provocations were presented every 75 seconds, on average. Each ratio completed on the B button delayed subsequent provocations for 500 seconds. A minimum of three sessions with this avoidance contingency were presented. Finally, provocations were presented every 300 seconds, on average. No avoidance or escape contingencies were in effect during the final schedule. As such, changes in aggressive responding produced no change in provocation density during subsequent dose-response determinations. When performance became stable, alcohol dose-response curves were measured.

Drug Administration: Prior to each session subjects were given twenty minutes to consume a cocktail containing 16 a of a ginger ale-ethanol mixture, using 95% ethanol, four drops of peppermint oil and crushed ice. with 1 ml of ethanol floated on top. Sessions began 30 minutes after receiving the cocktail. Breath samples were collected before and after each session to document blood-alcohol levels. Ethanol doses of 0.125, 0.25 and 0.5 g/kg were administered double-blind in an ascending/random sequence until three replications per dose were recorded. Drug administration was separated by a minimum of forty eight hours and delivered when preceding placebo session values were within variability ranges established prior to drug administration.

After completing the study, subjects responded to open-ended questions designed to solicit information about the validity of the cover story.

RESULTS

Blood alcohol levels were estimated by breath analysis immediately before and after each session. The smallest dose produced negligible blood levels prior to sessions (0.1 g/dl or less), and no alcohol was detected following sessions. The moderate dose produced slightly higher presession alcohol levels (approximately .03 g/dl) which decreased to negligible levels following the session. The highest dose (0.5 g/dl) produced higher presession levels (.04 to .08 g/dl) that remained slightly elevated following sessions.

The effects of alcohol on aggressive responding averaged over an entire session are presented in figure 1. Low doses of alcohol produced slight increases on aggressive responding in three of four subjects which is consistent with previously reported results. No systematic changes were observed on point-maintained responding following alcohol administration.

The relationship between concurrent fixed-ratio response requirement and aggressive responding was analyzed during control sessions. Figure 2 presents the number of aggressive responses, adjusted for provocation, emitted during fixed-ratio 50, 200 and 500 components (open circles). Three of four subjects emitted significantly more aggressive responses per provocation as concurrent response requirements were increased.

The effects of alcohol on aggressive responding were also analyzed as a function of concurrent fixed-ratio response requirement. Data in figure 2 suggest that the behavioral effects of the highest dose (0.5 g/kg) were enhanced as concurrent ratio response requirements increased in two subjects.

Written responses on a poststudy questionnaire and verbal responses during debriefing indicated that all subjects accepted the validity of the initial cover story.

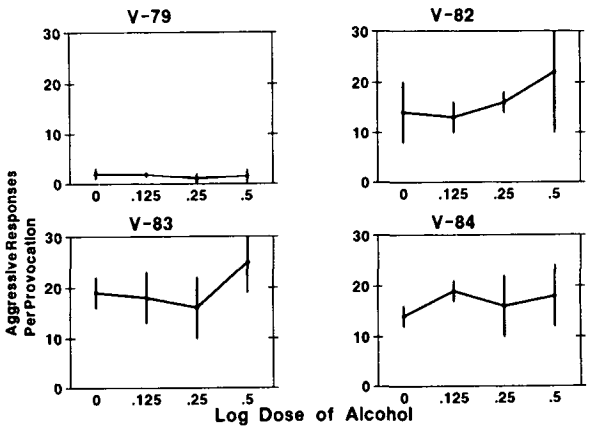

FIGURE 1. Aggressive responses per provocation as a function of the log dose of alcohol (g/kg). Vertical lines represent ± 1 S.E. Each point represents the mean of at least 3 sessions, and points above 0 g/kg represent the mean of at least 9 sessions.

DISCUSSION

Alcohol increased the tendency for subjects to respond aggressively when provoked. Alcohol had little effect on or decreased nonaggressive responding at these same doses, suggesting that the effects of the drug on aggressive responding were not produced by nonselective stimulation. Density of both point presentations and point subtractions remained constant across alcohol doses; therefore, the effects of provocations were not altered by drug-induced changes in responding. Running rate and pattern of responding were similar on point-maintained and aggressive response options, suggesting that the differential alcohol effects observed were not related to differences in control responding (Cherek et al., 1985). Instead, the relative probabilities of responding on the aggressive response option increased following alcohol administration.

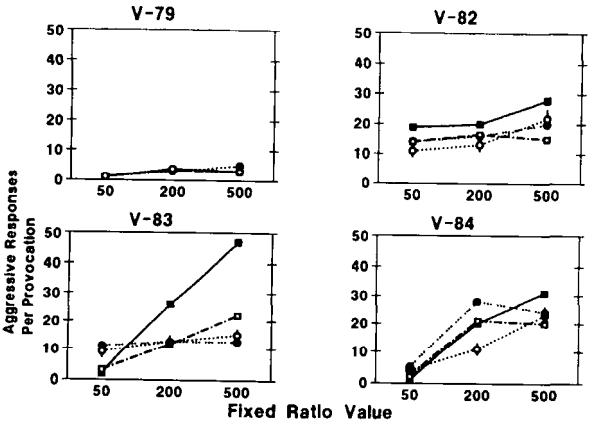

FIGURE 2. Aggressive responses, adjusted for number of provocation, emitted during three separate components of a mixed reinforcement schedule at four alcohol conditions. Open circles represent control sessions, open squares represent 0.125 g/kg, closed circles represent 0.25 g/kg, and closed squares represent 0.5 g/kg. Vertical lines represent ± 1 S.E. Each point represents the mean of at least 3 sessions. and control points represent the mean of at least 9 sessions.

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Small doses were studied to produce blood-alcohol levels below socially-determined levels of intoxication (see also Cherek et al., 1985). Other researchers (e.g., Taylor et al., 1976: Zeichner and Pihl 1979) have demonstrated the aggression-enhancing effects of high alcohol doses in a laboratory setting.

The probability of aggressive responding was linearly related to concurrent fixed-ratio response requirements. Hutchinson and co-workers (1977) studied the relationship between jaw clenching, a correlate of human anger, and concurrent fixed-ratio response contingencies. Their data also revealed a positive relationship between fixed-ratio response requirements, up to 500, and jaw clenches. As ratio requirements were increased beyond 500, however, jaw clenching decreased in intensity, resulting in an overall inverted-U shaped relationship between fixed-ratio response requirement and jaw clenches. Jaw clenches were also related to the temporal characteristics of point-maintained responding, closely paralleling the relationship between nonhuman adjunctive behavior and concurrent inducing schedules. This finding along with the results of the current study suggest that concurrent schedule contingencies influence the emotional and behavioral response to aversive stimuli. In the present study, concurrent reinforcement commodities (points on a counter) were also used as provoking stimuli. As such, the results should be related to the schedule-induction literature with caution.

Alcohol effects on aggressive responding were influenced by concurrent fixed-ratio. response requirements in two subjects. Concurrent reinforcement contingencies appear to be one factor determining the situation-specific nature of alcohol effects on human aggressive behavior.

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Naltrexone Stimulation of Pituitary, Adrenal, and Gonadal Hormones During the Luteal Phase of the Menstrual Cycle: Acute Effects of Alcohol

Jack H. Mendelson, Nancy K. Mello, Ulrike Lange, Siew Koon Teoh, Alice S. T. Skupny, Susan Palmleri, and James Ellingboe

Derangements in menstrual cycle and reproductive function of women are frequently associated with chronic alcohol abuse (Hugues et al. 1980; Ryback 1977; Moskovic 1975). Experimental animal studies have shown that chronic ethanol administration may adversely affect pituitary and gonadal hormone function (Van Thiel et al. 1978; Cicero 1980; Gavaler et al. 1980; Van Thiel and Gavaler 1982; Mello et al. 1983). However, studies of acute administration of alcohol to normal women and female rhesus monkeys did not produce any significant changes in plasma levels of luteinizing hormone (LH) or 17 beta estradiol (E_2) (Mendelson et al. 1981; McNamee et al. 1979; Välimäki et al. 1983; Mello et al. 1984). The absence of any acute alcohol effects on female pituitary gonadotropins and gonadal steroid hormones in these studies may have been related to relatively low and stable hormonal levels during the early follicular and mid-luteal phases of the menstrual cycle. The present study was undertaken to determine acute alcohol effects on pituitary, adrenal and gonadal hormones following perturbation by administration of the opioid antagonist Naltrexone.

We have recently reported that Naltrexone may be a safe and effective drug for assessing function of the hypothalamic-anterior-pituitary axis in women (Mendelson et al. 1986). We observed that Naltrexone induced an increase in plasma luteinizing hormone (LH), prolactin, ACTH and cortisol during the early follicular phase of the menstrual cycle. Naltrexone administration also induced stimulation of pituitary, adrenal and gonadal hormones during the luteal menstrual cycle phase. The purpose of this study was to determine if acute alcohol administration modified or attenuated Naltrexone stimulated anterior pituitary, adrenal and gonadal hormones in healthy normal cycling women during the luteal phase of the menstrual cycle.

METHODS

Ten healthy women between the ages of 22 and 33 (mean age 26) provided informed consent for participation in these studies. All women had normal physical and mental status examinations.

The women also had normal blood chemistry, blood hemogram and urinalysis studies. All women had normal menstrual cycle function, none were pregnant and none used contraceptive medication. Urinalysis for drug screening carried out when subjects were initially evaluated for participation in the study as well as on each study day were negative for opioids, barbiturates, benzodiazepines, cocaine and other stimulant, depressant, psychoactive and psychotropic drugs. No women had any past or current history of alcohol or drug abuse.

Each woman was studied on two occasions one day apart. Each subject received both alcohol and alcohol placebo (as described below) in a randomized counter-balanced, double-blind procedure. Half of the subjects received alcohol on the first study day, the other half received alcohol on the second study day.

Subjects reported to the Clinical Research Facility at 9 a.m. on each study day following a 12 h fast. An intravenous catheter was placed in the antecubital vein and connected to a slow infusion of physiologic saline. Subjects remained recumbent throughout the study and were not permitted to smoke.

Following collection of blood samples over a 30 min interval, subjects swallowed a 50 mg Naltrexone tablet. After collection of two more consecutive blood samples (at 30 min intervals), subjects drank either an alcohol or an alcohol placebo solution over a period of 15 min. The alcohol solution consisted of 1 ml of 95% ethanol/kg of body weight diluted in a total volume of 12 ozs. of ice cold fruit juice. Blood samples were collected at 15, 20, 25, 30, 45, 60, 90, 120, 150, and 180 minutes following initiation of alcohol or placebo intake. Both the ethanol solution and placebo alcohol were administered with a new device which has been described previously (Mendelson et al. 1984).

RESULTS

There were no significant differences in progesterone values for subjects during the two conditions. Naltrexone administration did not significantly affect plasma progesterone levels. Administration of alcohol or placebo did not produce any significant alterations in plasma progesterone values. A statistically significant increase in LH levels was found after both Naltrexone and placebo administration ($P < .001$) and Naltrexone and alcohol administration ($P < .001$). A significant increase in prolactin levels over baseline values was found for both the placebo ($P < .04$) and the alcohol ($P < .001$) conditions (Fig. 1). Plasma cortisol levels increased significantly following Naltrexone and placebo intake ($P < .001$) (Fig. 2). After Naltrexone and alcohol intake, a significant increase in plasma cortisol levels ($P < .01$) was found (Fig. 2). Plasma estradiol levels did not increase significantly following Naltrexone and placebo administration. However, plasma estradiol levels were significantly elevated following Naltrexone and alcohol intake ($P < .001$). Data from

representative subjects are shown in Figures 1, 2, and 3.

DISCUSSION

Naltrexone induced stimulation of the hypothalamic-pituitary-adrenal-gonadal axis observed in previous studies with women during the early, mid and late follicular phase of the menstrual cycle (Mendelson et al. 1986) was also found in this study carried out during the luteal phase of the menstrual cycle. The magnitude of LH stimulation was smaller in women studied during the mid-luteal phase of the menstrual cycle than those administered the drug during the early follicular phase. However, the magnitude of Naltrexone induced prolactin and cortisol stimulation during the mid-luteal phase of the menstrual cycle were the same as those observed in women studied during the early follicular phase.

Naltrexone administration did not alter progesterone levels during either placebo or alcohol conditions. Naltrexone administration did not induce any changes in plasma estradiol levels during the placebo condition. However, Naltrexone followed by alcohol administration resulted in a significant sustained elevation in plasma estradiol levels. This observation is consistent with findings we obtained in previous studies of acute alcohol administration following naloxone infusion to women during the mid-luteal phase of the menstrual cycle.

Acute alcohol intake alone had no effect on basal LH levels in women and Macaque monkeys (Mendelson et al. 1981; McNamee et al. 1979; Välimäki et al. 1983; Mello et al. 1984). The absence of an acute alcohol effect on LH following Naltrexone administration is consistent with previous studies we have carried out with concurrent naloxone and alcohol administration to women. These data are also consistent with previous findings obtained in studies with male Macaque monkeys where alcohol (BAL levels 250-350 mg/dl) did not effect naloxone induced stimulation of LH and T (Mello et al. 1985). Alcohol administration also did not suppress naloxone stimulated LH in rat when naloxone was administered in doses of 1-4 mg/kg (Cicero et al. 1982). However, following low-dose naloxone administration (0.75 mg/kg) alcohol (90 mg/dl) suppressed LH stimulation (Cicero et al. 1982).

Since naloxone is postulated to directly stimulate hypothalamic secretion of LHRH (Yen et al. 1985) these data suggest that alcohol does not impair hypothalamic regulation of LHRH secretion over the dose range studied. These findings, considered in the context of previous studies of alcohol effects on synthetic LHRH stimulation of pituitary release of LH, suggest that both hypothalamic and pituitary function may be resilient to the effects of acute alcohol administration. In female rhesus monkey, blood alcohol levels of 180 to 270 mg/dl did not suppress LHRH stimulated LH (Mello et al. 1986). Alcohol also

did not suppress LHRH stimulated LH in intact male rodents (Cicero et al. 1978) or normal males (Ylikahri et al. 1978). These studies of acute alcohol effects on LH under basal or naloxone or synthetic LHRH stimulation are discordant with studies of chronic alcohol effects where suppression of LH has been observed in alcoholic women (Hugues et al. 1980; Moskovic 1975; Välimäki and Ylikahri 1981). This suggests that repeated or sustained episodes of alcohol intoxication are required to suppress pituitary gonadotropin secretory activity in females.

Alcohol Effects on Naltrexone Stimulated Estradiol

After placebo control administration, estradiol levels were unchanged by Naltrexone administration. Lack of a Naltrexone-related increase in plasma E_2 following the placebo control is consistent with the finding that a single dose of synthetic LHRH, sufficient to stimulate an eight to tenfold increase in plasma LH, did not raise plasma levels of E_2 in normal women who were studied during the follicular phase of the menstrual cycle (Kletzky et al. 1982).

The significant and sustained elevation of plasma E_2 levels after concurrent administration of both alcohol and Naltrexone was unexpected. Although increased LH secretion would be expected to enhance gonadal steroidogenesis, one should not observe increased plasma E_2 levels unless E_2 metabolism were to become rate limiting. No alcohol-related changes in plasma E_2 have been observed in previous studies with normal, healthy women (Välimäki et al. 1983; McNamee et al. 1979; Mendelson et al. 1981) or female Macaque monkeys (Mello et al. 1984). However, basal E_2 levels but not Naltrexone stimulated E_2 was measured in these studies.

The rapid increase in plasma E_2 following concurrent Naltrexone and alcohol administration could be the consequence of increased E_2 production and/or decreased E_2 metabolism. Increased synthesis of E_2 from testosterone is probably not the explanation, because alcohol induction of aromatase activity has been demonstrated only during the long-term ingestion of alcohol (Gordon et al. 1976). A more plausible mechanism is that the decrease in NAD/NADH ratio, known to be caused by metabolism of ethanol in the liver (Forsander et al. 1958), might reduce the rate of oxidation of E_2 to estrone, a shift in equilibrium that has been demonstrated for other 17-hydroxy/17-ketosteroid pairs in plasma and gonadal tissue (Cronholm and Sjovall 1968, 1969; Murono and Fisher-Simpson 1984, 1985).

The biologic significance of increased plasma estradiol levels following alcohol intake during Naltrexone induced gonadotropin stimulation remains to be determined. Since an increase in endogenous levels of estradiol during the late follicular phase of the menstrual cycle is necessary for the normal periovulatory LH surge, it is possible that acute ethanol intake just prior to ovulation could effect the time of onset or duration of the LH

surge. Anovulatory cycles and luteal phase inadequacy are frequent concomitants of alcohol abuse in women (Hugues et al. 1980; Moskovic 1975).

Whether or not recurrent episodes of alcohol intoxication may change plasma estradiol levels during early pregnancy also remains to be determined. Such studies are currently in progress.

Alcohol and Naltrexone Induced Stimulation of Prolactin

Naltrexone induced stimulation of prolactin levels in women observed in this study was consistent with recent reports of naloxone stimulation of prolactin secretion (Snowden et al. 1984; Yen et al. 1985; Cetel et al. 1985). Naltrexone stimulation of prolactin during the placebo condition study was probably due to Naltrexone stimulation of gonadotropin releasing hormone (GnRH). Braund et al. (1984) reported increased prolactin levels following GnRH administration during the luteal phase of the menstrual cycle.

The large increase in prolactin levels following Naltrexone and concurrent ethanol administration was not anticipated since acute ethanol intake alone did not produce any significant changes in plasma prolactin levels in women studied during the follicular phase of the menstrual cycle (Mendelson et al. 1981).

Alcohol induced increases in plasma E_2 levels during concurrent Naltrexone stimulation of LH could facilitate Naltrexone induced stimulation of prolactin release. Estrogens have been reported to increase prolactin secretion in experimental animals (Neill 1974; Clemens and Meites 1977; Quadri et al. 1979) and humans (Frantz et al. 1972; Yen et al. 1974). Diefenbach and his associates (1980) have reported that E_2 treatment significantly increased the prolactin response to TRH in intact, ovariectomized and stalk-sectioned female rhesus monkeys.

A modulating effect of 17 β-estradiol on prolactin secretion is believed to occur at both hypothalamic and pituitary levels (McCann et al. 1968; Dufy et al. 1979). Direct action of estrogens on prolactin secreting cells has shown to be related to specific estrogen cytoplasmic receptors and transport mechanisms (Hang et al. 1978). Estrogens stimulate incorporation of precursors in prolactin biosynthesis (Stone et al. 1977; Lieberman et al. 1980), and there is also evidence that estrogens decrease sensitivity of prolactin cells to dopaminergic inhibition (Gala and Boss 1975; Labrie et al. 1978; Beaulieu et al. 1979; Ferland et al. 1979).

Cetel and co-workers (1985) have concluded that "a common neuroendocrine mechanism is involved in the opioidergic control of prolactin and LH secretion and that this effect of naloxone is manifested only during high ovarian steroid environments." Data obtained in this study are consistent with the hypothesis

that Naltrexone induced prolactin secretion is enhanced under conditions when E_2 levels are elevated. Further studies are necessary to determine the biologic significance of ethanol induced increases in plasma prolactin levels during concurrent gonadotropin stimulation.

Alcohol and Naltrexone Induced Stimulation of Cortisol

Alcohol administration following Naltrexone was associated with a prompt increment in plasma cortisol levels. The rate of increment as well as the peak plasma cortisol levels following Naltrexone and ethanol administration was significantly greater than after Naltrexone and placebo intake ($P < .01$). Acute alcohol administration alone has been found to induce an increase in plasma cortisol levels (Mendelson et al. 1971). Ethanol induced enhancement of Naltrexone stimulation of plasma cortisol levels is probably due to a direct effect of ethanol and Naltrexone on ACTH release from the pituitary since we have shown, in previous studies, that Naltrexone administration produces a prompt increase in plasma ACTH levels which is followed by subsequent increases in plasma cortisol (Mendelson et al. 1986).

In summary, the findings obtained in this study indicate that Naltrexone stimulates LH, prolactin and cortisol secretion in normal cycling women during the mid-luteal phase of the menstrual cycle. Naltrexone did not alter basal plasma levels of progesterone or estradiol. However, a significant increment in estradiol levels occurred following concurrent Naltrexone and ethanol administration. The mechanisms underlying this phenomena are probably due to ethanol's effects on hepatic biotransformation of estrogens following gonadotropin stimulation. The enhanced levels of anterior pituitary, gonadal and adrenal hormones following naloxone administration and ethanol suggests that acute alcohol intake stimulates rather than depresses the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary adrenal axis. Although depression of pituitary, adrenal and gonadal function may occur after chronic alcohol intake or alcohol abuse, we conclude that ethanol has a biphasic, dose related effect on hypothalamic, anterior-pituitary, adrenal and gonadal hormones.

FIGURE LEGENDS

FIGURE 1. Plasma prolactin levels for a representative subject prior to and following Naltrexone (N) and placebo (P) administration (top panel), and Naltrexone (N) and alcohol (A) administration (bottom panel).

FIGURE 2. Plasma cortisol levels for a representative subject prior to and following Naltrexone (N) and placebo (P) administration (top panel), and Naltrexone (N) and alcohol (A) administration (bottom panel).

FIGURE 3. Plasma estradiol levels for a representative subject

prior to and following Naltrexone (N) and placebo (P) administration (top panel), and Naltrexone (N) and alcohol (A) administration (bottom panel).

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FIGURE 1

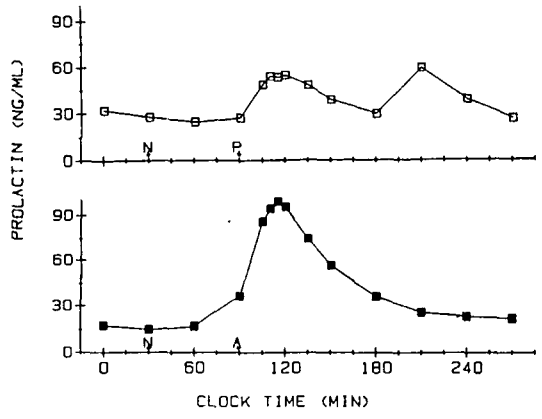


FIGURE 2

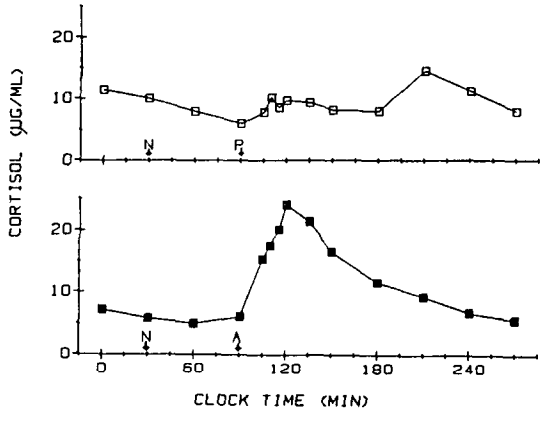
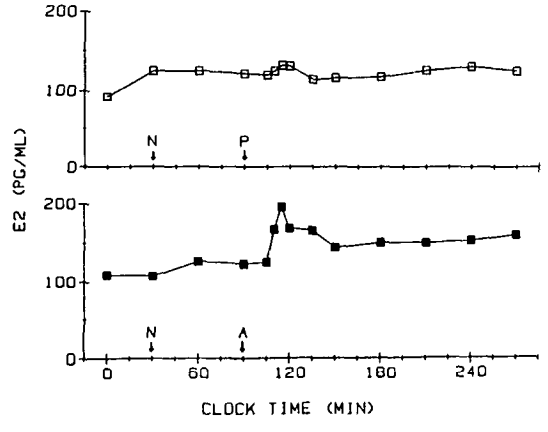


FIGURE 3



Alcohol Enhances LHRH-Stimulated LH in Ovariectomized Female Rhesus Monkeys

Nancy K. Mello, Jack H. Mendelson, Mark P. Bree, and Alice S. T. Skupny

INTRODUCTION

It is well-established that chronic alcohol abuse is associated with menstrual cycle abnormalities (persistent amenorrhea, anovulation and luteal phase defects) (Hugues et al. 1980; Moskovic 1975; Välimäki and Ylikahri 1981; Mello et al. 1983). But it has been difficult to determine if the toxic effects of alcohol occur primarily at the hypothalamus, the pituitary, or the ovary, or all in combination. Synthetic luteinizing hormone releasing hormone (LHRH) stimulates release of anterior pituitary gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), and can be used to assess pituitary function. We recently reported that alcohol (2.5 and 3.5 g/kg) prevented LHRH stimulation of FSH in normal female rhesus monkeys studied during the follicular phase of the menstrual cycle (Mello et al. 1986). However, alcohol did not prevent a significant increase in LH following LHRH stimulation. Under sucrose control conditions, LHRH-stimulated significant increases in both LH and FSH within 30 and 80 min, respectively (Mello et al. 1986).

A selective alcohol blockade of LHRH stimulated FSH is consistent with the hypothesis that FSH release is controlled by an interaction between endogenous LHRH stimulation and suppression by a gonadal peptide, inhibin (McCann et al. 1983; Channing et al. 1985). The differential effect of alcohol on LH and FSH during the follicular phase of the menstrual cycle suggested that FSH secretory cells may be more sensitive to alcohol than LH secretory cells. Because FSH is essential for normal follicular development and maturation (Goodman and Hodgen 1983; Ross 1985). an alcohol related inhibition of FSH could result in some menstrual cycle irregularities commonly seen in alcohol dependent females. Suppression of FSH could delay follicle maturation and ovulation or result in luteal phase dysfunction after timely ovulation (DiZerega and Hodgen 1981; Goodman and Hodgen 1983).

Since the release of pituitary gonadotropins in normal females, under basal or artificially stimulated conditions is necessarily

influenced by the ovarian steroid milieu, it seemed important to reexamine alcohol effects on LH and FSH in ovariectomized females. If alcohol also suppressed LHRH stimulated FSH, but not LH in ovariectomized females, this would indicate a primary effect of alcohol at the level of the pituitary. Alternatively, if alcohol's effects on pituitary hormones were different in ovariectomized and normal females, this would indicate that ovarian steroid or peptide modulation of the hypothalamic-pituitary-gonadal axis is one important determinant of alcohol's effect on FSH and LH. This study examined the acute effects of alcohol (2.5 and 3.5 g/kg) and an isocaloric control solution on LHRH stimulated LH and FSH in bilaterally ovariectomized monkeys.

METHODS

Subjects: Five sexually mature, bilaterally ovariectomized female rhesus monkeys (7.4 +1.1 kg) were studied. These monkeys had been ovariectomized for-over 440 days ($\bar{X}$ = 558 +105 days). Two monkeys were alcohol-naive and three monkeys had a history of low dose alcohol self-administration but had been alcohol-free for over 18 months when these studies began. Monkeys were maintained on ad lib food and water; monkey chow was supplemented daily with fresh fruit, vegetables and multiple vitamins. A 12-h light-dark cycle (7 a.m. to 7 p.m.) was in effect.

Animal maintenance and research was conducted in accordance with the guidelines provided by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facility is licensed by the U. S. Department of Agriculture. The health of the monkeys was periodically monitored by a consultant veterinarian from the New England Regional Primate Research Center.

Sequence of Conditions and Integrated Plasma Sample Collection: Acute effects of alcohol and sucrose control solutions on LHRH stimulated LH and FSH were evaluated in the same subjects under identical conditions. Basal levels of LH and FSH were measured for 80 min before alcohol or sucrose was administered. LH and FSH were measured for an additional 120 min before LHRH was administered, and for 180 min after LHRH administration. Samples were collected at 20 min intervals except during the first hour after LHRH administration when samples were collected at 15 min intervals to more accurately follow the timecourse of LHRH stimulated LH and FSH activity.

Details of the integrated plasma sample collection procedures have been reported previously (Bree et al. 1982). A complete description of the radioimmunoassay procedures for LH and FSH analysis and the enzymatic method used for plasma alcohol analyses appears in Mello et al. 1986. The LH assay sensitivity was 7.2 ng/ml. Intra- and inter-assay coefficients of variation were 6.4 and 7.1 percent, respectively. The FSH assay sensitivity was a2.0 ng/ml. Intra- and inter-assay coefficients of variation were

9.4 and 14.5 percent, respectively. The plasma alcohol assay sensitivity was 20 mg/dl. Intra- and inter-assay coefficients of variation were 2.7 and 3.8, respectively.

Alcohol Administration: Alcohol (2.5 and 3.5 g/kg), prepared as a 25 percent solution, was administered through a pediatric grade nasogastric tube. Alcohol effects were compared with a sucrose control solution, isocalorically equivalent to 2.5 g/kg alcohol. Monkeys were fasted for 18 to 20 h to ensure uniform absorption of alcohol.

LHRH Administration: Synthetic LHRH (Gonadorelin hydrochloride; Factrel®) (100 mcg i.v.) was injected into the saphenous vein 120 min after alcohol administration. LHRH was administered during the ascending phase of the blood alcohol curve to ensure that the maximal effects of LHRH stimulation occurred when blood alcohol levels exceeded 150 mg/dl.

Statistical Analysis: The effects of sucrose, alcohol and LHRH on pituitary hormones were evaluated with Analysis of Variance (ANOVA) and Least Square Difference (LSD) followup tests. If ANOVA revealed a significant main effect, the significance of group mean values at each sample period were compared with baseline means using Dunnett's test for comparison of multiple experimental groups with a single control group.

RESULTS AND DISCUSSION

LH levels were equivalent before and after administration of a sucrose control solution. Synthetic LHRH administration significantly increased LH levels ($P < .001$) as evaluated by ANOVA. LH increased significantly within 30 min after LHRH administration and remained above baseline levels for 105 min ($P< .01$). FSH levels were also equivalent before and after sucrose control administration. Synthetic LHRH stimulated a significant increase in FSH within 60 min ($P < .01$).

Acute Alcohol Administration Did Not Change Basal LH and FSH: Basal LH levels were lower after alcohol but these changes were not statistically significant. The pre-alcohol baseline LH values averaged 168 (±6) ng/ml and 162 (±8) ng/ml, respectively. After administration of 2.5 g/kg alcohol, LH averaged 142 (±6) ng/ml and after administration of 3.5 g/kg of alcohol, LH averaged 150 (+6) ng/ml.

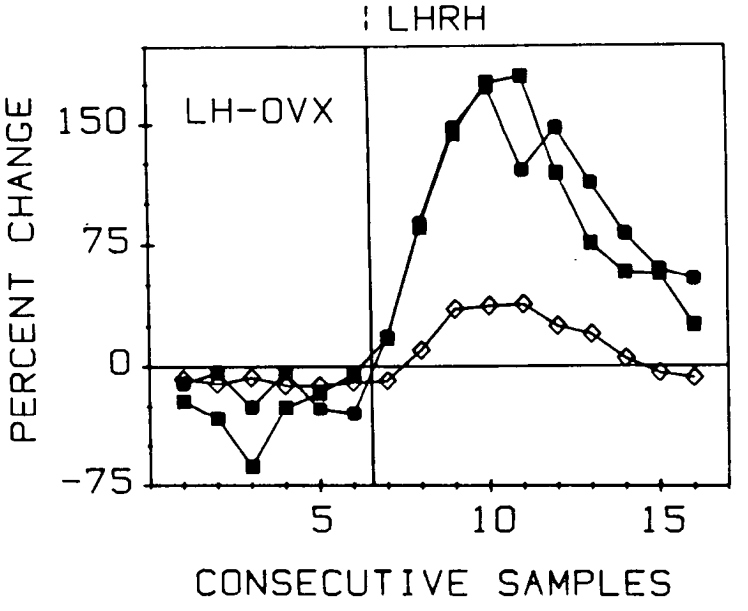
These data are consistent with previous studies of acute alcohol effects on LH in post-menopausal women (Mendelson et al. 1985) and in normally cycling women (Mendelson et al. 1981; McNamee et al. 1979; Välimäki et al. 1983) and female Macaque monkeys (Mello et al. 1984). However these findings are at variance with data obtained in ovariectomized female rats where alcohol (3.0 g/kg I.G.) suppressed LH but not FSH under basal (non-stimulated) conditions (Dees et al. 1985).

Basal FSH levels were unchanged by alcohol administration. Baseline FSH values averaged 48 (24) and 66 (±2.2) ng/ml, respectively. After administration of 2.5 and 3.5 g/kg alcohol, FSH averaged 49 (±3) ng/ml and 61 (±1.5) ng/ml, respectively.

These data are consistent with reports of unchanged FSH levels after acute alcohol administration in human males (Välimäki et al. 1984). But in human females, acute alcohol administration has been associated with a significant decrease in FSH over time (Välimäki et al. 1983).

Acute Alcohol Administration Enhanced LHRH Stimulated LH: LHRH stimulated a significant increase in LH ($P < .001$) after administration of 2.5 and 3.5 g/kg of alcohol. LH increased significantly within 30 min after LHRH administration ($P < .01$) when blood alcohol levels averaged 242 (±26) and 296 (±20) mg/dl. The magnitude of the LHRH stimulated increase in LH after alcohol was significantly greater than after sucrose administration ($P < .01$, .001). Moreover, there was an alcohol dose-dependent enhancement of LHRH stimulated LH. After 3.5 g/kg alcohol, LHRH stimulated LH levels were significantly higher than after 2.5 g/kg alcohol ($P < .001$) or sucrose control administration ($P < -001$). Baseline LH values were equivalent under all three conditions.

The alcohol related enhancement of LHRH stimulated LH in comparison to control conditions is clearly shown in data from a representative individual monkey (Fig 1). It is apparent that the rate of onset, magnitude and duration of the LH response to LHRH was greater after alcohol (closed squares = 2.5 g/kg; closed circles = 3.5 g/kg) than after sucrose control (open triangles) administration.



It is difficult to account for the significant alcohol dose-related increase in LHRH stimulated LH observed in these ovariectomized monkeys. Increased pituitary sensitivity to LHRH stimulation following alcohol administration could reflect alcohol's effects on endogenous LHRH or on other hormones known to modulate pituitary sensitivity to LHRH, such as estradiol. Ovariectomy reduces circulating estradiol by approximately 60 percent, but estrogens are produced in the adrenal and through peripheral conversion of androgens to estrogens (Ross 1985). Under LHRH stimulation conditions, increased estradiol could enhance the LH response, just as the mid-cycle LH surge in normally cycling rhesus females is dependent upon the peri-ovulatory increase in estradiol (Karsch et al. 1973). Two lines of evidence are consistent with the hypothesis that alcohol-induced changes estradiol may increase pituitary sensitivity to LHRH stimulation.

First, we found that alcohol significantly increased plasma estradiol levels in comparison to control ($P < .004$) following gonadotropin stimulation by naloxone in normally cycling women (Mendelson et al. 1986). We speculated that alcohol might increase estradiol production and/or decrease estradiol metabolism. Since intrahepatic ethanol metabolism decreases NAD availabililty for other coupled oxidative reactions (Cronholm and Sjovall 1968; Cronholm et al. 1969; Murono and Fisher-Simpson 1984, 1985), this in turn might reduce the rate of oxidation of estradiol to estrone and result in elevated estradiol levels (Mendelson et al. 1986). Alcohol also increases estradiol levels in ovariectomized rats (Gavaler et al., 1986).

Second, the sustained significant elevation in LHRH stimulated LH after alcohol (165 min) in comparison to control (105 min) is consistent with earlier studies of estradiol pretreatment in ovariectomized monkeys (Krey at al. 1973). Although chronic (13 day) estradiol treatment decreased the amplitude of the LH response to LHRH stimulation, LH remained elevated for over 3 hours whereas in untreated ovariectomized and intact rhesus females, LH levels decreased within 30 min (Krey et al. 1973). There is also evidence that estradiol pretreatment increases pituitary sensitivity to LHRH stimulation in normal and hypo-gonadal women (Jaffe and Keye 1974; Lasley et al. 1975). Estrogen pretreatment also increased pituitary responsivity to LHRH stimulation in the intact diestrous rat (Arimura and Schally 1971). Consequently, it is possible that if alcohol did increase estradiol, this could have sensitized the pituitary to produce an augmented LH response to LHRH stimulation. Unfortunately, we were unable to measure estradiol in ovariectomized females in these studies. Therefore, we cannot provide direct evidence to confirm or refute the hypothesis that alterations in steroid biotransformation, associated with intrahepatic ethanol catabolism, may have increased plasma estradiol and further sensitized pituitary sensitivity to LHRH stimulation.

Acute Alcohol Effects on LHRH Stimulated FSH: After administration of 2.5 and 3.5 g/kg alcohol, FSH increased significantly ($P < .01$) within 45 min after LHRH administration. Although FSH levels were significantly higher after 3.5 than 2.5 g/kg alcohol ($P < .001$), this probably reflects significant differences in both baseline and post-alcohol FSH levels ($P < .001$).

In contrast to parallel studies in normally cycling female rhesus monkeys (Mello et al. 1986), there was no differential effect of alcohol on LHRH-stimulated FSH and LH in these ovariectomized females. These data suggest that in the absence of antecedent ovarian steroid modulation, pituitary FSH secretory cells are more resistent to the acute effects of alcohol. In normally cycling females, ovarian steroid hormones exert a negative feedback effect on both LH and FSH during the early follicular phase. Yet after LHRH stimulation, alcohol had no suppressive effect on LH in normals or in ovariectomized females, and suppressed FSH only in intact, not in ovariectomized females. Further studies will be required to clarify the contribution of ovarian steroids or peptides (inhibin) to these effects.

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FOOTNOTES

[1]A complete list of references is available upon request

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Psychophysiological Effects of Alcohol-Related Stimuli

Mary E. McCaul, Jaylan S. Turkkan, and Maxine L. Stitzer

When the physiological and subjective effects of a drug such as ethanol are repeatedly paired with stimuli in the natural environment, drug-related conditioned responses may come to be elicited by these previously neutral stimuli. Such conditioned responses are thought to play an important role in triggering craving and precipitating relapse to drug use following periods of abstinence. While much of the objective evidence of conditioning during repeated drug administrations has been obtained in animal studies, there have been several demonstrations of the operation of conditioning in humans.

Conditioned responses to naturally occurring drug-related stimuli have been demonstrated in humans when slides and videotapes depicting drug rituals have been presented to opiate addicts (Teasdale, 1973; Ternes et al, 1980) or when drug addicts have been allowed to perform drug preparation and injection rituals (O'Brien et al, 1980). In alcoholics, differences in heart rate, skin conductance and subjective response measures were found when alcoholic subjects were given controlled exposure to the taste of alcoholic versus nonalcoholic beverages in doses that were insufficient to produce direct drug effects (Baker and Cannon, 1979; Cannon and Baker, 1981; Kaplan et al, 1985).

The present study extends previous research on physiological and subjective responses to naturalistic alcohol-related stimuli, i.e. tastes, sight and smell of liquor. First, it includes two comparison stimuli; one that is similar in gustatory intensity to whiskey and one that is a neutral gustatory stimulus. Second, it characterizes the components and magnitude of alcohol-related responses in subjects with different drinking histories, that is, social drinkers and alcoholics.

METHODS

Subjects. Twelve male volunteers participated in the study: six reported histories of alcohol abuse and six were moderate drinkers. Alcoholics met the following criteria for participation: five or more years heavy alcohol use, 10 or more drinks

daily (approximately 3 - 7 days per week), one or more dependence symptoms such as tremor, morning drinking or blackout, and either prior treatment for alcohol abuse or alcohol-related social, legal or employment problems. Social drinkers did not exceed four to six drinks daily (approximately 2 - 5 days per week) and reported no dependence symptoms, withdrawal or drinking-related problems. Subjects were excluded from the study if they reported recent regular use of any substance other than alcohol, caffeine or cigarettes.

All subjects were 25 - 40 years old; mean ages of social drinkers and of alcoholics were 29.5 and 30.8 years old, respectively. Mean years of regular alcohol use was not significantly different for the two groups; social drinkers had been drinking regularly for 12.2 years and alcoholics for 14.3 years. Average daily alcohol consumption and average number of drinking days per week were significantly different for the two groups. Alcoholics drank an average of 18.8 ounces of alcohol on 6.7 days per week, whereas social drinkers consumed an average of 5.6 ounces of alcohol on 3.3 days per week.

All subjects provided written informed consent prior to participation in the study. Subjects were paid $40 for the single day session.

Procedures. Subjects participated in a single outpatient experimental session of 4 hours duration. Subjects were checked for blood alcohol level and a urine specimen was obtained for later drug screening before the start of the session. Subjects were not allowed to smoke or drink caffeinated beverages for at least one hour before the start of the session.

Throughout the experimental session, subjects remained seated in a quiet laboratory room with the research technician. After subjects were instrumented, a 25-minute physiological stabilization period began; during this period, subjects received a practice stimulus presentation trial to acquaint them with the experimental procedures. For the remainder of the session, twelve four-minute stimulus presentation trials were conducted; trials were separated by a variable inter-trial interval averaging 10 minutes.

The three types of stimulus trials were alcohol, hot-pepper juice and water. Alcohol stimuli included a 2 ml sample of diluted whiskey to sniff and taste and a "Jack Daniels" whiskey bottle for visual display. Hot-pepper juice stimuli included a 2 ml sample of diluted pepper juice and an empty "Sons of Italy" peppers jar. Water stimuli consisted of 2 ml water and a pitcher for display. Each 2 ml sniff/taste stimulus was delivered in a test tube which the subject held under his nose for two minutes, after which he was instructed to drink. Stimuli were presented in a randomized block design; each type of stimulus was presented four times during the session.

Dependent Measures. Physiological measures included: heart rate,

continuously monitored using chest EKG electrodes; and skin temperature, continuously monitored from a skin surface thermister taped to the index finger of the left hand. Data were averaged over 10 second intervals for one minute following each stimulus presentation.

Subjective effect questions were displayed on a computer screen; a joy box next to the subjects' right hand was used to move a screen cursor arrow to respond to each self-report item. When subjects had moved the arrow to the desired position, they pushed a button on the joybox to advance to the next item. Analog questions included ratings of stimulus intensity, liking, feeling different, feeling high, feeling hungover, and craving for alcohol. Physiological and subjective data were analyzed using a repeated measures analysis of variance.

RESULTS

Subjects rated the alcohol and pepper juice stimuli as similar in intensity; both were rated as significantly more intense than water. Thus, subjects' ratings confirmed the stimulus selection of pepper juice as a control for the gustatory intensity of alcohol.

Figure 1 presents the effects of water, pepper juice and alcohol on mean heart rate for social drinkers and alcoholics. There was a significant effect of stimulus type ($p<.0001$) as well as a stimulus type X group interaction ($p<.02$) on heart rate. For both groups, heart rate was higher following pepper juice and alcohol presentations than following water. This increase was greater in

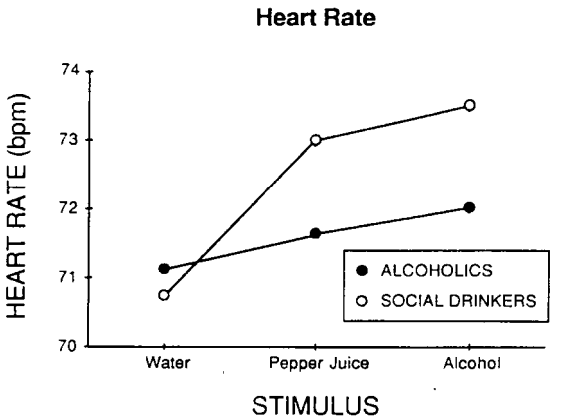

FIGURE 1. Mean heart rate during stimulus presentation trials as a function of stimulus type. Each point is an average of four stimulus presentation trials, averaged over six subjects. Trials were one-minute averages, pooled from six 10-second intervals following stimulus presentation.

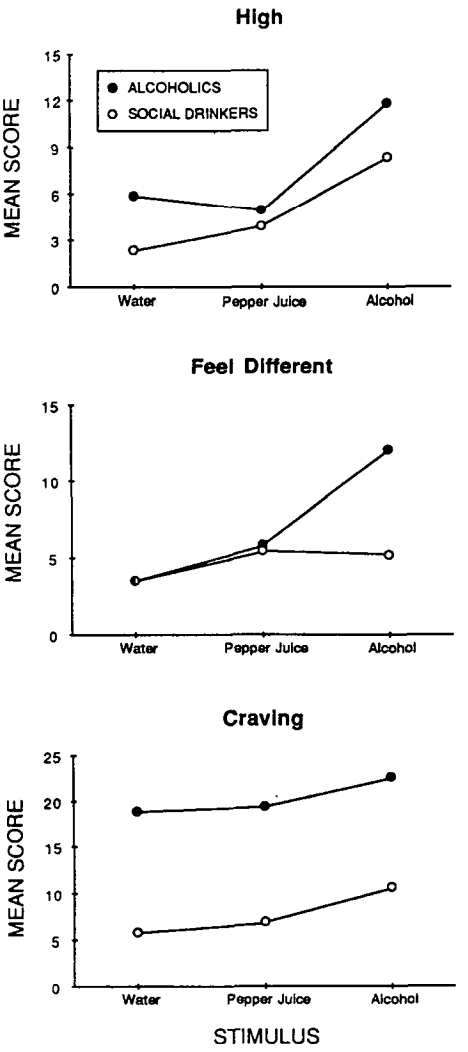

FIGURE 2. Mean scores for three subjective report items as a function of stimulus type. Scores were obtained one minute after the start of each stimulu presentation trial. Each point is an average of four stimulus presentation trials, averaged over six subjects.

social drinkers, who showed an average difference of 3.5 bpm between water and alcohol stimuli, than in alcoholics, who showed an average difference of approximately 1.0 bpm. There was no significant effect of stimulus type or of group on skin temperature.

There was a significant effect of stimulus type on three of the five subjective report items. On all three items, subject ratings were highest following alcohol stimuli and less following pepper juice and water. Figure 2 (top panel) presents the effects of stimulus type on mean analog high score for social drinkers and alcoholics. The effect of stimulus type on high ratings was highly significant ($p<.006$), with subjects reporting feeling higher following alcohol stimuli than following pepper juice and water stimuli.

As shown in Figure 2 (middle panel), there was a similar pattern in alcoholics' responses to the analog question "Do you feel any different now than before the stimulus"; that is, mean score was significantly higher following alcohol stimuli than following pepper juice and water ($p<.021$) However, social drinkers did not respond differently on this item to any of the three stimuli.

Finally, Figure 2 (bottom panel) shows that there was a significant effect of stimulus type and of group on craving, specifically "How badly do you want an alcoholic drink right now." Subjects in both groups reported significantly higher craving scores following alcohol stimuli than following pepper juice or water stimuli ($PC.021$) Alcoholics reported significantly higher craving following all three stimuli than did social drinkers ($p<.051$) There were no significant effects of stimulus type or group for analog questions on hangover and stimulus liking.

DISCUSSION

In summary, heart rate increases appeared to be determined by the intensity of the stimulus rather than by the stimulus relationship to alcohol; that is, heart rate was higher following the more intense stimuli of pepper juice and alcohol than following water. In contrast, subjective responses appeared to be determined by stimulus type rather than by intensity; high, feeling different and craving all were reported as higher following alcohol stimuli than following pepper juice and water stimuli. Generally, responses to the three stimulus types were similar in alcoholics and social drinkers; craving was the only measure on which significant group differences were evident.

Our findings for heart rate are in contrast with previous reports of differential responses of alcoholics to alcohol-related vs. alcohol-neutral stimuli (Kaplan et al, 1985). These differences may stem from the failure to control for stimulus intensity in some earlier research. However, our findings for the subjective analog measures are similar to other research that has reported magnitude of subjective responses to be a function of the stimulus relationship to alcohol (Kaplan et al, 1985; Pomerleau et al,

1983). Our findings suggest a divergence in physiological and subjective measures of conditioned responses to alcohol and demonstrate the importance of appropriate controls in understanding this research area. Further investigations on a number of variables including environmental context, length of pre-session alcohol abstinence and expectation of alcohol availability are currently in progress in this laboratory.

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Mood States Can Elicit Conditioned Withdrawal and Craving in Opiate Abuse Patients

Anna Rose Childress, A. Thomas McLellan, Michael Natale, and Charles P. O'Brien

In the course of clinical work with opioid abusers, we and others have often noted the apparent association between negative *mood states* (e.g., depression, anxiety) and subsequent *drug use* (Woody et al. 1975; Dorus and Senay 1980; Rounsaville et al. 1982). Most explanations of this association have focused on the ability of opioids or other drugs to reduce or temporarily "medicate" the patient's symptoms (Khantzian et al. 1974; Wurmser 1979). We have recently become aware of another, perhaps equally important, way in which moods and drug use may be linked. While studying the ability of drug-related stimuli to elicit conditioned *craving* and *withdrawal-like* responses in opioid abusers (Childress et al. 1984, 1985), we noticed that the effectiveness of our stimuli often seemed to vary as a function of the patient's mood: if angry or depressed, patients would sometimes report a stronger subjective response of craving or withdrawal. We began to suspect that the repeated pairing of certain mood states with drug administration (perhaps through attempts at self-medication) had caused the mood states themselves to become conditioned stimuli, capable of eliciting the conditioned phenomena associated with opioid use, particularly conditioned craving and withdrawal-like responses (O'Brien 1975).

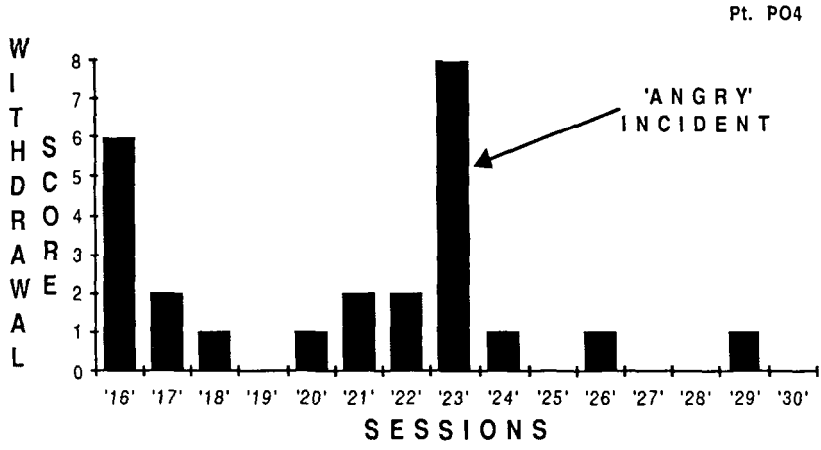
Our own work and that of others has shown that external drug-related stimuli (e.g., drug paraphernalia, drug talk, etc.) which have been repeatedly paired with drug administration can become conditioned stimuli capable of eliciting *high-like, craving or withdrawal-like* symptoms in opioid abusers (Teasdale 1973; Ternes et al. 1979; Sideroff and Jarvik 1980; Childress et al. 1984, 1985). The current study extends these earlier findings by investigating the ability of *internal* stimuli such as mood states to become conditioned stimuli capable of eliciting conditioned high-like, craving and withdrawal-like symptoms. If the link between mood states and these conditioned responses can be demonstrated, it would mean that problem moods in drug patients can lead to drug use in at least two ways: 1) by prompting self-medication of the uncomfortable mood symptoms

(negative reinforcement) and 2) by directly eliciting (through classical conditioning) responses such as conditioned craving and withdrawal which, in turn, trigger drug use. These two ways are, of course, not mutually exclusive and may even be mutually enhancing.

BACKGROUND

During the course of an earlier treatment study (Childress et al. 1984, 1985) attempting to extinguish conditioned craving and withdrawal-like responses in opioid abuse patients, we encountered a methadone outpatient, "Mr. B," who had received a series of 22 repeated exposures to drug- related stimuli, including 6 or more cook-up rituals. In the course of these treatment sessions, the drug-related stimuli had gradually become less evocative for this patient, as shown in Figure 1 (See sessions 16 through 22). Prior to session 23, Mr. B had been ejected from the hospital canteen for loitering and had engaged in an angry exchange with hospital security officers. Upon subsequently performing a cook-up ritual, the patient began to experience a cluster of *withdrawal-like symptoms,* including yawning, rhinorrhea, lacrimation, sweating, hot flashes and a bad (withdrawal-like) taste in his mouth. The patient himself was surprised at the number of symptoms he experienced commenting "Boy, Doc, this is the worst session we've had - I guess all my troubles are getting to me ..." Across the next sessions (24 - 30) the patient, no longer acutely angry, exhibited minimal response to the extinction stimuli.

Figure 1. C H A N G E I N S Y M P T O M S O V E R S E S S I O N S



This case and others like it led us hypothesize that mood states, alone or in conjunction with other drug-related stimuli, could be important triggers for drug craving, conditioned withdrawal and potentially, drug use. We chose to study three negative affective states common in our patients:

depression, anxiety and anger -- and, for comparison value, one positive affective state, euphoria.

METHOD

The subjects for this study were seven male veteran opioid dependent patients receiving inpatient detoxification. These patients were clinically screened to rule out diagnoses of schizophrenia or organic brain syndrome. The mean age of this patient sample was 38 years, with an average of 14 years of opioid use and 4 years of methadone treatment. After screening, patients were admitted to the inpatient detoxification unit for a four-week stay. Methadone dose was 25 mg on admission and dose was reduced to 20 mg the day after admission. Subsequent dose reductions were scheduled such that detoxification was completed by the end of the third week post-admission.

Prior to the initiation of treatment, patients were given two sessions which introduced psychotherapy and offered basic training in guided self-hypnosis. Guided self-hypnosis training was provided by two licensed doctoral-level psychologists according to the methods of Spiegel & Spiegel (1978). This technique enabled patients to briefly re-experience feelings of anger, euphoria, anxiety, depression or deep relaxation by recalling, in vivid sensory detail, a scene associated with those feelings. Before treatment began, patients were asked to select and to describe scenes associated with each of these mood states.

Each treatment session began with 30 minutes of cognitive-behavioral psychotherapy. Following therapy, patients entered a state of focused relaxation through guided self-hypnosis. After becoming relaxed (giving a self-rating $\geq$ 6 on **a** 1-10 scale) patients were then asked to shift their focused concentration to a predetermined mood scene. Patients remained focused on this particular mood scene until they re-experienced the feelings associated with the scene. After reaching sufficient absorption/involvement in a given mood (a self rating of $\geq$ 6 on a 1-10 scale), the patient was asked to open his eyes and proceed with a cook-up ritual. Following the cook-up, the session ended with a final period of deep relaxation. Each of the 4 mood states was induced on 4 separate, randomized occasions using a Latin square design.

Measures

Data were based on the subjective ratings of *overall high, craving,* and *wifhdrawal* using a 1-10 scale for each rating. In addition, the type and intensity of symptoms was probed through an accompanying list of 24 withdrawal- like and 24 high-like symptoms. Examples of withdrawal-like symptoms included “watery eyes,” “runny nose,” “pounding heart,” “chills,” etc. Examples of high-like symptoms included “skin itchy,” “drowsy, ” “coasting,” etc. The intensity of each symptom was rated by the

subjects on a 4 point scale, yielding total scores for *withdrawal* and *high* symptoms. Measures were collected 1) at Baseline, 2) after mood-induction (Post-Mood), and 3) after cook-up ritual (Post-Cook-Up).

RESULTS

Mean values for subjective *high, craving, withdrawal, ratings as well as high* and *withdrawal symptom* scores were derived for each of the four mood states, at each rating interval (Baseline, Post-Mood, Post-Cook-up). Two-way analyses of variance (patient x mood) were performed, using difference scores to directly assess the impact of the induced mood (Baseline to Mood), the additional impact, if any, of the cook-up ritual (Mood to Cook-Up), and finally, the net effect of mood induction and the cook-up ritual (Baseline to Cook-Up).

Effect of Mood Alone as a Conditioned Stimulus - The analyses assessing the effect of mood as a conditioned stimulus showed a direct main effect of mood state on both subjective withdrawal and the withdrawal symptom score ($p<.024$). Further paired comparisons (Bonferaroni t-test) showed the main effect of mood was due largely to the significant difference ($p<.05$) between euphoria and depression. induction of euphoria actually reduced withdrawal responses from baseline values, while depression triggered overall increases in both withdrawal measures. Though induced anger was a powerful elicitor of withdrawal symptoms in one study patient, there was no consistent effect of anger on withdrawal across the patient sample. The differential effect of the other induced mood states on withdrawal symptoms is illustrated in Fig. 2, using data from Pt. MOB, whose pattern of response parallels that of the group as a whole. In general, mood effects on *craving* were less consistent and less prevalent than the effects of mood on withdrawal, though the pattern was similar: euphoria reduced craving in a few patients, while the negative affective states, *depression, anger* and particularly, *anxiety* tended to increase subjective craving. There was essentially no effect ($p>10$) of mood on either the high rating or high symptom score.

Effect of Adding Cook-up Ritual - Assessment of Post Mood to Cook-Up difference scores yielded no further statistically significant changes in either *craving* or *withdrawal* as a function of the added cook-up ritual. Cook-up tended to further increase withdrawal symptoms when preceeded by induction of anxiety (in four of six patients), but the extent of change fell short of producing either a significant main ($p<.127$) or interaction effect ($p<.253$) of cook-up beyond mood. There were statistically significant main and interaction effects upon subjective high and high-symptoms, but these effects were due almost entirely to the contribution of one patient. In this patient, cook-up reversed both the euphoria-induced increase and the depression induced decrease in high/high symptoms.

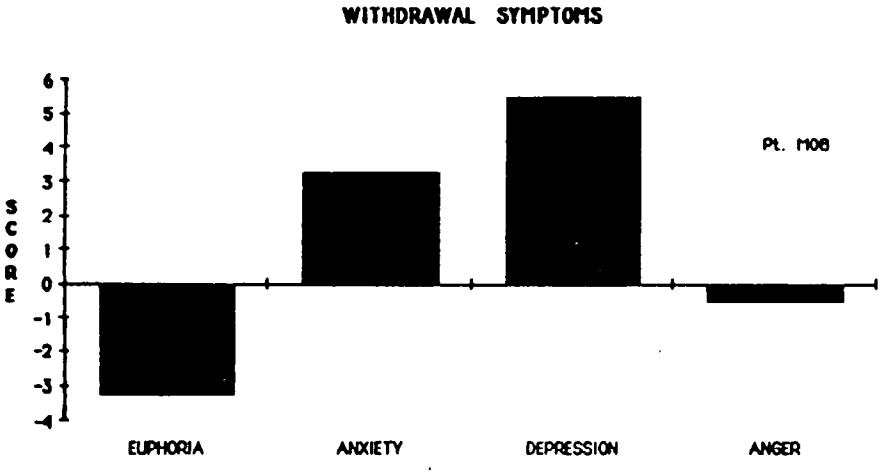


Figure 2. *Differential responsivity to drug-related stimuli (cook-up ritual) under different mood states in a methadone patient undergoing detoxification. Each bar represents the mean withdrawal symptom score for four stimulus presentations.*

Net Effect of Mood Induction Plus Cook-Up - Analyses of Baseline to Cook-Up difference scores yielded a significant main effect of patient ($p<.012$) and a significant (patient x mood) interaction effect ($p<.043$) of the net procedure (mood induction plus cook-up) on the *craving* rating and withdrawal symptoms. For most patients, the net effect of negative mood induction plus cook-up was to increase subjective ratings of craving and withdrawal and withdrawal symptoms above baseline values. When cook-up followed euphoria induction, however, both subjective withdrawal and withdrawal symptoms decreased in all five subjects who had withdrawal symptoms at baseline. There were no significant effects of the total procedure on either subjective high or high symptoms.

SUMMARY AND DISCUSSION

From these results, it is apparent that:

1) Certain negative mood states, particularly anxiety and depression, can act as reliable 'triggers' or CSs for conditioned withdrawal and craving in opiate abuse patients.

2) Approximately two-thirds of this small patient sample were "mood responders," showing relatively systematic changes in withdrawal and/or craving to one or more of the induced mood-states. This finding suggests that treatment programs featuring extinction should address both internal and external CSs in order to be maximally effective.

3) These internal mood states do not require the presence of external drug-related stimuli in order to exert their effects. In fact, with the present procedures, mood induction alone accounted for most of the changes in

withdrawal and craving. Addition of the cook-up ritual did not cause further significant change in either the magnitude or the direction of the conditioned responses.

4) Induction of the positive mood state, euphoria, actually tended to reduce the baseline withdrawal and craving.

Our interpretation of these data has been cast in terms of the ability of certain mood states to elicit conditioned opioid-related phenomena. It could, however, be argued that induction of certain moods may produce symptoms which resemble opioid withdrawal, but which in reality, reflect the basic profile of arousal, associated with the mood itself. Induction of anxiety for example, might cause "palpitations" or "restless, nervous feelings" which also occur in opioid withdrawal. This explanation, however does not explain the present pattern of results. For example, there is little overlap between the symptoms of opioid withdrawal and those of depression, yet depression was a potent elicitor of opioid withdrawal symptoms. Anger, on the other hand, can cause considerable sympathetic arousal, but it did not systematically produce increased reports of withdrawal. Euphoria, which can be associated with increased arousal, palpitations, etc. actually caused a reduction in reported withdrawal. Finally, symptom items associated more specifically with opioid withdrawal, such as stomach cramps, chills, nausea, watery eyes etc., were endorsed as often as the relatively non-specific items, e.g., "palpitations," "nervousness," etc. Given these patterns, we feel that most of the withdrawal symptoms experienced by these patients are a function of the conditioned association between certain moods and subsequent drug use, not just a mis-labeling of arousal associated with the mood itself.

The readiness with which most of these detoxifying patients experienced and labeled withdrawal symptoms is greater than we have found in either methadone-maintained or abstinent populations (Childress et al. 1984, 1986). This difference may be due to the ongoing detoxification procedure in the present sample, which may have increased the probability of experiencing conditioned withdrawal, and almost certainly increased the likelihood of noticing and labeling symptoms: most detoxifying patients are quite attuned to signs of withdrawal. These data would suggest that detoxifying patients may be particularly vulnerable to the "trigger" effect of negative mood states on withdrawal symptoms.

We are impressed that the impact of mood states on withdrawal and craving is robust enough, and systematic enough, to be evidenced even in this small subject sample under these artificial conditions. Given the variability in hypnotizability and in the mood induction procedure itself, less systematic results or even the lack of effects across subjects would not have been too surprising. Instead, these consistent results suggest that mood states are powerful "real life" modulators of craving and withdrawal for many patients and, as such, demand treatment attention.

From a therapeutic standpoint, two treatment approaches may be useful. First, professional psychotherapy focused on increased understanding and control over the frequency of negative moods has been shown to be helpful. and mood-related extinction, involving repeated exposure to the "trigger" mood(s) (with or without relevant drug-stimuli), not followed by drug. Second, since induction of euphoria through guided self-hypnosis helped alleviate baseline withdrawal symptoms in all patients who had such symptoms, self-guided positive mood induction could also be a useful tool for the detoxifying patient, as an adjunct to, or even a partial replacement for, the medications used to ease opioid withdrawal.

Fortunately, these methods are not mutually exclusive and might very well be mutually enhancing. We are currently conducting a treatment-outcome protocol in abstinent opioid abusers which employs both these features (Childress et al. 1986).

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Abuse Liability Assessment of Buprenorphine-Naloxone Combinations

George E. Bigelow, Kenzie L. Preston, and Ira A. Liebson

Buprenorphine is an opioid analgesic with partial agonist activity. Previous studies with buprenorphine in a post-addict population showed that while buprenorphine produced morphine-like subjective effects, it had lower intrinsic activity than morphine and produced a lesser degree of physical dependence than morphine (Jasinski et al., 1978). These investigators suggested that buprenorphine has the potential to be abused but that this potential is less than that of morphine.

One strategy used to decrease the abuse of opioid analgesics has been to combine the opioid analgesic with an opioid antagonist. Examples of combinations which have been investigated include: morphine and nalorphine (Fraser et al., 1956), methadone and naloxone (Nutt and Jasinski, 1974) and pentazocine and naloxone (Legros et al., 1984). The later combination, pentazocine and naloxone, is currently being marketed in the United States.

The purpose of this study was to evaluate the effects of combining naloxone with buprenorphine. This was done by assessing the combination product as well as buprenorphine alone in relation to two reference compounds, hydromorphone and naloxone, in opioid-dependent human volunteers. Measurements included both morphine-like effects and opioid abstinence signs and symptoms. Compounds which show a morphine-like profile of effects are viewed as possessing morphine-like abuse potential; this is a methodology which has received extensive use and validation (Jasinski, 1977). Measures of opioid abstinence were derived from those developed by Kolb and Himmelsbach (1938) and from other documented opioid abstinence signs and symptoms.

METHODS

The subjects were 6 adult male volunteers who were maintained on methadone HCl 30 mg/day. Methadone doses were administered once every 24 hours, approximately two hours after the

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experimental session. On the basis of physical examination, history, and laboratory chemistries, subjects were found to be without significant medical or psychiatric disturbance other than their drug abuse. Subjects gave their written informed consent prior to beginning the study and were paid for their participation. Subjects participated while residing in an 8-bed behavioral pharmacology research ward.

Seven drug conditions were tested in randomized order for each subject: placebo, hydromorphone HCl 6 mg; naloxone HCl 0.2 mg; buprenorphine 0.2 and 0.3 mg; buprenorphine 0.2 mg plus naloxone HCl 0.2 mg; and buprenorphine 0.3 mg plus naloxone HCl 0.2 mg. Commercial preparations of hydromorphone HCl and naloxone HCl were used. Buprenorphine HCl was supplied by Reckitt and Colman Pharmaceutical Div in ampules in the concentration of 0.2 and 0.3 mg (base) in 1 ml normal saline. The appropriate volume of drug solution from ampules was diluted to 1.5 ml with 0.9% normal saline. Normal saline (1.5 ml) served as placebo. All doses were given subcutaneously as two 0.75 ml injections, one in each upper arm, under double-blind conditions. Hydromorphone and naloxone doses were calculated on the basis of the salt. Buprenorphine doses were calculated on the basis of the drug base.

Each subject participated in 7 experimental sessions. Sessions were run at the same time of day for each subject and were separated by at least 48 hours. All subjective effect and psychomotor performance measures were presented on a computer screen. Subjects used a key pad and joy stick to respond to subjective effect questions and to perform the psychomotor tasks. The subject was seated in an experimental testing room for each 2.5 hour session. After a 15 min stabilization period, 10 min of baseline physiological recording was taken; a pupil photo was taken, and the subject answered subjective effect questionnaires and performed two psychomotor tasks. Approximately 30 min after the start of the physiological recording; subjects received subcutaneous injections of saline or active drug. The session continued in the testing room for 2 hours after drug administration. Physiological measures, except for pupil diameter, were monitored continuously throughout the session. Self-report, behavioral, and pupil diameter were collected at 15, 30, 45, 60, 90, and 120 min after drug administration.

Four physiological measures were monitored continuously: heart rate, blood pressure, skin temperature, and respiration. Data for each measure were collected and stored in one-minute intervals using a microcomputer. Pupil diameter was determined from photographs taken in ambient room lighting using a Polaroid camera with a 3X magnification. Subjective effect measures included visual analog scales, a pharmacological class questionnaire, and an adjective rating questionnaire. On the visual analog scales, the subject rated his current degree of "high" and "sick" and the degree of "any drug effect," "good effects," "bad effects," and "liking" of

the drug effects by placing a mark along a 10 cm line marked at either end with "none" and "extremely." On the pharmacological class questionnaire, the subject categorized the drug effect as being most similar to one of ten classes of psychoactive drugs; the questionnaire provided descriptive titles for and examples of each of the following classes: placebo, opiate, opiate antagonists, phenothiazines, barbiturates and sleeping medications, antidepressants, hallucinogens, benzodiazepines, stimulants, and other. The adjective rating questionnaire consisted of 40 items which the subject rated on a 5-point scale from 0 (no effect) to 4 (maximum effect). The items in the rating scale were divided into 3 subscales: the Fraser scale [adjectives previously shown by Fraser et al. (1961) to be sensitive to opioid effects], an opioid agonist-like scale [adjectives in the Fraser scale plus additional adjectives associated with morphine-like effects], and a withdrawal scale [adjectives derived from the Himmelsbach abstinence rating scale (Jasinski, 1977) plus other adjectives decribing withdrawal symptoms]. The subject was instructed to give answers describing how he felt at the time he filled out the questionnaires. Observer ratings were completed by a research technician who rated the subject's drug effect at the same times and using the same adjective list as the subject himself.

Data were converted to change from pre-drug baseline and were analyzed by analysis of covariance with repeated measures over time and as areas under the time course curves. Duncan's Multiple Range test was used to assess differences of the active drug conditions from placebo.

RESULTS

On the pharmacological class identification questionnaire subjects identified placebo as a blank in 100% of opportunities. Hydromorphone was identified as an opioid in 50% of opportunities. When given alone both doses of buprenorphine were typically identified as being placebo (78 and 86% of opportunities) with only occasional identifications as an opioid or other. Naloxone alone was identified primarily (67% of opportunities) as an opioid antagonist, as were both buprenorphine-naloxone combinations (81 and 67% of opportunities).

On the visual analog scales hydromorphone produced significant increases on the "liking," "good effects," "high" and "any drug effects" scales of the visual analog scales. Buprenorphine given alone had no significant effects on any of the visual analog scales. Naloxone and the buprenorphine-naloxone combinations increased the "any drug effects," "bad effects" and "sick" scales. On the subject-rated adjective scales hydromorphone produced increases in both the subject-rated Fraser and agonist adjective rating scales, but had no significant effects on the withdrawal scale. Naloxone alone

and in combination with buprenorphine produced little or no changes in the agonist scale. Naloxone alone produced significant increases in the withdrawal scale score. The withdrawal scale scores produced by the naloxone-buprenorphine combinations, though elevated, were not significantly different from placebo; these scores, however, were also not significantly different from naloxone. Buprenorphine alone produced no significant changes in scores on any of the subject-rated adjective rating scales.

On the observer-rated adjective rating scales, neither hydromorphone nor buprenorphine produced significant changes on any scale. Naloxone given alone and given in combination with buprenorphine significantly increased withdrawal scale scores.

On the physiological measures hydromorphone produced significant decreases in pupillary diameter and respiratory rate. Naloxone and naloxone plus buprenorphine increased pupil diameter and decreased skin temperature. Hydromorphone, naloxone and the buprenorphine-naloxone combinations all produced significant increases in blood pressure. None of the physiological measures were changed following either dose of buprenorphine tested as compared to placebo.

DISCUSSION

The results of this study, which compared hydromorphone, naloxone, buprenorphine, and two buprenorphine-naloxone combinations, show that hydromorphone produced increases in a number of opioid-sensitive subjective effect measures, decreases in pupil diameter and respiratory rate, and increases in blood pressure. At the doses tested, buprenorphine given alone had no significant effects on any subjective, behavioral, or physiological measure. Naloxone produced increases in subjective effect and behavioral scales indicating opioid abstinence effects and produced decreased skin temperature and increased blood pressure and pupil diameter. Both buprenorphine-naloxone combinations produced changes in the subjective, behavioral, and physiological effects which were in the same direction as those produced by naloxone, indicating that the combinations had qualitatively naloxone-like properties.

The purpose of combining buprenorphine and naloxone is to discourage diversion of buprenorphine for substitution for an opioid agonist in opioid-dependent abusers. The results of the present study showed that, in subjects maintained on a low dose of methadone (30 mg), clinically useful doses of buprenorphine plus naloxone 0.2 mg produced significant abstinence symptoms which were similar to those produced by naloxone 0.2 mg alone and which were perceived by subjects as being quite unpleasant. In our opinion, these combinations of buprenorphine and naloxone have a low potential for abuse in an opioid-dependent population.

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Effects of Passive Exposure to Marijuana Smoke

Edward J. Cone, John D. Roache, and Rolley E. Johnson

INTRODUCTION

At the present time, millions of screening tests for cannabinoids in urine are being administered to members of a large cross-section of our society to determine if recent active use of marijuana has occurred. The confirmed finding of a positive result can lead to severe legal and professional consequences for the individual tested. Recently, it has been reported that the passive inhalation of marijuana smoke can result in the production of urines which test weakly positive for cannabinoids and also result in low blood levels of delta-9-tetrahydrocannabinol (THC) immediately after exposure (Law et al., 1984; Mason et al., 1983; Morland et al., 1985; Perez-Reyes et al., 1983). It is not clear from these studies if longer periods of passive exposure or more intense exposures to marijuana smoke increase the likelihood of individuals to test positive for cannabinoids.

We assessed the effects of passive inhalation of sidestream marijuana smoke in a group of 5 experienced, but drug-free male marijuana users. A similar but more limited study was performed with two male staff members without a history of marijuana use. These studies addressed the following questions regarding the passive inhalation of marijuana smoke:

1. Under standardized conditions, does increased smoke exposure lead to increased frequency of "positive" specimens?
2. Do multiple passive exposures lead to accumulation?
3. Are acute behavioral, physiological and hormonal effects produced?
4. Do marijuana-naive subjects produce more or fewer "positive" specimens?
5. How does this study relate to the conditions of the "real world" in which marijuana is smoked?

METHODS

Subjects: Five subjects (A-E) were healthy, drug-free males with a history of marijuana use. Criteria to enter the study included a documented period of 14 consecutive days of cannabinoid "negative" urines. During the study, subjects were housed on a closed ward under close surveillance. Two additional male subjects (F,G) from the staff with no history of marijuana use participated in one phase of the exposure studies. The studies were conducted under the guidelines for the protection of human subjects (45CFR46).

Passive Exposure Conditions. Subjects A-E were passively exposed under double-blind conditions to sidestream smoke from 16 standard marijuana cigarettes (2.8% THC) for one hour each day (0830-0930 hr) for 6 consecutive days. Smoke exposure was carried out at the same time each day. Preceding and following the days of marijuana smoke exposure, the subjects were exposed for two days to the smoke of 16 placebo cigarettes (0% THC). During exposure, the subjects wore goggles and sat quietly in a small unventilated room (2.1 m x 2.5 m x 2.4 m). The approximate volume after adjustment for contents was 12,225 liters. One-half of the cigarettes were burned at the start of the session (0-12 min) and the remainder were burned 30-42 min into the exposure session. The cigarettes were burned in a smoking apparatus designed to release only sidestream smoke. Room air samples were analyzed at timed intervals for THC and carbon monoxide (CO). A second exposure study was performed with the subjects A-E for 6 days under similar conditions with 4 marijuana cigarettes (2.8% THC) but preceded and followed by only one day of placebo smoke exposure. A third exposure study was performed with 2 subjects F and G for six days to the smoke of 16 marijuana cigarettes (2.8% THC) but without blind conditions and without exposure to placebo smoke.

Collection and Assay of Biological Samples. All urine specimens were collected from the SubJects during their participation in the dies. All urines were screened with the EMIT[A] d.a.u.™ Cannabinoid 20 Assay (Syva Co) with a 20 ng/ml low calibrator (EMIT 20). Specimens with rates greater than that of the medium calibrator (75 ng/ml) were assayed by EMITR d.a.u.™ Cannabinoid Assay which employs a 100 ng/ml low calibrator (EMIT 100). Venous bloods were collected from subjects A-E 30 minutes prior and 20-30 minutes following each exposure session with 16 cigarettes. Only subject D was eligible to donate blood samples during the 4 cigarette exposure study. Analysis for THC was performed by radioimmunoassay. Analyses for hormones were performed with commercial kits.

Behavioral and Physiological Measures. Behavioral and physiological effects were assessed at 1 hr (0730) and 0.5 hr (0800) before smoke exposure and following exposure at 0930, 1030, 1130 and 1230. Subjective effects were measured with subscales of the Addiction Research Center Inventory (MAR 15,

MBG, LSD, PCAG), Single Dose Questionnaire (Feel Drug, Drug Identification, Symptoms, Liking) and a visual analog scale (VAS). The latter scale consisted of a 200 mn line on which subjects rated the "high" or positive effects and "bad" or negative effects of the test conditions. Psychomotor performance was measured by the circular lights task (CLT) and a computerized version of the digit-symbol-substitution task (DSST). Behavioral and physiological responses were analyzed as differences between the responses after smoke exposure and the mean of the two control responses prior to smoke exposure. Area under the curve (AUC) for the response over time was calculated by the trapezoidal rule. If the analysis of variance of AUC data showed a significant ($p < 0.05$) effect of study days, a Tukey test was applied to determine which marijuana smoke exposure days were different than the placebo day preceding marijuana smoke exposure.

RESULTS

Chemical Evidence for Passive Absorption of THC. Specimen screening by EMIT® assay revealed a large number of "positive" urines with rates exceeding the 20 ng/ml calibrator (EMIT 20) during the 24 hour period following passive exposure to the smoke of 16 marijuana cigarettes (Table 1). A much smaller number of the EMIT 20 positive specimens for subjects A-E also exceeded the 100 ng/ml calibrator (EMIT 100). EMIT 20 positive urines also were obtained following exposure to the smoke of 4 marijuana cigarettes with 4 of 5 participating subjects producing at least one positive urine. The assay rates for these specimens were consistently below that of the 75 ng/ml calibrator of the EMIT 20 assay.

The time course for the appearance and disappearance of cannabinoids in urine was similar for most subjects. Generally the urines obtained during the subsequent 4 hours after exposure were the most likely specimens to test positive and exhibited the highest reaction rate. Also, the specific gravity of the specimen appeared to be directly related to the number of "positive" specimens produced at the higher exposure level. EMIT 20 positive urines were not detected later than 24 hours following the 4 marijuana cigarette exposure for subjects A-E and with one exception (subject F), no later than 48 hours following the 16 marijuana cigarette exposure. Subject F produced occassional EMIT 20 positive specimens for 5 days following passive exposure.

Daily mean plasma levels of THC for subjects A-E following exposure to the smoke of 16 marijuana cigarettes ranged from 2.4 to 7.4 ng/ml. The highest mean concentration of MC was found to occur on the last day (sixth day) of exposure to active marijuana smoke. The individual peak concentration also occurred on this day (Subject E, 18.8 ng/ml). For the one subject who donated blood during the 4 marijuana cigarette exposure sessions, THC ranged from 0.8 - 2.5 ng/ml. Plasma samples obtained from subjects A-E before and after passive

Table 1 Room Air THC Levels, Breath Carbon Monoxide Levels and EMITR Assay of Specimens After Passive Marijuana Smoke Exposure.

		Four Cigarette Exposure			Sixteen Cigarette Exposure			
Day	Conditions	EMIT 20 Positive Urines (Negative Urines)	Room[1] Air THC, mcg/L	Mean Breath[2] CO, ppm	EMIT 20 Positive Urines (Negative Urines)	EMIT 100 Positive Urines	Room[1] Air THC, mcg/L	Mean Breath[2] CO, ppm
		SUBJECTS A-E (EXPERIENCED MARIJUANA USERS)						
2	Placebo	0(42)	0	15.2	0(38)	0	0	32.6
3	MJ	4(36)	2.0	15.2	12(28)	0	4.8	27.4
4	MJ	3(37)	1.8	14.8	24(15)	2	6.4	27.8
5	MJ	6(26)	1.5	17.8	32(5)	1	5.2	29.8
6	MJ	4(36)	1.9	18.6	32(7)	1	7.1	27.2
7	MJ	2(31)	1.0	15.8	31(8)	1	7.0	28.0
8	MJ	4(32)	1.6	16.0	29(10)	0	6.3	31.4
9	Placebo	0(36)	0	15.8	12(25)	0	0	36.2
10	Placebo[3]	0(39)	--	--	4(32)	0	0	39.6
		SUBJECTS F,G (MARIJUANA-NAIVE SUBJECTS)						
1	--	--	--	--	0(16)	0	--	--
2	MJ	--	--	--	9(13)	4	9.1	--
3	MJ	--	--	--	9(7)	4	8.2	--
4	MJ	--	--	--	13(10)	8	15.6	--
5	MJ	--	--	--	7(12)	4	10.5	--
6	MJ	--	--	--	11(8)	6	11.2	--
7	MJ	--	--	--	6(9)	4	10.7	--
8	--	--	--	--	7(9)	4	--	--
9	--	--	--	--	4(14)	0	--	--
10	--	--	--	--	3(11)	0	--	--
11	--	--	--	--	1(16)	0	--	--
12	--	--	--	--	1(4)[4]	0	--	--

[1]Room Air THC concentration at end of 1-hour exposure session. [2]Mean breath CO levels obtained after 45 minutes of smoke exposure. [3]Second day of Placebo exposure only following 16 cigarette treatment. [4]Urines collected only from subject F.

exposure to the smoke of 16 marijuana cigarettes over the 10 day exposure study showed no significant changes in levels of cortisol, prolactin, growth hormone, testosterone, luteinizing hormone or follicle stimulating hormone. Breath and room air CO levels and room air levels of THC were dose-related to the number of marijuana cigarettes burned during exposure. Room air THC levels appeared to be inversely related to the number of people present in the room during exposure.

Behavioral and Physiological Effects. Elevated responses were obtained on the MAR 15 LSD PCAG VAS, Feel Drug and Liking scales after exposure to marijuana smoke (Table 2). These responses reached significance on all but the PCAG scale after 16 marijuana cigarette exposure. Responses were slightly depressed with CLT and DSST after 16 marijuana cigarette exposure but were not significant. Responses on most scales were time-related with peak effects occurring immediately after passive exposure to marijuana smoke.

Physiological measures were highly variable under both the 4 and 16 marijuana smoke exposure conditions. Supine pulse was elevated after both test conditions but reached significance only on one day of the 4 marijuana smoke exposure sessions and did not reach significance during the 16 marijuana smoke exposure sessions. Standing pulse was significantly elevated on the second day of exposure to the smoke of 16 marijuana cigarettes but was significantly depressed on the sixth day of exposure. Other measures generally showed no effects from passive inhalation at either exposure level.

Table 2. Behavioral and Physiological Effects of Passive Exposure to Marijuana Smoke

Measure	EFFECT, SIGNIFICANCE (DAY WITH SIG. EFFECT) Four Cigarettes	Sixteen Cigarettes
Behavioral		
MAR15	↑,NS	↑,0.05 (2-6)
LSD	↑,NS	↑,0.05 (2-5)
PCAG	↑,NS	↑,NS
MBG	0	0
VAS	↑,NS	↑,0.05 (2-6)
FEEL DRUG	↑,NS	↑,0.05 (1-6)
LIKING	↑,NS	↑,0.05 (1-6)
CLT	0	↓,NS
DSST	0	↓,NS
Physiological		
SUPINE PULSE	↑,0.05(5)	↑,NS
STANDING PULSE	0	↑,0.05(2); ↓,0.05(6)
OTHER	0	0

DISCUSSION

Does Greater Passive Marijuana Smoke Exposure Lead To An Increased Frequency Of "Positive" Urine Specimens?

Unquestionably our results indicate that as the intensity of marijuana smoke increases, passive absorption of cannabinoids increases with a subsequent increase in the excretion of urinary cannabinoids. An increase also occurred in the degree of positivity of specimens and in the length of time positive specimens could be detected after passive smoke exposure. It also appeared that passive exposure to the smoke of 4 marijuana cigarettes for one hour approximated the "threshold" level of exposure necessary for the production of cannabinoid urinary metabolites detectable by the EMIT 20 assay.

Do Multiple Passive Exposures Lead To Accumulation?

Room air concentrations of THC during the first exposure session was generally less than those found on subsequent exposure days. Thus, accumulation does not account for the initial increase in number of positives specimens produced by subjects A-E during the 16 marijuana smoke exposure sessions. Over the remaining six days of exposure with 16 marijuana cigarettes, reaction rates for subjects C, D and E increased with exposure indicating some accumulation was occurring; however, this was not evident for subjects A and B. No accumulation was evident following exposure to the smoke of 4 marijuana cigarettes by any subject.

Are Acute Behavioral, Physiological And Hormonal Changes Produced by Passive Inhalation of Marijuana Smoke?

Significant subjective effects were produced by exposure to the smoke of 16 marijuana cigarettes. The magnitude of these effects approximated those observed when the same subjects (A-E) actively smoked one marijuana cigarette (2.8% THC)(Data not reported here). Exposure to the smoke of 4 marijuana cigarettes produced lesser but qualitatively similar effects. Also, a slight but non-significant performance impairment was observed with the CLT and DSST following exposure to the smoke of 16 marijuana cigarettes. These latter tasks have been shown to be sensitive measures of drug-induced. performance impairment by sedative/tranquilizer drugs. All of the behavioral effects were time related and had returned to baseline within four hours after exposure. Consistent with these observations of behavioral effects were the detection of blood levels of THC following exposure which occasionally exceeded the arbitrary limit of 10 ng/ml which has been suggested as evidence of functional impairment. Physiological and hormonal effects after exposure to marijuana smoke were highly variable and showed no clear trends at any exposure level.

Do Marijuana-Naive Subjects Produce More Or Fewer Positive Specimens Than Experienced Users?

Following exposure to the smoke of 16 marijuana cigarettes, subjects F and G produced a total of 48 and 23 EMIT 20 positive specimens. This closely overlaps the range of positive

specimens produced by subjects A-E (27-49 positive specimens). Also, subject F generally had the lowest EMIT 20 reaction rates and subject G had the highest reaction rates of the 7 subjects tested. These data appear to indicate no differences between these groups in the degree of absorption and excretion of cannabinoids following passive marijuana smoke exposure.

How Does This Study Relate To The Conditions Of The "Real World" In Which Marijuana Is Smoked? This study was conducted in a small unventilated room in which marijuana cigarettes were burned producing only sidestream smoke. Comparisons with "real world" conditions are purely speculative, however the following points can be made: 1) the factor which limits the acceptability of passive smoke exposure may be smoke irritation of mucous membranes and the eyes; 2) adequate room ventilation, regardless of room size, would greatly reduce the amount of cannabinoids passively absorbed and consequently the excretion of cannabinoid "positive" urine specimens; 3) the amount of cannabinoids in roan air available for passive absorption appears to be inversely related to the number of people present in the room; 4) subjects receiving approximately equivalent doses of passive marijuana smoke exposure can show great variations in the manner in which they excrete cannabinoid metabolites. The most critical factor in producing positive specimens following "threshold dose" exposure appears to be the specific gravity of the urine. These studies emphasize the need for caution regarding individuals who could potentially be exposed to heavy marijuana smoke environments and also caution regarding the interpretation of urine screening results.

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Butorphanol-Precipitated Withdrawal in Opioid-Dependent Human Volunteers

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Butorphanol is an opioid analgesic with agonist/antagonist activity (Pircio et al., 1976). It is a potent analgesic [5 to 8 times more potent than morphine (Houde 1979; Pircio et al., 1976)], but has been found to have mild antagonist effects in morphine-dependent subjects. In 14 hour withdrawn morphine-dependent rhesus monkeys, at low doses butorphanol failed to suppress withdrawal and, at higher doses, produced a mild exaccerbation of withdrawal signs (Woods and Gmerek 1985). Butorphanol produced only mild suppression of abstinence signs (not statistically significant) in withdrawn morphine-dependent humans, and, in doses up to 8 mg, butorphanol precipitated only mild, non-dose related abstinence signs (equivalent to nalorphine 1.5 mg) in non-withdrawn subjects (Jasinski et al., 1975).

In the present study the effects of butorphanol in opioid-dependent humans were re-evaluated using methadone-dependent human volunteers. The effects of butorphanol were evaluated in relation to two reference compounds, hydromorphone and naloxone.

METHODS

The subjects were 5 adult male opioid-dependent volunteers with histories of narcotic drug abuse. Subjects were maintained on methadone HCl 30 mg/day, given once every 24 hours approximately two hours after the experimental session. On the basis of physical examination, history, and laboratory chemistries, subjects were found to be without significant medical or psychiatric disturbance other than their drug abuse. Subjects gave their written informed consent prior to beginning the study and were paid for their participation. Subjects participated while residing on a behavioral pharmacology research ward.

Drug conditions tested were: placebo, hydromorphone HCI 4 and 8 mg, naloxone HCl 0.1 and 0.2 mg, and butorphanol tartrate 0.375, 0.75, 1.5, 3 and 6 mg. Drug conditions were presented in a mixed

order. Saline placebo, hydromorphone doses, and naloxone doses were scheduled in random order; butorphanol sessions were also scheduled at random positions in the experimental sequence, but, as a precaution, since the appropriate dose, range of butorphanol was not known, doses of butorphanol were presented in an ascending series. Neither the research technician conducting the study nor the subjects were aware of this dose schedule. Commercial preparations of hydromorphone, naloxone, and butorphanol were used. The appropriate volume of drug solution from ampules was diluted to 3 ml with 0.9% normal saline. Normal saline (3 ml) served as placebo. All doses were given intramuscularly as two 1.5 ml injections, one in each upper arm, under double-blind conditions. Doses were calculated on the basis of the salts.

Experimental sessions were run at the same time of day for each subject and were separated by at least 48 hours. Data were collected in a quiet experimental testing room separated from the ward. The subject was seated in the experimental testing room for a 2.5 hour session. After a 15 min stabilization period, 10 min of baseline physiological recording was taken; a pupil photo was taken, and the subject answered subjective effect questionnaires. Subjective effect questions were presented on a computer screen, and subjects used a key pad and joy stick to respond. Drugs were administered approximately 30 min after the start of the physiological recording. The session continued in the testing room for 2 hours after drug administration. Physiological measures, except for pupil diameter, were monitored continuously throughout the session. Subjective effect and pupil diameter measures were collected at 15. 30, 45, 60, 90, and 120 min after drug administration.

Four physiological measures were monitored: heart rate, blood pressure, skin temperature, and respiration. Data for each measure were collected and stored in one-minute intervals using a microcomputer. Pupil diameter was determined from photographs taken in ambient room lighting using a Polaroid camera with a 3X magnification. Subjects completed three questionnaires: visual analog scales, a pharmacological class questionnaire, and an adjective rating questionnaire. On the visual analog scales, the subject rated his current degree of "high" and "sick" and the degree of "any drug effect," "good effects," "bad effects," and "liking" of the drug effects by placing a mark along a 10 cm line marked at either end with "none" and "extremely." On the pharmacological class questionnaire, the subject categorized the drug effect as being most similar to one of ten classes of psychoactive drugs; the questionnaire provided descriptive titles for and examples of each of the following classes: placebo, opiate, opiate antagonists, phenothiazines, barbiturates and sleeping medications, antidepressants, hallucinogens, benzodiazepines, stimulants, and other. The adjective rating questionnaire consisted of 40 items which the subject rated on a 5-point scale from 0 (no effect) to 4 (maximum effect). The items

in the rating scale were divided into 3 subscales: the Fraser scale [adjectives previously shown by Fraser et al. (1961) to be sensitive to opioid effects], an opioid agonist-like scale [adjectives in the Fraser scale plus additional adjectives associated with morphine-like effects], and a withdrawal scale [adjectives derived from the Himmelsbach abstinence rating scale (Jasinski 1977) plus other adjectives decribing withdrawal symptoms]. The subject was instructed to give answers describing how he felt at the time he filled out the questionnaires.

Data were analyzed by analysis of covariance with repeated measures for areas under the time course curves for individual drugs.

RESULTS

There were large individual differences in sensitivity to the effects of butorphanol. Two subjects received the 6 mg dose of butorphanol. One subject showed an intense response to butorphanol 3 mg and, therefore, did not receive the 6 mg dose. Two subjects showed signs of intense abstinence signs and symptoms following the administration of butorphanol 1.5 mg; higher doses of butorphanol were omitted. The intensity of the effects of the highest dose of butorphanol administered to each subject was approximately equivalent. Therefore, doses of butorphanol were analyzed as low, medium and high doses with the high dose being the highest dose each subject received, the medium dose being one half the high dose, and the low dose being one quarter of the high dose.

In the pharmacological class identification questionnaire subjects identified placebo as a blank in 70% of opportunities. Naloxone 0.1 and 0.2 mg were identified primarily (50 and 67% of opportunities, respectively) as an opioid antagonist. Butorphanol produced dose related increases in opioid antagonist identifications (low dose- 7%, medium dose- 47% and high dose- 63% of opportunities). Hydromorphone 4 and 8 mg were identified as an opioid in 30 and 13% of opportunities (respectively). Both doses of hydromorphone were identified as placebo in approximately 50% of opportunities.

On the visual analog scales, naloxone and butorphanol increased the "any drug effects," "bad effects" and "sick" scales while hydromorphone produced no significant changes on any of the scales. Hydromorphone also produced no significant changes on any of the adjective rating scales. Naloxone and butorphanol produced little or no changes in the agonist scale. Butorphanol produced significant increases in the withdrawal scale score. The withdrawal scale scores produced by naloxone, though elevated, were not significantly different from placebo.

On the physiological measures naloxone and butorphanol significantly increased pupil diameter and decreased skin temperature. Naloxone and butorphanol produced increases in systolic and diastolic blood pressure though only the increases produced by butorphanol rearched statistical significance. Hydromorphone produced significant decreases in pupillary diameter and respiratory rate.

DISCUSSION

The results of this study, which compared butorphanol. naloxone and hydromorphone, showed that hydromorphone produced decreases in pupil diameter and respiratory rate, but had little or no effects on subject-rated measures in these methadone-dependent and tolerant subjects. Naloxone produced decreased skin temperature, increased pupil diameter and increases in subjective effect scales indicating opioid abstinence symptoms Butorphanol produced changes in subjective and physiological measures which were in the same direction as those produced by naloxone, indicating that butorphanol precipitated opioid abstinence in methadone-dependent humans.

Qualitatively the abstinence signs and symptoms produced by butorphanol and naloxone were quite similar though there were some differences between naloxone and butorphanol in the specific items of the withdrawal adjective rating scale which each affected. Anecdotally, some subjects claimed to be able to discriminate between the effects of the two antagonists. In general, subjects found the effects of butorphanol to be less tolerable than those of naloxone. Further work is needed, however, to determine whether there are real differences between the abstinence syndromes produced by these two drugs or whether differences in duration of action and/or magnitude of effects of the doses compared account for the minor differences seen here.

In previous studies conducted in morphine-dependent humans and monkeys, only mild abstinence symptoms and/or signs have been produced by butorphanol. The reason for difference between the results of these previous studies and the present study are unclear. The most apparent difference is that subjects in the present study were physically dependent on methadone while the other studies used morphine-dependent subjects. There may be other, less-apparent differences between study procedures which account for the disparity.

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Altered Dose Incentive Procedures: Effects on Polydrug Use Among Methadone Maintenance Patients

Maxine L. Stitzer, Warren K. Bickel, and George E. Bigelow

Some methadone maintenance patients continue to abuse an assortment of sedative drugs while enrolled in treatment (Stimmel et al., 1978; Stitzer et al., 1981). These patients are typically denied take-home and other program privileges while counselors attempt to dissuade them from continuing their polydrug use. If drug use does continue, a treatment termination contract may be presented which specifies a standard of improvement needed for continued treatment participation. Recent studies have shown that treatment termination contracting can be effective strategy for dealing with continuing illicit drug supplementation among methadone patients (Dolan et al., 1985; McCarthy and Borders 1985).

Methadone dose alterations might also be effectively used to promote reduced supplemental drug use among maintenance patients. Since the daily methadone dose acts as a mild reinforcer (McCaul et al., 1982), partial dose reductions could be scheduled as an aversive consequence of continued drug use. Alternatively, the opportunity to receive dose increases might be used as a positive incentive for evidence of improved behavior. The purpose of the present study was to compare the effects of a positive and a negative dose alteration incentive procedure on polydrug supplementation among a group of chronic abusers. In the positive incentive procedure, subjects were offered the chance to raise their methadone dose over its original stable level by providing drug-free urine samples. Drug-positive samples reduced the dose back to its original stable level but the dose could not decrease below this level. In the negative incentive procedure, drug-positive urine samples resulted in partial dose decrements. Drug-free samples resulted in gradual restoration of the original dose, but the dose could not increase above its original stable level. Results of the present study may shed light on the relative efficacy of positive versus negative incentive procedures and may have practical importance for developing optimal treatment procedures at methadone clinics.

METHODS

Subjects. All patients enrolled in our treatment research clinic signed informed consent at program entry which stated that incentive programs would at times constitute an important part of their treatment plan and that during these programs, clinic privileges and treatment parameters including the size of their methadone dose might be determined by their drug use and other behaviors. The seventeen patients selected for participation in this study delivered more than 50% drug-positive urine samples during a 10-week baseline evaluation period, with benzodiazepine tranquilizers being the most frequently detected drug type. Five study subjects were female and 12 were male. Average age was 32.7 years (range = 23 - 43 years). Extensive histories of methadone treatment were common, with an average of 3.2 prior treatment admissions. Subjects had been enrolled at the present clinic for an average of 12.4 months (range = 2 - 23 months) prior to the start of the study intervention with an average pre-study daily methadone dose of 51.5 mg (range = 40 - 60 mg).

General procedures. Urine samples were collected three times weekly, on Monday, Wednesday and Friday. All samples were analyzed at an outside testing laboratory using thin layer chromatography which detects a wide variety of opiate and nonopiate drugs including morphine, codeine, hydromorphone, propoxyphene, diazepam, lorazepam, oxazepam, barbiturates, phenothiazines, hydroxyzine pamoate (Vistaril), ethchlorvynol (Placidyl), and amitriptyline (Elavil). Following a 10-week baseline evaluation period, subjects were randomly assigned to one of the two dose incentive procedures described below and previously approved by the FSK Institutional Review Board for Human Research. The procedures were implemented after a two week warning period, and subjects were followed for 18 weeks.

Dose incentive procedures. Urine test results were received from the outside testing laboratory by Thursday afternoon for samples collected on Monday of that week and on Friday and Wednesday of the previous week. Doses were altered each Monday based on these three most recent test results (Wed, Fri, Mon). Thus, the delay between sample collection and the altered dose consequence was 1 - 1-1/2 weeks. Each urine sample that contained detectable quantities of any drug besides methadone (quinine positives excluded) resulted in a 5 mg dose decrease, while samples free of all supplemental drugs resulted in a 5 mg dose increase. Shown below are the dose changes that could be earned each week by all study participants.

ALTERED DOSE CALCULATION

Drug-free specimens	Drug-positive specimens	Net dose change (mg)
0	3	-15
1	2	- 5
2	1	+ 5
3	0	+15

Subjects in the positive incentive condition could raise their dose to 160% of its original value by providing drug-free urines. Dose increases were lost drug-positive specimens were obtained, but the dose could not decrease below its original stable value. Subjects in the negative incentive condition could lower their dose to 48% of its original stable value by providing drug-positive urines. The dose could be restored with drug-free urines but could not increase above its original stable level.

RESULTS

As shown in Figure 1, the average percentage of drug-free samples during baseline was similar for the two study groups and did not exceed 20% drug-free samples during any two-week pre-study baseline period.

Both interventions resulted in improved rates of drug-free sample delivery, with the average increasing to about 44% drug-free samples during the intervention [repeated measures analysis of variance, $F(1,15) = 16.2$, $p < 0.0011$. It is clear from Figure 1 that there was no difference between the two incentive procedures as far as the overall extent of improvement in drug use was concerned. Figure 1 shows that improvements occurred early in the incentive program, in some cases during the pre-intervention announcement weeks, and were generally sustained throughout the 18-week followup. One subject in the decrease incentive condition relapsed during study week 11, however, resulting in a lowered percentage of drug-free samples for that group during subsequent weeks.

Individual subject differences in study outcome were apparent. About half of the subjects in each group improved sufficiently to deliver 50% or more drug-free urine specimens on average during the intervention period. An interesting qualitative difference in outcomes for the two groups was also apparent. Four subjects exposed to the negative incentive condition dropped out of the study by the fifth intervention week (on-going treatment was arranged for these patients at this clinic or at another clinic), while no subjects assigned to the positive incentive condition dropped out of the study. Rather,

some positive incentive subjects stayed at the clinic and continued to abuse supplemental drugs.

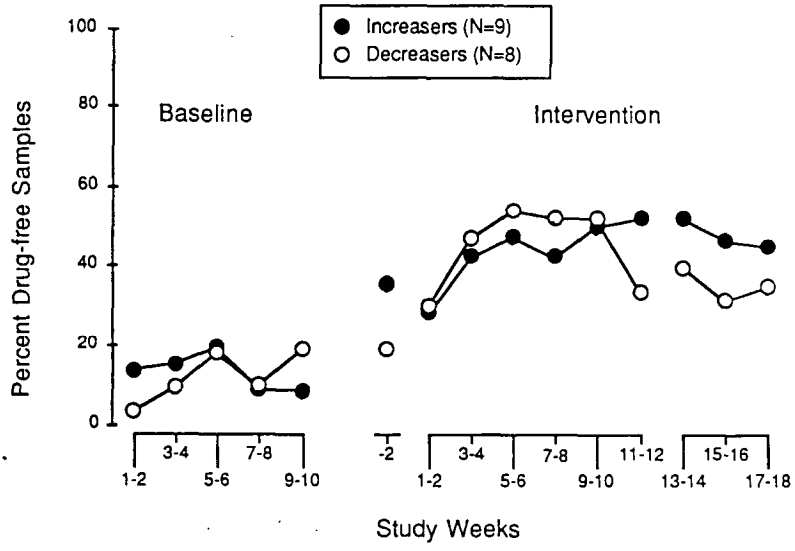

FIGURE 1. Percent drug-free urine samples delivered during successive study weeks by methadone maintenance patients assigned to a dose increase (N = 9) or a dose decrease (N- = 8) incentive procedure. Drug-free classification of urine samples is based on TLC testing that detects a wide variety of opioid and nonopioid drugs. Missing data from study dropouts have been replaced by average baseline percent of drug-free urines.

DISCUSSION

This study showed that polydrug abuse among methadone maintenance patients could be influenced by a program in which the size of the daily methadone dose was determined by recent urinalysis test results. Average percentage of drug-free urine reports increased about 30% -- from 13% during baseline to 44% during the period when the altered dose consequence was in effect. Previous research suggests that methadone dose alteration may in fact be a less potent reinforcer for methadone maintenance patients than take-home reinforcers (Stitzer, Bigelow and Liebson 1979) or menus offering a choice of reinforcers (Stitzer et al., 1982). Nevertheless, the present study suggests that interventions involving the methadone dose might be usefully applied in clinics where there is reluctance

to grant take-home privileges or other tangible rewards to patients with a history of chronic polydrug abuse.

Baseline data for these study subjects highlight the poor performance that can occur when no consequences are attached to urine results, while the improvement noted during the study intervention suggests that systematic consequences attached to urine results are desirable. That two different procedures involving methadone dose appeared equally effective, suggest that the specific content of the intervention used may be less relevant than the fact that some consequence is attached to urinalysis test results. Consistent with this notion is a recent report by McCarthy and Borders (1985) where improved treatment outcomes were obtained using a very simple set of structured consequences that required patients to be drug-free for at least 1 out of every 4 treatment months.

About half the subjects in each group showed marked improvement, delivering 50% or more drug-free urines during the intervention while the remaining subjects either left the clinic or showed only marginal improvement at best. These individual differences in outcome are consistent with other recent reports concerning effects of contingency management interventions including treatment termination contracting (Dolan et al., 1985) and take-home incentive procedures (Stitzer, Bigelow, Liebson and Hawthorne 1982). In general, it appears that substantial improvement can be expected in about half the subjects exposed to such interventions.

In the present study, a positive and a negative incentive procedure had differential effects on patient retention. Specifically, the negative incentive procedure involving dose decreases promoted study dropout, which in a nonresearch clinic might translate into treatment dropout. It is likely that treatment dropouts will have worse outcomes on measures of drug use, crime and mortality than patients who remain in methadone treatment. Results of the present study therefore support the use of positive as compared With negative incentive procedures since equivalent results may be obtained in those patients who will respond to contingency management interventions while treatment dropout among nonresponders will be avoided.

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Double-Blind Comparison of Parenteral Meptazinol and Morphine in Postoperative Pain

Abraham Sunshine, Howard Richman, Robert Cordone, Napoleon Estrada, Norma Robissa, and Nancy Olson

Maptazinol, m-(3-ethyl-l+ethyl-hexahydro-1-H-azepin-3-yl) phenol hydrochloride is a centrally active opioid analgesic with specificity for the mu-1 receptor[1]. Houde et al compared the effectiveness and safety of i.m. meptazinol (50, 100, and 200mg) to i.m. morphine (4,8 and 16mg) in cancer patients with moderate to severe postoperative pain and found meptazinol to be 1/15th to 1/20th as potent as morphine, and to provide a more rapid onset than morphine. Relative potencies for the hourly parameters were not presented, only those for time to Peak and the summary variables. Sleepiness was the most common side effect on morphine, while nausea was most common on meptazinol[2]. Forrest compared the same three dose levels of meptazinol to morphine 5 and 10mg in patients with moderate or severe general postoperative pain[3]. A valid bioassay was reported showing that 100mg of meptazinol is ap proximately eguivalent to 8mg of morphine for peak effect.

The present clinical trial was designed to assess the analgesic efficacy and safety of meptazinol (5Omg and 100mg) in comparison to morphine (5mg and 10mg) when administered parenterally for the treatment of postoperative pain. In addition to the relative potency estimates of meptazinol to morphine for the usual summary variables, hourly parameters were also assessed.

METHODS

Selection of patients: Adult male and female inpatients from the surgical services at New York Infirmary-Beekman Downtown Hospital in New York City who had moderate or severe postoperative pain and who required parenteral medications were subjects of this study. All patients were informed of the nature of this study and all gave written informad consent indicating their willingness to participate.

Study Design: This study utilized a double-blind, parallel group, single-dose design. Patients were randomly assigned to either morphine sulfate 5 or 10mg or meptazinol hydrochloride 50 or 100mg. When the patient's postoperative pain was moderate or severe, study medication was administered by

the research nurse as a single injection in the upper outer quadrant of the gluteal muscle. Pain intensity, relief, side effects, and concomitant medications were obtained by the research nurse at 1/2, 1 hour, and hourly thereafter for a total of 6 hours. If asleep at a scheduled interview, the patient was awakened. At each observation, patients were asked to rate their pain intensity on a four point scale using 0 = no pain; 1 = slight pain; 2 = moderate pain; and 3 = severe pain. At all but the baseline interview, they were also questioned to determine their percent relief from the initial pain. Relief was coded on a five point scale: 0 = no relief, 1 = 25% relief, 2 = 50% relief, 3 = 75% relief or 4 = 100% relief. Side effects reported by the patient or observed by the nurse were evaluated and reported. In order to obtain the patient's subjective estimate of the time to onset of drug effect each patient was asked to determine how long it took for the medication to provide them meaningful relief of pain. In addition, at the last interview, the patient was asked to assess his overall improvement and his overall rating of the test medication. These were respectively quantified on a seven-point scale ranging from: 1 = very much worse to 7 = very much better, and on a four-point scale: 0 = no help; 1 = fair; 2 = good; 3 = excellent.

Statistical Methods: Several measures of analgesia were derived from the interview data[4]. Pain Intensity Difference (PID) is the difference between the pain intensity score at an observation point and the baseline intensity. The sum of the Pain Intensity Difference (SPID) is the sum of the PID saxes, weighted by the time interval between observations and is an estimate of the area under the time-effect curve of the treatment. Percent SPID (%SPID) is defined for each patient to be the ratio of the patient's SPID score to the maximum possible SPID (depending on the baseline intensity) times 100. TOTAL is the sum of the relief values also weighted by the length of the time interval between observations. Time to Peak is defined for each patient as the time after drug administration that maximum PID occurs. Univariate bioassay analyses were done for each of the variables and yielded estimates of the relative potency of meptazinol to morphine with 95% confidence limits. The validity assumptions of parallelism of the log dose response lines, equality of the mean responses of the two test treatments and non-zero regression were tested in analysis of variance (ANOVA). Deviations from linearity could not be tested because no treatment was at three different dose levels. The statisical techniques for bioassay have been described by Finney[5] a comparison was made among the four treatments using a univariate one way analysis of variance to test the hypothesis of no difference between treatments for all parameters. When the ANOVA was significant at the 0.05 level, tests were performed to investigate pairwise differences between treatment using Peritz's modification of the Neuman Keuls procedure[6] and Fisher's protected least sig-

nificant difference test (LSD)[7].

RESULTS

One hundred and eighteen patients were enrolled in the study but one patient in the meptazinol 100mg group was dropped from the analysis because of adverse reactions which required treatment. The mean response for the hourly and sumnary variables and an indication of significant treatment differences are shown in Table 2. The time effect curve (Fig. 1) for pain intensity difference shows that the two doses of meptazinol are similar, with the higher dose resulting in more analgesia; the peak effect occurs at 30 minutes and thereafter there is a rapid decline in analgesic effect for both doses. The time effect curves for morphine are similar to each other but quite different from those of meptazinol in that the peak effect occurs at one hour, but the analgesic effect begins to decline thereafter at a far slower rate than is seen with meptazinol.

Dose response Lines of Meptazinol and Morphine: Statistically significant dose response lines and no significant deviations from parallelism were observed for most variables including SPID, %SPID, TOTAL, and Time to Peak as shown in Table 1. Relief variables at hour 4, 5, and 6, and PID variables at hour 2, 3, 4, 5, and 6 did not have a significant dose response.

Table 1. Relative Potency Estimate of Meptazinol to Morphine

Variable	Relative Potency Estimate of Meptazinol to Morphine	
	Estimate	95% Confidence Limits
Pain Relief Score		
0.5 Hour	0.19	0.11 - 2.03
1 Hour	0.12	0.08 - 0.21
2 Hour	0.06	0.00 - 0.11
3 Hour	0.04	0.00 - 0.07
PID Score		
0.5 Hour	0.23	0.13 - 11.85
1 Hour	0.13	0.07 - 0.36
SPID	0.04	0.00 - 0.08
%SPID	0.03	0.00 - 0.07
TOTAL	0.04	0.00 - 0.08
Subjective Onset of Relief	0.11	0.06 - 0.27
Time to Peak	0.25	0.12 - infinite
Overall Improvement	0.07	0.03 - 0.11
Global Assessment of Medication	0.08	0.05 - 0.11

Relative potency of Meptazinol to Morphine: Tests for the validity of the underlying assumptions necessary to estimate the relative potency of the two drugs were made in the analysis of variance. Estimates of the relative potency of meptazinol to morphine were 0.04, 0.03, 0.04, and 0.25 for SPID, % SPID, TOTAL and Time to Peak respectively and all had finite 95% confidence limits as shown in Table 3. For the summary variables SPID, %SPID, and TOTAL, 100mg of meptazinol is approximately equivalent to 4mg of morphine. The relative potency of meptazinol compared to morphine at 1/2 hour was .23 for PID and .19 for relief. At 1 hour, it was .13 for PID and .12 for relief. This would indicate that 100mg of meptazinol is approximately equivalent to 20mg of morphine when evaluated at 1/2 hour and approximately equal to 13mg of morphine at the first hour. By the second hour, 100mg of meptazinol is approximately equivalent to 6mg of morphine.

Table 2. Mean Values for Measures of Analgesic Efficacy

Variable	Morphine 5mg N = 29	Morphine 10mg N = 30	Meptazinol 50mg N = 30	Meptazinol 100mg N = 27
Baseline Pain Relief Score	2.5	2.5	2.5	2.5
1/2 hour	1.8	2.3	2.2	2.9 M,L
1 hour	2.0	2.9 M	2.3	3.0 M,L
2 hour	2.2	2.7 L	1.8	2.4
3 hour	1.7	2.5 M,L,H	1.1	1.5
4 hour	1.6 L,H	2.0 L,H	0.6	0.9
5 hour	1.4 L,H	1.9 L,H	0.5	0.7
6 hour	1.1 L	1.3 L,H	0.4	0.6
PID Score				
1/2 hour	1.1	1.4	1.5	1.9 M,S
1 hour	1.3	1.8 M	1.5	1.9 M
2 hour	1.3	1.7 L	1.1	1.4
3 hour	1.1 L	1.4 L,H	0.6	0.8
4 hour	0.9 L,H	1.2 L,H	0.3	0.6
5 hour	0.7 L	1.0 L,H	0.3	0.4
6 hour	0.6 L,H	0.6 L,H	0.1	0.2
SPID	5.8	7.5 L,H	3.9	5.2
%SPID	40.5 L	52.1 L,H	25.8	35.1
TOTAL	9.9 L	13.1 M,L,H	6.6	8.9
Subjective Relief Onset (min)	63.4	10.7 M	47.4	8.7 M
Peak %	64.9	82.7 M,L	64.4	80.9 L
Time to Peak (min)	126.2	83.0	71.0 M	33.3 M
Global Improvement	5.5	6.3 M,L,H	5.4	5.8
Global Assessment of Medication	1.7	2.4 M,L	1.5	2.1 L

M = Significantly better than morphine 5mg, $p < 0.05$
L = Significantly different than meptazinol 50mg, $p < 0.05$
H = Significantly better than meptazinol 100mg, $p < 0.05$
S = Significantly better than morphine 10mg, $p < 0.05$

Differences Between The Two Doses of Morphine: A dose response was seen between the low dose and high dose of morphine with the high dose having better mean efficacy scores for all efficacy variables. Statistically significant differences between the two dose levels were seen for pain relief and PID at 1 hour, relief at 3 hours, and for the summary variables, TOTAL Relief, Patient's Subjective Onset of Relief, and Peak %. The two global assessments, i.e. patient's overall improvement and patient's rating of the test medication, also indicated that morphine 10mg was significantly more efficacious than morphine 5mg.

Differences Between The Two Doses of Meptazinol: A dose response was also seen between the two dose levels of meptazinol with the higher dose having overall better mean efficacy saxes; however, significant pairwise differences between the 50 and 100mg dose were seen only for relief scores at 1/2 and 1 hour, Peak %, and the patient's global assessment of the medication.

Differences Between Maptazinol and Morphine: In general, morphine 10mg was the most effective treatment and meptazinol 5Omg was the least effective. Meptazinol 100mg was the most effective in terms of mean efficacy scores at 1/2 and 1 hour, patient's subjective onset of relief and Time to Peak. Meptazinol 1OOq was significantly superior to morphine 5mg for relief and PID at 1/2 and 1 hour, patient's subjective onset of relief, and Time to Peak. In addition, meptazinol 100mg was significantly better than morphine 10mg for PID at 1/2 hour. Morphine 10mg was significantly more efficacious than both dose levels of meptazinol for the hourly PID and relief scores beginning with the second hour for the low dose, and the third hour for the high dose and continuing throughout the sixth hour. Morphine 10mg was also significantly superior to the two levels of meptazinol for SPID, %SPID, TOTAL and the patient's assessment of overall improvement. In addition, morphine 10mg was significantly better than the low dose of meptazinol for Peak % and for the patient's global rating of the medication.

Morphine 5mg was significantly more effective than meptazinol 50mg for for relief at hours 4, 5, and 6, PID at hours 3, 4, 5, and 6, %SPID, and TOTAL. In addition, morphine 5mg was significantly better than meptazinol 100mg for relief at hours 4 and 5, and PID at hours 4 and 6.

Adverse Reactions: Twenty patients reported adverse reactions, of which 9 received meptazinol 100mg, 6 received morphine 10mg, 3 received meptazinol 50mg and 2 patients received morphine 5mg. One patient in the meptazinol 100mg group became diaphoretic and nauseated 1/2 hour after study drug administration, was administered Benadryl 50mg and was discontinued from the study. Meptazinol 100mg had the greatest incidence of adverse effects followed by morphine

10mg. Some adverse effects reported with meptazinol 100mg include apprehension, diaphoresis, dizziness, lightheadedness, nausea, and vomiting. Some adverse effects reported with morphine 10mg include dizziness, drowsiness, sleepiness and flushing.

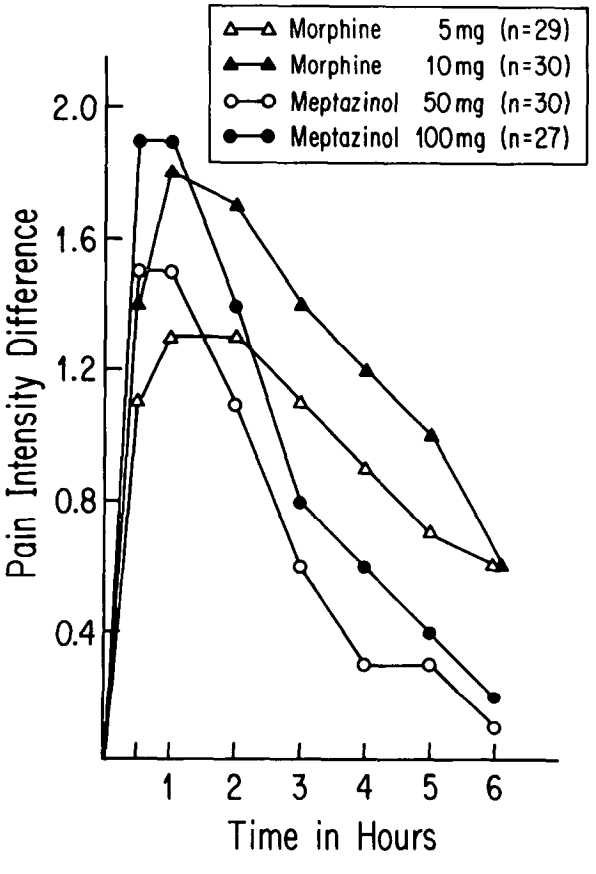

Figure 1: Time effect curve for mean PID scores are calculated by subtracting the mean pain intensity score for each observation point from the pain intensity at 0-hour.

DISCUSSION AND CONCLUSION

The estimate of the relative potency of meptazinol to morphine for SPID and TOTAL were 0.04, while for Time to Peak it was 0.25. Thus 100mg of meptazinol is approximately equivalent to 4mg of morphine based on the summary variables SPID and TOTAL. However, based on the Time to Peak variable, 100mg meptazinol is approximately equivalent to 25mg of nor-

phine. In addition, the relative potency estimates were greatest at the 1/2 and 1 hour points; thereafter, *they* decreased considerably, so that by the third hour 100mg of meptazinol was approximately equivalent to 4mg of morphine. In summary, meptazinol 100mg is an effective, rapidly acting analgesic of short duration. It could be of great value in ambulatory outpatient surgical procedures including endoscopy and colonoscopy, where the pharmacological effects are required for only a brief period and the patient then goes home. It's non-sedating effect make it safer in ambulatory patients and in debilitated patients such as those with pulmonary disease. The percentage of patients with adverse effects reached 32% for the 100mg dose of meptazinol. It would be of interest to study the 75mg dose for efficacy and adverse effects.

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Treatment Effectiveness: Medical Staff and Services Provided to 2,394 Patients at Methadone Programs in Three States

John C. Bail, Eric Corty, S. Paul Petroski, Henrietta Bond, Anthony Tommasello, and Harold Graff

INTRODUCTION

Daily intravenous heroin use and addiction continues to be a major social and medical problem in the United States with some 500,000 addicts concentrated in metropolitan areas. The major treatment modality serving this addict population is methadone maintenance therapy which currently treats some 75,000 patients. The issue of whether this relatively new mode of treatment should be expanded, maintained or restricted continues to be a subject of controversy both within the medical community and the public arena.

The controversy about methadone maintenance treatment not only is beset with the uncertainties and ambiguities which tend, generally, to accompany new modes of treatment, but it involves a fundamental ambivalence about the role of medicine in the rehabilitation of intravenous opiate addicts. This ambivalence about how the contemporary problem of opiate addiction should be addressed is reflected in the spectrum of opinions found in the scientific and medical literature (Musto, 1973; Eddy, 1973; Ball and Graff, 1975; Dole and Joseph, 1978; Lowinson, 1981; Trebach, 1982; McLellan et al., 1982; Woody et al., 1983; Wilson and Herrnstein, 1985).

METHOD

A comprehensive evaluation of methadone maintenance treatment involves description and measurement of four fundamental entities: (1) the patients and their presenting problems, (2) the programs that provide treatment, (3) the treatment provided and, (4) the desired goal or outcome of treatment. (Meyer, 1983; Jaffe, 1984; Tims and Holland, 1984).

The present paper addresses the question of what constitutes methadone maintenance treatment. Specifically, we address the issue of what specific types of medical services are provided to patients in methadone maintenance treatment. (A collateral paper describing counseling and other non-medical services is in

preparation.)

In the summer and fall of 1985, a research team from the Methadone Research Project interviewed the entire treatment staff at the seven selected programs. The interviews in each program took place on a single day during a designated week. The interviewers collected data about employment, demographics, treatment duties, and actual services provided to patients in the prior week. It is these data that make up the basis of the present paper.

THE MEDICAL STAFF AT THE 7 METHADONE PROGRAMS

The medical staff at these seven methadone maintenance programs are divided into four categories. (Table 1) These are, A) physicians, B) nurse practitioners and physician's assistants, C) nurses and, D) pharmacists. All programs have at least one physician; these seven programs are served by a total of 15 physicians. However, the actual amount of time that these programs are served by the physicians range from .23 FTE to 2.52 FTE. (An FTE is a Full Time Equivalent and represents from 35 to 40 hours per week, depending upon the program:) The average program has a mean of .84 FTE of physician coverage per week.

Two programs are served by physician's assistants (PAS) and two by nurse practitioners (NPs). Two of these programs have two full time PAs/NPs; the other two have only part time coverage. For the four programs, there is a mean of 1.12 FTE of NP/PA coverage per week.

All but two of the programs have coverage by dispensing nurses. These two programs ('P' and 'E') have coverage by pharmacists. Nurses comprise the largest category of medical staff with a total of 21 RNs and LPNs. The five programs with nurses have from three to six nurses on staff with total FTE ranging from 2.60 to 5.13. For the five programs, there is a mean of 3.48 of nurse FTE per week. The seven pharmacists are split between two programs with the total FTE ranging from 1.68 to 3.0. Thus, for the two programs there is a mean of 2.34 pharmacist FTE per week.

Role and Medical Services Provided by the 21 Nurses

The principal role of nurses in most of the programs was to dispense methadone to patients. Thus, in the five programs which employed nurses for this purpose (two employed pharmacists) most of their time was devoted to this task. At the same time, most of the nurses also provided general medical care to patients. Thus, the 21 nurses provided treatment to 288 patients during the designated week. The nurses, then, commonly had a dual treatment role in the programs - dispensing methadone and providing medical care. Each of these roles can be briefly described.

The dispensing role of the nurses usually involved continuous

dose preparation, record keeping, checking patients for intoxication, keeping attendance, urine scheduling and similar tasks. Sometimes, the nurses also collected urine specimens and collected fees.

The second role of nurses was to provide general medical care. In this role, nurses tested patients (e.g., for alcohol intoxication), provided liaison to physicians and other staff, gave medical advice and education and otherwise assisted patients in numerous ways. Although the role of nurses in particular programs varied somewhat, in most cases they fulfilled both the dispensing and treatment roles.

Role and Services Provided by Physicians Assistants and Nurse Practitioners

The role of the 3 physicians assistants and 3 nurse practitioners was quite different from that of the dispensing nurses as these staff did not engage in dispensing. Their role, therefore, was principally devoted to providing general medical care. These medical services included intake and other physical examinations, determination or recomnendation of methadone dosage change (as well as that of other medication), case management, treatment referrals both within and outside the program and other related duties. In general, these PA and NP staff worked closely with the staff physicians.

With respect to output of medical services, the PA/NP staff saw more patients and provided more services per FTE than the nurses, but this was to be expected as they did not have a dispensing role along with a medical care role.

It is pertinent to note that only four of the seven methadone maintenance programs had either physician assistants or nurse practitioners on the staff. Furthermore, these highly trained medical staff were only in full-time status at two programs.

The Role of Pharmacists in the Methadone Programs

Two of the seven programs employed pharmacists rather than nurses to dispense methadone. The role of the seven pharmacists was primarily to dispense methadone. They provided, for the most part, little other medical service to patients. Indeed, one of the notable differences between the pharmacists and nurses was that the latter group also provided general medical care and education to patients.

Of the two programs which employed pharmacists to do the dispensing of methadone, one also employed two full-time nurse practitioners while the other had no nursing staff. Thus, one program had neither dispensing nurses, nor nurse practitioners nor physicians assistants.

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Role and Medical Services Provided by Physicians in the Methadone Programs

All fifteen of the staff physicians provided medical care to patients in the methadone maintenance programs. Although the nature of this care varied somewhat from program to program, especially where psychiatrists were employed, in general the physicians provide general medical coverage through physical examinations, case reviews, direct patient care, consultation and referral to outside medical facilities. Other services provided included psychiatric evaluation and therapy, dosage change, admission screening and examination, advice on rehabilitation, legal and family problems as well as medical education of various kinds.

In analyzing the extent of medical services provided by physicians in the seven programs, it was evident that there ware marked differences in the amount of treatment available and provided. Thus, physicians in some programs treated ten times more patients per week than in others; the range in number of patients treated per week was from 8 to 94. Similarly, there were comparable variations in the percentage of patients seen by physicians in the programs on a weekly basis - from a high of 25.4 percent of the patient population to a low of 3.1 percent of the patient population. (Table 2).

These marked differences in the amount of medical services provided by physicians in the different programs ware partly due to variation in the availability of staff. Thus, when the above differences in services provided (i.e., patients treated) were analyzed with regard to full-time physician equivalents, the variation was greatly diminished. Thus, the range in number of patients seen by physicians per week was from 27.6 to 90.0, with four of the programs treating over 50 patients per full-time physician equivalent.

Still, two points seem noteworthy with regard to the medical services provided by the physicians at these programs. First, and most significant, there ware vast differences in the medical coverage provided by physicians. Thus, ten times more patients were seen by physicians in some programs than others. Also, in some programs only 3 or 5 percent of the patients ware seen by physicians during a week.

Second, it is important to note that the major reason why patients in some programs receive treatment from physicians while others in different programs do not is that there are extraordinary differences in the staffing patterns in these methadone maintenance programs. Thus, it has been found that some programs have ten times more coverage by physicians than others.

Medical Services Provided Per Week By the Staff Groups Combined

In order to ascertain the overall extent of medical services provided patients in these seven methadone maintenance programs, it is efficacious to combine the separate services provided by dispensing nurses, physicians assistants-nurse practitioners and physicians. When this is done, it may be seen that there are still considerable variations in the provision of medical care. Thus, the number of patients seen per week by the medical staff varies from 36 to 185 in the seven programs. Although this figure involved some duplication (as some few patients are seen by more than one type of medical staff), it does reflect the number of treatment sessions and it is a rather accurate enumeration of the extent of medical services actually provided on a weekly basis.

It is relevant to analyze how the available medical staff provide services on a comparative basis; that is, on the basis of full-time staff equivalents (FTEs). When this is done (Table 3, column 5), some of the prior differences dissipate. Although the number of patients seen in the seven programs per full-time staff (FTE) varies from 9.7 to 38.5, in 5 of the 7 programs each full-time staff treats from 22 to 32 patients per week. But, the program with the fewest medical staff sees the most patients per FTE. Therefore, the main reason for marked differences in treatment services provided is that some programs have more treatment staff than others.

Analysis of the provision of treatment services can be undertaken with respect to the percentage of the patient population seen per week by medical staff. These figures show notable differences among the seven programs. Thus, the range in percentage of patients who received medical services is from 14 to 53 percent on a weekly basis. Thus, patients in some methadone programs are three times as likely to receive medical services as those in others.

CONCLUSION

In this report, we have investigated the type and frequency of medical services provided to 2,394 patients by 49 medical staff in seven methadone maintenance programs. Data were collected by a four person research team at each site by means of face-to-face interviews with the entire treatment staff.

The research findings lend support to four major conclusions. First, it is evident that there are marked differences in both the number and type of medical staff which serve these methadone programs. Some programs have continuous daily medical coverage by physicians, others have only part-time coverage on certain days with no coverage on other days. Some programs have highly qualified full-time physicians assistants or nurse practitioners, but some do not. Most of the programs have nurses, but not all. Two programs have pharmacists, but five do not. So, we have

found notable differences in the medical staff available to provide treatment.

Second, we have found considerable variation in the medical services provided patients in these seven programs. In some programs, less than 10 percent of the patients see a physician on a weekly basis, while in others 20 percent or more see a physician. Similar variations in the frequency of treatment were observed for the entire treatment staff.

Third, it was found - as might be expected - that the provision of medical services to patients was related to the number and type of available staff. This finding is important. For it indicates that the available staff - whatever this is - is actively engaged in providing medical services to these needy patients. It is not that patients fail to seek these services. Rather, the findings indicate that there is a pressing demand for medical treatment in these programs.

Lastly, from an evaluation perspective, this analysis of medical staff and the treatment services they provide is only a first step. Next, we need to investigate whether the provision of these and other treatment services in methadone maintenance programs are related to patient improvement and rehabilitation. How, for example, does length, type and intensity of methadone treatment affect patient outcome?

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TABLE 1

Medical staff at seven methadone maintenance programs and patient population of and during designated week*

Program	MD's No.	MD's FTE	PA & NP No.	PA & NP FTE	Nurses No.	Nurses FTE	Pharmacists No.	Pharmacists FTE	Total Medical Staff No.	Total Medical Staff FTE	Pt. Population in Program
P	4	2.52	2	2.0			4	1.68	10	6.20	350 +
R	2	0.86			3	2.64			5	3.50	350 +
O	2	0.29	1	0.28	4	3.80			7	4.37	250 +
J	2	0.23	1	0.20	5	3.25			8	3.68	200 +
E	2	0.69					3	3.0	5	3.69	250 +
C	1	0.50			3	2.60			4	3.10	200 +
T	2	0.80	2	2.00	6	5.13			10	7.93	350 +
Total:	15	5.89	6	4.48	21	17.42	7	4.68	49	32.47	2,394

* Patient census is presented by range in order to maintain confidentiality. Calculated values, however, are derived from the actual figures.

TABLE 2

Medical services provided by physicians at seven methadone maintenance programs during designated week.

Program	No. of MD's.	FTE	Patient Census	Pts. Seen/Wk by MD's	Pts. Seen/Wk by MD's, Per FTE	% of Pts Seen/Wk by MD's
P	4	2.52	350 +	94	37.3	25.4
R	2	.86	350 +	65	75.6	17.3
O	2	.29	250 +	8	27.6	3.1
J	2	.23	200 +	19	82.6	8.7
E	2	.69	250 +	36	52.2	13.8
C	1	.50	200 +	45	90.0	20.1
T	2	.80	350 +	35.5	44.4	5.2
Total:	15	5.89	2,394	302.5	51.4	12.6

TABLE 3

Patients seen by the total medical staff of each program during designated week

Program	Patient Census	Total Medical Staff No.	Total Medical Staff By FTE	Pts./Seen in Week	Pts. Seen Per FTE	% of Pt. Census Seen/Wk by Medical Staff
P	350 +	10	6.2	166.5	26.9	45.0
R	350 +	5	3.5	81.5	23.3	21.7
O	250 +	7	4.4	96.0	21.8	36.8
J	200 +	8	3.7	116.5	31.5	53.4
E	250 +	5	3.7	36.0	9.7	13.8
C	200 +	4	3.1	119.5	38.5	53.3
T	350 +	10	7.9	184.5	23.4	26.9
Total	2,394	49	32.5	800.5	24.6	33.4

A Clinical Trial of Buprenorphine: I. Comparison with Methadone in the Detoxification of Heroin Addicts II. Examination of its Opioid Blocking Properties

Warren K. Bickel, Rolley E. Johnson, Maxine L. Stitzer, George E. Bigelow, Ira A. Liebson, and Donald R. Jasinski

A new synthetic opioid, buprenorphine, has shown promise as a treatment drug for opioid addiction that possesses many of the benefits of the standard agonist and antagonist treatments methadone and naltrexone) while possessing few of their liabilities (Jasinski et al 1978). Like methadone and naltrexone, buprenorphine has a long duration of action. Buprenorphine, in a fashion similar to methadone, supports drug self-administration and therefore may be associated with good treatment retention (Lukas et al., 1983; Mello et al., 1981). Like naltrexone, buprenorphine is very safe across a broad range of doses (Lewis et al., 1982). Moreover, buprenorphine produces only a minimal degree of physical dependence which may permit rapid detoxification (Lukas et al., 1984). The limited withdrawal associated with the discontinuation of buprenorphine treatment may render buprenorphine as the preferred pharmacotherapy for opioid detoxification. Additionally, buprenorphine via the mechanism of pharmacologic blockade reduces opioid self-administration (Mello et al., 1982). Although the dose-effect relation of buprenorphine's blockade has yet to be determined, pharmacologic blockade has been demonstrated in man (Jasinski et al., 1978 Thus, buprenorphine has many properties that may be beneficial in the treatment of opioid addiction.

One undesirable feature is buprenorphine's ability to precipitate withdrawal in opioid-dependent individuals in a fashion similar to that of naltrexone. For example, Lukas et al. (1984), found that subjects maintained on methadone when abruptly placed on buprenorphine maintenance exhibited mild withdrawal signs and symptoms lasting from three to five days. Buprenorphine's withdrawal producing properties may result in poor retention during treatment induction and may represent its greatest impediment as a treatment of opioid addiction.

The purpose of the present study was to conduct, what is to the authors' knowledge, the first controlled clinical trial of buprenorphine. Two experiments were conducted. First, buprenorphine was compared with methadone in the detoxification

of heroin addicts. This study will delineate the efficacy of buprenorphine relative to methadone and indicate whether buprenorphine's withdrawal producing properties will affect treatment outcomes. Second, an experiment was conducted in which the opioid blocking properties will affect treatment outcomes. Second, an experiment was conducted in which the opioid blocking properties of a range of buprenorphine doses were examined.

METHODS

General Methods. The basic protocol for these studies will be described in this section, while any deviations from the basic protocol will be explained under specific methods for subsequent studies.

Subjects. Subjects were enrolled in a 90-day (13 week) detoxification program. Individuals qualified for treatment by showing physical indications of recent intravenous drug use. All subjects underwent a standard medical evaluation prior to enrollment and only healthy individuals were permitted to participate. In order to obtain a homogenous sample of opioid addicts, individuals were also excluded from study participation if there was urinalysis evidence of recent methadone use. Subjects were instructed to abstain from opioid use for a total of 10 hrs prior to receiving their first dose of the treatment drug. All subjects provided written informed consent prior to participation in the study.

Clinic Procedures. Patients reported to the clinic daily and ingested their medication under nursing supervision. Urine samples were collected on Monday, Wednesday and Friday with an additional urine sample collected every other weekend on either Saturday or Sunday according to a random schedule. All samples were collected under staff observation and immediately tested on site via an Emit system (Syva Corp.) for the presence of opiates and benzodiazepines. Additionally, randomly selected samples were sent out each week to an independent laboratory and were analyzed for a wide variety of both opioid and nonopioid drugs using thin-layer chromatography (TLC) analysis.

Self-Report Measures. During each clinic visit in which a urine sample was collected, three self-report questionnaires were filled out by the patients. The first questionnaire was an opioid drug effect adjective rating scale composed of 20 times describing typical opioid drug effects (e.g., nodding, skin itchy). The second questionnaire was a withdrawal adjective rating scale composed of 20 items describing common withdrawal symptoms (e.g., painful joints, hot or cold flashes). On both scales, patients rated each item on the degree to which they experienced the symptoms during the past 24 hours on a scale of 0 (not at all) to 9 (severe). The third questionnaire was the Addiction Research Center (ARC) chronic dosage questionnaire

composed of a checklist of withdrawal symptoms, a yes-no question asking "are you kicking?", and a global question asking "how do you feel?" which could be answered on a 6 point scale ranging from very bad to very good.

Experiment I. Comparison of Buprenorphine and Methadone during the Detoxification of Heroin Addicts.

Subjects. Forty-five males with an average age of 30 yrs were enrolled in the study. Participants were randomized, stratifying for race, to either the buprenorphine or methadone treatment groups. No significant differences were found between the treatment groups on any demographic variable.

Dosing Procedures. Patients ingested both an oral medication (methadone or methadone placebo) and a sublinqual medication (buprenorphine or buprenbrphine placebo) under nursing supervision at each clinic visit. Both the subjects and the nurses had no information about which medication was active or of the dose reduction schedule. Patients were stabilized on either 2 mg of buprenorphine or 30 mg of methadone for weeks 1-3 of the study. After this stabilization period, the doses were reduced over weeks 4 to 7 with placebo being administered during weeks 8-13. The dose-reduction schedule for buprenorphine during weeks 4-7 was 1.34, 0.67, 0.34 and 0.17 mg. The methadone dose reduction schedule for the same period was 20, 10, 5 and 2.5 mg. Buprenorphine prepared in a 20% ageous alcohol solution and administered sublingually. Methadone was suspended in cherry syrup (Methadose) and administered orally. The placebo for each drug was the same vehicle without the active drug.

Measures. Measures included retention, the number of days of subject participation prior to the first of three consecutive absences or the end of the 90 day detoxification period, attendance, the number of days the subject received scheduled medication during the retention period, drug positive uriness and the self-reported measures described above.

Data Analysis. Data were analyzed for the clinical measures of detoxification which included: (1) opioid positive urines, (2) benzodiazepine positive urines, (3) clinic attendance, (4) treatment retention, and (5) self-report measures of opioid drug effects and withdrawal symptomatology. The mean duration of treatment retention for the two groups was analyzed including data from all subjects via a t-test (two-tailed). Only patients who remained in treatment at least six weeks were included in the analysis. Additionally, only data obtained during the first six weeks were analyzed due to subject attrition.

Urinalysis results obtained from the EMIT opiate assay (opiate positives) were analyzed in two ways; that is, (1) all opiate positive urines and (2) all opiate positive and missing urines obtained during the first six weeks were analyzed via a two way

repeated measures analysis of variance (treatment, time, treatment by time). This latter approach of replacing missing data makes the conservative assumption that during a clinic absence patients were taking illicit opioid drugs. Urinalysis from the Emit benzodiazepines assay, self-report measures of opioid drug effects, withdrawal symptomatology, and daily clinic attendance were analyzed via a two way repeated ANOVA (treatment, time, treatment by time).

Experiment II. Examination of the Opioid Blocking Properties of Buprenorphine.

Subjects. Five white males served as subjects over a nine month period with an average age of 32.5 yrs. Patients were told that they would receive buprenorphine.

Clinic Procedures. Patients received a dose of buprenorphine (sublingual) at each clinic visit. Both the subjects and the nurses dispensing the medication were blind to the dose.

Buprenorphine Doses. Patients received first an ascending series of buprenorphine doses followed by a descending series of doses. The patients were chronically maintained on each dose of buprenorphine for a two week interval. The ascending and descending doses of buprenorphine were 2, 4, 8, 16, 4, and 2 mg, respectively. Patients were maintained on buprenorphine placebo during the last week of the study.

Hydromorphone Challenge Session. Between the 10 and 14 day of chronic administration of each buprenorphine dose, the patient participated in a 5 hour challenge session approximately 24 hours after the last administration of buprenorphine. During that session, subjects received three injections administered intramuscularly. The first injection consisting of saline was administered after a 30 minute stabilization period. The remaining two injections consisting of 6 and 12 mg of hydromorphone, respectively, where administered at 1.5 hr intervals. The session was terminated 1.5 hours after the last injection. Physiological measures were taken continuously throughout the session. Pupil photographs were taken every 30 minutes. Five analog scale self-report questionnaires were administered every 15 minutes throughout the session. A larger batter of questions were administered every 30 minutes. The daily medication was administered to the subject at the completion of the challenge session. Subjects were paid $25 for each session they completed and $100 upon completion of all the challenge sessions.

Measures. The physiological measures consisted of diastolic and systolic blood pressure, heart rate, respiration rate and skin temperature, as measured by an Apple II Psychophysiological Station. Pupil diameter was measured from a photograph taken with a Polaroid close-up camera with 3X magnification.

Self-report questions were presented by the Apple II Station. The five analog scale questions presented the subject with a 100 point line anchored by "not at all" on one end and "extremely" at the other. The patients moved an arrow to the extent they felt the drug. The patients were asked to indicate the subject rates "drug effect", "drug liking", "good" and "bad" effects, as well as subjective "high." Withdrawal and opiate drug effect questionnaires, described above, were administered in the larger battery.

Data Analysis. The self-report and physiological measures were averaged across the five subjects and presented in a cumulative dose-effect fashion. A dose effect for each buprenorphine dose is presented.

RESULTS

Experiment I. Mean duration in treatment was analyzed using a t-test wit no significant differences being found between the buprenorphine (N = 22) and methadone (N = 23) groups. The remaining analyses were conducted on the data obtained from patients staying in treatment for at least six weeks which was 17 for th buprenorphine group and 14 for the methadone group. The repeated measures ANOVA failed to reveal any significant differences between the groups. Only the withdrawal symptoms from the ARC Cronic Dosage Attitude questionnaire approached significance [$F(1,29) = 3.44$, n.s.]. On that measure the methadone group reported fewer withdrawal symptoms than the buprenorphine group. Daily attendance, opiate positive urines, opiate positive or missing urines, benzodiazepine positive urines, withdrawal symptoms, and global feelings showed significant time effects. However, significant time effects are to be expected and reflect the gradual reduction in dose throughout the detoxification. Only withdrawal symptoms showed a significant group by time interaction [$F(5, 145) = 2.70$, $p < .05$].

Experiment II. Table 1 presents subject rated high as a function of the cumulative hydromorphone challenge dose across the ascending buprenorphine doses. A steep hydromorphone dose effect curve was obtained on subject rated "high" with subjects maintained on 2 mg of buprenorphine. Less hydromorphone effect is seen under the 4 and 8 mg of buprenorphine. Maximal suppression of hydromorphone's effects occurred when patients were maintained on the 16 mg dose of buprenorphine.

Table 1
Subject Rated "High"

Hydromorphone (mg)	Buprenorphine Dose (mg) 2	4	8	16
0	0.45	0.6	0.28	0.6
6	3.0	1.67	1.26	1.0
12	4.24	2.63	2.33	1.82

DISCUSSION

On the majority of clinic measures from Experiment 1, 2 mg of buprenorphine is as effective as 30 mg of methadone in the detoxification of heroin addicts. Overall, the results from both groups are poor with respect to illicit opioid use and retention. However, this is not a surprising result since most studies of detoxification show poor retention and relapse to opioid use during dose reduction. Nonetheless, buprenorphine is acceptable to patients and can be used in the treatment of narcotic addiction. This is remarkable because a drug with antagonistic properties would not be expected to as effective as methadone, an opioid agonist, in the treatment of heroin addicts.

An important result from Experiment I was the lack of significant group differences on the subject retention suggesting that buprenorphine precipitated withdrawal may not have occurred. This may be due to our instruction to patients that they should remain abstinent from opioids for 10 hr prior to receiving their first medication; that is, buprenorphine may be less likely to precipitate withdrawal when the patient is already experiencing mild withdrawal symptomatology. This view is supported by a study by Aceto (1984) which examined buprenorphines ability to precipitate withdrawal in morphine-dependent monkeys. Buprenorphine was found to precipitated withdrawal in a dose-related fashion when the monkeys were administered morphine one-hour prior to the buprenorphine challenge. Most importantly, buprenorphine was found to suppress withdrawal in a dose-related fashion when administered to monkeys who were 15 hr abstinent and already in withdrawal. The necessity of having patients remain abstinent to the extent that they experience withdrawal may be necessary for successful induction onto buprenorphine treatment.

The examination of the opioid blocking properties of buprenorphine indicates that this drug can produce substantial blockade. The most efficacious dose appeared to be the 16 mg dose, which is approximately equivalent to the 8 mg (SC) used by Jasinski et al., (1978) (R.E. Johnson, personal communication). Although the 16 mg dose of buprenorphine produced maximal blockade, the 4 and 8 mg dose may provide clinically significant blockade.

In summary, buprenorphine is a drug that can be used to treat narcotic addiction. Whether buprenorphine will prove to be a more effective treatment than methadone will require additional studies in which a variety of doses and buprenorphine's various properties such as opioid blockade are examined in clinical trials. Even though Experiment I does not indicate that 2 mg of buprenorphine is more efficacious than methadone, there are two other properties of the drug that may favor its use over methadone. First, buprenorphine is safer than methadone; that is, buprenorphine overdoses are unlikely to result in death because there is a ceiling on buprenorphines ability to cause respiratory depression. Second, buprenorphine is less likely to be diverted for illicit use than methadone because it will precipitate withdrawal in addicts not currently in withdrawal, and therefore, may be less likely diverted for use in that population.

References available upon request.

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Extinction of Conditioned Responses in Abstinent Cocaine or Opioid Users

Anna Rose Childress, A. Thomas McLellan, Ronald N. Ehrman, and Charles P. O'Brien

In 1948, Wikler observed that opioid addicts who had been detoxified and maintained in a drug-free state would sometimes report symptoms of opioid withdrawal when talking about drugs during group therapy sessions, or when they returned to areas where they had previously used drugs. Wikler labeled this phenomenon *"conditioned withdrawal"* speculating that environmental stimuli had acquired the ability (through classical conditioning) to elicit many of the signs and symptoms of actual opioid withdrawal. He further hypothesized that these "conditioned withdrawal" phenomena might play an important role in triggering *relapse* to drug use in the *abstinent* opioid abuser.

In the years since Wikler's original observations, there have been several studies demonstrating the existence of conditioned withdrawal in animals (Wikler & Pescor 1967; Goldberg & Schuster 1970) and in humans (O'Brien 1975; O'Brien et al 1977). We and others have also reported withdrawal-like symptoms and/or signs in opiate addicts exposed to drug-related stimuli (Teasdale 1973; Ternes et al 198O; Sideroff & Jarvik 1980). Given this evidence, the existence of conditioned withdrawal is generally accepted, but its actual clinical importance remains uncertain. For the past several years our research center has engaged in treatment-outcome projects to help determine the clinical importance of conditioned responses such as craving and withdrawal. The general approach in most of these projects has been to first select conditioned "trigger' stimuli (eg. sight of syringe, drug-talk, cook-up paraphernalia) which reliably elicit conditioned drug responses in the target population, and then to attempt to reduce these responses through repeated, non-reinforced exposure (extinction).

In a large scale treatment-outcome study employing extinction trials in *methadone* patients (McLellan et al 1986), we found that these drug-related stimuli were reliable elicitors of conditioned opioid-related responses, particularly conditioned craving and conditioned withdrawal.

With 20 or more extinction sessions, conditioned craving was significantly reduced, but conditioned withdrawal was still in evidence.

In the past year we have been studying conditioned drug-related responses in *abstinent,* formerly dependent abusers of either cocaine or opioids. The two groups of patients were studied following completion of a 30-day in-patient rehabilitation program. As with our previous study in methadone patients, this project involved extensive pre-treatment, post-treatment and follow-up measures of the patients' clinical status. Data from these sessions has permitted us to examine the question of whether, and to what extent, conditioned drug-related responses are seen in *abstinent addicts following 30 days of inpatient treatment.* The current paper is an interim report on two sources of data collected in the course of the long-term treatment outcome study: 1) *Pre-treatment laboratory measurement* of the conditioned responses associated with opioid dependence and 2) attempted ***extinction*** of the conditioned responses associated with either cocaine or opioid use.

METHODS - Study I - Pre-Treatment Assessment of Conditioned Responses in Abstinent, Opioid-Dependent Subjects

Subjects - for the opioid study were 13 formerly opioid-dependent males who had just completed at least 30 days of abstinence-oriented rehabilitation in a therapeutic community. They were then transferred to the research facility for a three-week inpatient stay, followed by an eight-week outpatient treatment phase. Interested candidates were clinically screened to rule out diagnoses of major thought disorder (schizophrenia) or organic brain syndrome. Patients participated in the study with the understanding that they would be randomly assigned to one of four treatment packages: 1) Supportive-expressive psychotherapy-plus extinction (SE-E), 2) Supportive-expressive psychotherapy plus control activities (SE-C), 3) Drug counseling plus extinction (DC-E) and 4) Drug counseling plus control activities (DC-C). Eventual inter-group comparisons will thus permit assessment of the contribution of professional psychotherapy and/or extinction to treatment outcome. The average age of these subjects was 34 years, having an average of 14 years heroin use and three years of methadone treatment.

Pre-treatment measurements. Prior to treatment each patient's responsivity to drug-related stimuli was assessed in a go-minute laboratory session. These laboratory test sessions were conducted in an environmentally-controlled, electrically-shielded recording chamber. Both physiological and subjective measures were obtained.

Physiological measures included peripheral skin temperature (TEMP), galvanic skin resistance (GSR), heart rate (HR), and respiration (RESP). These physiological measures were simultaneously recorded on a

polygraph and a Bio-Med computer software package for later analyses.

Subjective measures were obtained by asking each abstinent patient to rate, on a 1 to 10 scale, the degree of subjective high, craving, or withdrawal experienced under each set of stimulus conditions. Both neutral and drug-related stimulus conditions were employed, with each patient experiencing both conditions, acting as his own control. The following stimulus sequence was used: 1) Neutral Baseline, 2) Neutral Videotape (a nature story), 3) Neutral Activity (video pong game), 4) Drug Baseline, 5) Drug-related Videotape (buy-sell and cook-up-shoot-up rituals) 6) Drug-related Activity (handling drug paraphernalia and performing rituals appropriate to the drug e.g., cook-up, tie off or prepare pipe for free base.) and 7) Recovery Baseline. Both Neutral and Drug-related stimuli had been developed through our previous large scale study of conditioned phenomena in a methadone-maintained patient sample.

RESULTS - Study I

Pretreatment Laboratory Testing. First, mean values for five minute stimulus periods representing each stimulus period (e.g., Neutral baseline, Neutral video, Neutral activity, etc.) were determined for each of the physiological variables (GSR, HR, RESP, and TEMP), for each patient. Since baseline values on some of these physiological variables sometimes show drift in the course of a session, we used **a** difference score to represent the response to each Neutral or Drug-related stimulus condition. The difference score was derived by subtracting the appropriate baseline value from a given stimulus condition, e.g. (Neutral video) - (Neutral baseline): (Drug Video) - (Drug baseline) etc. These difference scores were then compared between Drug and Neutral conditions using the paired t-test procedures. T-test results for both GSR and TEMP physiological variables differed significantly for the Drug vs. Neutral activity ($p<.05$, one-tailed test). These physiological results, showing greater arousal (indexed by GSR) and greater reductions in skin temperature to Drug vs. Neutral activities, parallel our earlier findings in methadone maintained patients (McLellan et al 1986).

Subjective measures. As with the physiological measures, we first derived difference scores to represent the change in craving for each stimulus compared to its baseline and then compared the Neutral vs. Drug-related conditions using paired t-tests. Results of these comparisons showed a highly significant difference in craving between Drug vs. Neutral activity ($p<.005$, one-tailed test). No significant differences were seen between neutral and drug-related stimuli on the high or withdrawal subjective measures. Interestingly, craving increases upon presentation of drug-related stimuli have occurred in 11 of 13 abstinent patients tested thus far; markedly greater than the 50% rate shown by methadone

patients tested under the same conditions (McLellan 1986).

METHODS - Study II - Extinction of conditioned Responses in Abstinent Opioid-Dependent and Cocaine-Dependent Subjects

Subjects - *Opioid* subjects were six of the original 13 subjects (described in Study I) who had been randomly assigned to one of the extinction groups. *Cocaine* subjects were six male veterans who had completed inpatient treatment for cocaine dependence at the same therapeutic community program described in Study I. These patients were all pilot subjects in our developing study of conditioned drug responses associated with cocaine use. They were all males with an average age of 34 and four years of cocaine use. All cocaine subjects were freebasers.

Extinction Sessions - All subjects in both groups received 20, hour-long extinction sessions during their three-week inpatient stay. Three types of conditioned drug stimuli were presented during each session: audiotape segments of a drug "buy;" videotaped segments of an individual using the drug; and a drug activity in which the subject actually went through the drug preparation procedures with paraphernalia identical to those they had used in their home environment. Each of the three types of stimuli were repeated three times during each session, thereby providing nine drug-related stimulus exposures per session for a total of 180 exposures over the course of 20 sessions. This intensive inpatient extinction offered four to five times as much drug-related stimulus exposure as our previous procedures with methadone patients.

The stimuli for the opioid subjects have been described previously (Mclellan et al., 1986) and the cocaine stimuli were developed in the same way using language, scenes and paraphernalia directly from the patients' street experience. Most extinction sessions were conducted on the inpatient treatment ward. During all sessions subjective measures were taken at baseline and following stimulus exposure. Data were based on 10-point ratings of the intensity of feelings of *high, craving* and *withdrawal* responses that a patient may experience upon exposure to drug-related stimuli (CS's). More detailed measures of the type and intensity of symptoms was then probed through a list of 48 symptoms.

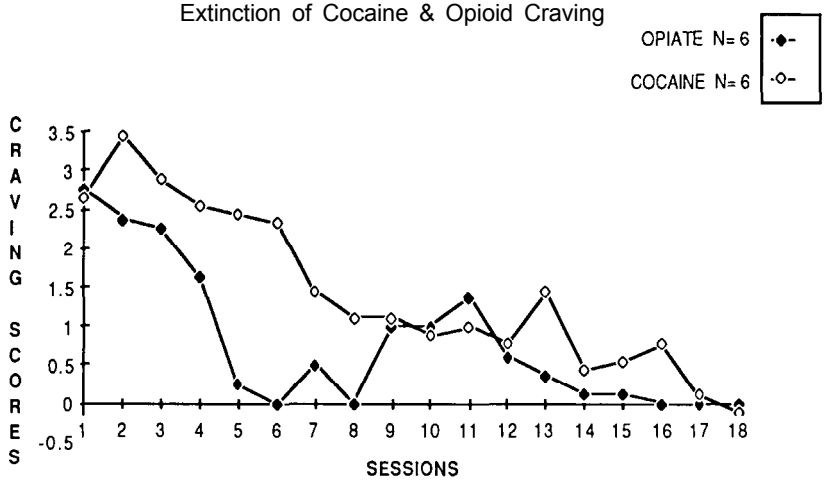
RESULTS - Study II

Difference scores were calculated for all three rating measures as well as the two symptom scores for each session, by subtracting the baseline measure from the post-stimulation score. These difference scores were examined in a repeated measures ANOVA for both opioid and cocaine subjects. Despite the small number of subjects there were significant ($p<.01$) reductions shown in withdrawal symptoms and in the craving ratings for both groups. High ratings and high symptom scores were never

elevated during the intervention and were therefore not significantly changed over sessions (p>.10). Reduction in craving symptoms as a function of extinction trials is illustrated for both groups in Figure 1. As seen, most of the reduction in craving occurs by the tenth session of the intensive extinction regimen.

FIGURE 1

Extinction of Cocaine & Opioid Craving



SUMMARY AND DISCUSSION

Though data collection in our treatment-outcome study of abstinent former opioid dependent patients is still in progress and the cocaine study is in an early stage, two significant findings are already apparent: 1) Drug dependent patients who have just completed 30 or more days of *abstinence* in a therapeutic community setting are extremely vulnerable when exposed to drug-related stimuli. The opioid patients show increased arousal (GSR) and decreased peripheral skin temperature (TEMP) to Drug-Related, as compared to Neutral activities. Our preliminary evidence suggests similar signs of arousal in abstinent former cocaine users. Subjectively, 11 of 13 opioid patients showed increased *craving* to drug-related stimuli, a rate more than twice that found in methadone-maintained patients tested under the same conditions. 4) The current extinction procedures were effective in virtually eliminating both craving and withdrawal symptoms in response to the stimuli within 20 treatment sessions (a 3-week inpatient stay).

Ironically, all these abstinent patients had just finished a “complete” course (30 days) of therapeutic community (TC) treatment and were considered ready for discharge. Most of these patients had felt the TC experience was

quite beneficial, and had prepared them to stay drug-free. They were subsequently dismayed to experience intense feelings of craving and vulnerability upon direct exposure to drug-related stimuli. Several patients spontaneously commented, “It’s like I was never there (at the TC) - this (craving) is so strong...” The TC had provided strong support for these patients’ resolution to remain drug-free, but had left intact the conditioned responses which could so readily undermine their best intentions.

The results in the cocaine subjects are preliminary, but they represent the first systematic examination of this aspect of cocaine dependence. Since results of standard treatment with this powerful drug are poor, the incorporation of extinction procedures seems to be warranted in a controlled trial. A procedure similar to our current extinction protocol might be a particularly useful adjunct to other more standard treatment programs for the abstinent patient. In its current evolution, our extinction program is quite intensive, but can easily fit into a 30-day stay. It can be administered by trained *counselors or research assistants* and does not require physician time. The stimuli and procedures are easily adapted to a clinic setting. Finally it appears to be effective in virtually *eliminating* conditioned craving and withdrawal to the drug-related stimuli which we present. Of course, how-long these reductions will last and how well these reductions in response will generalize to other drug-related stimuli in the patient’s post-discharge world remains the crucial question. We hope generalization will be enhanced by supplemental, outpatient extinction with *individualized* conditioned stimuli.

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Source of Income as a Predictor in Opioid Addicts: 2.5 Year Follow-Up

Thomas R. Kosten and Bruce J. Rounsaville

INTRODUCTION

Although several studies have suggested that drug abuse treatments have had limited success in helping former addicts become self supporting (1-3), a more recent study by McLellan et al. (4) suggested that an inability to become self-supporting during methadone maintenance treatment may be limited to patients who are supported by public assistance before applying for treatment. In their study, addicts were grouped according to three primary sources of pretreatment income - employment, public assistance and illegal activities - and then analyzed separately at follow-up. The public assistance group demonstrated not only an inability to legally support themselves, but globally poor outcomes with no significant improvements in any area, while the other two groups made significant improvements in several psychosocial outcomes including self support through legal employment. From a recently completed 2.5 year follow-up of treatment seeking addicts (5,6), we were able to assess the generalizability of the McLellan study using data from a longer follow-up that included females, and to compare addicts treated with detoxification alone to those getting methadone maintenance.

METHODS

Study subjects were opiate-addicted applicants for treatment at the Substance Abuse Treatment Unit (SATU) of the Connecticut Mental Health Center and the Yale University Department of Psychiatry in New Haven, Connecticut. One hundred and fifty-one out of 204 consecutively admitted subjects participated in a follow-up evaluation 2.5 years after the initial evaluation (5,68). Sociodemographic and clinical characteristics of the subjects lost to follow-up were compared to those interviewed, and the two groups were demographically comparable, but were found to differ on several indicators of antisocial behavior. Fewer of those lost to follow-up had school behavior problems as children (38% vs 55%) or met DSM-III criteria for antisocial personality disorder as adults (37% vs 63%). Those lost also

had fewer arrests (7.5 vs 11.5) and less previous drug abuse treatment (1.5 vs 2.4 previous predominantly male (76%), 41% were white, 27% were currently married, 51% were employed, and the mean age was 28 ± 5 years. They had used opiates for 9.8 ± 4.3 years.

Both the admission and follow-up evaluations included the Addiction Severity Index (ASI) (7,9), the Social Adjustment Self-report scale (SAS) (10), Beck Depression Inventory (11) and Maudslay Personality Inventory (12,13). The AS1 is a structured interview yielding ten-point clinical ratings of problem severity in six areas: medical, legal, substance abuse, employment, family and psychological. On each of these scales higher scores indicate more impairment.

Patients were classified into three groups based on their sources of income during the 30 days before seeking treatment, as described by McLellan et al. (4). This division resulted in 48 employed (E) patients, 46 welfare (W) patients and 57 illegal income or criminal (C) patients. The three groups differed in age and the percentage of males. More males were in the employed group (90%-E vs 65%-W vs 70%-C), and the welfare group was younger than the other two groups (28-E vs 26.5-W vs 29-C). Because of these demographic differences, sex stratified and covariance analyses were also performed. Illegal income and employment at follow-up were compared within the nine subgroups formed by "source of income" (employed, welfare, criminal) and treatment type (methadone, drug-free, detoxification) using analysis of variance for income change and chi squared for percent employed.

RESULTS

On the ASI, substance abuse, family, legal and psychological problems significantly decreased for all three source of income groups, but the amount of improvement across the three income groups was significant only for employment and legal problems. On these two scales the welfare group showed relatively little change compared to the other two source of income groups. For employment, the E group went from 2.2 at intake to 2.1 at follow-up, the W group from 4.0 to 3.2, and the C group from 4.2 to 3.2 ($F=7.4$, $P<0.0001$). For legal problems, the E group went from 3.8 to 2.1, the W group from 3.5 to 2.5, and the C group from 5.0 to 2.4 ($F=5.6$, $P<0.01$).

For the total sample, income from illegal activities was substantially reduced, but income from other sources did not significantly change. Illegal income decreased from a mean of $1058 to $246 monthly during the 2.5 year follow-up ($t=5.8$, $P<0.0001$). Mean employment earnings increased slightly from $356 to $ 383, and mean welfare income increased from $48 to $79 for the whole sample. Within source of income groups, significant changes in income were evident for the employed and criminal groups, but not for the welfare group. Decreases in

employment earnings were demonstrated for the employed group ($741 to $483), but no change for the other two groups (F=5.8, P<0.01). Illegal income substantially decreased for the criminal group ($2552 to $446), but did not change for the other two groups (F=25, P<0.0001). Thus, in illegal income and legal problems, as well as employment, the welfare group showed less improvement than the other two groups.

To examine whether welfare patients benefited from treatment with methadone maintenance, we compared methadone maintenance to detoxification alone. During this 2.5 year follow-up 83 patients received methadone maintenance, and 40 patients received detoxification alone. Based on McLellan's findings, we expected that the welfare patients would do no better with methadone maintenance than with the minimal treatment of detoxification alone. In comparison, we expected that methadone patients in the other two income groups would show more improvement than did the detoxification patients.

The amount of illegal income at intake and follow-up for the three source of income groups was compared between the methadone and detoxification patients. For the methadone maintained subjects, all three income groups reduced their illegal income at follow-up, although the welfare group had the least percentage reduction (26%-W vs 74%-E and 81%-C). In comparison for the detoxification alone subjects, the employed group showed an increase in illegal income of 35% compared to reductions by the welfare (48%) and criminal groups (84%). In terms of our major question, welfare predicted a poor illegal activities outcome, since when treated with methadone maintenance, the welfare group reduced their illegal income substantially less than the patients in the other two income groups (F=7.0, P<0.0001). Furthermore, for the employed group, methadone maintenance had a substantial impact in reducing illegal income compared the increase reported by those getting detoxification alone.

In terms of employment status among the methadone maintained patients, the welfare patients were less often employed at follow-up (50%) than were the other two groups (E-83% and C-66%) (chi squared = 3.4, P<0.05, one tailed). Among the detoxification patients, employment for the welfare group at follow-up (50%) did not differ from that for the other two groups (E-60% and C-33%) (chi squared = 0.03, n.s.). Compared to detoxification patients, methadone maintained patients were employed more often within both the employed (83% vs 60%) (chi squared = 2.6, P<0.05, one tailed) and criminal groups (66% vs 33%) (chi squared = 3.9, P<0.05), but not within the welfare group (50% vs 50%) (chi squared = 0.0, n.s.). Thus, as pretreatment source of income, welfare predicted a relatively poor employment outcome during methadone maintenance treatment.

DISCUSSION

Treatment outcome 2.5 years after intake was examined within three subgroups of patients categorized by their primary source

of income when applying for treatment: employment, welfare and criminal activities. A wide variety of outcomes were examined including several psychosocial assessment scales, the Addiction Severity Index, monthly income from the three primary sources and employment rates as follow-up. Because the previous short-term study of this issue included only methadone maintained patients and did not have a minimal treatment control group (4), we compared the methadone maintenance patients to those receiving only detoxification in the present follow-up. Two questions were addressed: 1. did pretreatment welfare do better with methadone maintenance than with detoxification alone?

The answer to the first question was that pretreatment welfare support did not predict globally poor outcome, and the most striking finding was the general improvement of subjects in all three "source of income" groups. The differences in outcome among the three groups were consistent with the areas of identified problems at intake, and the group with the most severe problem showed the most improvement in that problem area. In contrast to the previous six month follow-up by McLellan (4), our welfare group improved in most areas, and in depressive symptoms and social adjustment they showed more improvement than the other two groups. The differences from McLellan et al. suggest that the previous study of male veterans populations, and source of income may be much better as a short-term than long-term predictor of outcome.

In answer to the second question, treatment with methadone maintenance appeared to have little impact beyond detoxification in reducing unemployment and illegal income among addicts whose initial source of income was primarily public assistance (welfare). This finding is consistent with the previous study by McLellan et al. (4), although McLellan did not have a comparison group for their methadone maintenance sample in order to control for non-specific effects of seeking treatment. Future follow-up studies will need to include minimal treatment or other suitable comparison groups before we conclude that compared to other income groups, addicts on welfare obtain minimal benefits from methadone maintenance, and that they need other forms of treatment such as residental programs.

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AIDS Virus Exposure Behavior in Long-Term Addicts: A Preliminary Study

M. Douglas Anglin and Mary-Lynn Brecht

ABSTRACT

Data is presented on the prevalence of clinical AIDS and antibodies to the AIDS virus in intravenous drug users. In addition, data from subjects followed over twenty years in a California study on the natural history of narcotics addiction is reported. Information about AIDS attitudes and high risk behavior is related to sample characteristics and narcotics use patterns. The possibility for heterosexual transmission is assessed by report of marital or common-law status and by the extent of drug use by long-term sexual partners.

INCIDENCE OF AIDS

Through April 7, 1986, 19,181 diagnosed cases of acquired imiunodeficiency syndrune AIDS) had been reported to the Centers for Disease Control (CDC 1986); 274 of these were children under 13 years of age, and 81 were adolescents between 13 and 19. The epidemic has grown geunetrically since the first few cases were identified in 1979-1981. Cases have been reported in 46 states and the District of Columbia, with the highest prevalence in New York (31% of cases). California has reported 23% of the cases; Florida and New Jersey represent 7% and 6%, respectively. Among U.S. cities New York leads with 5,796 cases, followed by San Francisco with 2,014, and Los Angeles with 1,555. When cases am standardized to a per-million-population rate, New York is first with 573 cases per million, followed by San Francisco (554), Miami (308), Newark (210), and Los Angeles (185).

AIDS also has been reported in many other countries. By March, 1985, 940 cases were reported from Europe (CDC 1985a). Although relatively few AIDS cases have been officially reported in Africa, it is estimated that the incidence is considerably higher (Piot et al., 1984). The disease itself appears to follow a different course in Africa, and understanding that pattern could provide information important for the rest of the world (Fauci et al., 1985).

DRUG ABUSERS AND AIDS

Intravenous (IV) drug use is the primary risk factor in 17% of the AIDS cases reported in the United States, and IV drug users constitute the second largest risk group after homosexual men. IV drug users constitute 14% of male and 53% of female adult AIDS cases nationwide (CDC 1986). In NEW York 30% of AIDS cases as of August 1985 had IV drug use as the major risk factor (Bureau of

Communicable Disease Control 1985). AIDS victims from other risk groups also have IV drug use as a secondary risk factor; for example, national figures indicate that 14% of homosexuals with AIDS also have a history of parenteral drug abuse (Ginzburg 1984; Ginzburg et al., 1985).

Table 1 shows the shows of AIDS cases through about April 6, 1985, that have IV drug as a contributing or sole risk factor.

Among the 231 pediatric AIDS cases nationwide, 76% are associated with having a parent with or at risk for AIDS (CDC 1986). Many cases have teen documented in which one or both parents am IV drug users (Rubinstein et al., 1983; Aleske et al., 1983; Kaplan et al., 1985).

Isolated studies also show substantial increases in the HTLV-III serum positive rate among IV drug users. Weiss et al. (1985) found that 46% of the sample of 56 drug abusers were HILV-III positive in 1982, while Spira et al. (1984) found at least 58% of a sample of 86 were confirmed positive. Table 2 illustrates the serious increase in seropositive rates in major cities of the United States and Europe.

AIDS appears to spread among drug abusers through sharing of needles and drug paraphernalia. It can also be transmitted from drug abusing mothers to infants. Furthermore, AIDS can be sexually transmitted by male IV drug users to non-using females (Pitchenik et al., 1983; Masur et al., 1932; Redfield et al., 1985) or by incarcerated drug using males to other males by homosexual activity in institutions.

In a preliminary study, Los Angeles County has reported only 7 of 350 (2.0%) of admissions to methadone maintenance and methadone detoxification programs to be positive for HILV-III antibodies (Mascola 1985). This is quite low compared to the approximately 50% rate reported for homosexual men (Detels 1986). The potential for a rapid increase in the rate for IV drug users exists because 12% of the men in the Los Angeles sample reported being gay or bisexual. Thus a "window" of exposure of a significant size exists between the two highest risk groups.

Of further concern is the 18% of women in the Los Angeles sample who reported prostitution. If the HlLV-III exposure rate in IV drug users rises to a high level, a second window of exposure exists for transmission to the heterosexual community.

The risk factors within the parenteral substance abusing group affect the development of the disease after exposure are not known. However, repeated exposure may be necessary, because few health care workers have contracted AIDS in spite of needlestick injuries (CDC 1985b). Use of innunosuppressant drugs or infections such as hepatitis-B or Epstein-Barr Virus (EBV) are possible cofactors (Marmor et al., 1985). Evidence of immunosuppression has been found in IV drug users without AIDS (Fiorini et al., 1985).

Opportunistic infections are more common than Kaposi's sarcoma in IV drug users (Des Jarlais et al., 1984). Shorter survival times are associated with opportunistic infections than with Kaposi's sarcoma (Riven et al., 1984). IV drug abuse was the major risk factor in a study of AIDS patients with central nervous system involvement (Koppel et al., 1985).

Relatively few data am available on prevalence and transmission of AIDS in parenteral drug abusers. Studies only recently have progressed from observations and case studies to more epidemiological ones. More data are needed to trace the course of AIDS infection and disease in this population. Such data are critical to public awareness, policy decisions, and provisions for prevention and treatment resources.

ATTITUDE AND BEHAVIOR SURVEY

An ongoing longitudinal study on the natural history of narcotics addiction gave us an opportunity to collect attitudinal and behavioral data from 458 heroin addicts. The original study was designed to evaluate the outccme of treatment for 581 heroin addicts admitted to the California Civil Addict Program during 1962-1964 (McGlothlin et al., 1977). A 20-year followup is now in progress on the 458 addicts still alive. Interviewing began for the 20-year postadmission follwup in November 1985. Because of the increasing prevalence of HTLV-III antibodies, AIDS-related complex (ARC), and AIDS among the IV drug users, we included four AIDS-related questions in the natural history interview:

- How concerned are you about your physical health?
- How many times have your shared a needle with someone in the last five years?
- How concerned are you that you might have been exposed to AIDS?
- How many intravenous drug users do you know who have or had AIDS?

To obtain early information on our findings, we analyzed the first 107 interviews completed as of January 15, 1986. Although this sample is biased towards subjects who were easy to locate (in prison or with a stable mailing address), we believe the results provide minimm estimates and am thus conservatively generalizable to the total sample. The estimte should be interpreted conservatively since subjects who are still active "street" addicts are less likely to be represented in these first 107 interviews and are more likely to be at risk for AIDS exposure.

RESULTS

Table 3 summarizes the results of the AIDS-related and other selected questions from this preliminary sample. We have divided the sample into a high-AIDS risk group and a low-AIDS risk group. The high risk group (N = 74) contains those subjects reporting IV heroin use after January 1, 1980, the date we chose as a starting point for AIDS exposure risk. The low-risk group (N = 33) reported their last IV use of heroin as occurring before January 1, 1980. Although 15% of the high-risk group did not admit to having shared needles in the five years preceding the interview, we have retained them in the high-risk category because of possible underreporting of the behavior. We are more confident of the validity of the report of heroin use since a urine specimen is obtained for analysis at the time of interview.

Although both high-risk and low-risk groups indicated moderate to very great concern for personal health (83% and 68%, respectively), only 33% of the high-risk group and 6% of the low-risk group were personally concerned about AIDS. Since only 5% of the high-risk group and none of the low-risk group had personally know of anyone with AIDS, this lack of concern is not surprising. On the other hand, the reported level of concern implies either that information and education efforts directed toward IV drug users, especially those most

recently involved, are reaching only a minority or that a strung capacity for denial exists in this group. It is apparent that the high-risk group needs greater education or other intervention, for 85% of them report having shared needles with other users in the last five years--55% report having done so 99 or more times.

The recent and current heroin involvement by the high-risk group is considerable, because 72% had used heroin and 49% had been addicted in the year prior to intewiew. Some 23% reported daily use at the tine of interview. As the general prevalence of AIDS virus increases in the next few years, the risk to this group will increase in an epidemic manner.

Not only is the high-risk group itself likely to be exposed to the AIDS virus because of past and current needle sharing, but their sexual partners are also, especially if they too are addicted. Thirty-one percent of the high risk group were married, and 7% were involved with a common-law partner at the tine of interview. Thirty percent of those reporting themselves married also reported having wives addicted after January 1, 1980. Sixty-six percent of those reporting a common-law relationship indicated that their partners had been addicted after January 1, 1980.

In summary, these results, together with those reported earlier for the Los Angeles County study, point to the serious AIDS education and prevention needs in heroin addicts generally, both short-term and long-term. Even 20 years after admission to treatmant, 69% of our preliminary sample reported heroin involvement in the AIDS high-risk period after January 1, 1980; 50% reported heroin involvement in the year before interview. The majority of the sample exhibited little or no personal concern abort AIDS exposure and reported extensive needle sharing. Finally, a large number of heterosexual women may also be at risk for AIDS exposure through sexual contact or neddle sharing because of marriage or common-law relationships with long-term addicts. More remotely, but still at risk, are the children of women intimately involved with long-term addicts.

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(May be obtained from the authors.)

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TABLE 1. Clinical AIDS and IV Drug Use

Sample	N	%
All AIDS cases*	18,883	100.0
IV drug history	4,680	24.8
IV drug use (sole risk)	3,184	17.0
Location of IV AIDS cases*	2,673	100.0
New York	1,921	60.3
New Jersey	507	15.9
Florida	171	5.4
California**	74	2.3

		IV History	
Selected populations*	N	N	%
Prisoners	766	728	95
Adult women	1,208	777	64
Children†	273	148	54

Sources: *New York Times, April 6, 1986. New Fear on Drug Use and AIDS
**California Department of Health Services (1986)

TABLE 2. Seroprevalence of AIDS virus antibodies in IV drug users

Area/Sample	Year	Positive (%)
United States		
New York (Des Jarlais)	1985	50-60
New Jersey (Rutledge)	1985	50-60
San Francisco (Moss)	1985	5-15
Los Angeles (Mascola)	1985	1-2
Europe		
Italy (Angarano, Aiuti)	1985	44-76
Edinburg (Robertson)	1986	51
Spain (Rodrigo)	1985	37
Greece (Papaevangelou)	1985	2

TABLE 3. Preliminary results from CAP* followup: AIDS risk, attitudes, and behaviors

Variable	High-AIDS risk 1980-1986 %	Low-AIDS risk before 1980 %	Total %
N	74	33	107
	CHARACTERISTICS		
Age at interview:			
40 - 45 years	47	39	45
46 - 50 years	37	34	35
51 - 55 years	11	18	13
56 - 61 years	5	9	7
Year born:			
1930 or before	7	15	9
1931 - 1940	54	52	54
1941 - 1950	39	33	37
Ethnicity:			
Black	8	12	9
Hispanic	64	39	56
Anglo	28	49	35
Status at interview:			
Incarcerated	12	0	8
On MM	18	9	15
Using heroin daily	23	0	16
Irregular user	27	0	19
No use or on MM	11	88	35
Other	10	3	8
	AIDS-RELATED VARIABLES		
Concerned about personal health:			
Not at all	10	19	12
Very little	7	13	9
Moderate	26	16	23
Much	19	13	17
Very much	38	39	38
Number of times shared needles last 5 years:			
None	15	100	41
1 - 49 times	26	-	18
50 - 98 times	4	-	3
99 + times	55	-	38
How concerned about AIDS:			
Not at all	43	82	55
Very littled	22	12	19
Moderate	12	0	8
Much	5	0	4
Very much	16	6	13

(continued)

TABLE 3 (continued)

Variable	High-AIDS risk 1980-1986 %	Low-AIDS risk before 1980 %	Total %
Knows IV user with AIDS:			
None	95	100	96
One	3	0	2
Two or more	2	0	2
	IV DRUG USE PATTERNS		
Number of months since last heroin use:			
0 - 12 months	72	0	59
13 - 36 months	18	0	12
37 - 72 months	10	0	9
> 72 months	0	100	20
Frequency of use per month during last use:			
Less than 5 times	32	50	37
6 - 20 times	23	10	19
21 - 60 times	9	19	11
61 - 90 times	17	7	14
91 or more	19	14	19
Number of months since last addiction:			
0 - 12 months	49	0	34
13 - 36 months	20	0	14
37 - 72 months	17	0	12
> 72 months	14	100	40
Frequency of use per month during last addicted period:			
30	14	55	26
31 - 60	19	21	20
61 - 90	32	6	24
91 or more	35	18	30
	TRANSMISSION LIABILITY		
Marital status at interview:			
Single	26	8	21
Married	31	74	44
Common-law	7	0	5
Divorced	24	12	21
Widowed	3	0	2
Separated	10	6	8
Spouse addicted after 1-1-80:			
Yes	30	16	25
Common-law addicted after 1-1-80:			
Yes	66	N/A	46

*CAP California Civil Addict Program

Social Support and Relapse to Tobacco, Alcohol, and Opiates: Preliminary Findings

Barbara E. Havassy, Sharon M. Hall, and, Jeanne M. Tschann

Social support is often defined as resources provided by other persons (Cohen and Syme, 1985). It is described in terms of the structure and patterns of relationships with others, for example, whether one is married, and in terms of its functional aspects, for example, the extent to which relationships provide emotional resources (such as a sense of being understood). Negative qualities of functional support, for example the constraints supportive relationships may exert over one's choices, have also been described (see Lehmann et al., 1983, Schaefer et al., 1981).

Methods of measuring social support as well as the variables differ depending on which aspect of support is measured. Despite this variability and a lack of understanding about just how social support operates, both structural and functional aspects of support have been shown to play a positive role in health outcomes. Outcomes of drug treatment especially maintenance of abstinence, may also be facilitated by both aspects of support (see Cohen and Willis, 1985).

Consideration of social support is not common in the drug treatment literature. When examined, however, social support has generally predicted outcomes (e.g. Finney et al., 1980; Foster et al., 1971; Mermelstein et al., in press, Moos et al., 1979; Orford et al., 1976).

In considering outcomes of drug treatment, it may be important to distinguish general (or global) social support from support for cessation of drug use and maintenance of abstinence. The concept of support specific to drug-use and its potential relationship to relapse has received little attention. Mermelstein et al. (in press) attempted to differentiate global social support from that specific to quitting smoking, however, results were unclear. Cohen et al. (1985) suggested that support from intimates rather than from casual acquaintances may be an important determinant of abstinence.

Several consistencies emerge from the social support, drug abuse, and treatment literatures. (1) There is evidence of the

importance of social support in preventing relapse. (2) There is indication of gender differences in social support (see Cohen and Willis, 1985) and in relapse rates (for example, see Davidson, 1976, U.S. Public Health Service 1980). Nevertheless, knowledge is lacking about processes underlying these differences. (3) There is indication that general social support functions differently from support specific to drug use.

This paper reports the results of a preliminary study about social support and relapse. The sample consists of the first 77 subjects to enter a study of commonalities in relapse in three groups of treated drug users: smokers, alcoholic, and opiate users. (The completed sample consists of 230 subjects in roughly equal proportions. Data on the 230 are being analyzed currently.) The work reported here tested two hypotheses on social support. These hypotheses were: (1) Higher levels of social support will be related to a lower probability of relapse. (2) Women will receive less social support for maintaining abstinence than men. Relapse was defined as one week of daily use of the problem drug.

METHOD

Subjects - Subjects were drawn from three drug treatment groups: opiate addicts completing 21-day methadone assisted detoxification or 28 day residential treatment, alcoholics completing 28 day residential treatment, and smokers completing 28 day cessation treatment. Subjects were employed within the six months before beginning treatment, were of low to middle socioeconomic status, and gave data adequate for follow-up contact. Primary source of income for subjects was legal. Descriptive statistics for this sample are found in Table 1.

TABLE 1
Descriptive Statistics

		TOBACCO (n=30)	ALCOHOL (n-29)	OPIATES (n=18)	TOTAL (n=77)
SEX	M	18	25	11	54
	F	12	4	7	23
ETHNICITY	WHITE	27	22	14	63
	BLACK	3	7	4	14
MARITAL STATUS	NEVER MARRIED	18	8	5	31
	MARRIED	6	9	6	21
	SEPARATED	6	12	7	25
	DIVORCED				
EMPLOYMENT STATUS	EMPLOYED	26	13	5	44
	NOT EMPLOYED	4	16	13	33
AGE	X	35.4	38.3	34.8	36.3
	SD	7.6	5.5	7.3	6.9

Treatment Facilities - The treatment facilities from which subjects were recruited met the following criteria: 1) The

treatment goal was abstinence. This goal and the similarity of goal across sites allowed us to better assess the common effects of central variables. 2) The treatment episodes were approximately one month and all episodes had a well-defined ending date. This similarity eliminated length of treatment as a confounding variable. 3) The treatment facilities served clients who were sufficiently representative of the populations within the drug group so that the data obtained would be meaningful, while allowing us to equate drug groups on demographic characteristics.

Smokers were recruited from an outpatient smoking treatment program offered by our research group at San Francisco General Hospital, a large public teaching hospital. Alcoholics were recruited mostly from two residential programs. Both used Alcoholics Anonymous (AA) extensively. Opiate users were recruited from four sources, three 21 day detoxification clinics, and one 28 day residential facility. The outpatient clinics provided methadone detoxification and supportive counseling. The inpatient program was based on an adaptation of the AA model.

Procedures - Subjects were recruited at their treatment site during the last third of treatment. Only clients who had acceptable attendance and were abstinent were recruited. Abstinence was verified by two biochemically validated self-reports at least 72 hours apart. Clients agreeing to participate underwent a screening procedure and completed an informed consent. A study intake assessment battery, including treatment history, drug and alcohol use, as well as measures of mood, withdrawal symptoms, and life events, was administered prior to treatment completion. Follow-up assessments were completed once a week for 12 weeks (beginning with the first week following treatment end), or until the subject relapsed. Subjects' drug and alcohol use, moods, and withdrawal symptoms were monitored during follow-up assessment. We recognized this procedure could confound perceived social support with short-term treatment outcomes. Nevertheless, because all of the alcohol and some of the opiate subjects were recruited from inpatient facilities they could not report on social support from their environment until they returned to it.

Measures - (1) Social Network and Support Inventory (SNSI). This instrument, developed this study, contains three measures of social support: functional social support, support specific to drug use, and structural social support (social networks). The functional support measure was predominantly influenced by the work of Sarason et al. (1983). Our measure taps emotional and instrumental support and negative aspects of social support. (2) The Interaction Questionnaire (IAQ). This measure was adapted from the Partner Interaction Questionnaire (PIQ) originally used with smokers (Mermelstein et al., 1983). The original questionnaire is a measure of the frequency of responses by one's spouse about smoking and of the perceived

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helpfulness of these behaviors to the subject in maintaining abstinence. We revised the questionnaire to be appropriate for any intimate dyadic relationship between adults who live together. (3) Drug-Use. Self-report of abstinence was verified for smokers by expired air carbon monoxide readings of 9 ppm or less. Self-report of abstinence for alcoholics was verified by absence of alcohol in urine specimens. Report of abstinence for opiate addicts was verified by absence of morphine in urine specimens. Urinary alcohol and morphine were assayed by a commercial laboratory licensed and monitored by the State of California. Subjects who were lost to follow-up were coded as having relapsed.

RESULTS

The small and incomplete sample suggests that a generous probability level, for example $p<.10$, would be appropriate. On the other hand, because of the small sample size, conservative statistics including hierarchical regression analyses by sets, could not be used. Data analysis therefore required more tests than ideal. As a compromise, we retained the $p<.05$ significance level. Given these circumstances, interpretation of the results should be considered tentative. For all of the reported analyses, save one four-fold chi-square, prototypic analyses are hierarchical linear regression for continuous dependent variables and logistic regressions for dichotomous dependent variables. In all cases the effects of treatment class (TC) were entered first. We did not include an interaction effect of TC and the independent variable (IV) of interest when the small and unbalanced cell sizes led us to expect an interaction would not be meaningful.

Social Support and Relapse - The hypothesis that greater perceived social support would predict longer time before relapse was confirmed. Higher levels of emotional and instrumental support predicted longer times to relapse for all three drug groups. The third measure of global social support, negative support, did not predict relapse. The measure of abstinence support, IAQ experienced helpfulness scale, only approached significance ($p>.11$). These results are shown in Table 2.

TABLE 2
Measures of Social Support (SS) as Predictors of Time to Relapse

	Emotional	Instrumental	IAQ
Model	F(5,69)=2.94*	F(5,68)=4.81***	F(5,39)= 5.61***
TC	F(2,69)=4.73*	F(2,68)=4.95**	F(2,39)=10.02***
SS (IV)	F(1,69)=4.29*	F(1,68)=5.59*	F(1,39)= 2.69NS
TC x SS	F(2,69)=<INS	F(2,68)=4.29*	F(2,39)= 2.66NS

*p<0.5; **p<0.01; ***p<0.001

Having a "partner" (operationally defined as currently living with "a spouse, intimate partner, or in a household with someone you are close to"), predicted weeks to relapse and relapse vs. not. Those with partners (n=45, 58%) had a longer time to relapse (mean=10.51 weeks) than those who did not (mean=6.22 weeks). Only 33% of subjects with partners relapsed, while 78% of subjects without partners relapsed. These data imply the presence of a partner facilitates abstinence. These results are shown in Table 3.

TABLE 3
Effects of a Partner as a Predictor of Relapse

	Time to Relapse	Relapse vs. Not
Model	$F(3,73)=14.08^{***}$	$X^2_2(2)=23.19^{***}$
TC	$F(2,73)=7.43^{***}$	$X^2_2(1)=7.40^{**}$
Partner	$F(1,73)=27.38^{***}$	$X^2(1)=15.79^{***}$

**p<0.01; ***p<0.001

Greater drug use of spouses and their encouragement of drug use predicted shorter time to relapse (spouse drug use and time to relapse $F(1,25)=5.82$, $p<.05$, model $F(3,25)=5.68$ $p<.01$, TC $F(2,25)=5.61$ $p<.01$ (spouse encouragement of drug use and time to relapse $F(1,25)=6.79$, $p<.05$; model $F(3,25)=6.12$, $p<.005$, TC $F(2,25)=5.79$, $p<.01$).

The drug use of network members and their encouragement of subjects' drug use also predicted relapse and time to relapse. Again, greater drug involvement of network members and encouragement of drug use predicted shorter time to relapse. These results are shown in Table 4.

TABLE 4
Social Network (SN) Drug Use and Encouragement for Drug Use as Predictors of Time to Relapse and Relapse vs. Not

	Network Drug Use (Time to Relapse)	Encouragement Drug Use (Time to Relapse)	Encouragement Drug Use (Relapse vs. Not)
Model	$F(5,63)=5.04^{***}$	$F(5,63)=3.53^{**}$	$X^2_2(3)=11.73^{**}$
TC	$F(2,63)=6.06^{**}$	$F(2,63)=5.54^{**}$	$X^2_2(1)=6.36^{*}$
SN (IV)	$F(1,63)=8.84^{**}$	$F(1,63)=6.06^{*}$	$X^2_2(1)=5.07^{*}$
TC x SN	$F(2,63)=2.11$NS	$F(2,63)=<1$NS	$X^2(1)=<1$NS

*p<.05; ** p<.01; *** p<.001

Social Support and Gender - Preliminary data confirmed the hypothesis that women experienced less support for abstinence than men. On the IAQ, independent of drug group, women reported they experience less helpfulness in maintaining abstinence than did men [male mean=.83; female mean=.30]. It is unlikely that

this difference reflects only a tendency for women to be more open about negative experience. Independent of drug group, women reported receiving more instrumental support (male mean=4.25, female mean=5.05). These results are shown in Table 5. Women also reported receiving more emotional support than men, regardless of drug group (male mean=4.26, female male= 4.83), but this difference did not achieve accepted levels of significance.

TABLE 5
Social Support and Gender

	IAQ	Instrumental Support
Model	F(3,41)=2.87*	F(3,70)=4.61**
TC	F(2,41)=1.98NS	F(2,70)=4.59*
Sex	F(1,41)=4.64*	F(1,70)=4.64*

*p<.05; **p<.01

Independent of drug group, women's networks were different from men's, though these results narrowly missed p<.05. They were more likely to have a partner (X (1) = 3.68, p<.06), 75% of women had partners vs. 52% of men. They were more likely to have2a best friend [X2 (1)=3.51, p<.07; model X2 (2)=9.05, p<.02; TC X2 (1)=5.54, p<.021.

DISCUSSION

These data suggest social support aids abstinence across the three drug groups. They also indicate that sources and types of support may be different for men and women but that these differences do not mitigate the value of social support in preventing relapse.

Several findings of importance emerge. First, general social support predicts time to relapse. Second, "partners" play a critical role in early relapse. Subjects with partners were significantly less prone to relapse in the 12-week post-treatment period. They also had significantly longer periods of time before relapse. Among subjects who had partners, relapse was predicted by partners' behaviors. The greater the experienced helpfulness of the partner the longer was the period before relapse. Not surprisingly, greater drug involvement of partners and more encouragement of subjects' drug use predict relapse and shorter time periods before relapse. These findings may describe a two-stage risk process: to avoid early relapse, it is valuable to have a partner, and, given a partner, risk of relapse is decreased if the partner is not drug-involved and does not encourage drug use. Third, women reported more general social support than men. Also, they experienced abstinence-specific support to be less helpful than did men. It appears the "paths" to abstinence may be different for men and women.

Woman appear more likely to have general social support as a resource. Because of this support, they may not find partner provided specific support to be helpful. Men may have less general social support and may find support for abstinence from their partners to be more helpful than do women.

These data are among the first to indicate that naturally occurring social support, that is, support available in the routine of daily life, can help persons who have problem use of tobacco, alcohol, and opiates to maintain abstinence. General and drug-use specific social support both appear to influence abstinence.

Our study is based on the assumption that indentification of variables associated with relapse that span the addictions is important. It can lead to better understanding of relapse processes and to the developments of an integrative and unified model of relapse. That social support operates across drug groups to aid abstinence reinforces our research approach. The findings of sex differences may assist in further articulating a model of relapse.

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The Discriminative Stimulus Properties of Histamine H1 Antagonists in d-Amphetamine-Trained and Midazolam-Trained Pigeons

Suzette M. Evans and C. E. Johanson

Antihistamines, specifically H1 histamine receptor antagonists, have been shown to have central nervous system (CNS) effects, possessing both depressant and stimulant properties (Douglas, 1985). Since the most common side effect of these drugs is drowsiness and sedation, the majority of studies have focused on the sedative properties of this drug class, particularly in combination with other sedative compounds such as barbiturates and ethanol (Winter, 1948; Hughes et al., 1964; Smith et al., 1974). Despite the fact that H1-antihistamines are also known to possess CNS stimulant properties, few experimental studies concerning these actions have been reported (Douglas, 1985; Wyngarden and Seevers, 1951; Caplan et al., 1982; Poling et al., 1983). Although as a class, antihistamines have both stimulant and depressant effects, within the class, there are differences that appear to be related to the type of antihistamine. For instance, the ethanolamines, of which diphenhydramine is prototypic, have the greatest sedative properties. Promethazine (a phenothiazine), chlorcyclizine and hydroxyzine (piperazines) have sedative properties and hydroxyzine has been used clinically as an anxiolytic (Rickels, 1978). In contrast, the alkylamines (e.g., chlorpheniramine) are reported to produce CNS stimulation (Douglas, 1985) and tripelennamine and pyrilamine (ethylenediamines) also produce relatively more CNS stimulation.

In spite of the recognition that antihistamines have CNS effects, there are few laboratory studies on their behavioral effects. One way of evaluating different Hl-antihistamines with respect to their relative sedative/stimulant properties is by using drug discrimination methods which have proven useful in terms of classifying pharmacologically related drugs (Schuster and Balster, 1978). Although there have been a few studies on the discriminative stimulus properties of antihistamines (Overton, 1978; White, 1985; Winter, 1985; Karas et al., 1985), only one of these studies (Winter, 1985) have not been designed to evaluate their sedative/stimulant properties. Therefore, the purpose of the present study was to examine the discriminative stimulus properties of a wide variety of types of H1-antihistamines in terms of their ability to substitute for the psychomotor stimulant &amphetamine or the sedative-hypnotic midazolam in pigeons. The HI-antihistamines tested were prototypic for their type and care was

taken to select compounds that were reported to be at the extremes of the sedative/stimulant continuum. The sedative compounds tested included diphenhydramine, promethazine, hydroxyzine, and chlorcyclizine. The less sedative H1-antihistamines tested included chlorpheniramine, pyrilamine, and tripelennamine. The HZ-antagonist, cimetidine, which does not act centrally, was also tested as a control.

METHODS

Animals. The animals used in this study were eight white Carneaux pigeons maintained at 80% of their free-feeding weight and housed individually with water and grit freely available. To supplement food obtained during the experimental session, Purina Pigeon Checkers were provided after the session to maintain reduced weights. Three of the pigeons (#408 428 and 3380) had previously been trained to discriminate 2.0 mg/kg intramuscular (i.m.) d-amphetamine from saline (Evans and Johanson, submitted) and had been tested with a variety of Drugs. The other five pigeons were trained to discriminate 1.0 (#2779) or 3.0 mg/kg (#1490, 1859, 3315, 7227) midazolam i.m. from saline and had been tested with a variety of drugs other than antihistamines.

Apparatus. The experiment was conducted in two ventilated custom-made operant chambers, each equipped with two translucent response keys which were transilluminated during the experimental session. Purina Pigeon Checkers were made available from a food magazine which was illuminated during food delivery.

Training. The discrimination training procedure used has been described in detail previously (de la Garza and Johanson, 1985). During each experimental session each pigeon was injected intramuscularly with the training drug (d-amphetamine or midazolam) or saline in a 1 ml/kg volume 10 minutes before the session. Following this pretreatment period, the experimental session began, signalled by the illumination of both keys and the houselight. Thirty consecutive responses (fixed-ratio 30; FR 30) on the injection-appropriate key resulted in 3-see access to food. The left key was correct after drug administration for 2 d-amphetamine pigeons and 3 midazolam pigeons. The right key was correct after drug for the remaining pigeons. The opposite key was designated correct following saline injections. The drug-key-reinforcer relationship was maintained throughout the experiment. Responses on the incorrect key reset the fixed-ratio requirement on the correct key. Each session lasted until 50 reinforcers were delivered or until 30 minutes had elapsed, whichever occurred first.

Training sessions continued until the percent of total responses on the correct key was above 90% and the number of responses emitted on the incorrect key before the first reinforcer was delivered was less than 30 for seven consecutive sessions. The injection preceding each session was selected from a pseudorandom sequence, with the restriction that no condition would occur for three consecutive sessions.

Testing. In order to evaluate the stimulus properties of various antihistamines, these compounds were administered instead of drug (d-amphetamine or midazolam) or saline during test sessions. Throughout a test session, 30 consecutive responses on either the drug-appropriate or saline-appropriate key resulted in food delivery. In all other respects test sessions were identical to training sessions. Between test sessions, training sessions continued. The training drug and saline under training conditions were administered in a double alternation sequence with test sessions inserted every third session, i.e., saline, drug, test, drug, saline, test, etc. If an animal failed to meet the training criteria during a training session, further testing was postponed until the animal met these criteria on at least two consecutive training sessions.

Initially a dose-response function for the training drug was established during test sessions. Subsequently, the discriminative stimulus properties of several antihistamines were tested in a similar manner. In general, each dose of a test compound was tested once and three or four doses of each compound were tested in a mixed order. The dose-response function for each compound was completed before another compound was tested. Dose-response functions for each drug were determined in at least three pigeons. After the completion of each dose-response function, pigeons were given test sessions with either the training drug or saline.

Data analyses. A drug was considered to produce discriminative stimulus effects similar to those of the training drug if at least 80% of the total responses during the test session were emitted on the drug-appropriate key. The discrimination data are presented as the percentage of total responses emitted on the drug key (&amphetamine or midazolam) for individual pigeons or as the percentage of animals tested at a particular dose that reached the 80% criterion. In addition, response rate (resp/sec) on the two keys was determined for each session. A test compound was tested until a dose was given that resulted in at least 80% of the responses occurring on the drug-appropriate key or until a dose was reached which substantially reduced response rate.

Drugs. The following drugs used in this experiment were gifts: d-amphetamine sulfate (National Institute on Drug Abuse), tripelennamine hydrochloride (CIBA-Geigy Corp., Summit, NJ), hydroxyzine hydrochloride (Pfizer Inc., Brooklyn, NY), cimetidine hydrochloride (Smith, Kline and French Laboratories, Philadelphia, PA), chlorcyclizine hydrochloride (Burroughs-Wellcome and Co., Research Triangle Park, NC), chlorpheniramine maleate (Whitehall Laboratories Inc., Hammonton, NJ), midazolam maleate (Hoffman-La Roche, Inc., Nutley, NJ), diphenhydramine hydrochloride and promethazine hydrochloride (Wyeth Laboratories, Inc., Philadelphia, PA). The drugs were dissolved in 0.9% saline and the doses are expressed in terms of the salt.

RESULTS

Control performances. In the d-amphetamine-trained group, all three pigeons responded above 90% on the correct key thoughout most of

the experiment. During test sessions the rate of responding across the three pigeons ranged from 1.31 to 2.89 resp/sec following saline and from 1.27 to 2.79 resp/sec following d-amphetamine (2.0 mg/kg). d-Amphetamine produced a dose-related increase in the percentage of responses emitted on the d-amphetamine-appropriate key. At the lowest dose tested (0.3 mg/kg), less than 4% of responding occurred on the d-amphetamine-appropriate key in all three pigeons whereas at 1.0 and 3.0 mg/kg more than 90% of responding occurred on the d-amphetamine-appropriate key. d-Amphetamine also produced a dose-related decrease in rate of responding.

Similar results were obtained in the midazolam-trained group. All five pigeons responded above 90% on the correct key throughout most of the experiment. During test sessions the rate of responding across the five pigeons ranged from 1.06 to 2.39 resp/sec following saline and from 0.33 to 1.98 resp/sec following the training dose of midazolam. Midazolam produced a dose-related increase in the percentage of responses emitted on the midazolam-appropriate key. At the lowest dose tested (0.3 mg/kg), less than 25% of responding occurred on the midazolam-appropriate key in all five pigeons whereas at the training dose (1.0 or 3.0 mg/kg) more than 95% of responding occurred on the midazolam-appropriate key. A higher dose (10.0 mg/kg) produced greater than 95% midazolam-appropriate responding in the 3 pigeons tested. Midazolam also produced a dose-related decrease in response rate.

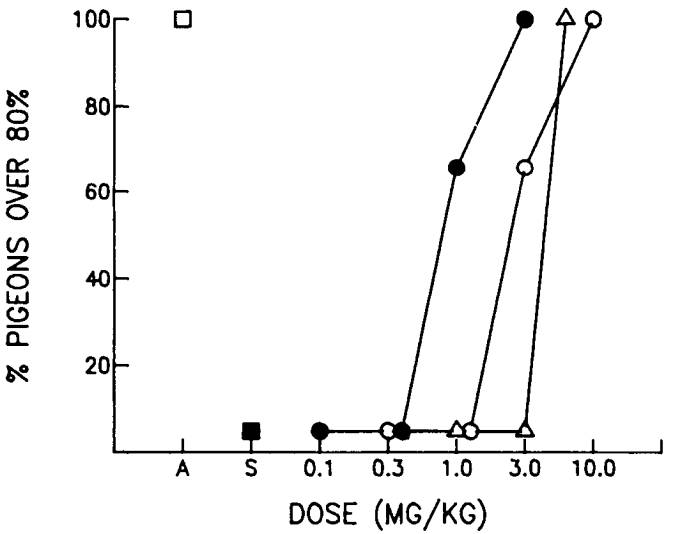

Figure 1. The percentage of d-amphetamine-trained pigeons responding at least 80% on the d-amphetamine-appropriate key when tested with diprenhydramine ( ), chlorpheniramine ( ), tripelennamine ( ) or test sessions of 2.0 mg/kg d-amphetamine ( ) or saline ( ).

Antihistamines. Of the seven H1 receptor antagonists tested in the d-amphetamine group, the ethylenediamine, tripelennamine (1.0 to 3.0 mg/kg), the ethanolamine, diphenhydramine (3.0 to 10.0 mg mg/kg) and the alkylamine, chlorpheniramine (5.6 mg/kg) substituted for d-amphetamine in all pigeons tested (Fig. 1). There was little effect on response rate at doses that substituted for d-amphetamine.

Two other antihistamines did not share discriminative stimulus properties with d-amphetamine in all pigeons tested (Fig. 2). Pyrilamine, an ethylenediamine, produced more than 80% d-amphetamine-appropriate responding in 2 of the 3 pigeons tested at 5.6 or 10.0 mg/kg (Fig. 2). Response rate was substantially reduced in the pigeon given 10.0 mg/kg whereas 5.6 mg/kg substituted for d-amphetamine in another pigeon without affecting response rate relative to d-amphetamine rates. Only partial substitution was observed with #3380 (57% at 5.6 mg/kg and 54% at 10.0 mg/kg) and these doses substantially reduced response rate. Chlorcyclizine, a piperazine, produced greater than 80% d-amphetamine-appropriate responding with no effect on response rate at 10.0 mg/kg in one pigeon. However, chlorcyclizine failed to substitute for d-amphetamine in 2 pigeons up to doses that substantially reduced response rate.

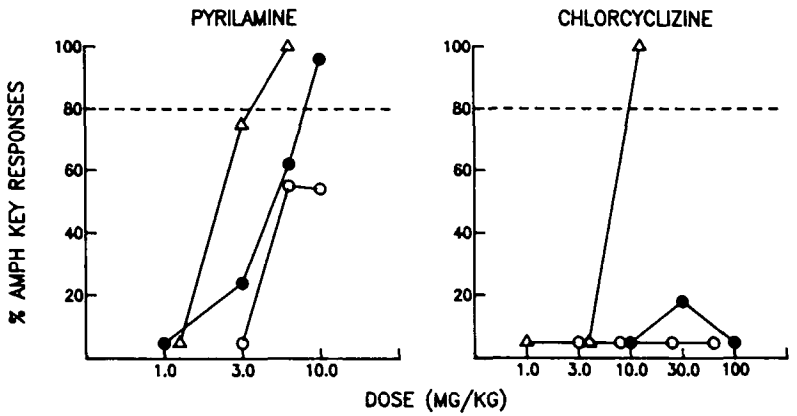

Figure 2. Percent of d-amphetamine responses as a function of dose during substitution test sessions with pyrilamine and chlorcyclizine for pigeons #408 (△), #428 (●), and #3380 (○).

In contrast, the phenothiazine, promethazine (1.0-17.0 mg/kg) and hydroxyzine (10.0-56.0 mg/kg), a piperazine, were not similar to d-amphetamine since neither of these compounds substituted for d-amphetamine up to doses that substantially reduced or completely

suppressed responding. Furthermore, the peripherally acting H2-receptor antagonist, cimetidine (3.0-100.0 mg/kg), failed to substitute for d-amphetamine, although response rate was not reduced at 100.0 mg/kg.

Every antihistamine tested in the midazolam-trained group, which included diphenhydramine (1.0-56.0 mg/kg), hydroxyzine (1.0-56.0 mg/kg), pyrilamine (1.0-17.0 mg/kg) and tripelennamine (0.3-10.0 mg/kg), failed to substitute for midazolam in all pigeons tested. Doses were tested until response rate was substantially reduced or completely suppressed. The doses tested in the midazolam-trained group were comparable to or slightly higher than the doses of these antihistamines tested in the d-amphetamine-trained group.

DISCUSSION

The results of the present study demonstrate that H1-receptor antagonists have differential discriminative stimulus properties in the pigeon. In pigeons trained to discriminate d-amphetamine from saline, tripelennamine, chlorpheniramine, diphenhydramine and to a certain extent, pyrilamine, substituted for d-amphetamine. These results are consistent with other studies indicating that tripelennamine, chlorpheniramine and pyrilamine have less sedative properties than other types of antihistamines. In contrast, the antihistamines, promethazine, hydroxyzine and chlorcyclizine, reported to have more pronounced sedative properties, failed to substitute for d-amphetamine, with the exception that chlorcyclizine substituted for d-amphetamine in one pigeon. Furthermore, the H2-receptor antagonist, cimetidine, also failed to substitute for d-amphetamine, although this negative result is most likely due to its inability to penetrate the CNS as suggested by high doses (100.0 mg/kg) having no effect on response rate. Interestingly, diphenhydramine, one of the more sedative antihistamines, also substituted for d-amphetamine. At this time there is not enough information regarding the discriminative stimulus properties of antihistamines to adequately interpret this finding.

While the sedative/stimulant properties of H1-antihistamines have not been previously investigated in groups trained to discriminate a stimulant (&amphetamine) or a sedative-hypnotic (midazolam), other studies have demonstrated that H1-antihistamines can serve as discriminative stimuli. In pigeons trained to discriminate tripelennamine from saline (Karas et al., 1985) pyrilamine and diphenhydramine substituted for tripelennamine. Although chlorpheniramine only showed a partial substitution for tripelennamine, the authors concluded that higher doses should have been tested. On the other hand, promethazine only partially substituted and cimetidine failed to substitute for tripelennamine as did compounds from other drug classes including diazepam, morphine and phenobarbital. d-Amphetamine was also tested in this study and a dose of 1.0 mg/kg produced some tripelennamine-appropriate responding. However since higher doses were not tested it difficult to interpret these findings. In summary, the results of Karas et al. (1985) as well as the present study clearly indicate that within the class of antihistamines there are differences in discriminative stimulus properties.

However, in contrast to drug discrimination results in pigeons, other studies using H1-antihistamines as discriminative stimuli in rats report that antihistamines do not show differential discriminative stimulus effects. For instance, promethazine, a more sedative antihistamine, produced drug-appropriate responding in both diphenhydramine as well as chlorpheniramine-trained rats, even though chlorpheniramine is a less sedative antihistamine (Winter, 1985). In addition, H1-receptor antagonists tested in pyrilamine-trained rats, including diphenhydramine, promethazine and tripelennamine, substituted for pyrilamine even though they differ in their ability to induce sedation in humans (White, 1985).

Although the H1-antihistamines tested in the midazolam-trained group varied in their sedative/stimulant properties as well as in their a ability to substitute for d-amphetamine, none of these compounds substituted for midazolam. This included hydroxyzine, which was chosen because of its use clinically as an anxiolytic (Rickels, 1978).

In summary, the present study demonstrated that the antihistamines known to produce more "CNS stimulation" share discriminative stimulus properties with &amphetamine in pigeons. With the exception of diphenhydramine, other sedative H1-antihistamines did not substitute for d-amphetamine. However, even those more sedative-like H1-antihistamines tested failed to substitute for midazolam in pigeons.

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The Effects of 12-Hour Limited Access to Cocaine: Reduction in Drug Intake and Mortality

Steven I. Dworkin, Nick E. Goeders, John Grabowski, and James E. Smith

INTRODUCTION

Studies investigating contingent cocaine presentation have shown the drug to be a very efficacious reinforcer. The drug is self-administered by a number of species including humans under both simple and complex schedules of reinforcement. However, very few studies have reported any toxic consequences of cocaine administration (for review see Johanson 1984). Moreover, the primary condition necessary for observing a severe toxic effect (high subject mortality) appears to be not restricting access to the drug. Most studies investigating cocaine self-administration have used limited-access conditions (i.e. session durations of 6 hours or less). Limited-access conditions have been favored because they result in consistent daily-drug intake and little or no signs of drug toxicity (for review see Pickens, Meisch, and Thompson 1978). However, parametric investigations of the effects of experimentor controlled access to the drug with respect to eliminating or reducing mortality have not been reported. This study reports the effects of restricting drug intake to every other hour during 24 hour sessions. Twelve-hour access to cocaine resulted in a significant decrease in drug intake and eliminated subject fatality observed under conditions of unlimited access.

METHOD

Subjects

Male Fisher-344 rats between 90-150 days old at the beginning of the study were used in these experiments. All twelve subjects were continuously housed in operant-conditioning chambers containing three retractable levers and maintained on a reversed 12 hour light/dark cycle. Each chamber contained a food dispenser, a water dipper and a motor driven syringe pump.

Food and Water

The rats were initially trained on a two, lever concurrent chained schedule which has been previously described (Dworkin et al 1984). Briefly, two, levers were made available and a single response on either lever resulted in the retraction of the other lever. Nine additional responses resulted in the presentation of a 45 mg food pellet if the food lever was selected or 10 s of access to an 0.1 ml dipper of tap water if the other lever was selected. A 100 s limited hold contingency timed from the first response and a 30 s time out after reinforcer presentation or after the elapse of the limited hold were also scheduled.

Surgical Procedure

Following training on the food and water schedule the rats were implanted with chronic jugular catheters using previously described methods (Weeks 1962, 1972). The animals were then given unrestricted access to food and water and received hourly 0.2 ml infusions of heparinized saline for 2 days.

Cocaine

Following recovery from the surgical procedure the rats Were again placed into the three-lever conditioning chambers. A third lever along with the food and water levers was extended into the chamber and responses on this lever resulted in cocaine infusions via the jugular catheter. Cocaine injections consisted of a 0.5 mg/kg/inj dose administered over a 4.9 s period with each infusion paired with a 30 s tone. Limited access to cocaine was scheduled by withdrawing the cocaine lever for one hour periods every other hour. The food and water levers were continuously available.

RESULTS

Food and Water Intake before Cocaine

Responding maintained by the food and rater contingencies before the addition of the cocaine was similar to patterns previously reported using this schedule (Dworkin et al 1984). Once responding was initiated on either the food or water lever a high rate of responding was observed until delivery of the selected reinforcer. A large number of consecutively completed ratios occurred on both levers. The mean daily food intake (271 ± 39) was almost 2 1/2 times the mean daily rater intake (106 ± 41), resulting in a mean daily intake of 12 gms of food and 11 gms of water which is close to the 1 to 1 ratio of food to water intake usually observed in 24 hour studies.

TABLE I

Mean Daily Food, Water and Drug Intake

	Number of Reinforcers		
	Before Cocaine	Unlimited Access	Limited Access
Food	271 ± 39	57 ± 85	259 ± 46
Water	106 ± 41	62 ± 72	88 ± 37
Cocaine		84 ± 51	21 ± 17
	N=11	N=7	N=4

Values are means ± standard deviation for the last three days under each condition.

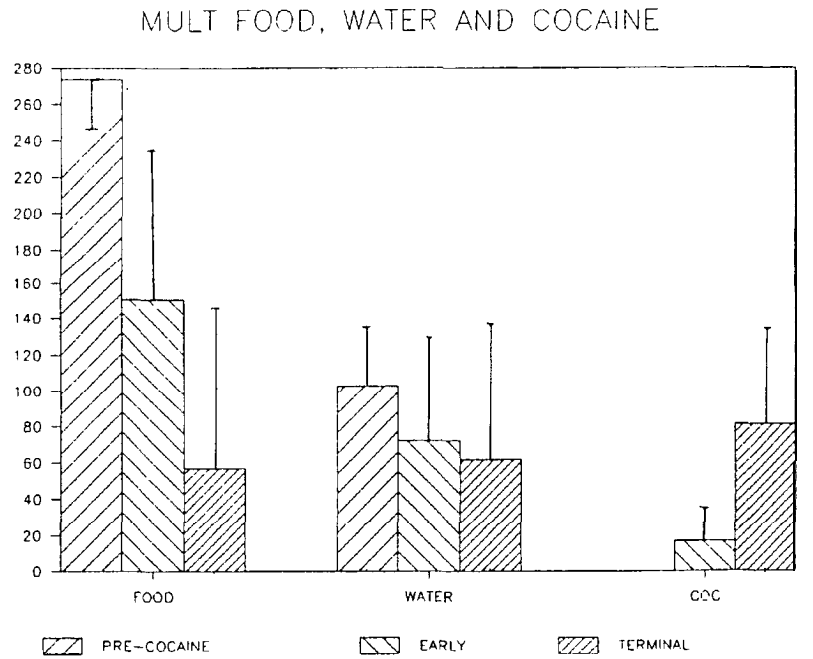

Figure 1. Mean number of reinforcers delivered per day for the last three sessions before drug was introduced (pre-cocaine), three sessions <5 days after the drug contingency was added (early), and the last three sessions (terminal). Vertical Lines indicate 1 Standard Deviation.

Continuous Access To Cocaine

Seven subjects rare given continuous access to food, water and cocaine. Six of these rats (86%) died within 20 days. The mean food, water and drug intake for these rats are shown in Figure 1. As show in Table 1 drug intake increased from a mean daily intake of 18 ± 17 injections (first 3 days of access) to 84 ± 51 injections (last 3 days before death). Food intake was also affected by unlimited access to cocaine. Responding on the food lever decreased 79%, resulting in a mean daily food intake of 2.6 gms. Continuous access to cocaine increased water intake in one rat and decreased water intake in the others.

Limited Access to Cocaine

The access to cocaine for 4 rats was reduced to 12 hours/day. Responding on the cocaine lever was initially engendered using unlimited access. However, when significant drug intake was observed these animals were placed on a schedule allowing alternate hour access to cocaine. Thus the cocaine lever was retracted during odd hours and extended during even hours. All four subjects exposed to this limited access procedure survived for 90-120 days at which time they were sacrificed. The limited access to cocaine did not result in significant changes in responding on either the food or water levers (See Table 2). however, drug intake was reduced compared to intake during unlimited access days.

DISCUSSION

Continuous access to cocaine has been reported to result in a high subject fatality rate in rats (Bozarth and Wise 1985) and monkeys (Deneau et al 1969; and Johanson et al 1976). This study further supports these observations. A similar schedule of food, water and morphine presentation (Dworkin et al 1984) did not result in any fatalities for up to 6 months of continuous exposure. Thus, this study further suggest that the toxicity associated with short-term continuous access to cocaine is greater than drug toxicity with continuous 1 long-term access to morphine.

Twelve-hour limited access to cocaine did not result in any fatalities. Cocaine appears to be extremely toxic under conditions where drug intake is totally under the subject's control. However, even minimal restraints on drug availability (access every other hour) severely attenuates the toxicity associated with the drug.

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Effects of Diazepam and Delta-9-THC on the Discrimination of Speech Sounds in the Baboon

Robert D. Hienz and Joseph V. Brady

INTRODUCTION

Numerous studies have documented the effects of drugs on various aspects of cognition or attention; relatively little is known, however, about drug effects on basic sensory processes. An assessment in terms of sensory function can be important, since impairment of sensory function may contribute to a drug's overall abuse liability either directly, or indirectly through effects on more complex behavioral processes (Brady and Lukas, 1984). In extending the assessment of the effects of drugs of abuse on sensory function in nonhuman primates, we have developed and applied a psychophysical procedure for evaluating the discriminability of human speech sounds in baboons. Speech sound discriminations using periodic, spectrally complex vowel sounds can provide a relatively simple method for assessing frequency resolving power in the auditory system, and deficits in such discriminations may be readily applicable to human situations. The present report describes the results of experiments designed to establish discriminations between five different vowel sounds, and to evaluate the effects of two commonly abused drugs, diazepam and delta-9-THC, on the discriminability of these vowels.

METHOD

Subjects: The subjects were three dog-faced baboons (Papio anubis), housed in individual cages and maintained on a 22-hr restricted feeding schedule with supplemental monkey chow and fresh fruit provided daily after each experimental session. Each animal had a previous drug history with diazepam, but not with delta-9-THC.

Apparatus: The testing apparatus consisted of a modified baboon squeeze cage fitted within a double-walled sound attenuating chamber (IAC Inc., Model 1201 A). A 76 x 97 cm intelligence panel attached to one side of the cage contained a primate lever (BRS/LVE Model PRL-003), a red light-emitting diode used as a cue light, and a tube feeder for delivery of banana pellets. Vowel stimuli were delivered through a wide-range speaker suspended outside the cage and located directly over the animal's head, approximately 20 cm above ear level.

Steady-state vowels were produced via an Echo][speech synthesizer controlled by an Apple IIE computer. The vowel sounds used were /a/ /ae/, /ɔ/, /U/, and /e/. Fundamental pitch frequency was 122 Hz. All stimuli were 120 msec in duration, and were presented at a rate of 2/set. The average intensity of the vowels was 83 dB.

Procedure: Vowel discriminations were conducted using a standard reaction time procedure. Throughout the entire session a background train of sounds of one vowel was presented (e.g., / / as in "caught"). This standard vowel was presented at a rate of 2/sec. A flashing red cue light (5/set) signalled the start of each trial. In the presence of the cue light a lever press changed the flashing red light to a continuous red light which remained steady as feedback as long as the animal held the lever down. At intervals ranging from 1.0 to 7.3 sec after initiation of this maintained holding response, a stimulus change occurred which consisted of two alternations of the standard vowel with one of the four comparison vowels. Release of the lever during this alternation period was considered a correct detection of the change in vowel sounds, and resulted in delivery of the reinforcer (two 190-mg banana-flavored pellets). A 3-sec intertrial interval (ITI) followed reinforcement; during this time lever responses re-initiated the ITI. Lever releases prior to comparison stimulus onset produced a 3-sec timeout, then reinstated the 3-sec ITI without reinforcement. If an animal failed to release the lever during a stimulus change, the red cue light was turned off following the second comparison stimulus offset, and lever release then returned the animal to the ITI. Following the ITI, the flashing cue light signalled initiation of the next trial in the series of several hundred which comprised each daily two to three hour experimental session.

Vowel detection scores were determined by randomly selecting on each trial one of the four comparison vowel sounds to alternate with the standard vowel sound, and examining detection frequencies (i.e., percent correct lever releases) for each comparison vowel. To measure the false alarm or "guessing" rate of each subject, "catch" trials were interspersed among the normal trials during testing sessions. During these catch trials no comparison vowel sounds were presented, and lever releases during catch trials were punished with a 3-sec timeout.

Test sessions were divided into blocks of 140 trials with each of the four comparison vowels plus catch trials presented randomly approximately 28 times during each block. Four to five such blocks of trials occurred within each session to provide a number of separate within-session estimates of detection frequencies. Performances were considered stable when false alarm rates were below 30% for each block of trials within a session, and no systematic changes in detection frequencies were evident. Each animal was tested with two different vowel sets; for the first set /ɔ/ was the standard vowel, while for the second set /ae/ was the standard vowel.

Drug Administration, During diazepam testing, a single i.m. injection was given at the beginning of each experimental session, immediately after placing an animal in the chamber. On test days, the animals received either drug vehicle or one of the doses of drug; i.m. saline injections were given on all non-test days. Diazepam was administered i.m. in the gluteal region at doses of 0.32, 1.0, 3.2, and 10.0 mg/kg. Diazepam was dissolved in a vehicle containing propylene glycol, polyethoxylated vegetable oil, and ethanol (20:20:60). The alcohol was evaporated before injection. All drug doses were prepared immediately before injection and concentrations were adjusted to an injectable volume of 0.5 to 1.0 ml. Delta-9-THC was given orally by injecting the drug into a slice of orange, and watching the animal consume the orange slice at the start of the session. Doses of delta-9-THC given were 0.32, 1.0, 3.2, and 5.6 mg/kg. On non-drug days animals were given either an orange slice alone or an orange slice injected with ethanol (vehicle). Doses of both drugs were given in mixed order, and subsequent drug administrations were scheduled only after vowel detection frequencies returned to baseline values and no further changes were evident.

RESULTS

Figure 1 shows the basic effects of diazepam and delta-9-THC on the discriminability of the four comparison vowels /a/, /e/, /ae/, and /U/ from

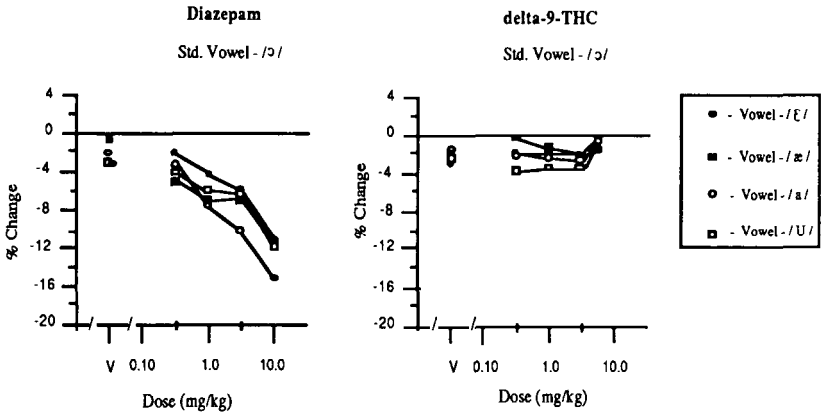

Figure 1. Mean percent changes in vowel discriminability for diazepam (left) and delta-9-THC (right) as a function of drug dose. Each curve is for the indicated comparison vowel, and represents the mean of three animals.

the standard vowel /ɔ/ (first vowel set). Plotted are the percent changes in vowel discriminability as a function of drug dose averaged across the three animals. These averages were based on differences between the peak drug effect and the mean of all blocks of the preceding saline control day averaged across replications at each dose, where the peak drug effect was defined as the lowest percent correct found across all blocks of a drug session. Vehicle control data were derived in an identical manner. Diazepam produced clear decreases in vowel discriminability for all four comparison vowels, with greater decrements occurring at the larger diazepam doses (Figure 1, left). Delta-9-THC, on the other hand, had no effect on vowel discriminability at any of the doses tested. Higher doses of both drugs produced cessation of responding in all animals. Significantly, the effects of diazepam were most prominent with the vowel /a/, the vowel that had the smallest changes in formant frequency structure from the standard vowel /ɔ/. Changes in formant frequency for the 4 comparison vowels, compared to the standard, were 198, 280, 635, and 685 Hz for the second formants, and 56, 130, 43,

and 133 Hz for the first formants of vowels /a/, /U/, /e/, and /ae/, respectively.

Figure 2 shows the effects of diazepam and delta-9-THC on vowel discriminability for the second vowel set (/ae/ as the standard vowel), plotting the mean percent change in vowel discriminability for each

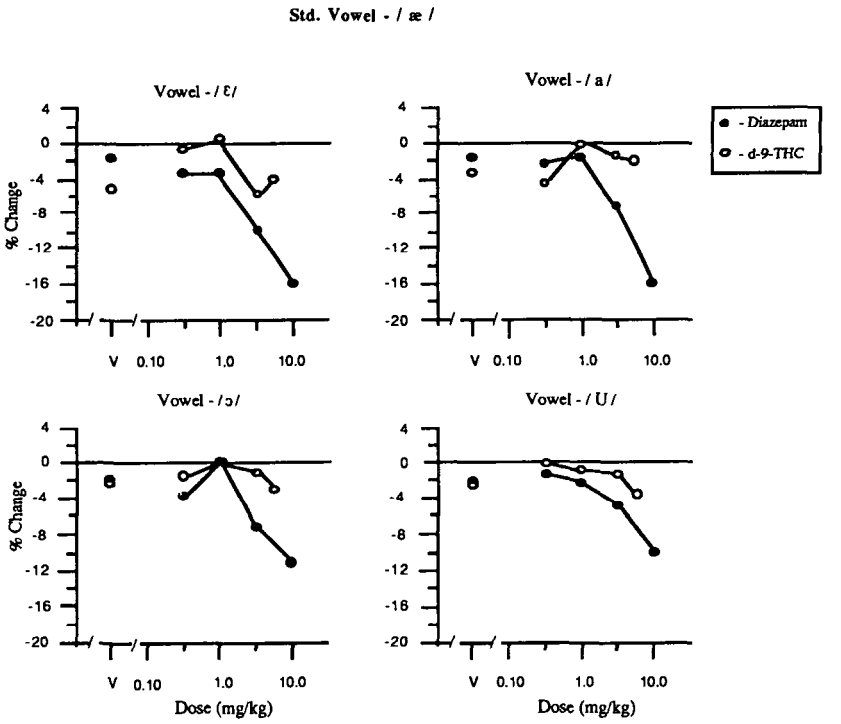

Figure 2. Comparisons of mean changes in vowel discriminability for diazepam (filled symbols) and delta-9-THC (open symbols) for the second set of vowels, with /ae/ as the standard vowel in each case. Each data point is a mean for three animals.

comparison vowel averaged across all three animals. Each comparison vowel is graphed separately to show more clearly the differences between diazepam and delta-9-THC. Again, diazepam produced clear, dose-dependent decreases in vowel discriminability for all 4 comparison vowels, while such changes did not occur with delta-9-THC. For this second set of vowels, changes in formant frequency from the standard vowel were -77, -90, -133, and -203 Hz for first formants, and -487, -50, -685, and -405 Hz for second formants

of vowels /a/, /e/, /ɔ/, and /U/, respectively. Thus the smallest formant frequency changes occurred for /e/ and /a/, the vowels showing the largest decrements in discriminability following the higher doses of diazepam.

DISCUSSION

The present results clearly differentiate between the effects of diazepam and delta-9-THC on the discrimination of vowel sounds in baboons. Diazepam produced consistent dose-related decrements in vowel discriminability, while delta-9-THC did not produce such consistent changes. These results are in accord with previous findings that diazepam also affects auditory function by elevating auditory thresholds over the same dose range (Lukas et al., 1985). It is unlikely, however, that the presently observed decreases in vowel discriminability could be attributed solely to the concomitant elevations of only 4 to 8 dB in auditory thresholds that have been reported to occur at diazepam doses of 3.2 and 10.0 mg/kg, (Lukas et al., 1985). Since auditory thresholds in the baboon range from 0 to 10 dB SPL for pure tones between 500 Hz and 2 kHz (Hienz et al., 1982), the present vowel intensities would have been 73-83 dB above threshold level. Previous studies have shown that animals can easily make vowel discriminations with vowel stimuli ranging from 50-70 dB above threshold levels (Dewson et al., 1969; Hienz et al., 1981). Thus even taking into account the effects of diazepam on auditory thresholds, the present stimuli were well within an intensity range over which such discriminations are readily made.

The lack of consistent changes in the discriminability of vowels following administration of delta-9-THC suggests the possibility that the discriminations were simply not difficult enough to show decrements in performance following drug administration. Evidence in favor of this interpretation is suggested by the fact that delta-9-THC has been previously shown to produce decrements at similar doses (2 and 4 mg/kg) in the accuracy of an auditory click frequency discrimination (click rates of 10.0 Hz vs. 9.4 Hz) in monkeys when baseline performance levels were less than 100% (Elsmore, 1972). Thus it may be that delta-9-THC can be shown to affect more difficult speech sound discriminations. It is nonetheless clear from the present data that diazepam more readily affects these types of discriminations than does delta-9-THC over the indicated dose ranges.

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Potentiation of Pentobarbital Sleeping Time by the Acute and Chronic Administration of Morphine

Robert N. Pechnick, Gregory W. Terman, and John C. Liebeskind

INTRODUCTION

During preliminary experiments examining the effects of pentobarbital anesthesia on morphine-induced analgesia in the rat, it was noted that pentobarbital sleeping time was greatly lengthened after the acute administration of morphine. While previous studies have concluded that the enhancement of barbiturate-induced sleeping time is due to inhibition of hepatic drug metabolizing enzyme activity (Bousquet et al. 1964; Ho et al. 1976a and 1976b), the exact mechanism of this interaction is not known. The present study examined the role of opiate receptors in the mediation of morphine-induced potentiation of pentobarbital sleeping time by testing two criteria for opiate receptor involvement: reversal by opiate antagonists, and the development of tolerance.

METHODS

Male Sprague-Dawley rats (370-420 g; Simonsen Laboratories, Cilroy, CA) were housed 6 per cage at 22-24°C under a 12-12 hr light-dark cycle (lights off 08:00-20:00 hr) for at least 14 days prior to the experiment. Standard rat chow and water were available ad libitum.

The first series of experiments evaluated the effect of acute morphine administration on pentobarbital sleeping time. Rats were randomly assigned to treatment groups. All injections were administered between 11:OO and 12:00 hr with a volume of 1.0 ml/kg. In the first experiment rats were injected s.c. with either saline (0.9%) or morphine sulfate pentahydrate (5.0 or 10.0 mg/kg), immediately followed by an i.p. injection of sodium pentobarbital (Nembutal; 50.0 mg/kg). Sleeping time, defined as the period from the moment of loss of the righting reflex until its return, was determined. In the second experiment rats were injected s.c. with either saline, morphine (10.0 mg/kg), naltrexone hydrochloride (2.0 mg/kg), or both morphine (10.0 mg/kg) and naltrexone (2.0 mg/kg). All

groups then received an i.p. injection of pentobarbital (50.0 mg/kg).

The final experiment assessed the effect of chronic morphine administration on pentobarbital sleeping time. Rats were anesthetized with halothane and implanted s.c. with either a placebo pellet, a morphine pellet (75.0 mg base), a naltrexone pellet (30.0 mg base), or both a morphine and a naltrexone pellet. Three days later pentobarbital (50.0 mg/kg) was injected i.p. and sleeping time recorded.

The data were analyzed by one-way analysis of variance followed by Student's t test. The criterion for rejection of the null hypothesis was $p < 0.01$.

RESULTS

The acute administration of morphine produced dose-dependent increases in pentobarbital-induced sleeping time (table 1); the administration of 10.0 mg/kg of morphine increased the mean duration of sleep by 73.8%. The acute injection of naltrexone alone had no effect on pentobarbital-induced sleeping time (table 2): however, naltrexone pretreatment completely blocked the acute morphine-induced potentiation of sleeping time.

Table 1

The effect of the acute administration, of morphine on pentobarbital-induced sleeping time

Group	Sleeping Time (min)
Saline	111.5 ± 4.8
Morphine (5.0 mg/kg)	162.7 ± 6.8*
Morphine (10.0 mg/kg)	200.8 ± 15.3*

Values represent means ± the standard errors of the mean (N=5-6 per group).
**p < 0.01 compared to saline controls.*

The mean sleeping time of naltrexone-pelleted rats was the same as that of placebo-pelleted controls (table 3). Morphine-pelleted rats showed a 134.0% increase in pentobarbital-induced sleeping time compared to placebo-pelleted subjects. This potentiation was not seen in rats that were also implanted with a naltrexone pellet.

Table 2

The effect of naltrexone pretreatment on the morphine-induced increase in pentobarbital sleeping time

Group	Sleeping Time (min)
Saline	99.5 ± 6.6
Naltrexone (2.0 mg/kg)	97.5 ± 5.1
Morphine (10.0 mg/kg)	153.7 ± 12.3*
Morphine (10.0 mg/kg)/ Naltrexone (2.0 mg/kg)	96.3 ± 6.5

Values represent means ± the standard errors of the mean (N=6 per group).
**p < 0.01 compared to saline controls.*

Table 3

The effect of chronic administration of morphine and/or naltrexone on pentobarbital sleeping time

Group	Sleeping Time (min)
Placebo Pellet	142.8 ± 11.1
Naltrexone Pellet (30.0 mg)	140.5 ± 6.9
Morphine Pellet (75.0 mg)	*342.6 ± 28.5**
Morphine Pellet (75.0 mg)/ Naltrexone Pellet (30.0 mg)	156.9 ± 13.7

Values represent *means ± the standard errors* of *the* mean *(N=8-11).*
**p < 0.01 compared to either placebo-pelleted or morphine-naltrexone pelleted groups.*

DISCUSSION

The results of the present study suggest that the morphine-induced potentiation of sleeping time is mediated by opiate receptors. The effect of acute morphine was completely blocked by the administration of naltrexone, although naltrexone alone had no effect on sleeping time. This latter finding confirms the results of Bhargava (1979) who failed to observe any effect of naltrexone on pentobarbital sleeping time; however, Fürst et al. (1977) found that naloxone decreased the duration of the loss of the righting reflex after either pentobarbital or methohexital.

While the finding of antagonism of the morphine-induced potentiation of sleeping time by naltrexone suggests the involvement of opiate receptors, these receptors do not necessarily have to be located within the central nervous

system. Garty et al. (1985) and Hurwitz et al. (1985) have recently reported the acute administration of opiates can inhibit the renal clearance as well as the hepatic metabolism of various drugs, and these effects can be reversed by naloxone. Thus, opiate effects on hepatic metabolism as well as renal clearance could be specifically mediated by opiate receptors. In support of a peripheral mechanism of action, opiate receptors (Dave et al. 1985) and opiate-like substances (Neidle et al. 1979) have-been found in the liver and kidney.

However, there are several lines of evidence suggesting that the potentiation of pentobarbital sleeping time is centrally mediated. Fatherazi et al. (1985) found that central injections of morphine into the septal area could increase pentobarbital-induced sleeping time, and this effect could be antagonized by intraseptal injections of naloxone. However, Stevenson and Turnbull (1974) reported that morphine given i. p. did not potentiate the effects of pentobarbital given directly into the lateral cerebral ventricles. Cherksey and Altszuler (1974) found that morphine also potentiated thiopental sleeping time in rats. Since the termination of action of thiopental is thought to be due to redistribution out of the brain rather than peripheral metabolism (Goldstein and Aronow 1960), Cherksey and Altszuler (1974) concluded that "morphine lowers the brain threshold for thiopental induced sleep". Moreover, they also reported that the injection of nalorphine would block morphine-induced potentiation of thiopental sleeping time in the dog. However, Cherksey and Altszuler (1974) noted that the half-life of thiopental in the brain was increased by morphine pretreatment; therefore, there may also be a dispositional component to this phenomenon.

Using a morphine pelleting procedure identical to that utilized in the present study, Cochin et al. (1979) found that tolerance to the analgesic effects of morphine is maximal 3 days after pellet implantation, and Ary and Lomax (1977) reported that the temperatures of rats return to baseline by 48 hr after pellet implantation. However, in agreement with previous reports (Bousquet et al. 1964; Ho et al. 1976a and 1976b; Lesher and Spratto 1978; Howd and Pryor 1980), the results of the present study indicate that at this time point no tolerance is observed to morphine-induced potentiation of pentobarbital sleeping time. The lack of tolerance to the potentiation of sleeping time may relate to the inhibition of the expression of tolerance to the analgesic effect of morphine reported in the pentobarbital-treated rat (Terman et al. 1985).

The simultaneous implantation of a naltrexone pellet completely blocked the potentiation of sleeping time in morphine-pelleted rats, providing further evidence that this phenomenon is mediated by opiate receptors. Chronic naltrexone treatment alone did not affect pentobarbital sleeping time in the present study, although Lehman et al. (1979) found that chronic naltrexone did potentiate hexobarbital sleeping time in the

mouse. This discrepancy could be due to the different species and/or the chronic drug administration procedures used. It is interesting to note that Axelrod (1956) found that the rats chronically given nalorphine along with morphine did not show the depression of hepatic N-demethylation that was observed during chronic morphine treatment.

In summary, the results of the present study suggest that morphine-induced potentiation of pentobarbital sleeping time is mediated by opiate receptors and is still present in morphine-tolerant subjects. It is not clear if this effect is centrally or peripherally mediated, or if the changes in hepatic drug metabolizing enzyme activity and drug distribution that have been observed by other investigators contribute to this phenomenon or merely correlate with it. However, unless these differences in metabolism and/or distribution are mediated by opiate receptors (Carty et al. 1985; Hurwitz et al. 1985), they must play a minor role in morphine-induced potentiation. The finding that the potentiation of pentobarbital sleeping time is even greater during chronic morphine treatment suggests that the use of barbiturates by individuals chronically taking opiates may have profound adverse consequences.

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Effects of Chlordiazepoxide on Intravenous Cocaine Self-Administration in Rats

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The nor-iatrogenic use of cocaine has rapidly increased during the last decade. Behavioral studies have demonstrated that the response-contingent administration of the drug is reinforcing and will maintain long and complex sequences of behavior by animals including humans under various schedules of reinforement (Fischmen and Schuster 1982; Goldberg et al., 1981; Pickens and Thompson 1968). Although the effects of the drug on neurotransmission are complex, the primary neurochemical action appears to be an inhibition of biogenic amine neurotransmitter uptake into presynaptic nerve endings. While it has not been conclusively elucidated whether or not these neurotransmitters are involved in the neurobiology of cocaine reinforcement processes, intravenous self-administration experiments suggest an important function for dopaminergic neurons. The administration of pimozide (DeWit and Wise 1977), haloperidol (de la Garza and Johanson 1982), alha-flupenthixol (Ettenburg et al., 1982) and sulpiride Roberts and Vickers 1984) modulate cocaine-maintained responding by rats and rhesus monkeys in a dose-related manner suggesting an attenuation of reinforcing efficacy. Moreover, 6-hydroxydopamine lesions of the nucleus accumbens disrupt cocaine self-administration (Roberts et al., 1977; Roberts et al., 1980) as do similar lesions of the ventral tegmental area where the cell bodies for the mesolimbic/mesocortical dopaminergic neuronal system are localized (Roberts and Koob 1982).

The pharmacological strategies currently under investigation for the treatment of the chronic non-medical use of cocaine have focused almost entirely on direct menipulations of dopaminergic or noradrenergic neuronal activity. The administration of neuroleptic drugs which attenuate self-administration in rats and non-human primates has been reported to be effective in the management of cocaine-

induced auditory and visual hallucinations and paranoia in humans (Kleber and Gawin 1984; Wesson and Smith 1985), but these drugs have no effect or may actually increase subjective reports of "cocaine craving" (Dackis and Gold 1985). The most promising pharmacological interventions appear to involve those drugs which mimic some of the neuropharmacological properties of cocaine. For example, preliminary data suggest, that direct receptor stimulation by the specific dopaminergic agonist, bromocriptine, may allleviate "cocaine craving" (Dackis and Gold 1985). In addition, open clinical trials have reported that treatment with tricyclic antidepressants (e.g., desipramine hydrochloride) for two to three weeks results in marked decreases in "cocaine craving" (Baxter 1983) and abstinence from further cocaine use regardless of whether an affective disorder was also present (Gawin and Kleber 1984).

The accepted therapeutic value of diazepam or related benzodiazepines in the treatment of chronic cocaine use has been limited to the management of seizures and acute anxiety following a massive overdose (Gay 1982; Wesson end Smith 1985). However, animal studies have suggested that a complex behavioral and neuropharmacological interrelationship exists between stimulants and benzodiazepines. Amphetamine and cocaine have been reported to augment the effects of diazepam and chlordiazepoxide in conflict and avoidance paradigms (Sansone 1975; Ford et al., 1979; Lerner et al., 1986), while diazepam and chlordiazepoxide can potentiate cocaine and amphetamine-induced increases in locomotor activity in mice (D'Mello and Stolerman 1977; Sansone 1980). Recent data suggest that these effects may be related to interactions between dopaminergic neuronal activity and brain benzodiazepine receptors. Intraventricular injections of 6-hydroxydopsmine result in significant reductions in the number of benzodiazepine receptors in the rat cerebral cortex (Sabato et al., 1980) and cerebellum (Doble et al., 1981) without affecting endogenous levels of GABA or GABA receptor labeling. In addition, the administration of buspirone or other neuroleptic agents results in pronounced increases in benzodiazepine receptor labeling measured "ex vivo" with flunitrazepam (Oakley and Jones 1983) or in vivo with Ro 15-1788 (Goeders and Kuhar 1985). This investigation was designed to evaluate the involvement of benzodiazepines in cocaine reinforcement by assessing the effects of chlordiazepoxide on intravenous cocaine self-administration in rats.

METHODS

Male Fischer 344 90 to 150 day old rats were implanted with chronic jugular catheters using previously described procedures (Weeks 1962). The catheter was inserted into the

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right posterior facial vein and continued subcutaneously to the back where it exited immediately behind the scapulae through a teflon-stainless steel harness. The catheter was contained within a spring-covered leash and attached to a leak-proof swivel connected to an infusion pump. The animals were housed in individual cages in ventilated housing chambers on a reversed 12 hr light/dark cycle with free access to food and water. While in the housing chambers, the animals received 200 ul infusions of heparinized saline every 2 hours to maintain the patency of the catheters.

The rats were tested for self-administration during daily 3 hour sessions five days a week. The cages were transferred to sound-attenuating operant conditioning chambers, and a response lever and stimulus light were installed in each cage. The animals were initially trained to self-;administer cocaine hydrochloride (0.5 mg/kg/200 ul infusion) on a continuous schedule of reinforcement. The response requirement was gradually increased to a fixed-ratio 10 limited hold 5 min (FR10 LH5) schedule where the animal had 5 minutes from the first lever-press to complete nine additional responses. A maximum of 40 infusions were allowed during each session to minimize the toxic effects of unlimited drug access. When responding stabilized (variations < 10%), the rats were tested for self-administration with duplicate probes of 0.25 and 1.0 mg/kg/infusion of cocaine. Each dose was tested during a 3 hour session with at least three days of baseline responding allowed between each dose probe.

The effects of chlordiazepoxide (CDP) on cocaine self-administration were investigated following the completion of the dose-response gradients. The rats were pretreated with CDP (3.8, 5.6, 7.5 and 10 mg/kg, i.p.) 15 minutes before the start of the experimental session and were allowed to self-administer cocaine (0.5 mg/kg/infusion) as described above. Each dose was tested twice, and at least three days of stable baseline responding were required between each CDP challenge. A separate group of rats were trained on a discrete trial fixed-ratio 10 schedule of food presentation with the intertrial interval yoked to the interinfusion interval of a self-administration animal to access the non-specific effects of CDP on lever pressing.

RESULTS

Intravenous injections of cocaine rapidly engendered self-administration in rats, with stable baselines of responding on the FRlO LH5 schedule of reinforcement obtained within three to four weeks. Decreasing the dose of the drug shortened the interinfusion intervals and increased the number of infusions per session while the higher dose had

the opposite effect (Figure 1). Pretreatment with the low dose of CDP (3.8 mg/kg) resulted in moderate decreases in drug-intake with 0.5 mg/kg cocaine while higher doses (7.5 to 10 mg/kg) virtually eliminated cocaine self-administration (Figure 1). However, regular patterns of self-administration were observed in two rats following pretreatment with 7.5 or 10 mg/kg CDP when the cocaine dose was increased to 1.0 mg/kg/infusion. Response rates were increased (+56%) by 3.8 mg/kg CDP in the food reinforcement animals while only the highest dose (10 mg/kg decreased responding (-49%).

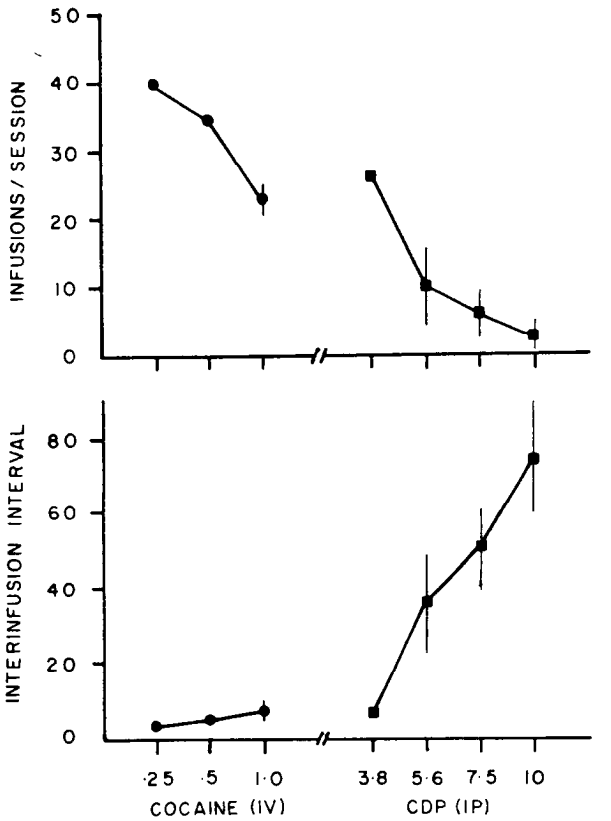

FIGURE 1. Effects of cocaine dose (mg/kg/infusion) and chlordiazepoxide (mg/kg) intravenous cocaine self-administration. Values are the means (± SEM) for double determinations in 5 rats. The effects of CDP on the self-administration of 0.5 mg/kg/infusion of cocaine were assessed following a 15 min pretreatment.

DISCUSSION

The results of this investigation suggest that chlordiazepoxide can influence the reinforcing efficacy of intravenous cocaine injections in rats. With the training dose of cocaine, CDP decreased drug-intake at low doses and eliminated self-administration at higher doses suggesting that CDP reduced the reinforcing efficacy of the training dose of cocaine. However, when the cocaine dose was increased, regular rates of self-administration were observed and the patterns of drug-intake indicated that the effects of CDP had been partially reversed.

The effects of CDP on cocaine self-administration could have resulted from the actions of the drug on rates of responding independently of effects on reinforcement. However, this is unlikely since the animals effectively completed the response requirement long before the limited hold had elapsed following pretreatment with CDP. In fact, low doses of CDP only moderately decreased drug-intake, and stable rates of responding were even observed with the higher doses when the cocaine dose was increased. Moreover, the doses of CDP used in this investigation are consistent with those which increase responding in conflict or punishment paradigms without affecting performance in non-punished controls (Rawlins et al., 1980; Sansone 1975) and which have little or no effect on spontaneous locomotor activity (D'Mello and Stolerman 1977; Sansone 1980). Furthermore, the effects of CDP on rats responding on a food reinforcement schedule yoked for rate with cocaine self-administration animals demonstrated that only the highest dose (10 mg/kg) affected the ability of these animals to respond.

The mechanisms though which CDP produced the effects on cocaine self-administration reported in this investigation are not clear, but other data suggest an important role for dopaminergic neurons. First of all, dopamine receptor antagonists have been reported to attenuate the reinforcing efficacy of cocaine (DeWit and Wise 1977; de la Garza and Johanson 1982; Ettenburg et al., 1982; Roberts and Vickers 1984). In addition, depletion of dopamine with intraventricular injections of 6-hydroxydopamine decreases the number of benzodiazeoine binding sites in the rat brain (Doble et al., 1981; Sabato et al., 1980) while neuroleptic drug which may increase the synthesis and release of dopamine) increase benzodiazepine "receptor binding in vivo (Goeders and Kuhar 1985; Oakley and Jones 1983) . These data suggest that benzodiazepines may influence the reinforcing efficacy of cocaine through direct or indirect interactions with dopaminergic neuronal systems. Therefore, since a significant proportion of the population

using cocaine for non-medical purposes may actually be "self-medicating to regulate painful feelings and psychiatric symptoms via their drug use" (Kleber and Gawin 1984), benzodiazepine therapy may provide a beneficial alternative strategy for the treatment and management of chronic cocaine use.

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Diazepam Preference in Subjects Seeking Treatment for Anxiety

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Johanson and Uhlenhuth (1980) reported that diazepam, a commonly-prescribed tranquilizer, was not preferred over placebo by most normal healthy subjects in a laboratory test of choice. Low doses of the drug (2 or 5 mg) were chosen about as often as a placebo, whereas a higher dose (10 mg) was chosen significantly less often than placebo. Subjective effects, measured at regular intervals after drug ingestion, showed that the drug produced predominantly sedative effects.

Similar results were found in subsequent studies using special subject groups who were thought 'to be at risk for excessive use of anxiolytics. For example, older individuals, who represent the highest proportion of prescription users of tranquilizers, showed no greater preference for diazepam than a younger comparison group (de Wit et al., 1985). In addition, anxious subjects, who might be expected to prefer the drug because of its anxiolytic properties, also did not choose diazepam over placebo (de Wit et al, 1986). Some of these subjects had anxiety levels high enough to meet criteria for a diagnosis of Generalized Anxiety Disorder (GAD; APA, 1980), and showed measurable decreases in anxiety after diazepam administration. Nevertheless, they did not prefer the drug.

Thus, even in individuals with high baseline anxiety levels a reduction in anxiety was not a sufficient condition for the drug to serve as a positive reinforcer. However, it was observed that most of the participants in the above study showed little interest in receiving treatment for their anxiety, suggesting that they were not in severe distress. The subjects had responded to ads which specified that the study was research- and not treatment-oriented, and during the debriefing interview they declined offers of referrals for treatment. In the present study, therefore, we recruited subjects who were highly anxious, and, more importantly, were seeking treatment for their anxiety.

METHOD

Subjects. Thirteen subjects between 21 and 47 participated in this study. They were recruited from the university and surrounding community through posters, word-of-mouth referrals and newspaper

ads. The posters and ads were headed "Are you anxious, tense, nervous?" and specified that treatment would be offered in return for participation in a research study. Subjects were accepted if they had experienced symptoms meeting criteria for GAD for at least one month prior to the interview. They were not excluded if they also met criteria for Social Phobia, Panic Disorder, or Obsessive-Compulsive Disorder, as long as these postdated the GAD. They were excluded if they had medical problems, if they reported a history of drug abuse or psychosis, or if they had a concurrent Axis I disorder other than an anxiety disorder. All subjects reported clinically significant levels of anxiety. They completed psychiatric self-report questionnaires including the Hopkins Symptom Checklist (HSCL; Derogatis et al., 1974), and the Spielberger Trait Anxiety Inventory (STAI; Spielberger, 1970), and were assessed with several clinician rating forms.

Instructions and consent. Prior to participation subjects signed a consent form which outlined the study in detail and indicated all possible side effects of any drug they might be given. They were informed that they would not be told what drug they ingested at the time, except that it would be either a psychomotor stimulant, a minor tranquilizer, or a placebo, and that the dose would be within the daily therapeutic range. Each subject agreed not to take other drugs except their normal amounts of coffee and cigarettes, 12 hours before and 6 hours after taking a capsule. Except for the actual drug ingested, subjects were completely informed of all other procedural details as outlined below.

Procedure. The 9-session choice experiment was conducted over a period of 3 weeks. Subjects reported to the laboratory between 9 and 10 am on Mon, Wed and Fri, at which time they completed mood questionnaires (see below) and ingested a capsule. This took approximately 5 minutes, after which they were free to leave the laboratory, taking with them further questionnaires to be completed 1, 3 and 6 hours later. The first four sessions were sampling sessions, on which the subjects ingested one of two different colored capsules containing placebo or 10 mg diazepam. Half the subjects received the drug on sessions 1 and 3 and the placebo on sessions 2 and 4. The order was reversed for the other half. The last 5 sessions were choice sessions, on which subjects were allowed to choose the capsule they preferred. The number of times the drug capsule was chosen was the primary dependent variable and the measure of the drug's reinforcing properties. After completion of the experiment subjects were debriefed and offered treatment for their anxiety. Response to treatment was also studied in relation to the subjects' choice behavior, and will be reported separately. Drug tablets (Valium 10 mg, Hoffman-LaRoche) were placed in opaque gelatin capsules (size 00) which were filled with dextrose powder. Placebo capsules were identical in size and contained dextrose powder only.

Subjective Effects. The scales used to assess mood were an experimental version of the Profile of Mood States (POMS; McNair et al., 1971) a shortened version of the Addiction Research Center Inventory (ARCI; Haertzen, 1966) and a Visual Analog Scale (VAS).

For purposes of brevity, only the POMS results will be presented here. The POMS consists of 72 adjectives commonly used to describe momentary mood states. Subjects indicate how they feel at the moment in relation to each of the 72 adjectives on a five-point scale

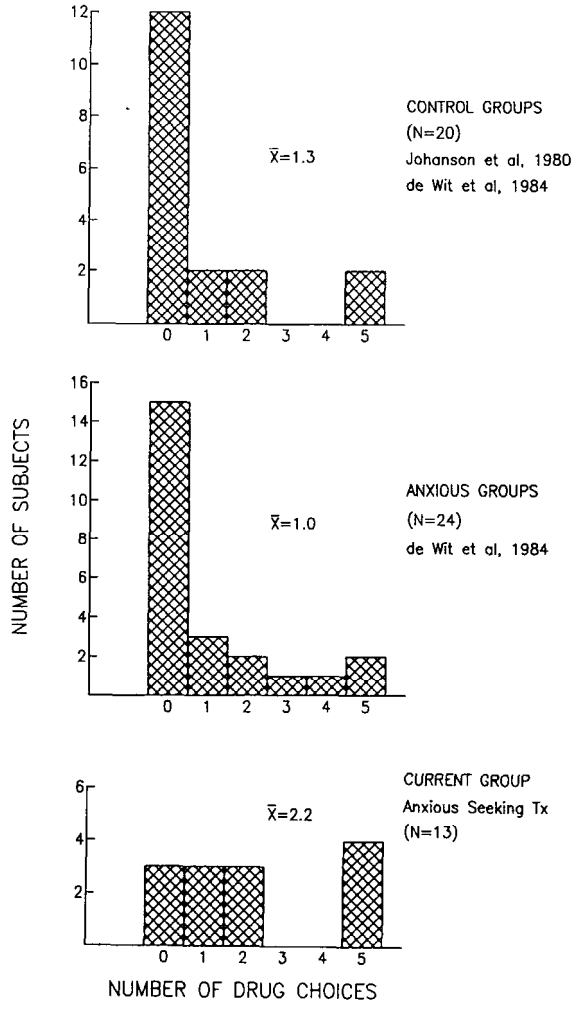

Figure 1. Number of subjects from previous and current studies who chose diazepam (10 mg) over placebo 0-5 times. Mean (x) number of drug choices for each group of subjects is indicated.

ranging from "not at all" (0) to "extremely" (4). There are eight clusters of items (subscales) which have been separated empirically using factor analysis (Anxiety, Depression, Anger, Vigor, Fatigue, Confusion, Friendliness, and Elation). The value of each subscale is determined by adding the numbers checked for each adjective in the cluster and dividing the total by the number of adjectives. Two additional subscales. Arousal and Positive Mood, were derived from the other subscales as follows: Arousal = (Anxiety + Vigor) - (Fatigue + Confusion): Positive Mood = Elation - Depression. POMS data from sampling sessions only will be presented: Subjects completed an additional questionnaire at hour 6 indicating whether they liked the drug, what they thought it was (stimulant, placebo, tranquilizer) and whether they had experienced any unusual reactions.

Results. Figure 1 illustrates the frequencies of drug choices (0 through 5) for the 13 subjects in the present study and, for comparison, the results from the previous studies. Whereas in the previous studies the large majority of subjects preferred placebo over diazepam, 30 percent of the subjects in the present study chose the diazepam on all five choice sessions. The average drug choice for the present group was 2.2 out of 5, while for the previous groups it was 1.3 or less.

TABLE 1. EXTRA-EXPERIMENTAL VARIABLES
(Mean values (± standard error) or frequencies)

	Subjects Choosing Diazepam 0-2 times	Subjects Choosing Diazepam 5 times
N	9	4
AGE	32.5 (+ 2.2)	27 (+ 2.1)
SEX M:F	2:7	4:0
HSCL: Anxiety	1.13 (+ .10)	1.45 (+ .35)
HSCL: Depression	1.03 (+ .12)	1.15 (+ .05)
STAI	55.7 (+ 2.8)	49.2 (+ 3.3)
Alcohol (drinks/wk)	4.4 (+ 1.7)	5.6 (+ 3.4)
Caffeine (drinks/wk)	18.4 (+ 4.3)	10.5 (+ 4.0)
Marijuana (never used)	2/9	1/4
used over 50 times	1/9	0/4
Stimulants (never used)	8/9	3/4
Hallucinogens (never used)	7/9	4/4
Tranquilizers (never used) Non-prescription use	8/9	3/4
Tranquilizers (never used) Prescription use	1/9	2/4

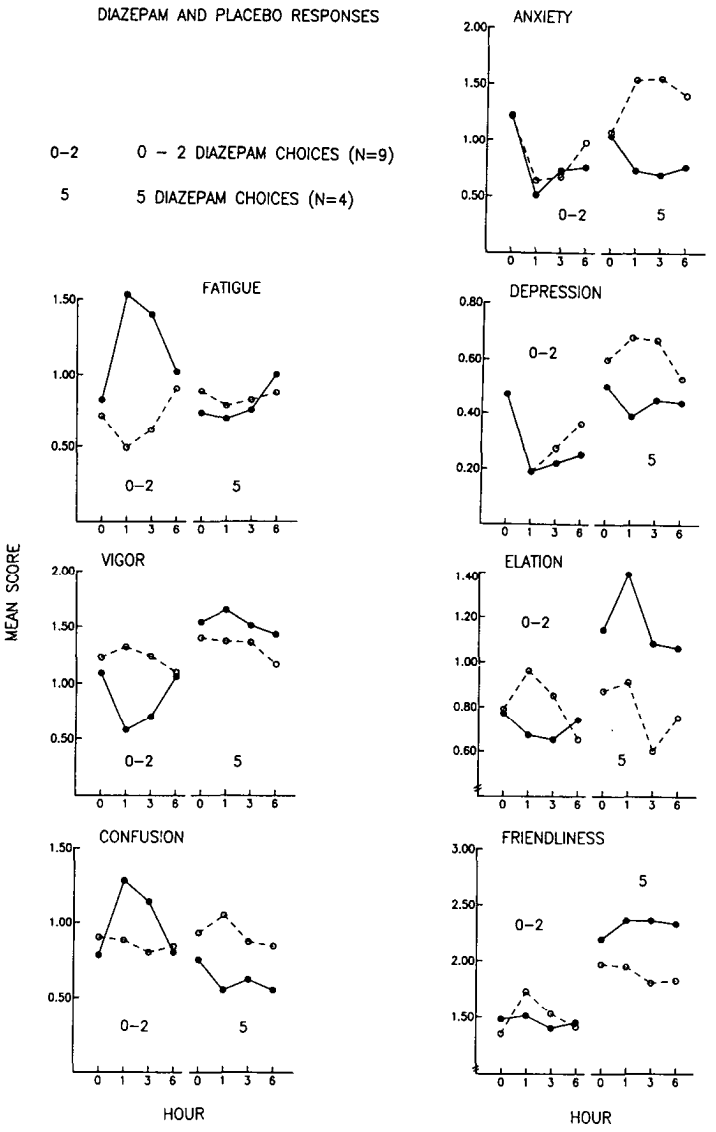

Figure 2. Average diazepam (solid line) and placebo (dashed line) POMS scores from sampling sessions only, for subjects who chose diazepam 0-2 times (N=9) and for subjects who chose diazepam 5 times (N=4).

Table 1 shows demographic characteristics, questionnaire scores and drug use histories of the 13 subjects, presented separately for the nine subjects who chose diazepam 0-2 times and the four who chose it five times. The groups were similar on all variables except ratio of males to females.

Liking ratings were consistent with choice behavior. Whereas the overall difference between placebo liking ratings and drug liking ratings was -5.7, the difference for low drug choosers was -17.6 (s.d. 5.8) and for high drug choosers it was +20.8 (s.d. 12.2).

Most of the subjects, regardless of choice behavior, labelled the diazepam correctly as a tranquilizer (10 out of 13 correct on both sampling sessions). Less than half of the subjects correctly identified the placebo. Errors in placebo identifications were about equally distributed between "stimulant" and "tranquilizer".

Subjective effects. When data from all 13 subjects were pooled, typical sedative-like subjective effects were obtained. These effects included decreased Anxiety and Arousa1, and increased Fatigue and Confusion. However, when POMS scores were calculated separately for those subjects who consistently chose the diazepam and those who chose it infrequently, marked individual differences in mood responses to the drug became apparent (Figure 2). On Fatigue, Vigor and Confusion subscales, a sedative-like drug effect was observed in the 0-2 choice group but not in the 5 choice group. On Anxiety and Depression subscales the 0-2-time choosers were not affected by the drug, whereas the 5-time choosers showed decreased scores after diazepam. Placebo session Anxiety and Depression scores for the 5-choice group were markedly higher than for the 0-2-choice group. Finally, Friendliness and Elation scores were elevated on drug sessions in the 5-time choosers but not the 0-2-time choosers. Pre-drug differences make the elevations in the 5-time choice group difficult to interpret, however. Although the small number of subjects precluded statistical comparisons, these results suggest that diazepam produced qualitatively different mood effects in the 5-time choosers compared to the 0-2 time choosers.

DISCUSSION

In contrast to the previously-tested groups of anxious and control subjects, a sizeable proportion of the subjects in the present experiment did consistently choose diazepam over piacebo (Figure 1). Thirty percent of these subjects chose the diazepam on all five choice trials, whereas in previous studies only 10% (control subjects) and 8% (anxious subjects) did so. The subjects in the present study differed from previously-studied anxious subjects in that they were sufficiently distressed by their level of anxiety to be seeking treatment for it, although they scored only slightly higher on self-report measures of anxiety.

The subjective effects of diazepam in the 0-2-time choose subjects were similar in quality, magnitude and course to the effects observed with this drug in previous studies (Johanson and

Uhlenhuth, 1980; de Wit et al., 1983; 1985). Drug liking scores and accuracy of drug labelling in this group were also not different from previous experiments. The 5-time diazepam choosers showed different subjective responses to the drug, however. They reported relatively less sedative effects (e.g., on Fatigue and Vigor scales), and did not report the previously-observed increase in Confusion. Anxiety and Depression scores were decreased in the 5-time choosers but not in the 0-2-time choosers in this study. Finally, there was some indication that Elation and Friendliness scores were higher on drug sessions in the 5-time choosers, an effect never previously observed (de Wit et al, 1986), although pre-drug differences between drug and placebo sessionsmade these findings difficult to interpret. The low and high choice subjects were similar on most extra-experimental variables, although proportionately more males chose the diazepam frequently. Whether this was related to the differences in behavioral and subjective responses to the drug or due to chance is not clear.

The conclusions that can be drawn from these data are limited because of the small number of subjects involved. Nevertheless, these findings suggest a logical relationship between drug preference and mood. In this and previous studies (de Wit et al, 1986), subjects who experienced predominantly sedative effects from diazepam were less likely to choose the drug over a placebo, and reported liking it less than placebo. The few subjects who did choose the diazepam in this study reported minimal sedative effects, some decrease in Anxiety and Depression, and possibly an increase in Friendliness and Elation. Whether any of these subjective effects play a causal role in the determination of choice behavior must await further research. Despite the small number of subjects involved, however, these results suggest that diazepam can be an effective positive reinforcer in some highly anxious subjects. As a result, this effect might make these individuals at risk for excessive of the drug.

REFERENCES ARE AVAILABLE UPON REQUEST

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The Dependence Syndrome Across Different Psychoactive Substances: Revised DSM-III

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INTRODUCTION

Despite mounting evidence in support of the dependence syndrome with alcoholism (1-12), there have been no similar investigations into patterning of the hypothesized syndrome elements among frequent users of other psychoactive substance. In part, the lack of empirical work with other substances has been related to the absence of instruments which tap dependence syndrome elements for drugs other than alcohol. Recently, the Substance Dependence Disorders section of the Structured Clinical Interview for DSM-III-R (SCID) has included a structured set of questions covering the elements of the dependence syndrome.

The symptoms hypothesized as clustering together in the dependence syndrome are the following: 1. narrowing of substance use repertoire such that substance use becomes stereotyped around a regular schedule of almost continuous or daily consumption; 2. salience of substance taking behavior such that, despite negative consequences, substance use is given higher priority than other activities which had previously been important; 3. increased tolerance; 4. withdrawal symptoms; 5. substance use to avoid withdrawal; 6. subjectively experienced compulsion to use substance; and 7. readdiction liability (1,13).

In the present study, two postulates of the dependence syndrome were evaluated: (a) the prediction that syndrome elements cluster into a unidimensional scale and (b) the relative independence of this syndrome from legal, occupational, medical, family and psychological problems associated with substance use.

METHODS

Subjects were interviewed at two settings: (a) 41 patients from an inpatient unit of a community mental health center, which treats patients with a wide range of diagnoses for 28 days, and (b) 42 patients applying for treatment at an ambulatory

Substance Abuse Treatment Unit. For the inpatient unit the sample was predominately male (54%), white (51%) with a high school education or more (80%) and with an average age of 34 (s.d. 10 years). For the outpatient setting the sample was predominantly male (74%), white (62%), with a high school education or more (81%) and with an average age of 29 (s.d. 7 years). Thus, both samples were demographically comparable. As anticipated, the rates of substance use disorders were considerably higher, particularly for opioids and cocaine, in the outpatient sample who identified themselves as substance abusers by seeking specialized treatment. Although the mean number of drugs abused was 2.3, 14 subjects had no history of drug abuse and 17 reported abuse of only one type of drug.

Two instruments were employed in the present study: 1. the Substance Dependence Disorders section of the Structured Clinical Interview for DSM-III (SCID) (18), and 2. the Addiction Severity Index (20,21). The SCID provides an interview guide for determining whether subjects meet DSM-III-R criteria for a range of psychiatric disorders. In the SCID, subjects were asked to describe substance using during the most severe episode in their lifetime, regardless of the presence or absence of a current episode. The Addiction Severity Index (ASI) was also completed for all inpatients (one had incomplete data) and a pilot sample of 15 of the 43 outpatients. The AS1 covers six major problem areas: legal, employment, medical, family, psychological, and substance abuse (20,21).

RESULTS

As a test for unidimensionality, the dependence syndrome items were scored as 0 (not present) and 1 (present) and then added together for each drug type to form seven dependence syndrome scales with scored ranging from 0 to 10. The values for Cronbach's alpha (23) ranged from 0.83 (cannabis) to 0.98 (opiates) indicating excellent internal consistency for all seven scales, and the item scale correlations were quite good overall. A second test of unidimensionality involved Guttman scaling for each type of drug based the 10 items. As indicated by Guttman reproducability coefficients ranging from 0.85 (alcohol) to 0.97 (opiates), most of the drugs demonstrated good approximations of "perfectly" unidimensional and cumulative scales. This suggested that the severity of the dependence syndrome was indicated by the total number of items endorsed.

To further test for unidimensionality, we factor analyzed the 10 items within each type of drug and found that all 10 items loaded onto single factors for opiates, cocaine and alcohol. These single factors accounted for a substantial part of the variance: 83% for opiates, 68% for cocaine and 56% for alcohol. Stimulants formed two factors that accounted for 67% of the variance. The factors were labeled "compulsion" and "problematic use" based on the items loading above 0.5. Sedatives and cannabis each had three factors with eigenvalues above 1. Based

on the items loading on each factor, two common factors were labeled "can't stop" and "compulsion/salience". The other factor was different for the two drugs and was labeled "problematic use" for sedatives and "withdrawal consequences" for cannabis. The three factors accounted for 69% (sedatives) and 58% hallucinogen dependence syndrome items were reported by less than four respondents, these infrequent items were removed. Including all 10 items resulted in three factors of which one included all five infrequent items. When these items were deleted, the other five items loaded together as a single factor that accounted for 69% of the variance.

Finally, to test our second postulate, the seven dependence syndrome scales were correlated with five composite problem severity scales from the ASI - legal, work, medical, family, psychological. Five of the 28 correlations between the seven dependence scales and the ASI scales were significant, but even for significant associations the shared variance was less than 10%.

DISCUSSION

Based on the newly revised DSM-III-R substance dependence criteria (15), we found that the 10 items in the draft of DSM-III-R formed internally consistent scales for all seven drugs under study. For alcohol, opiate and cocaine abusers the dependence syndrome scale met several criteria for unidimensionality including all items loading onto a single factor and forming good approximations to "perfect" Guttman scales. For the other drugs - stimulants, sedatives, hallucinogens and cannabis - a clear unidimensional structure was not confirmed by every type of analysis, but even for these drugs simple cumulative scales seemed to provide satisfactory measures of a dependence syndrome that was independent of medical-psychosocial problems. The factor analyses raised the most significant questions about the unidimensionality of the dependence syndrome for stimulants, sedatives and cannabis, and these drugs will need further study in larger samples. Thus, our findings supported the utility of the dependence syndrome for other drugs besides alcohol by specifically demonstrating that the syndrome elements clustered into unidimensional scales for most drugs and that this syndrome was relatively independent of medical-psychosocial problems.

The new DSM-III-R substance dependence criteria tested in this study were designed to solve difficulties in previous criteria. The increased coherence of using similar criteria across all substances has great appeal with the general trend toward polydrug abuse. For every drug examined in this study some abusers reported most of the 10 criteria for that drug. Furthermore, all of the criteria were useful for at least several drugs, so that no particular criterion seemed to be so rarely reported across all seven drugs that it merited being dropped. In a companion report, we also found that these new criteria

showed remarkable agreement with the current DSM-III criteria in identifying substance abusers (19). Thus, it agrees well with our current system in identifying substance abusers while being considerably simpler to elsewhere (15) including an ability to denote distinctions in severity of disorder and the removal of excessive emphasis of physiological aspects of dependence. Overall, the present study provides encouragement for undertaking more intensive work with other substances besides alcohol using the newly revised DSM-III-R.

REFERENCES: May be obtained from the author on request.

ACKNOWLEDGEMENTS

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Effects of Marijuana, Cocaine, and Task Performance on Cardiovascular Responsivity

Richard W. Foltin, Richard M. Capriotti, Margaret A. McEntee, Marian W. Fischman, Joseph V. Brady, and Julia J. Pedroso

Many drugs of abuse have significant effects on the cardiovascular system. Marijuana has been shown to produce reliable increases in heart rate with no change or only small decreases in blood pressure in humans (e.g., Weiss et al, 1973). Cocaine, on the other hand reliably increases both heart rate and blood pressure (e.g., Fischman et al, 1976). Although many studies have investigated the effects of these drugs on the cardiovascular system, all have focused upon drug effects under resting baseline or nonstressful conditions, and none have investigated the interactive effects of drug administration and task performance. This report describes the results of a study on the interactive effects of task performance, in combination with either smoked marijuana or intranasal cocaine, on heart rate and blood pressure.

METHODS

Subjects. Healthy adult male volunteers, 23 to 38 years of age participated. All subjects were experienced in the use of both marijuana and cocaine and had been screened in accordance with extensive physical and psychological assessment procedures. Informed consent was obtained and subjects were paid daily based on their individual task performance. Additional bonus payments were earned for completion of a study.

Performance Task. A modified repeated acquisition task (Fischman, 1978) was presented on a CRT screen and subjects were provided a 3 button response manipulandum interfaced with an Apple IIe microcomputer. A random sequence of up to 25 correct position responses (left, center, or right) was possible during each session. The sequence of correct responses was changed for each session.

Subjects were required to respond on one of three response keys (left, center, or right) and each correct response produced an asterisk on the CRT. Incorrect responses were followed by a one-second timeout when the screen was blank. When a correct sequence was achieved the subject was prompted to repeat that sequence. Upon completion of the sequence a second time, a cumulative counter registered an increment in points earned. Each trial required the subject to complete a sequence of responses one response longer than the previous trial (from 1 to 25). The task ended after 10 minutes.

PROCEDURE. Subjects were seated in a comfortable reclining chair with one arm kept level with the heart. Heart rate was continuously monitored via chest electrodes and both heart rate and blood pressure (systolic, diastolic and mean arterial) were recorded every two minutes using a Dinamap 825XT automated Vital Signs monitor (Critikon Inc., Tampa, FL).

Subjects remained in the laboratory for at least one hour after each experimental session to allow drug effects to dissipate.

Experiment 1 - Marijuana: Nine subjects completed five consecutive daily sessions. Each session consisted of: 1) a one hour monitoring period to insure that no caffeine, nicotine or other drugs were consumed in association with testing, 2) a 10 minute rest period, 3) 5 minutes of paced smoking of a 1.84% THC (w/w) or placebo, 1 gram marijuana cigarette, 4) 10 minutes of post-drug monitoring, 5) 10 minutes of task performance (Group I) or resting baseline (Group II), and 6) 10 minutes of resting baseline. All subjects smoked active marijuana on days 2, 3, and 4 and placebo on days 1 and 5. Group I (n=5) performed the task each day whereas Group II (n=4) rested during the task period. Thus, data on cardiovascular changes were collected under active and placebo marijuana conditions in the presence (Group I) or absence (Group II) of task performance. These data were compared with data collected under baseline conditions prior to drug administration.

Experiment 2 - Cocaine: Seven subjects completed six experimental sessions. Each session consisted of: 1) a one hour monitoring period to insure that no caffeine, nicotine or other drugs were consumed in association with testing, 2) a 14 minute rest period, 3) 10 minutes of task performance , 4) administration of cocaine hydrochloride (4, 48, or 96mg combined

with lactose to yield a 100 mg of powder which was inhaled within a two minute period), 5) 14 minutes of resting baseline, 6) 10 minutes of task performance, 7) 34 minutes of resting baseline, 8) 10 minutes of task performance and 9) 14 minutes of resting baseline. Thus, each session consisted of task performance once prior to drug administration and then twice (15-25 and 60-70 minutes) after cocaine administration.

Six of the seven subjects were tested with each of the three doses of cocaine twice. One subject was tested only once at each dose level. The first series of drug doses was administered in ascending order, while the doses in the second series were randomly assigned.

RESULTS

Experiment 1: Figure 1 compares the changes in heart rate following active marijuana under task performance (Group I) and no task performance (Group II) conditions. Marijuana-induced heart rate increases were sustained at peak levels 10-20 minutes after drug administration under task performance conditions (Group I). In the absence of task performance, peak level increases were not sustained (Group II). After completion of the task (20-30 minutes post drug), there was no difference in heart rate between the two groups. Figure 2 summarizes the effects of active and placebo marijuana on mean arterial blood pressure(MAP) under task performance (Group I) and no-task performance (Group II) conditions. Active marijuana alone (Group II, bottom panel) produced a small but consistent increase

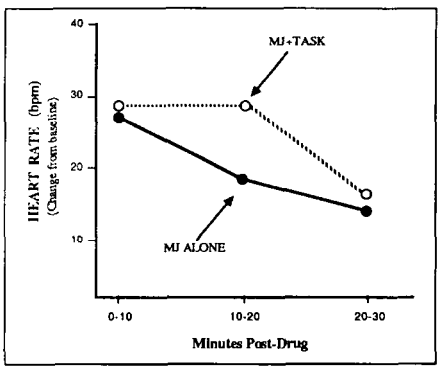

Figure 1. Change from baseline heart rate following marijuana alone (n=4) and marijuana + task performance (n=5).

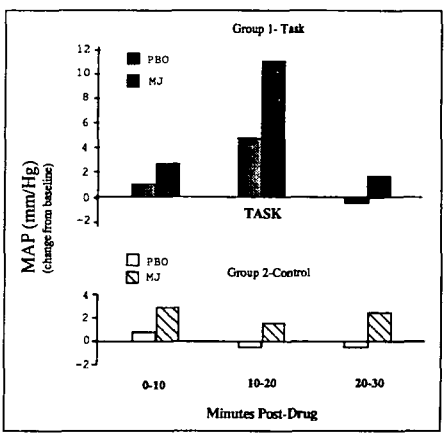

Figure 2. Change from baseline mean arterial pressure (MAP) following marijuana and task performance (Top Panel: Group I and marijuana (Bottom Panel: Group 2).

in MAP (2-3 mm/Hg) compare to placebo. In contrast, substantial increases in MAP levels (10-12 mm/Hg) were observed during task performance following active marijuana administration (10-20 minutes post-drug) as compared to task performance under placebo conditions (Group I, top panel). This incremental increase in MAP with task performance plus active marijuana was a consistent finding with all five subjects in Group I. After completion of the task (20-30 minutes post drug) MAP returned to baseline. Subjects who performed the task after smoking active marijuana thus showed substantially elevated blood pressures and sustained heart rate increases under such conditions. If an additive model of interaction is hypothesized and the effect of task alone is added to the effect of marijuana alone, an expected value for the effects of marijuana plus task performance can be derived. Figure 3 presents the results of subtracting the mathematically derived expected values from the observed values presented in Figures 1 and 2. For all measures, the observed values were greater than the expected values, indicating that the response was greater than that predicted by an additive model. Although the differences were small for both heart rate and systolic blood pressure, the results summarized in Figure 3 indicate clearly that the effects on diastolic and mean arterial pressure of marijuana in combination with task performance were significantly greater than predicted by an additive model of interaction (DP, $t(5) = 2.61$, $p < .05$; MAP, $t(5) = 3.12$, $p < .05$).

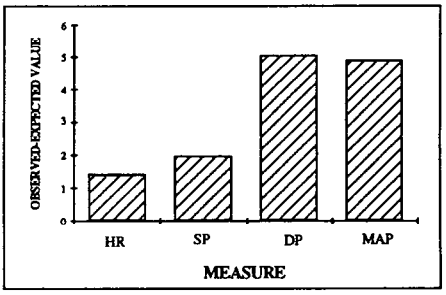

Figure 3. Observed expected values for each cardiovascular measure following marijuana administration, HR = heart rate, SP = systolic pressure, DP = diastolic pressure, MAP = mean arterial pressure

Experiment 2: Figure 4 compares the changes in heart rate following task performance alone and task performance in combination with inhaled cocaine at the indicated dose levels. The difference scores represent changes from the pre-drug resting baseline obtained prior to

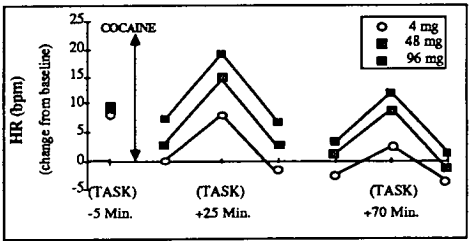

Figure 4. Mean change baseline heart rate after 4, 48, and 96 mg cocaine under task and resting conditions. Drug adminisrered at 0 minutes.

the first task performance for the 13 observations at each dose level, i.e., six subjects tested twice at each dose and one subject tested once at each dose. Performance of the task alone (prior to drug administration) increased heart rate by 8 to 10 beats per minute, and cocaine alone (0-15 minutes after drug administration) produced dose-dependent increases in heart rate. When the task was performed following cocaine administration (15-25 minutes post-drug), peak heart rate increases were consistently sustained above task alone or cocaine alone baseline levels. Heart rate levels returned immediately to baseline following the task performance (25-30 minutes after drug administration). The effects of cocaine interacting with the task-elicited heart rate response were attenuated 60-70 minutes after drug administration, as indicated by the smaller changes in heart rate shown in the right-hand section of Figure 4.

Figure 5 compares the changes in mean arterial blood pressure (MAP) following task performance alone and task performance in combination with each of the administered doses of cocaine. Once again, both task performance alone (pre-drug) and cocaine alone (0-15 minutes after drug) produced substantial elevations in blood pressure (10-12 mm/Hg). In combination (15-25 minutes after drug administration), only the 96mg dose increased blood pressure above levels produced by task performance alone. Baselines were again recovered rapidly (25-30 minutes after drug), and the interactive effect on blood pressure of cocaine with task performance were similarly attenuated 60-70 minutes after drug administration (far right section, Figure 5).

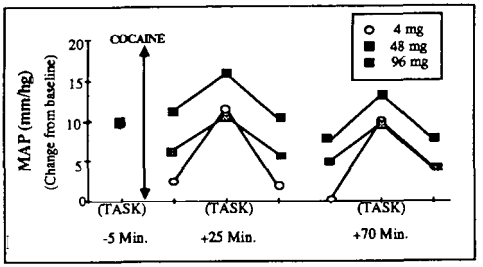

Figure 5. Mean change from baseline blood pressure after 4, 48, and 96 mg cocaine under task and resting conditions. Drug administered at 0 minutes.

An additive model was hypothesized for the cardiovascular effect of cocaine in combination with task performance. For each of four measures (heart rate, systolic blood pressure, diastolic blood pressure, and mean arterial pressure), an expected value was derived by adding the effect of the task alone to the effect of 4, 48 or 96 mg cocaine alone. Figure 6 presents the results of subtracting this mathematically derived expected value from the

observed values (Figures 4 and 5). Positive values indicate that the observed response was greater than predicted by an additive model, while negative values indicate that the observed response was less than predicted by an additive model. cocaine and task performance had a greater effect on heart rate than predicted by an additive model of interaction. Conversely, cocaine and task performance in combination had a lesser effect on blood pressure than predicted by an additive model of interaction.

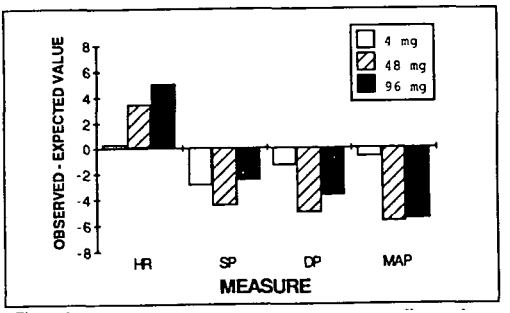

Figure 6. Observed minus expected values for each cardiovascular measure as a function of dose of cocaine. HR = heart rate, SP = systolic pressure, DP = diastolic pressure, MAP = mean arterial pressure

DISCUSSION

The results of the present experiments show clearly that the cardiovascular effects of smoked marijuana and inhaled cocaine can be substantially exacerbated by environmental interactions. Task performance combined with smoked marijuana sustained drug induced heart rate elevations substantially above control levels and consistently elevated mean arterial blood pressure when compared to marijuana or task performance alone conditions. The substantial increases in blood pressure during task performance under active marijuana conditions was greater than predicted by an additive model of drug-by-task interaction. Task performance in combination with inhaled cocaine also sustained drug induced heart rate and blood pressure elevations substantially above cocaine alone and task performance alone conditions. In contrast to the effect of marijuana, however, only the elevated heart rate values observed under combined drug and task performance conditions were greater than those predicted by an additive model of drug by task interaction.

The finding of increased heart rate and heightened blood pressure responsivity produced by the interactive effects of smoked marijuana or inhaled cocaine and prevailing environmental circumstances would seem to require reevaluation of the cardiovascular consequences of such drug use and the conditions under which it occurs. The present findings indicate that the potential cardiovascular

effects of drugs can be exacerbated by a wide range of circumstances, and these circumstances may include common stressful events that occur regularly, e.g., rush hour driving.

Interestingly, the marijuana and cocaine using subject population represented in this study was extensively screened to eliminate all hypertensive volunteers. Almost 50% of all those volunteering to participate in this research were rejected because of elevated pressure levels. Although the relationship between prior cocaine and/or marijuana use and hypertension is unknown, the difficulty in obtaining normotensive marijuana and cocaine experienced subjects points to the importance of the data collected in the present study. Further investigation of the complex interaction between drug combinations and prevailing environmental circumstances will provide valuable information on the adverse health consequences of drug abuse.

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Intravenous Cocaine Self-Administration by Human Volunteers: Second-Order Schedules of Reinforcement

Jack E. Henningfield, Ro Nemeth-Coslett; Jonathan L. Katz, and Steven R. Goldberg

One effect of cocaine that may contribute to its abuse liability is its efficacy in establishing previously neutral environmental. stimuli as both discriminative stimuli and conditioned reinforcers. These stimuli can then function to strengthen and control behavior in their own right (Goldberg, 1973; Goldberg, Spealman and Kelleher, 1979; Johanson and Schuster, 1981; Katz, 1979; Kelleher, 1966; Katz and Goldberg, 1986). Second-order schedules of drug injection have been used to systematically study conditioned reinforcing effects of stimuli associated with drugs in laboratory animals (cf. Goldberg, Kelleher and Morse, 1975). Under second-order schedules, responding under one schedule is treated as a response unit which is reinforced according to another schedule. Often completion of each response unit produces a brief stimulus that also accompanies the drug injection. Second-order schedules can be used to establish and maintain extended sequences of behavior maintained by relatively intermittent drug injections, and have been used in the experimental analysis of the degree to which a previously ineffective stimulus can maintain behavior by virtue of its association with drug delivery.

For example, in one condition of an earlier study of cocaine self-administration by squirrel monkeys, every 10 to 30 presses on a lever produced a brief flash of light; after five minutes had elapsed from either the start of the session or the last drug injection, 10 responses again produced the light and also an intravenous injection of cocaine (Goldberg, Kelleher and Goldberg, 1981). Two functional consequences of the second-order schedule contingency were that (1) control over behavior was established that was appropriate to the schedule of presentation of the brief visual stimulus, and (2) overall rates of responding markedly increased when the secondary stimuli were omitted although frequency of cocaine injections remained unchanged.

The present report describes the results obtained in a preliminary study of intravenous cocaine self-administration in two human volunteers. The purpose of the study was to assess the viability of maintaining behavior of human subjects under a second-order schedule in which effects of environmental stimuli associated with

drug injections could be objectively assessed. Conventional self-reports of interoceptive drug effects were also assessed (Jasinski, 1977). The plausibility of using such a procedure was suggested by the results of earlier studies which had demonstrated that intravenous drug self-administration by human subjects could be safely studied under controlled laboratory conditions, and that such studies could provide data complimentary to conventional studies of abuse liability and dependence potential (e.g., Fischman and Schuster, 1982; Mello, Mendelson and Kuehnle, 1982; Mello, Mendelson, Kuehnle, and Sellers, 1981; Henningfield, Miyasato and Jasinski, 1983; Henningfield and Goldberg, 1984). These studies permit the collection of both self-administration (i.e., behavioral) data as is typically collected in studies with animals as well as the collection of self-report data typically collected in human studies. Thus the present approach provides a method of comparing the two types of data in addition to information not available with either experimental design alone.

METHODS

Two male volunteers, ages 27 (H-743) and 50 (H-689) resided on a residential research ward for the duration of the study. The subjects were cigarette smokers who had histories of abuse of a variety of drugs including opioids, stimulants and sedatives; both had histories of intravenous cocaine use. Except for one hour prior to and during experimental sessions, subjects were free to smoke their usual brand of cigarettes but they were not given access to illicit or therapeutically used drugs. They were not permitted to consume caffeinated substances for 12 hours prior to the start of, or during, sessions.

Experimental sessions were approximately three and one-half hours in duration and were scheduled one to three days apart. Prior to a session, a catheter was inserted into a forearm vein and its patency was maintained with a gravity fed dextrose solution (12 ml per hr). Subjects were comfortably seated with access to an operant test panel equipped with two levers and attendant stimulus lights. Reading materials were also available. The subject's electrocardiogram was on constant display to a research nurse who sat out of sight of the subject and also monitored behavior.

The reinforcement schedule was programmed and data were collected using a PDP8 computer system. Responding on only one of the two levers on the operant panel had any programmed consequences and this lever was distinguished by the illumination of a green light immediately above it. The schedule was as follows: Each 100 responses (lever-presses) on the designated lever produced a 10-sec illumination of a light (red light over the lever) and sounding of a tone (fixed-ratio or FR 100 schedule); the first FR 100 completed after the lapse of a one-hour interval (fixed-interval or FI 60-min schedule) produced the 10-sec light-tone combination and a 1 ml injection of either cocaine or saline. During some sessions the light-tone stimulus was only presented when an injection was obtained, and responding within the one-hour intervals had no programmed consequences. Sessions were terminated

when either three injections had been delivered or 3.5 hours had elapsed. A session light (house light) at the top of the panel was illuminated for the entire duration of the session, but for one minute following each injection the stimulus light over the lever was extinguished and responses had no scheduled consequences (timeout). During the timeout, subjects were required to rate any positive and/or negative effects, produced by the injection, on 100 mm visual line analog (VLA) scales.

Immediately following each session, two questionnaires were administered to quantitate interoceptive effects produced by injections. The first was a short form (40 items) of the Addiction Research Center Inventory (ARCI) which contains empirically derived scales sensitive to the effects of several classes of psychoactive drugs. The second was the Single Dose Questionnaire which contains a scale of drug liking, a drug identification list with the names of 12 commonly used drugs, and a symptom check list. Additionally, the attending nurse filled out an observer-version of the Single Dose Questionnaire.

All lever pressing and drug self-administration was "voluntary". The following instructions were read to the subject immediately prior to each session:

> During this test you are free to press the left lever as often as you like. Only presses on the left lever will produce injections; any responses on the right lever will have no effect. However, you do not have to press the left lever at all, but you must remain seated, awake and equipped with the catheter for the duration of the session. During the session certain lever presses will turn off the green light, turn on the red light and sound a tone. Other lever presses will result in the light, tone, and an i.v. injection of either some dose of cocaine or placebo. Doses of cocaine or placebo will remain unchanged throughout an individual session. After each i.v. injection, the lights will go out for about a minute and the equipment will not operate. If you miss an injection, the lights will also go off for about a minute. When the lights come back on, you will need to check your responses on form IVN-5, and then you may continue to lever press if you like. If you reach the injection limit programmed into the computer, the lights will go off until the program decides that it is safe for YOU to continue. You may end a session or injection immediately by turning off the AC power switch.

Subject H-689 was the first subject tested in this study. He was initially tested during 8 sessions in which cocaine injections (25 mg) were available for self-administration and 7 sessions in which only saline was available for self-administration. Subject 743 was tested for a total of 37 sessions under a variety of conditions in which either cocaine or saline were presented and in which the secondary stimuli were either

presented or omitted during the 60-minute intervals. For H-743, cocaine doses were either 25, 7.9 or 2.5 mg per injection, and the dose was held constant across sessions until behavior stabilized for at least three sessions.

RESULTS

Self-report measures indicated that 25 mg cocaine injections produced interoceptive effects which were reliably different from those produced by saline. Specifically, scores were elevated on the Morphine Benzedrine Group (MBG) and Lysergic Acid Diethylamide (LSD) scales of the Addiction Research Center Inventory (ARCI), and the Liking scale of the Single Dose Questionnaire. Observer ratings of drug liking were also elevated following cocaine injections when compared to placebo. Scores on the positive VIA scale averaged 28 mm for H-689 and 34 mm for H-743 during their initial series of cocaine injections. Occasionally, a negative rating of a few mm occurred after a cocaine injection but the average negative rating was only 1 mm or less. Saline injections also produced average ratings of 1 mm or less on either VLA scale. For subject H-743 the lower dose of 7.9 mg produced weak but consistent ratings of about 3 mm on the positive scale and 0 mm on the negative scale; at 2.5 mg ratings were 0 mm on either scale after the first session. Interestingly, VLA scale scores tended to decline within sessions across the three injections: second injection scores were about 77% of first injection scores and third injection scores were about 71% of first injection scores.

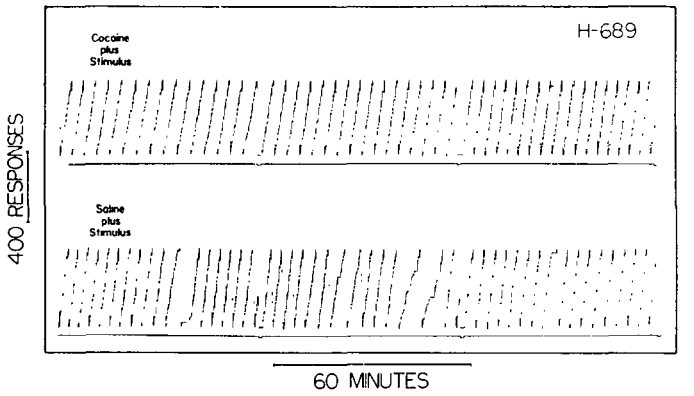

Fig. 1. Representative cumulative records from H-689 with cocaine (upper record) and saline (lower record) injection. The records show that high rates of responding occurred under both conditions.

Both subjects pressed the active lever and self-administered at least one injection per session. whether cocaine or saline was available. Subject H-689 pressed the lever at a high rate throughout the sessions, pausing only during injections. Figure 1 shows representative cumulative records of responding from both a cocaine and a saline sess ion. As shown in the

records, saline maintained high and monotonic rates of responding comparable to those maintained by cocaine and there was no evidence of a trend in rate of responding across sessions. Also shown in the figure, there was little evidence of the development of patterns of responding characteristic of those obtained in of cocaine self-administration in animal subjects, or when other reinforcers are used in behavioral studies with human volunteers. That is, there was no evidence of the "break and run" responding on the fixed-ratio components, nor was there evidence of positively accelerated response rates ("scalloped" response rate patterns) within the fixed-interval schedules.

During initial sessions, subject H-743 also pressed the lever at high rates that did not appear to be under explicit control of either the stimuli presented according to the fixed-ratio schedule, or by the injection of cocaine that followed completion of the fixed-interval schedule. However, by about the sixth session, the subject began to reliably pause after each injection, and to develop patterns of responding characteristic of those seen with animal subjects under fixed-interval schedules of food or drug reinforcement. Furthermore, the cocaine-paired stimuli appeared to be maintaining patterns of responding characteristic of those obtained under fixed-ratio schedules of food or drug reinforcement. These patterns of responding are illustrated by the representative cumulative records shown in Figure 2. As shown in the figure, overall rates of responding were substantially lower when saline was substituted for cocaine. However, the within-session patterns of responding were somewhat more interesting. For instance, during the first interval of each session, there was no reliable difference between rates of responding when cocaine or saline were delivered. However, during subsequent intervals, if cocaine had been delivered at the end of the first fixed-interval, response rates were similar or somewhat higher; if saline had been delivered following completion of the first fixed-interval, response rates generally diminished considerably. Within each fixed-ratio, rates were high and monotonic and did not vary widely across conditions.

Diminished rates of responding occurred at lower doses of cocaine; however, on each day that this subject was tested at the lower doses, all three available injections were self-administered. When saline was scheduled only the first injection was taken on one-half of the sessions. Interestingly, at low doses, VLA scale scores were very low or 0, and no drug effects were indicated on the Single Dose Questionnaire, although all available injections were obtained and overall rates of responding exceeded saline values.

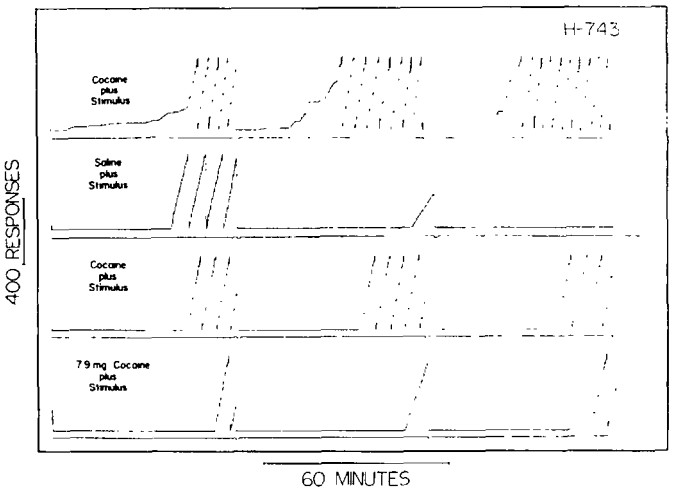

Fig. 2. Representative cumulative records from H-743 under each of the indicated conditions. The records show that rates of responding maintained by 25 mg of cocaine exceeded those maintained by either saline or 7.9 mg.

DISCUSSION

The present results indicate that human subjects can be safely used to study cocaine self-administration behavior under second-order schedules of reinforcement. The study also confirmed that self-administered cocaine produced similar interoceptive effects as cocaine administered independently of the behavior of the subject (e.g., Fischman, Schuster, Resnekov, Shick, Krasnegor, Fennell and Freedman, 1976). For one subject (H-743), the number of cocaine injections exceeded the number of saline injections, and response rates maintained by cocaine exceeded those maintained by saline. These data suggested that cocaine served as a positive reinforcer relative to saline. However, subjects always took the first available injection of the session and there was a tendency to take injections even in the absence of reliably identified subjective effects. Similarly, whereas rates of lever pressing were either stable or increasing within the sessions when cocaine was administered, self-reported effects tended to diminish across subsequent injections. It is possible that the stimuli presented according to the second-order schedule contingency contributed to the persistance of the behavior. As suggested above, the results from procedures most commonly used to assess abuse liability in humans (self-reported interoceptive effects) did not perfectly correspond to the results from procedures most commonly used to assess abuse liability in animals. Identification of the factors that lead to such behavioral persistence may help to better understand and treat cocaine dependence; for here, as is the case with other drugs of abuse, a treatment obstacle is that the degree of behavioral persistence often appears disproportionate to the effects of

the drug. Of more theoretical interest, further investigation will be required to assess the conditions under which patterns of behavior leading to human drug self-administration are similar to those which have been obtained with animals. In a similar vein, it is also important to study the role of the drug associated stimuli in the mediation of drug self-administration behavior as well as the possibility that such stimuli may come to produce cocaine like interoceptive effects in their own right.

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The Influence of Pharmacotherapy on the Neonatal Sleep Patterns of Infants Born to Opiate-Addicted Mothers

M. Guilia Torriolo, M. Chiara Steffanini, P. Mariotti, S. Calzolari, C. Fundaro, and E. Tempesta

The sleep cycles of 24 infants (15 male, 9 female) born at term (39-41 wks) to opiate-addicted mothers were studied. All infants weighed >2500 grams and, with the exception of neonatal abstinence (NAS), were considered healthy infants. In accordance with the various pharmacotherapies recommended for withdrawal, the infants were divided into four groups (6 infants each): Gr.l-treated with phenobarbital (Pb); Gr.2-treated with methadone (Me): Gr.3-treated with Pb and Me: Gr.4-infants without abstinence and needing no treatment. The NAS Scoring System (Finnegan) was used to assess severity of withdrawal. Neurological examinations were performed at one week of age and upon discharge from the hospital. Sleep studies consisted of three hour polygraphic recordings obtained from each infant during the second week of life and/or following withdrawal. These included 6 EEG channels (Fp2-C4; C4-T4; T4-02; Fp1-C3; C3-T3; T3-01) and channels of electroculogram (EOG) and electromyogram (EMG) from the chin surface. Each EEG polygraphic record was independently evaluated by two investigators. *A* two tailed t-test (student's t) was used for statistical analyses. Findings included 1) poor sleep organization in infants born to opiate-addicted mothers: 2) decrease of Quiet Sleep (Q.S.) in Gr.1 (Pb); 3) alterations in Active Sleep (A.S.) for all treated groups, but not for the control infants. The possible mechanisms involved in these sleep cycle abnormalities are discussed.

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Contributions of Maternal Alcohol, Caffeine, and Cigarette Use to Neonatal Status

Juliana S. Lancaster, Claire D. Coles, Kathleen A. Platzman, Iris E. Smith, and Arthur Falek

The teratogenic effects of maternal alcohol use on prenatal growth and neonatal neurobehavioral status have been well documented. There has been some debate about the real consequences of alcohol and other drug use when such use is confined to relatively small amounts or is described as social in nature. Recent work has shown increased neurobehavioral alterations in the newborns of women who drank throughout pregnancy as compared to the infants whose mothers did not drink or stopped mid-pregnancy. The present analysis was undertaken to examine the individual and/or interactive contributions of prenatal exposure to alcohol, nicotine, caffeine and marijuana to neonatal size and neurological status. On the third day of life, 143 full-term infants were examined by a developmental psychologist using the Prechtl Neurological Examination for Newborn Infants. Infants whose mothers drank throughout pregnancy were smaller and had lower Prechtl scores than did those whose mothers did not drink or stopped mid-pregnancy. Regression analyses were conducted on alcohol-exposed and non-exposed infants separately using measures of maternal health. Other drug use, and alcohol use were appropriate as predictors of birthweight, head circumference, and Prechtl score. Results showed that roughly 10 percent of the variance in Prechtl scores was accounted for by the regressions for exposed and non-exposed infants alike. For non-exposed infants, 3-4 percent of the variance could be accounted for by the regression. However, for the exposed infants, 20 percent of the variance in each growth measure could be accounted for by the regression. Results indicated that neurological status and prenatal growth, are measures of different aspects of infant health. The results strongly support the teaching that pregnant women should avoid consumption of alcohol and other drugs.

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Perinatal and Developmental Outcome of Infants Exposed to Methadone In-Utero

Karol Kaltenbach and Loretta P. Finnegan

The purpose of this research is to delineate the effects of methadone exposure in-utero. Subjects were 141 infants born to drug dependent women maintained on methadone during pregnancy and 127 non-drug exposed comparison infants matched for race, maternal age, and socioeconomic status. The average daily methadone dose for mothers maintained on methadone was 39 mg. Methadone exposed infants had smaller birth weights than comparison infants. The mean birth weight for methadone infants was 2963 gm and the mean birth weight for comparison infants was 3210 gms ($t=4.09$, $p<.001$). Differences were also found in head circumference. Methadone exposed infants had a mean head circumference of 33.2 cm and the mean head circumference found in comparison infants was 33.94 cm ($t=3.23$, $p<.01$). However, this difference was not clinically significant but rather reflects the relationship between birth weight and head circumference. No difference was found between groups in mental development. One hundred sixty infants (100 methadone exposed infants and 60 comparison infants) were evaluated with the Bayley Scale of Mental Development at 6 months of age. Mean Bayley Mental Development scores for methadone exposed infants and comparison infants were 103 and 105 respectively. These data suggest that while methadone exposure in-utero is associated with lower birth weight, by six months of age, these infants do not exhibit any remarkable developmental sequelae.

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Prenatal Cocaine Use Associated With Down Regulation of Receptors in Human Placenta

Ching H. Wang and Sidney H. Schnoll

Cocaine use during pregnancy has been shown to be associated with increased risk of abruption placentae and spontaneous abortion. The effects may be due to its vasoconstrictive action. The vascular effects may be mediated through the increased in catecholamines caused by cocaine. Opioid and adrenergic receptors have been identified on placental tissue, and prenatal exposure to opiates and ethanol results in a significant decrease in α_1- and β-adrenergic receptors. Since cocaine affects catocholamine levels, we studied whether the transient increase in catecholamines occurring during cocaine use in pregnancy caused changes in receptor systems in the human placenta.

Placental tissues were collected from 8 women with histories of cocaine use during pregnancy and age-matched controls. β-adrenergic receptors were labeled with ^{3}H-dihydroalprenolol, μ-opiate receptors with ^{3}H-naloxonc and δopiate receptors with ^{3}H-D-ala-D-leu-onkephalin.

Cocaine users showed a significantly lower number of ȝ-adrenergic receptor binding sites and μ- and δ-opiate binding sites (Bmax). In 2 patients who were also on methadone during pregnancy, the changes in μ-opiate receptor binding were abolished. Inclusion of these two placentas did not alter the significant reduction of μ-opiate receptor sites among the cocaine users ($p<.05$). There was no reduction in binding affinities at the three receptor sites, reflecting a true down regulation of the receptor population.

Down regulation of adrenergic receptor binding sites has been associated with increased levels of agonists. This would be anticipated with cocaine's blocking of catecholaminc reuptake. Cocaine may also cause release of endogenous opiates, and the transient increase in opratc agonists may cause the down regulation of opiate receptors. Why the patients on methadone did not have an alteration in the μ-opiate receptors is unknown.

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Cocaine- and Methadone-Exposed Infants: A Comparison

Ira J. Chasnoff

In conjunction with the increased use of cocaine in the general population of the United States, the number of cocaine-using pregnant women presenting to the Perinatal Center for Chemical Dependence at Northwestern University has continued to escalate. The present study of a population of cocaine-exposed infants was undertaken to investigate perinatal morbidity and mortality in infants born to cocaine-using mothers.

From January 1976 to January 1986, 52 infants were born to cocaine-using women enrolled in the Perinatal Center for Chemical Dependence. A comparison group (N=73) was selected from the population of the Perinatal Center representing women of a similar racial distribution who conceived while addicted to heroin and were converted to low-dose methadone maintenance for at least the last two trimesters of their pregnancies. These women had no history or evidence of cocaine use. Methadone-maintained women were selected as a control group in order to be able to compare two groups of women who were similar in social, demographic and environmental backgrounds as well as being comparable for cigarette, alcohol and marijuana use during pregnancy.

As in other substance-abusing populations, the cocaine-addicted women had a high incidence of infectious disease complications, especially hepatitis (24%) and venereal disease (10.5%). There was an increase in complications of labor and delivery (premature labor, precipitous labor, abruptio placentae and fetal distress) in cocaine-using women as compared to heroin/methadone-addicted women. There was no difference in fetal growth parameters between the two groups. Cocaine-exposed infants showed a greater deficiency in' state control, and on follow-up there appeared to be an increased rate of sudden infant death in the cocaine-exposed infants.

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Sexual Activities in Drug Dependent Women

Robert Smith, Dianne Regan, Saundra Ehrlich, and Loretta P. Finnegan

Drug dependent women tend to lead chaotic lives. No where is this more evident than in relationships, particularly with men. Unable to deal with the conflicts, addicts frequently find solace in drugs. Since drugs may substitute for sex or be used to mask feelings of sexual incompetence, a link has been suggested between sexual activities (behavior, performance, desire) and the use of such drugs as heroin, marijuana, opiates, LSD and amphetamines. To investigate further, 98 women were asked to complete a Sexuality Questionnaire. Subjects included 51 drug dependent women (DDW) enrolled in a methadone program and a group of 47 drug-free control women (CW) matched for age, race, and socioeconomic status. The groups were compared on adolescent and adult sexual parameters. In many respects, the DDW were similar to the CW in sexual behaviors, despite high depression scores, lower self-esteem and sexual and physical abuse. Differences in the groups included: less frequent sexual activity than CW following first intercourse (55 vs 80%); tendency to fewer relationships (76 vs 87%); less current sexual activity (50 vs 60%): and drug abusing partners with difficulties in sexual performance. Although first intercourse in both groups occurred at approximately age 16, for 3 of the DDW, this event was the result of rape. The counselors felt that for many of the women, the drugs were initially used to cope with the trauma of sexual abuse. First intercourse for the majority of DDW occurred less as a result of an ongoing relationship and more as an impulsive event. Fewer Family Center women reported current sexual relationships/activities. These findings suggest an association between drug abuse and difficulty in forming stable, satisfying sexual relationships.

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Cocaine Abuse in Pregnancy: Effects on the Fetus and Newborn

Lynn Ryan, Saundra Ehrlich, and Loretta P. Finnegan

A dramatic increase in cocaine use is occurring among pregnant drug-dependent women (DDW), yet few studies exist regarding the potential effects of this abuse on the neonate. Within a methadone maintenance program providing pre- and postnatal services for DDW, the outcome of infants born to cocaine-using DDW was compared to that of infants of non-cocaine using DDW and non-DDW. The study population included 150 pregnant women: 50 women used heroin and methadone plus cocaine, 50 used heroin and methadone minus cocaine, and 50 were non-DDW. All were matched for age, race, gravidity, parity, socioeconomic status, and cigarette smoking. Significant differences were found between the cocaine and drug-free groups in infant birth weight, length, head circumference, and Apgar scores, with the cocaine group having lower values for each variable. Mean gestational age did not vary between groups. The cocaine group included 1 spontaneous abortion and 4 fetal deaths; non-cocaine DDW had 2 fetal deaths: there were no fetal deaths in the control group. Abstinence scores, incorporating 21 parameters to quantify symptoms in infants born to DDW, were studied. Mean scores for 19 of the parameters were lower in the cocaine group than in the non-cocaine DDW, with the exception of vomiting and convulsions. These data suggest that: 1) infants born to drug dependent women, both cocaine and non-cocaine users, have poorer general outcomes than those born to drug free women; 2) maternal cocaine use does not appear to increase the incidence of neonatal abstinence: 3) pregnancies complicated by cocaine abuse are at greater risk for fetal loss from spontaneous abortions and later from fetal death; 4) infants born to cocaine abusing women had infants with decreased birth weight, head circumference, length and Apgar scores: and 5) there may be a greater incidence of Sudden Infant Death Syndrome in infants exposed to cocaine.

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The Law of Hyperbolas: A Model of Drug Action Applied to Opiate Dependence and Abstinence

Julian E. Villarreal, Luis A. Salazar, Jorge E. Herrera, and Silvia L. Cruz

INTRODUCTION

Experimental analysis has produced evidence that opiate dependence is the hypertrophy of a special opiate receptor system which operates in rate mode. A formal definition of the nature of such hypertrophy has been offered (Villarreal et al. 1985 a,b). The alterations in function that constitute opiate abstinence are mediated by the hypertrophied rate-coupled system. Thus, to illustrate, opiate abstinence precipitated by opiate antagonists in early dependence occurs not when a given absolute number of receptors are occupied by opiate antagonist but when the rate of occupation by antagonist molecules is sufficiently high to reach suprathreshold excitation in opiate dependent neurons.

A formal model receptor system capable of operating in rate-coupled mode, which will be described further on, was studied by mathematical computer analysis. Our group previously showed that such model is remarkably adequate to reproduce the diversity of features of experimental opiate dependence. The purpose of the present work was to examine, under a complete range of conditions, the quantitative relationships between the main variables of the rate-coupled system and the characteristics of its responses. The result is a mathematical law of drug action that provides a broad basis of formal theory applicable to the analysis and description of the mechanisms of opiate dependence, specially as these are revealed in the complexity of changing action patterns exhibited by the abstinence-precipitating actions of opiate antagonists.

The new mathematical formulation is called the law or the rule of hyperbolas. It defines drug potency and mode of responding in simple algebraic terms, as functions of the main variables of the receptor system. It applies to any type of drug-receptor-effector structure which mediates the actions of either drugs, hormones or other humoral agents acting under the scheme of Fig. 1. Such agents must only fulfill the following two conditions: 1) they must bind with receptors obeying the law of mass action; 2) they must produce primary pharmacologic microevents which have an exponential decay, statistical or analogic, as an approximation at least.

Although the law of hyperbolas is likely to have useful application in other areas of pharmacology, it is examined now mainly in the concrete sense of a theory applied to the abstinence-precipitating actions of opiate antagonists in the moving baseline of progressive dependence. Therefore, all drug action treated in this paper should be regarded as referring primarily to those excitatory actions of antagonists that mediate the precipitation of abstinence. The experimental evidence clearly demonstrates that abstinence produced by opiate antagonists is not the result of the simple occlusion of receptors by silent chemical occupation. The onset of antagonist occupation of receptors causes excitatory subthreshold events which rapidly fade away, leaving afterwards the receptors chemically occupied but physiologically inactive.

A diagram of the model of rate-coupled receptor system employed is shown in figure 1. In this type of system, the duration of the primary pharmacologic stimulus is determined not by the entire duration of the chemical bond between drug and receptor but by the dynamics of the primary biological effector associated with the primary chemical receptor (e.g., by the opening and closing of ionic channels in excitable membranes, or by stimulation of enzyme systems producing second messenger substances). A good example is given by opiate abstinence precipitated by the antagonist naloxone in early dependence. Here the number of receptors that appear to be participating in the process of neuronal excitation (the DRact of Fig. 1) at any moment after drug administration is smaller than the total number of occupied receptors (the DRact plus the DRinact). The disparity between the number of receptors contributing to cellular activation and the number occupied by drug molecules limits pharmacologic sensitivity with a mathematically predictable temporal course (Pardo, 1959) and gives rise to self-antagonism as well as to the other features that characterize the behavior of rate-coupled systems (Villarreal, et al., 1985 a,b; Cruz, 1986).

The experimental evidence indicates that the hypertrophy of the opiate rate-coupled system of opiate dependence consists in an increased duration of the primary pharmacologic microevents caused by the onset of binding of opiate antagonists to their receptors. These changes in duration of the primary excitatory microevent were simulated through mathematical means by varying the numerical value of the decay rate constant kt of the active state of the receptor (Villarreal et al., 1985 a,b; Cruz, 1986). It was found that progressive decreases in the kt of the system cause a hypertrophy of the abstinence response system which reproduces all the defining phenomenological characteristics of opiate dependence considered in specific detail. These characteristics are: 1) the emergence of a capacity of the organism that becomes opiate dependent to produce abstinence syndromes in response to opiate antagonists; 2) the progressive increase in severity and duration of the abstinence syndromes that can be produced in the course of dependence; 3) the response of precipitated abstinence requires antagonist administration at high rates in early dependence but this requirement is progressively reduced as dependence increases in magnitude; 4) there is a progressive transformation of the mode of responding of the abstinence receptor

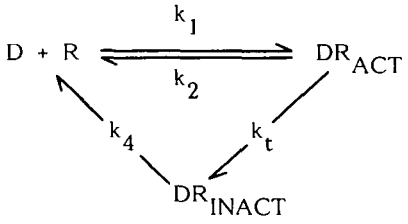

FIGURE 1. Model receptor system capable of rate-coupled behavior. the drug-receptor-effector complex is initially in a condition of activation as DRact. Afterwards, the effector becomes inactivated but the receptor remains occupied as DRinact. The rate constant for decay of the activated effector is kt. Modified from Gosselin (1977).

system from rate-coupled to occupation-coupled behavior; 5) in the course of dependence, there is an enormous supersensitivity to the abstinence-precipitating actions of single doses of antagonists, with parallel shifts to the left in their dose-abstinence curves, with very large changes in their ED50s but without any changes in their chemical binding properties.

The law of hyperbolas is given as the algebraic equation that relates kt and the other basic variables of the rate-coupled receptor system to: 1) the dose of drug required for the production of a fixed reference level of pharmacologic response (the ED50 is employed here); 2) the mode of pharmacologic responding (i.e., whether rate- or occupation-coupled). The equations discussed in this paper refer only to peak responses produced by the instantaneous addition of single drug doses. We chose to deal with these responses first because they are the most interesting part of the abstinence response and also because peak responses have previously eluded a satisfactory systematic treatment in any theory of drug action.

It must be noted that the development of the law of hyperbolas is a consequence of the full logical and mathematical derivation of propositions that we learned from Pardo (1959) and of the differential equations elaborated by Cosselin (1977), both of which contributions can now be. amply worked out because of the availability of electronic computers.

METHODS

We studied computer-generated solutions of the set of simultaneous differential equations derived by Gosselin (1977) for receptor systems operating as in Fig. 1. These solutions are formal simulations of the temporal course of events in the receptor system in response to the administration of a simulated drug dose. Such solutions were obtained with Gosselin's analytic integrations and also by numerical methods of integration. Next are the simultaneous differential equations employed.

$$d[ACT]/dt = k_1 (D)[R] - k_2[ACT] - k_t[ACT] \quad (1)$$

$$d[INACT]/dt = k_t[ACT] - k_4 [INACT] \quad (2)$$

Where [ACT] = DRact, and [INACT] = DRinact, and the other symbols as in Fig. 1. In actual computation, the term k1 (D)[R] of equation I was transformed to k1 (D)[1 - DRact - DRinact] because the total of the receptors is assumed to be conserved as: R total = 1 = R + DRact + DRinact.

Complete series of simulated pharmacologic responses to different values of the drug dose parameter were produced under each tested set of conditions of the system. Dose-response curves were then constructed where the response taken was the peak quantity of receptors in the active state generated by each simulation of dose administered (the peak number of DRact). Finally, ED50 values were obtained from dose-response curves for a wide diversity of combinations of kt, k1, and k2. In all these studies, the constant k4 was fixed at 0.0001. Peak responses are affected only by extreme values of k4; this constant affects mostly stationary state responses.

RESULTS

For any given simulated pharmacologic agent, defined with fixed k1 and k2, variations in log kt produce changes in log ED50 along lines with the form of a hyperbola as in Fig. 2. Each hyperbola represents a line of pharmacologic isoactivity (ED50s) as a function of variations in kt; All drugs can be represented by identical hyperbolas. The drugs vary in the position of their hyperbolas in the plane defined by log kt and log ED50.

All drug hyperbolas have similar asymptotes. One is a horizontal asymptote whose numerical value on the y axis corresponds precisely to the value of the log of the chemical dissociation constant, log (k2/k1), of the drug in question (see Fig. 2). Thus, in the space of the hyperbolas, when the active state is long-lived (with a small decay rate constant kt), occupation becomes the limiting factor and the value of log ED50 is primarily determined by the horizontal asymptote log (k2/k1); the chemical dissociation constant is then the primary determinant of drug potency. Furthermore, as Fig. 2 shows, the character of the responses defined by the horizontal arms of the hyperbolas is that of occupation-coupled modes of pharmacologic behavior. Therefore, we can designate the horizontal branches of the hyperbolas as the domain of occupation-coupled modes of pharmacologic responding.

When the duration of the primary pharmacologic microevent is short (large kt), the duration of the active state rather than occupation becomes the limiting factor in the determination of drug potency. With short microevents, the value of log ED50 is primarily determined by the second, oblique, asymptotes of the hyperbolas. One form of the equation of the oblique asymptotes is the following:

$$\log \ ED_{50} = \log \ k_t + \log \ (2/k_1) \qquad (3)$$

The oblique branches of the hyperbolas are the domain of rate-coupled modes of responding (Fig. 2). As equation 3 shows, in this rate-coupled domain, the log ED50 is determined by log kt, with

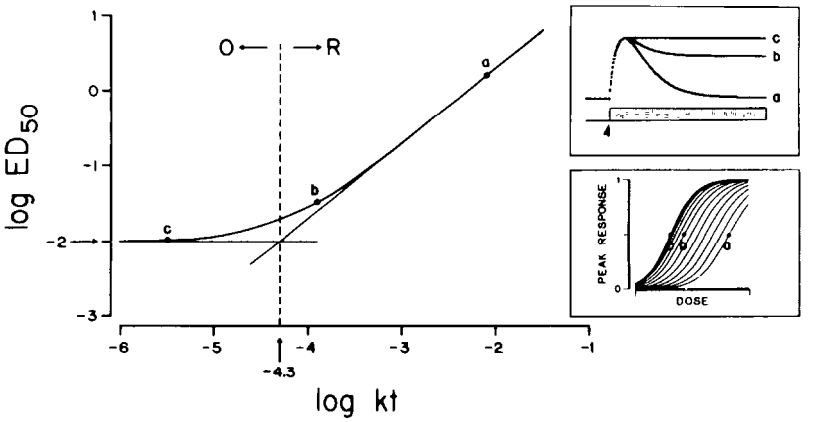

FIGURE 2. An example of a hyperbola relating log kt to log EL50 for a drug with kl = 0.01 and k2 = 0.000l; log (k2/k.l) = -2. One inset shows three sample simulated responses to each of three points a, b, and c on the hyperbola. The time bases of the three records are different. The other inset show the complete set of dose-response curves that produced the hyperbola.

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a slope of one, and with the chemical association rate constant k1 playing a role in determining the vertical intercept of the oblique asymptote, on the plane of log kt vs log ED50.

For any hyperbola in general, the point at which its two asymptotes intersect is called its center. Such center is a convenient point to place the borderline between the domains of occupation-coupled behavior and of rate-coupled behavior. The x coordinate of the center of any hyperbola corresponds to the value of log (k2/2) of the drug in question (see for instance Fig. 2 where log (0.0001/2) = -4.3). It may be said that occupation-coupled modes of responding will be observed with any drug in a system whose kt falls to the left of k2/2.

Systematic mathematical simulations provided the material to define the general equation of the pharmacologic hyperbolas. Starting from the cannonical equation for a hyperbola with vertical major axis, an equation was found with a rotation and shape fitting the characteristics of the pharmacologic hyperbolas:

$$y^2\text{-}xy\text{-}0.085=0 \qquad (4)$$

This equation, however, is centered at the origin of the coordinate axes. The pharmacologic hyperbolas have centers outside the origin, with coordinates given by x = log (k2/2) and y = log (k2/kl). Equation (4) can then be rewritten, replacing x and y by the correction terms for translation of the origin in the plane defined by log kt

and log ED50. Expanding the corrected version of (4), rearranging it and solving for log ED50, we obtain the general equation of the pharmacologic hyperbolas:

$$\log \quad ED_{50}=((D+E)/2)+[((D+E)/2)^2-DE+C]^{1,2} \tag{5}$$

Where D = log (k2/kl), E = log (2kt/kl), and C is a numerical constant equal to 0.085. There are only two variable terms in equation (5). One is "D", the log of the chemical dissociation constant, KD. The other term "E" contains kt, the decay constant of the primary pharmacologic stimulus. The new term 2kt/kl may be called the "pharmacologic evanescence constant", KE. In view of this, we can rewrite the equation of the oblique asymptotes (3) as follows:

$$\log ED_{50} = \log (2kt)/k1 = \log KE \tag{6}$$

It is then worth noting that just as the horizontal asymptotes are defined by the chemical dissociation constant KD, the oblique asymptotes are defined by a hybrid pharmacochemical term KE = 2kt/k1, which is the pharmacological analogue of the chemical dissociation constant.

DISCUSSION

The 3-component receptor model presented here is based on Gosselin's receptor inactivation model (1977). We chose this model as a starting point because it is the simplest cyclical receptor model discussed in the literature which includes a refractory step and which has formal features of broad applicability. Our model retains Gosselin's equations intact but reformulates its physiologic content to suit the demands of the experimental evidence concerning opiate dependence as well as that produced by direct studies on the physiology of excitatory microevents in excitable membranes. The model receptor system presented in this paper describes our reformulation and the interested reader may consult Gosselin's paper for a detailed comparison of both models. The major difference lies with the variable to which Gosselin attributes the role of primary pharmacologic stimulus. In our model the primary pharmacologic stimulus is the total number of receptor-effector units in the activated state (DRact) at any one time. Gosselin, however, proposes that the primary pharmacologic stimulus should be proportional to the rate at which receptor units in the active state turn to the inactive state; quantitatively, this means that the pharmacologic stimulus should be proportional to the mathematical product of what we here call kt times DRact. Such proposal would immediately imply absurdities for our experimental problem. To assume with the full Gosselin model that the pharmacologic stimulus were kt times DRact would mean that in very early dependence (large kt) there would be large abstinence responses, and that in well developed dependence (small kt) there would be small abstinence responses. On the other hand, this paper and previous communications of our group provide abundant evidence of the validity of Gosselin's mathematical kinetics in accounting for the processes of opiate abstinence and dependence. Gosselin's kinetics give mathematical form to basic processes in

the microphysiology of excitable membranes. For instance, there are many recent reports showing that the active states of excitatory single ionic channels in a variety of membranes have a statistically exponential decay, or at least a close approximation. We have begun work searching for a direct demonstration of the equivalence of kt with the rate of decay of depolarizing ionic channels in the membranes of opiate-sensitive neurons.

The law of hyperbolas is specially well suited as a quantitative framework for the complex set of phenomena presented by opiate dependence and abstinence. The law is also well suited as a conceptual base for the comparative analysis of antagonists with regard to their abstinence-precipitating actions. Finally, the law can serve as the basis for a theory of interactions between antagonists, including the cases of self-antagonism and of specific cross-antagonism. The relative positions of the hyperbolas of two antagonists will indicate if and when one of them will act as antagonist to the abstinence-precipitating actions of the other antagonist.

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New Approaches to the Evaluation of Opioid Agonists and Antagonists Upon the Isolated, Electrically Stimulated Mouse Vas Deferens Preparation

Charles B. Smith

INTRODUCTION

Opioid receptors are classified in part by the relative potencies of agonists and the selectivity of antagonists in a variety of preparations (Martin et al., 1976; Lord et al, 1977). The isolated, electrically stimulated guinea-pig ileal and mouse vas deferens preparations have been used widely to evaluate the pharmacological actions of opioid agonists and antagonists. In the past a comparison of the actions of such drugs upon the two preparations has been used to evaluate receptor selectivity. Mu, kappa and delta receptors are present in the mouse vas deferens preparation. The development of new, highly selective antagonists such as the irreversible mu receptor antagonist beta-funaltrexamine (beta-FNA, Portoghese et al, 1980; Ward et al., 1982) and the reversible delta receptor antagonist ICI-174864 (Cotton et al., 1984; Smith et al., 1984) have made possible the evaluation of selectivity of opioid agonists and antagonists by use of the mouse vas deferens preparation alone. The purpose of this report is to describe new procedures used by the Drug Evaluation Unit at the University of Michigan to study opioid agonists and antagonists upon the mouse vas deferens preparation and to show results obtained with various standard opioid agonists and antagonists by the use of these procedures.

METHODS

Male, albino ICR mice, weighing between 25 and 30 g, were used. The mice were decapitated, the vasa deferentia removed, and 1.5 cm segments were suspended in organ baths which contained 30 ml of a modified Kreb's physiological buffer. The buffer contained 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 HC1, 0.3, tyrosine, 0.2; ascorbic acid, 0.1; and disodium edetate,0.03. The buffer was saturated with 95% O_2 - 5% CO_2 and kept at 37°C. The segments were attached to strain gauge transducers and suspended between two platinum electrodes. After a 30-min equilibration period, the segments were stimulated once every 10 sec

with pairs of pulses of 2 msec duration, 1 msec apart and at supramaximal voltage.

The following antagonists were studied: naltrexone HCl, ICI-174864 [N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH] and beta-FNA. Naltrexone and ICI-174864 were added to the organ baths 15 minutes before the determination of cumulative concentration-effect relationships for the various agonists. beta-FNA was added to the organ baths after the initial equilibration period. Thirty min later the beta-FNA was removed from the organ baths by repeated washings with fresh buffer. The tissues were washed three times every 5 min for 15 min. Cumulative concentration-effect relationships for the various agonists were then determined 20 min after the last wash (i.e. 30 min after the beta-FNA was removed from the organ baths). The following agonists were studied: DADLE [D-Ala^2-D-leu^5 enkephalin], DSLET [Tyr-D-Ser-Gly-Phe-Leu-Thr], DPDPE [D-pen-D-pen enkephalin], FK-33824 [Tyr-D-Ala-GlyaePhe-Met-CO)-01], LY-123502 [Tyr-D-Ala-Gly-4-F-Phe-phenylgylcinamide acetate, morphine sulfate, MR-2033 [(±)-5,9-alpha-dimethyl-2',-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan], tifluadom, sufentanil and U50, 488H [trans-3,4-dichloro-N-methyl-N(2-(l-pyrro-lidinyl)cyclohexyl)benzeneacetamide methanesulfonate hydrate]. EC 50's were calculated by probit analysis, and pA_2 values were determined to assess relative potencies of antagonists. All values are the means of at least 6 determinations ± the standard error of the mean.

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 naltrexone. The concentration of the unknown drug is varied from the lowest with activity to that which is maximally effective. 2) If the submitted drug inhibits the twitch, the ability of naltrexone to reverse the inhibition is determined. 3) The submitted drug is assessed for its ability to antagonize the actions of morphine on the vas deferens. 4) The drug is assessed for its ability to reverse the inhibition produced by a maximally effective concentration of morphine. 5) Finally, if the drug has opioid agonistic activity, studies are conducted to determine the receptor type upon which it acts. If it has antagonistic activity upon the vas deferens or upon any of the other preparations used in the Drug Evaluation Unit, the type of antagonism (competitive, noncompetitive) and the receptor selectivity is determined.

The procedure for determining the type of receptor upon which an agonist might act is outlined in FIGURE 1. A drug which acts primarily upon delta opioid receptors will be blocked by both ICI-174864 and beta-FNA. A drug which acts upon kappa receptors will be blocked by naltrexone, but not by beta-FNA. A drug which acts primarily upon mu receptors will be blocked by naltrexone and beta-FNA but not by ICI-174864.

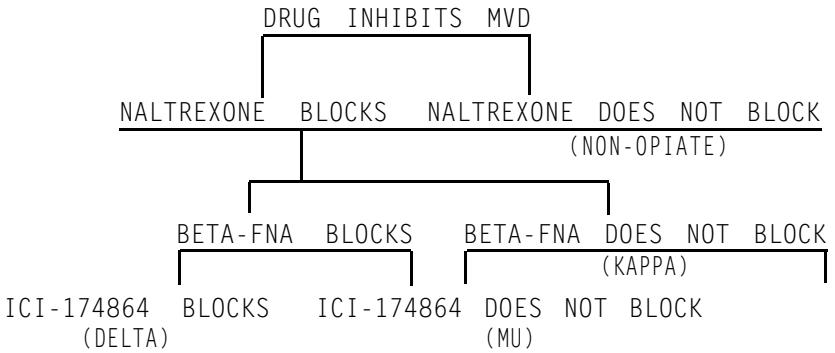

FIGURE 1. Schematic diagram of procedure for evaluating opioid agonists.

The procedure for the further evaluation of opioid antagonists is shown in FIGURE 2. A drug which is selective for mu receptors will block the actions of morphine or sufentanil. One which is selective for delta receptors will block the actions of DSLET, DADLE or DPDPE, and one selective for kappa receptors will block the actions of U50,488H. A naltrexone-like antagonist which has activity at all three receptors will block the actions of the three types of agonist although the pA_2 values for the antagonist will differ when determined with the various receptor-selective agonists.

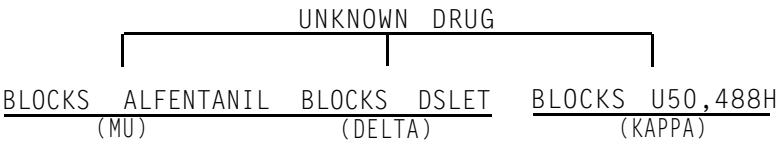

FIGURE 2. Schematic diagram of procedure for evaluating opioid antagonists.

RESULTS

Naltrexone caused parallel shifts to the right in the concentration-effect curves for mu, kappa and delta receptor agonists. pA_2 values for naltrexone determined with the mu agonists morphine and sufentanil were 8.59 ± 0.15 and 8.60 ± 0.13 respectively. pA_2 values for naltrexone determined with the kappa agonists MR-2033 and tifluadom were 8.20 ± 0.24 and 8.34 ± 0.15, and pA_2 values determined with the delta-agonists DSLET, DADLE and LYZ-123502 were 7.60 ± 0.10, 7.65 ± 0.05 and 7.57 ± 0.05 respectively. The slopes of the Schild plots did not differ from unity for any of the agonist-antagonist interactions. These results indicate that naltrexone has differing affinities for mu, kappa and delta opioid receptors. This type of analysis, however, is

not practical in the routine evaluation of new compounds for opioid activity because of the large number of experiments required to determine individual pA_2 values.

As reported previously (Smith et al., 1984), ICI-174864 caused parallel shifts to the right in the concentration effect curves

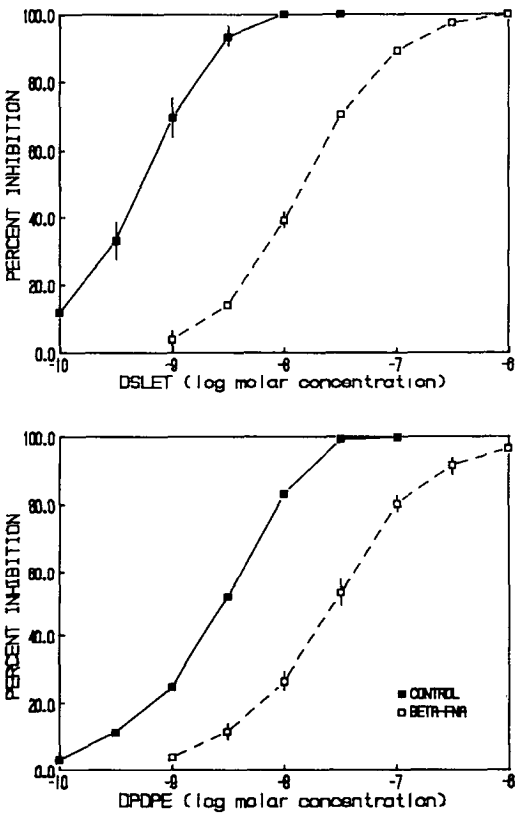

FIGURE 3. Antagonism by beta-FNA of the effects of DSLET and DPDPE upon the isolated, electrically stimulated mouse vas deferens preparation. Ordinate: inhibition expressed as a percent of the baseline contraction; abscissa: concentration of agonist. Solid squares: control preparations. Open squares: preparations were exposed to beta-FNA, 1 microM, for 30 min and then washed repeatedly with fresh buffer for a further 30 min after which concentration-effect relationships for the agonists were determined. Each point represents the mean of at least 6 determinations. Vertical lines represent standard errors of the mean.

for delta receptor agonists. pA_2 values for ICI-174864 determined with DSLET, DADLE and LY-123502 were 7.90 ± 0.06, 7.60 ± 0.03 and 7.70 ± 0.51 respectively. ICI-174864 did not shift the concentration-effect curves for morphine, sufentanil, U50,488H, Mr-2033 or tifluadom in concentrations as high as 30 uM. Thus, as reported previously, ICI-174864 is a very selective antagonist of delta opioid receptors.

In addition to its ability to irreversibly block mu opioid receptors, beta-FNA antagonized the actions of delta receptor agonists such as DSLET and DPDPE upon the mouse vas deferens preparation (FIGURE 3). In contrast beta-FNA at a concentration of 1 µM did not alter responses to either U50,488H (FIGURE 4) or to Mr-2033. Thus, in the mouse vas deferens preparation, beta-FNA is an irreversible antagonist at delta as well as at mu opioid receptors.

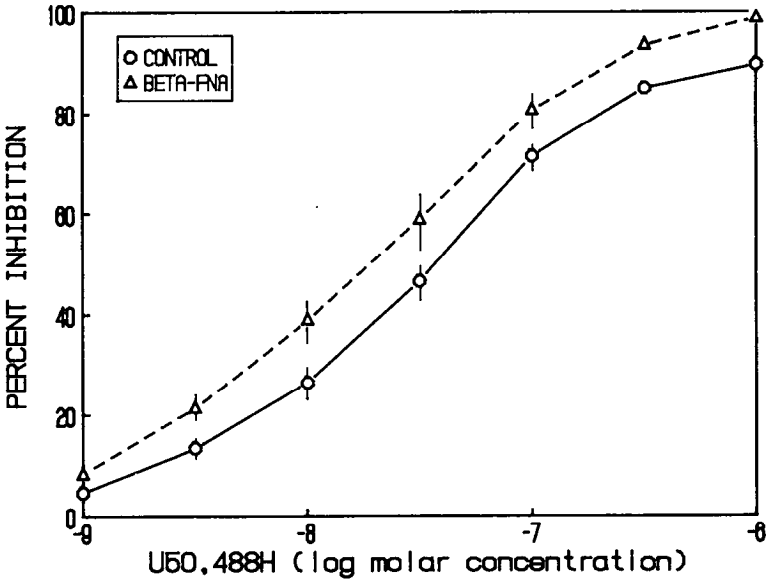

FIGURE 4. Failure of beta-FNA to antagonize the effects of U50, 488H upon the isolated, electrically stimulated mouse vas deferens preparation. See FIGURE 3 for details.

DISCUSSION

In the past it has been difficult to determine upon a single smooth muscle preparation whether an opioid agonist acted primarily at mu, kappa or delta receptors. In addition, the assessment of the selectivity of opioid antagonists has been equally difficult. The development of new and more selective agonists and antagonists has led to new strategies for the evaluation of opioids upon the isolated, electrically stimulated mouse vas deferens

preparation. Previously we reported that exposure of the vas deferens preparation for 15 min to beta-FNA results in a blockade of both mu and delta opioid receptors (Smith et al., 1984). The present study shows further that beta-FNA is an irreversible antagonist at both mu and delta receptors, but not at kappa receptors in the vas deferens preparation. Thus, an opioid agonist which is blocked by naltrexone, but which is not blocked by beta-FNA would appear to act primarily at kappa opioid receptors. ICI-174864 is a reversible opioid antagonist which over a wide range of concentrations only blocks the actions of opioid agonists upon delta receptors. Therefore, this antagonist can be used to identify drugs which have significant activity upon delta receptors. An opioid agonist which is blocked by naltrexone, beta-FNA and ICI-174864 would be likely to act at delta receptors.

A similar approach can be taken to the evaluation of opioid antagonists. A selective delta receptor antagonist will block only the actions of drugs such as DSLET, DPDPE and DADLE. A selective mu receptor antagonist will block selectively drugs such as morphine and sufentanil which in the vas deferens appear to act almost solely upon mu receptors. Finally, a kappa receptor antagonist would be expected to block the actions of a drug such as U50, 488H. At this time we have not found an antagonist highly selective for the kappa receptor. These new procedures for the evaluation of opioid agonists and antagonists upon the mouse vas deferens preparation should facilitate greatly the evaluation of new opioid drugs.

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A Cyclic Somatostatin Analog That Precipitates Withdrawal in Morphine-Dependent Mice

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ABSTRACT

We evaluated the ability of the mu selective, peptidic, opioid antagonist CTP to precipitate withdrawal in morphine-dependent mice after intracerebroventricular (i.c.v.) and subcutaneous (s.c.) administration. The withdrawal syndrome evoked by i.c.v. CTP was different in some respects from that observed after i.c.v. naloxone. Naloxone, given i.c.v., produced shakes and tremors, defecation, diarrhea, wet dog shakes, jumping and weight loss. In contrast, the prominent signs following i.c.v. CTP were grooming, tremors and shakes, defecation, wet dog shakes and weight loss. CTP treated mice exhibited a greatly reduced incidence of jumping behaviors and diarrhea. While s.c. naloxone evoked similar effects to i.c.v. naloxone, CTP given S.C. stimulated defecation and modest weight loss only. The differences in the profile of withdrawal signs between naloxone and CTP may be related to their differences in receptor selectivity or possibly to their respective alkaloidal and peptidic natures. The relative lack of behavioral effects seen after s.c. CTP probably reflects the inability of CTP to pass through the blood brain barrier, and indicates that although the majority of withdrawal signs are mediated by centrally located opioid receptors, the gastrointestinal tract can be withdrawn independently of the central nervous system.

INTRODUCTION

We have previously reported that the cyclic somatostatin analog CTP (D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH2), produces selective opioid mu-receptor antagonism in binding assays with homogenized rat brain (Pelton et al., 1985; Gulya et al., 1986) as well as in the guinea pig ileum longitudinal muscle-myenteric plexus and

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in the mouse vas deferens (Shook et al., 1986a) (delta/mu ratio ≈ 6000). In analgesic tests in mice, CTP was found to produce potent, long-lasting competitive antagonism of mu-selective agonists and it also antagonized delta selective ligands, but in a noncompetitive fashion. The mutual antagonism of the analgesic actions by CTP of both mu and delta ligands probably reflects a functional coupling of certain analgesic mu and delta receptors, and does not indicate that CTP loses its mu selectivity in vivo. This is supported by the finding that CTP does not block the effects of delta agonists on the gastrointestinal tract (Shook et al., 1986b). Due to its peptidic nature, CTP does not readily cross the blood brain barrier (Shook et al., 1986c). These results indicate that CTP is an effective mu-receptor antagonist both in vitro and in vivo which exerts delta analgesic antagonist properties in vivo. In this study we evaluated the ability of CTP to precipitate withdrawal in morphine-dependent mice after intracerebroventricular (i.c.v.) and subcutaneous (s.c.) administration. Naloxone HCl (given i.c.v. and s.c.) was used as a standard for comparison.

METHODS

Mice were made morphine dependent and tested for signs of opioid withdrawal as previously described (Way et al., 1969). Briefly, male ICR mice weighing 20-25 g were surgically implanted with 75 mg morphine-base pellets or placebo pellets (1 pellet/mouse) under ether anesthesia. After implantation of pellets, mice were housed 3 per cage under standard 12 h light, 12 h dark conditions , and received food and water ad libitum. After 72 h exposure to the pellets, mice were weighed, and then administered i.c.v. or s.c. distilled water, CTP or naloxone. Mice were observed for behavioral and physiological signs of withdrawal for the 15 min period immediately following i.c.v. injection, and for 2 consecutive 15 min periods following s.c. administration. All signs were noted, including the following characteristic signs of withdrawal: shakes and tremors, defecation, diarrhea, wet dog shakes and jumping. Body weights were measured again at 15, 30 and 60 min following injection in order to determine if weight loss had occurred.

Naloxone HCl was purchased from Sigma (St. Louis, MO.). Morphine-base pellets (75 mg each) were received from the National Institute on Drug Abuse. CTP was synthesized by methods previously described (Pelton et al., 1986).

I.C.V. injections were made in a total volume of 3.0 or 3.3 ul according to the modified methods of Haley and McCormack (1957) under light ether anesthesia. S.C. injections were made on a 10 ml/kg basis.

RESULTS

Administration of naloxone by both i.c.v. (Table 1) and s.c. (Table 2) routes of administration resulted in distinct opioid withdrawal syndromes. The syndrome produced by i.c.v. naloxone was characterized by dose-related increases in the occurrence of shakes and tremors, defecation, diarrhea, wet dog shakes, jumping and weight loss. Those mice treated with i.c.v. CTP exhibited a pronounced withdrawal syndrome which was different in some respects from that of naloxone (Table 1). Their withdrawal syndrome was characterized by shakes and tremors, grooming, defecation, wet dog shakes and weight loss. CTP (i.c.v.) treated mice showed a greater incidence of wet dog shakes and a greatly reduced occurrence of diarrhea and jumping compared to naloxone (i.c.v. or s.c.) treated mice. Grooming occurred after i.c.v. CTP both in morphine dependent and morphine naive mice. Administration of s.c. naloxone (Table 2) elicited effects similar to those produced by i.c.v. naloxone (Table 1). In contrast, s.c. CTP produced defecation and modest weight loss only, without causing any behavioral effects (Table 2).

The results presented in Table 2 include behavioral observations taken from the first 15 min following s.c. injections only, because naloxone activity peaked during this time, and there was no difference in the effects produced by s.c. CTP in either period. Administration of distilled water to morphine dependent mice caused no behavioral effects and only minimal weight loss (mean±sem, % weight loss was 0.7±0.4 g for i.c.v. and 0.3±0.2 g for s.c.).

TABLE 1 Occurrence of Withdrawal Signs following intracerebroventricular administration of Naloxone or CTP

Withdrawal Sign	Naloxone (ug, i.c.v.)[a] 0.1	1.0	10.0	CTP (ug, i.c.v.)[a] 0.1	1.0	10.0
Shakes & Tremors	4/8	8/8	8/8	7/8	8/8	8/8
Grooming	0/8	0/8	0/8	8/8	8/8	8/8
Defecation	3/8	8/8	8/8	4/8	7/8	8/8
Diarrhea	0/8	3/8	8/8	0/8	0/8	1/8
Wet Dog Shakes	1/8	8/8	8/8	8/8	8/8	8/8
(mean±sem)[b]	13	8±3	16±4	25±4	52±8	48±8
Jumping	0/8	0/8	8/8	0/8	0/8	2/8
(mean±sem)[b]			40±12			19±14
>3% weight loss	0/8	6/8	8/8	4/9	6/8	8/8
(mean±sem)[c]	1.2±.2	4.2±.4	5.5±.5	3.3±3.1	4.3±.6	7.8±.5

[a] number of mice displaying sign/total number of mice tested within 15 min after injection

[b] mean±sem, number of times that sign was displayed (does not include those mice which did not show the sign)

[c] mean±sem, % weight loss of all mice tested

TABLE 2 Occurrence of Withdrawal Signs following subcutaneous administration of Naloxone or CTP

Withdrawal Sign	Naloxone (mg/kg, s.c.)[a] 0.01	0.10	1.0	CTP (mg/kg, s.c.)[a] 0.01	0.10	1.0
Shakes & Tremors	0/5	3/5	5/5	0/5	0/5	1/5
Grooming	1/5	0/5	1/5	1/5	0/5	0/5
Defecation	5/5	5/5	5/5	3/5	5/5	5/5
Diarrhea	0/5	5/5	5/5	0/5	0/5	1/5
Wet Dog Shakes	1/5	5/5	5/5	0/5	1/5	0/5
(mean±sem)[b]	2	10±2	25±4	-	2	-
Jumping	0/5	4/5	5/5	0/5	0/5	0/5
(mean±sem)[b]	-	63±20	88±28	-	-	-
>3% weight loss	0/5	3/5	5/5	1/5	0/5	0/5
(mean*sem)c	1.8±.6	3.2±.2	4.7±.5	3.3±2.2	1.2±.6	1.2±.3

[a] number of mice displaying sign/total number of mice tested within 15 min after injection

[b] mean±sem, number of times that sign was displayed (does not include those mice which did not show the sign)

[c] mean±sem, % weight loss of all mice tested

DISCUSSION

The results presented herein demonstrate that CTP, given i.c.v., precipitates withdrawal in morphine-dependent mice, as would be expected of an opioid receptor antagonist. The differences in the profile of withdrawal signs elicited by i.c.v. CTP and naloxone (differential stimulation of diarrhea, wet dog shakes and jumping) may be explained by differences in their receptor selectivities. Naloxone can interact with all known opioid receptors (i.e. mu, delta and kappa). In contrast, CTP has no kappa antagonist actions and except for analgesia, has no delta antagonist actions. (CTP blocks delta analgesia through a noncompetitive mechanism which may reflect a functional link between certain mu and delta receptors). If CTP retains its mu-receptor selectivity in the morphine-dependent mouse, then it should precipitate only those effects associated with the mu receptor. Due to its relative nonselectivity for the different types of opiold receptors, naloxone may precipitate effects associated with all opioid receptors rendered dependent by chronic morphine treatment. According to this hypothesis, shakes and tremors, defecation, wet dog shakes and weight loss may be mu receptor mediated, while diarrhea and jumping may not be mu mediated. Since it is not clear what receqtors are effected by this method of inducing morphine-dependence, it is difficult to determine which receptors mediate the other effects, but possible candidates may include the delta, kappa and epsilon receptors. Alternatively, it is possible that a peptide opioid antagonist, such as CTP, exhibits a somewhat different spectrum of withdrawal than an alkaloid type antagonist, such as naloxone.

The relative inability of CTP to cross the blood brain barrier affords the unique advantage of separating centrally from peripherally mediated mechanisms. Results from this study indicate that while the centrally located opioid receptors are largely responsible for mediating withdrawal responses, peripherally located opioid receptors can undergo withdrawal while the brain is not withdrawn.

The grooming seen after i.c.v. CTP (not s.c.) in morphine naive and dependent mice suggests that this effect is not a withdrawal sign; its mechanism is unknown.

In summary, CTP, a putative mu receptor antagonist, precipitates a withdrawal syndrome in morphine-dependent mice which is somewhat different from that of naloxone. Unlike naloxone, CTP can be used to separate centrally and peripherally mediated opioid effects.

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Comparison of the Effects of Cocaine and D-Amphetamine on the Pharmacological Actions of Methadone

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Polydrug abuse and the increasing popularity of cocaine abuse represent potentially serious clinical problems in the chronic use of methadone. Our previous studies have shown that many centrally acting drugs given concurrently with methadone can affect the analgesic response and the development of physical dependence on methadone (Liu and Wang 1975, 1978, 1981). Severe side effects and death among methadone maintenance patients are rarely attributed to methadone alone (Concool et al. 1979; Longwell et al. 1979). Indeed, cocaine has been found to contribute to the death of methadone-treated patients (Mittleman and Wetli 1984).

It is generally believed that many similarities exist between cocaine and amphetamine in terms of subjective behavioral, pharmacological and toxicological effects (Jaffe 1980; Fishman and Schuster 1982). These two central nervous system (CM) stimulants have been widely abused among drug addicts enrolled in methadone maintenance programs. Cocaine addicts describe the euphoric effects of cocaine in terms similar to those used to describe amphetamine effects. Although there are many similarities of actions, the mechanisms of actions and toxicity may not be the same. For example, the effects of cocaine are brief, lasting only a few minutes after iv administration, whereas the effects of amphetamine may last for hours (Jaffe 1980). The phenomenon of increased amphetamine toxicity in aggregated mice as compared to isolated mice has been well documented (Gunn and Curd 1940) and was confirmed in our experimental conditions reported previously (Wang et al. 1969). To our knowledge no laboratory studies exist demonstrating aggregated toxicity of cocaine. The mechanisms of cocaine toxicity and lethality, long considered a safe drug, are poorly understood. One report indicates hyperthermia as an important contributor to cocaine death in dogs (Catravas and Waters 1981). The purpose of the present studies was to compare the effects of cocaine and d-amphetamine administration on methadone analgesia and withdrawal and to evaluate cocaine toxicity and hyperthermic response in rodents.

METHODS

Animals and Chemicals. Male Swiss Cox mice (Harlan Lab, Indianapolis IN) and male Sprague-Dawley rats (Sasco Lab, Madison WI) were used. The initial body weights of the mouse and rat were 24-28 g and 100-200 g, respectively. Doses of cocaine·HCl, d-amphetamine SO_4, dl-methadone·HCl and naloxone·HCl are expressed in terms of their salts. The experiments for mortality were carried out using cages of standard size (24 cm x 18 cm x 18 cm) in air-conditioned room maintained between 22° and 24°C. Mice were placed individually for isolation or 9-10 per cage for aggregation after injection of cocaine. Before the acute experiments, all animals were fasted for 18 hr with free access to water.

Measurement of Analgesia and Rectal Temperature. Analgesia was measured by a hot-plate method as described previously (Liu and Wang 1975). No reaction on the hot plate (maintained at 58 ± 0.5°C) within 30 sec was considered as maximal response. Rectal temperature was recorded by a thermistemp thermometer (Yellow Spring Instrument Co, Yellow Spring OH).

Development and Assessment of Physical Dependence on Methadone. Rats were rendered dependent on methadone by sc implantation for 7 days with an Alzet osmotic minipump (Alza Corp, Palo Alto CA) containing 200 μℓ of 180 mg/ml methadone water solution. The degree of physical dependence was assessed by administering a challenging dose of naloxone (5 mg/kg, sc) following the 7-day implantation. The incidences of naloxone-precipitated jumping and wet-dog shakes were counted for 20 min and the degree of severity of ptosls and teeth chattering were rated on a scale of 0-4 (with 4 being the most severe) during the 20-min observation period. Body weight loss was calculated as a percent change of body weight 3 hr after administration of naloxone from the body weight taken before naloxone injection.

Analysis of the regression line and calculation of LD_{50} were made according to the computer programs of Tallarida and Murray (1981) using an Apple II Computer. Significance was attributed at $P<0.05$ using Student's *t* test.

RESULTS

Effect of Acute Cocaine and D-Amphetamine on Methadone Analgesia. The intensity and duration of methadone analgesia were not affected by cocaine (20 mg/kg, ip) given 30 min prior to administration of methadone (4 mg/kg, sc). In both control and cocaine-treated rats the maximal analgesic effect persisted for 30 min. Methadone analgesia persisted for less than 2 hr in both groups of rats.

Similarly, d-amphetamine (5 mg/kg, ip) given 30 min before administration of methadone (4 mg/kg, sc) did not significantly change the analgesic response to methadone. The intensity and duration of methadone analgesia in control and acute d-amphetamine-treated groups were comparable.

Cocaine Lethality in Isolated and Aggregated Mice. The 0-1 hr and 0-20 hr mortality data of cocaine are presented in Table 1. In both aggregated and isolated mice, most deaths occurred within 1 hr after administration of cocaine. Death was preceded by clonic and tonic convulsions. There was a tendency towards enhanced acute lethality under aggregated conditions as compared to isolated conditions. However, the LD_{50} of cocaine at 0-1 hr or 0-24 hr in aggregated mice was not significantly different from the LD_{50} in isolated mice as shown in Table 1.

TABLE 1. COCAINE MORTALITY IN AGGREGATED AND ISOLATED MICE[a]

	Aggregated			Isolated		
		% Mortality			% Mortality	
Dose mg/kg)	Number of Mice	0-1 hr	0-20 hr	Number of Mice	0-1 hr	0-20 hr
60	20	10.0	10.0	18	11.1	11.1
80	21	47.6	47.6	18	33.3	44.4
100	22	77.2	86.3	20	35.0	50.0
120	24	95.8	95.8	17	82.3	82.3
LD_{50} and 95% confidence limits (mg/kg)		81.9 (73.9-90.7)	80.5 (73.0-88.9)		96.6 (84.2-110.8)	90.6 (79.5-103.4)

[a] *Cocaine·HCl was injected ip. The mortality was counted 1 and 20 hr after administration of cocaine.*

Effects of Cocaine and d-Amphetamine on Rectal Temperature of Rats. Acute cocaine (25 mg/kg, ip) treatment did not induce hyperthermia during the 2-hr experiment on Day 1 in naive rats. After 7 days of continuous infusion of cocaine (70 mg/kg/day, ip) through osmotic minipump, there was no effect on rectal temperature. Rectal temperature remained unchanged from 0-min control value during the 2-hr experiment on Day 8 after administration of cocaine (25 mg/kg, ip). In contrast, the rectal temperature of naive rats was increased from 0-min control value 15 min after administration of a single dose of d-amphetamine on Day 1 (Table 2). The rectal temperature stayed high during the 2-hr experiment. After 7-day implantation with d-amphetamine, rats developed tolerance to the hyperthermic effect of d-amphetamine. This phenomenon was evidenced by the fact that the rectal temperature did not go up but stayed the same as the 0-min control value after the same challenging dose of d-amphetamine used on Day 1 was given on Day 8 (Table 2).

TABLE 2. EFFECTS OF ACUTE AND CHRONIC d-AMPHETAMINE ON RAT RECTAL TEMPERATURE[a]

	Rectal Temperature (°C) After d-Amphetamine Injection				
Day	0-min	15-min	30-min	60-min	90-min
1	37.9±0.33	38.9±0.23*	39.6±0.20**	39.6±0.18**	39.8±0.19**
8	38.1±0.13	38.3±0.07	38.4±0.23	38.3±O.14	38.3±0.19

[a] *Rats were given d-amphetamine (15 mg/kg, is) on days 1 and 8 for measurement of rectal temperature. After the 90-min temperature was taken on Day 1, all rats were implanted ip for 7 days with osmotic minipump containing 200 µl of 150 mg/ml d-amphetamine. Figures are mean ± S. E. for 7 rats.*

* *P<0.05; **P<0.01 compared with 0-min value.*

Effects of Acute and Chronic Treatment with Cocaine or d-Amphetamine on Naloxone-Precipitated Withdrawal Signs in Methadone-Dependent Rats. Injection of naloxone to chronic methadone-implanted rats elicited abstinence signs of precipitated wet-dog shakes and escape attempts (jumping). Other abstinence signs included teeth chattering, ptosis, diarrhea and body weight loss. The effects of acute treatment with cocaine and d-amphetamine on naloxone-precipitated withdrawal signs are presented in Figure 1. Acute cocaine treatment did not cause any significant changes on jumping, wet-dog shake, diarrhea and body weight loss, but significantly decreased the degree of teeth chattering and ptosis elicited by naloxone challenging. In contrast to the effect of cocaine treatment, acute d-amphetamine treatment greatly increased the number of jumping and decreased the number of wet-dog shakes. Acute d-amphetamine treatment also greatly decreased the degree of teeth chattering, ptosis, diarrhea and body weight loss. When the doses of d-amphetamine were 10 mg/kg or higher, most of the rats jumped continuously right after administration of naloxone. The rats then died preceded with very high rectal temperature and convulsion.

The effects of chronic pretreatment with cocaine and d-amphetamine on naloxone-precipitated withdrawal signs in methadone-dependent rats are summarized in Figure 2. Similar to the effect of acute cocaine treatment, chronic cocaine pretreatment produced no significant effects on naloxone-precipitated jumping and wet-dog shake but decreased the degree of ptosis and diarrhea as compared to control group. In contrast to the effect of chronic cocaine pretreatment, chronic d-amphetamine pretreatment greatly increased the number of jumping. In addition, chronic d-amphetamine pretreatment almost completely abolished teeth chattering and ptosis precipitated by naloxone. Similar to the effect of chronic cocaine pretreatment, chronic d-amphetamine pretreatment caused smaller degree of diarrhea as compared to control group.

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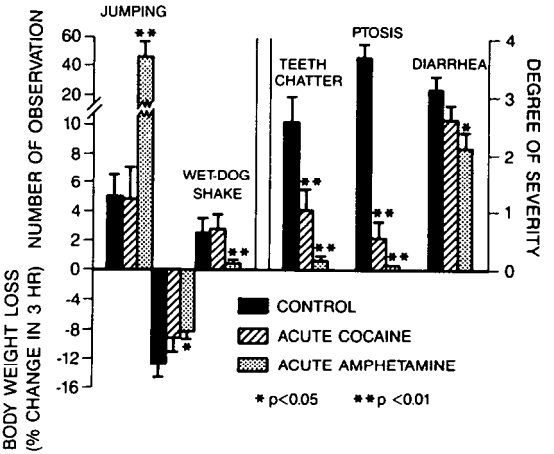

*Figure 1. Effect of acute treatment with cocaine or d-amphetamine on naloxone-precipitated withdrawal signs in methadone-dependent rats. Rats were made dependent on methadone via sc implantation for 7 days with osmotic minipump containing 200 µl of 180 mg/ml methadone water solution. On Day 8, they were injected with saline (2ml/kg, ip), cocaine (20 mg/kg, ip) or d-amphetamine (7.5 mg/kg, ip) 30 min before a challenging dose of naloxone (5 mg/ml, sc) was given. The withdrawal signs were counted or observed for 10 min. Each bar and vertical line is mean ± S.E. of at least 6 rats. ** P<0/01 from control.*

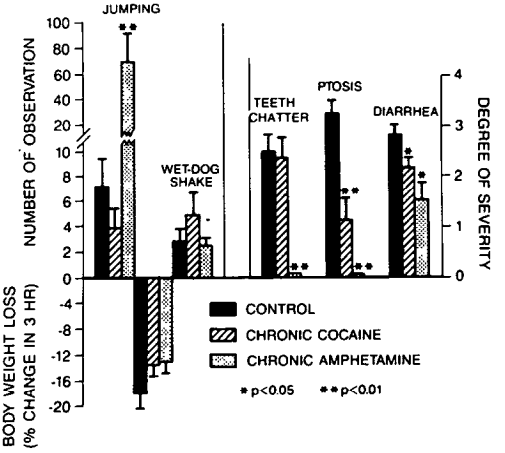

Figure 2. Effect of chronic pretreatment with cocaine or d-amphetamine on naloxone-precipitated withdrawal signs in methadone dependent rats. Rats were implanted for 7 days with osmotic mini-

*pumps containing methadone (200 µl of 180 mg/ml, sc) plus either cocaine (200 µl of 240 mg/ml, ip) or d-amphetamine (200 µl of 50 mg/ml, ip). Control rats were implanted with methadone and placebo minipump. After 7 days implantation, all rats were given a challenging dose of naloxone (5 mg/kg, sc) and withdrawal signs were counted or observed for 20 min. Each bar and vertical line is mean ± S.E. of at least 6 rats. *P<0.05 and ** P<0.01 from control.*

DISCUSSION

The results of the present studies indicate some distinguishable actions of cocaine from d-amphetamine in terms of toxicity and their effects on methadone withdrawal signs. Amphetamine given before administration of morphine has been shown to enhance morphine analgesia in rats (Carenzi 1978). Our data showed that acute administration of d-amphetamine or cocaine shortly before administration of methadone exerted no significant effect on methadone analgesia. Additionally, in a preliminary experiment, we found that administration of cocaine 20 min before or after administration of a sublethal dose of methadone (40 mg/kg sc) did not significantly change the mortality of methadone in naive rats.

Aggregated toxicity, a phenomenon with amphetamine, was not demonstrated with cocaine. However, mortalities in the aggregated and isolated mice tended to be higher than in that of isolated mice when the doses of cocaine were higher than 60 mg/kg. It is interesting to note that the majority of deaths occurred during the first 1 hr following cocaine administration. All deaths were preceded by severe tonic and clonic convulsions.

Some investigators (Greenblatt and Osterbert 1981; Askew 1982) have suggested that amphetamine-induced hyperthermia, causing damage to the CNS. contributed to the increased lethality of amphetamine in aggregated mice. It has been reported that tolerance developed to the hyperthermic effect of the amphetamine in rats (Lewander 1971; Gotestam 1976). The present studies confirmed this phenomenon and also showed that acute and chronic administration of cocaine produced no effect on rectal temperature of rats. This observation is not in agreement with Catravas and Waters (1981), who found that acute cocaine administration in dogs produced a severe hyperthermic response. However, it should be noted that lethal doses of cocaine were used. Similarly, Wilson et al. (1976) reported that increases in body temperature, respiratory rate and heart rate were seen only after large iv doses of cocaine were given to rhesus monkeys. Therefore, it is not surprising that changes in rectal temperature were not observed in the present studies since we used nonlethal doses. Whether cocaine produces hyperthermia in man appears to be controversial. Resnick et al. (1977) reported that 100 mg by insufflation had no effect on body temperature or respiratory rate but raised heart rate and blood pressure. Fishman et al. (1976) reported that core temperature remained constant during the 60-min period following iv injection of 25 mg cocaine. Others, however, have reported that cocaine decreases skin temperature measured by thermocouple taped on the left index fingertip (Rowbotham et al. 1984).

Acute administration of cocaine or chronic pretreatment with cocaine together with methadone did not modify the naloxone-precipitated characteristic methadone withdrawal signs. This result suggests that cocaine neither affected the expression of abstinence signs nor affected the process of development of physical dependence to methadone. These results are in agreement with the report of Steinberg (1978) that previous exposure to cocaine failed to influence the development of morphine dependence. Contrastly, Steinberg (1978) showed that d-amphetamine did have an effect on the development of morphine dependence. Results of our data indicate an enhancement of characteristic abstinence signs when rats were given a single dose of d-amphetamine or pretreated chronically with a combination of d-amphetamine and methadone before administration of naloxone. We have previously shown that desipramine given to methadone-dependent rats also intensified methadone withdrawal jumping precipitated by naloxone (Liu and Wang 1981). The failure of cocaine to elicite this response may indicate a difference in mechanism or site of action.

Based on the results of our previous and present studies, it can be concluded that the following differences exist between cocaine and d-amphetamine with respect to their mechanisms of toxicities and their effects on the pharmacological actions of methadone. Cocaine differs from amphetamine in that there is no increased toxicity of cocaine in aggregated mice as compared to isolated mice; it does not produce hyperthermia in rats at nonlethal dose levels; it does not enhance the naloxone precipitated withdrawal in methadone-dependent rats; it does not increase the lethality of methadone in naive rats; it does not increase the incidence of jumping and does not inhibit wet-dog shakes in methadone-dependent rats; and cocaine (25 mg/kg, ip) given shortly before a challenging dose of naloxone does not cause death in methadone-dependent rats whereas all the rats given d-amphetamine (10 mg/kg, ip) died within 40 min after administration of naloxone. Cocaine resembles amphetamine in that both drugs do not affect the analgesic response to methadone.

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Due to space limitations, a complete list of references may be obtained from the senior author.

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Analgesics 4. Studies on the Effects of the Introduction of Methyl at C-17 of N-Cyclopropylmethyl-Normorphine: Synthesis, Receptor Binding, *In Vivo* Activity, Conformation Energies

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INTRODOCTION

Our interest in the origins of the antagonist activity that is introduced when the N-methyl group of morphine is replaced by the ally1 group of nalorphine (6), Table 1, led us to propose (DeCraw et al, 1978) that different conformations of the N-ally1 group could ellicit different receptor responses, i.e., agonism or antagonism. Thus, if more than one N-ally1 conformation for nalorphine were energetically available, then the known "mixed" activity profile might be explained.

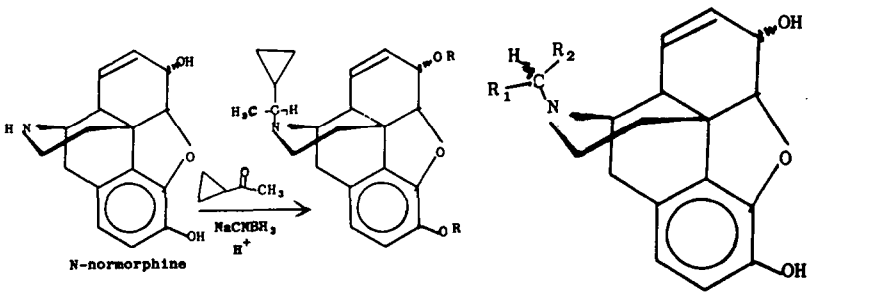
Calculations at that time confirmed that, indeed, nalorphine (6) has two low energy conformers as the N-ally1 group is rotated. In order to test our proposal, we decided to replace one hydrogen on the allyl-methylene carbon with one methyl group, since conformation energy calculations suggested that, for 3R and 3S (Table 1), only one low energy rotamer would remain of the two found for the parent nalorphine (6), and that the single low energy rotamers for R- and S-configurations would be different, each corresponding to one of the two found for nalorphine. If agonism/antagonism activity were modulated by the conformation of the N-substituent, then perhaps 3R and 3S would no longer demonstrate "mixed" activities and possibly manifest different receptor selectivities. Our earlier paper described the subsequent synthesis of mono-methylated analogs of N-n-propyl-normorphine (2 R, 2 S) and of nalorphine (3 R, 3S). All were active compounds with good opiate receptor affinities. We were disappointed to find that 3R and 3S, as well as 2R and 2S, when diastereomerically separated, demonstrated very little difference in activity and affinity. In the continued exploration of the effect of methyl addition to C-17 of the N-substituents of morphine, we report here studies of N-cyclopropylmethyl-normorphine (5) and the two diastereomers 1R and 1S, shown in Table 1.

METHODS

Chemistry

A diastereomeric mixture of 1R, 1S was prepared in nearly quantitative yield from N-normorphine and cyclopropyl methyl ketone under reductive alkylation conditions (Borch 1972) with sodium cyanoborohydride (Scheme 1). The desired N-alkylated product, as a diastereomeric mixture 1R, 1S, was recovered as an off-white solid (92% yield). 1R, 1S could not be separated by chromatography or crystallization, unlike the related analog mixtures of 2R, 2S and 3R, 3S. However, the bis-benzoate ester derivatives 4R, 4S of 1R, 1S were separable by both HPLC and fractional crystallization with better than 99% purity by NMR. A detailed report of this synthesis and complete high resolution NMR analysis of 1R and 1S will be reported later, as will the details on the X-ray analysis. The configuration at C-17 of 4R was confirmed as "r" by X-ray analysis by Dr. Camerman, see Figure 2.

Scheme 1

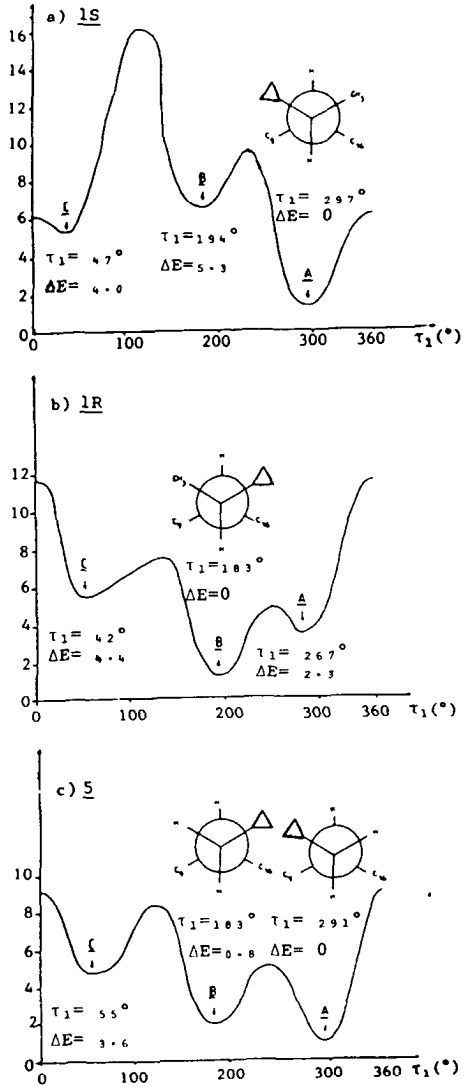


1R,1S; R = H
4 ; R = benzoyl

1R,1S; R_1=CH_3; R_2=CPM
2R,2S; R_1=CH_3; R_2=Et
3R,3S; R_1=CH_3; R_2=vinyl
5 ; R_1=H ; R_2=CPM
6 ; R_1=H ; R_2=vinyl

Pharmacology

Pure diastereomers 1R and 1S, together with the parent compound N-cyclopropylmethyl-normorphine (5), were tested for analgesic agonist and narcotic antagonist activities in the mouse tail-flick test. (Harris et. al., 1969.) For subcutaneous dosing the chemicals were dissolved in 0.9% saline solution. Morphine sulfate, nalorphine (6), and cyclaxocine (7) were evaluated as reference drugs. In a dose-response study, conducted at the peak time, percent agonism was calculated for each of the three dose levels. The percentages were plotted against the log-dose to compute the median effective dose (ED_{50}) and 95% confidence limits. (Litchfield and Wilcoxon, 1949.) For the evaluation of antagonist activity, the tail-flick assay developed by Dewey, et al. (1969) was modified



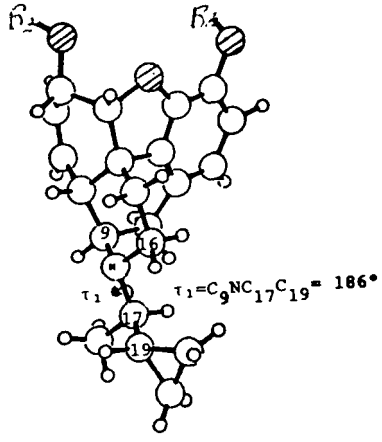


Fig. 2. X-ray structure for 4R. (dibenzoate ester of 1R).

Fig. 1 Rotational energies (kcal/mol) for a) 1S, b) 1R, and c) 5 about N-C_{17} bond (as function of τ_1, with Newman projection view along N-C_{17} bond for lowest rotamer minima.

to include retesting at several intervals.

Conformational Energy Calculations

Energy-conformational analyses of the N-substituents for isomers 1R, 1S and the parent 5 (Fig. 1) have been performed using the empirical energy program MOLMEC. (Christoffersen, et al, 1981.) The charges for the coulomb term in the seven-term energy formula were taken from MNDO calculations. (Dewar and Thiel 1977.)

As shown in Figure 1, two dihedral angles, τ_1=C9NC17C19 and $\tau 2$=NC17C19H, define the N-substituent conformation. The energy of rotation around N-C17 was calculated for fixed values of $\tau 1$ between 0° and 330° at intervals of 30°. For each value of $\tau 1$, $\tau 2$ was optimized, starting with several different values of $\tau 2$. Rotational energies reported in Figure 1 were obtained by constrained optimization of all geometric parameters. holding fixed at the lowest energy values for each $\tau 1$. Minima found by these constrained optimizations were refined by total geometry optimization of all bond lengths, bond angles and torsion angles.

Receptor Binding

Binding studies and data analysis were conducted as described previously. (Toll et al, 1984.) The tissues used were rat whole brain, and cerebellum from frozen guinea pig brain (Pel Freeze). Briefly, the tissues were homogenized in Tris buffer and washed twice by centrifugation. Incubation volumes were 2.0 mL with final tissue concentrations of 6.0 mg/mL original wet weight for rat brain and 4.5 mg/mL for guinea pig cerebellum. Incubations were for 1 h at 25°C prior to filtration and scintillation counting.

Results

Diastereomers 1R and 1S as well as 5 are all pure agonists in the mouse tail-flick test,as shown in Table 1. An X-ray structure analysis of the higher affinity diastereomer 1R shown in Figure 2, indicated it is the C17-R isomer. 1R and 5 are about equally potent, both being 5-10x more potent analgesics than morphine, while 1S is about 30 times less potent than 1R and 5, Table 1. This result is in surprising contrast to the results for the related analogs cyclazocine (7) and nalorphine (6), which were found to be pure antagonists in mice when the tail-flick procedure was administered.

Studies (Aceto et al., 1968, and Archer et al., 1964) have shown that both the species and the test used will affect apparent agonism and antagonism. Analogs 5, 6 and 7 are reported to have little or no analgesic agonist activity in the rat tail-flick and be potent antagonists in the same test against meperidine or morphine induced analgesia. Analogs 6 and 7 are also potent agonists in the mouse writhing test and in humans.

Table 1

ANALGESIC AGONIST AND NARCOTIC ANTAGONIST POTENCIES
(MOUSE TAIL-FLICK)

Compound	Agonism[a] ED_{50}[b]	Antagonism[c] of Morphine[a] AD_{50}[b]
1R	0.57 (0.28 - 1.14)b	>213
1S	15.18 (7.59 - 30.36)	>213
5	0.32 (0.13 - 0.80)	>221
6 (nalorphine)	828.0 (548.9 - 1,250.36)	2.04 (1.44 - 2.87)
7 (cyclazocine)	294.8	2.84 (1.54 - 5.22)
morphine	2.95 (1.84 - 4.72)	

[a] subcutaneous, [b] μmole/kg (95% confidence limits), [c] Antagonism of mouse tail-flick inhibition which is induced by 21.08 μ mol/kg (s.c.) of morphine sulfate.

Binding studies were conducted with three [^{3}H] ligands in two tissues in order to determine affinities at μ-, δ-, and κ-receptor sites. Shown in Table 2 are the affinities of 1R, 1S, 5 and a number of compounds well characterized at μ, δ, and κ sites. The affinities were obtained by analysis of receptor binding data using the curve-fitting program LIGAND. (Munson and Rodbard 1980) In guinea pig cerebellum the [^{3}H] ligands used were [^{3}H]EKC and [^{3}H] naloxone. In rat brain [^{3}H]EKC, [^{3}H]DADL, [^{3}H]DSLET and [^{3}H]DHM were used. In both tissues, the data for inhibition for each [^{3}H] ligand by each of the other compounds listed were analyzed simultaneously giving self-consistent affinity constants and maximum binding capacities.

In guinea pig cerebellar membranes, a two site fit was preferred. One site represents affinities at κ receptors. This site is characterized by high affinity for EKC, U-50,488, 1R and 5. The second site has moderate affinity for all the ligands tested. This indicates μ and δ receptors are not present in guinea pig cerebella. In rat brain membranes, a 3-site fit was preferred. These sites can best be labeled μ and δ, and again a low affinity site possibly unrelated to opioid activity. If κ-receptors are present in rat brain, the number of sites is sufficiently low so that they were not discerned using these ligands.

K_D values shown in Table 2 confirm that 1R and 5 have quite similar affinity profiles. A 20-30 fold superiosy of 1R and 5 over 1S in binding is consistently found at all sites. As reflected by their affinities in rat brain and guinea pig cerebellum, 1R and 5 are similar to the other cyclopropylmethyl analog (EKC) and have a high affinity at κ- as well as at μ-sites.

Table 2
AFFINITIES OF VARIOUS OPIOIDS
K_D nM

	Guinea Pig Cerebellum		Rat Brain		
	Site 1 "κ"	Site 2	Site 1 "μ"	Site 2 "δ"	Site 3
EKC	4.72	172	1.32	20.4	164
NAL	52.6	47.6	----	----	---
DADL	----	----	4.35	2.50	23,000
DSLET	---	----	16.1	1.56	12,600
DHM	333	52.6	0.53	151	18.2
U-50,	1.07	2500	270	14,900	3030
1R	1.10	71.4	0.12	9.09	13.3
1S	35.7	62.5	4.00	164	76.9
5	0.67	66.7	0.19	12.0	66.7
Bmax (pmol/g)	18.4	93	13.8	8.9	74

The rotational profiles found for rotation around N-Cl7 ($\tau 1'$) are shown in Fig.1. For each isomer the existence of three local minima indicated by the rotational curves. The resulting local minima (A, B, C) are indicated by arrows in Figure 1. The Newman projections along the N-Cl7 bond for the lowest of these minima are also shown in fig. 1, together with the relative energies and optimized τ^1 values.

For analog 5, two low-lying minima A and B are found with only 0.8 kcal/mol difference in energy, which; are separated by a rotational barrier of ~4 kcal/mol. The third rotamer C is 3.6 kcal/mol higher in energy than A, and the barrier of rotation towards A and B is > 7 kcal/mol. For 1R and 1S, only one low-lying rotamer for each is found corresponding to either the minimum B or A of 5, respectively. We find that all low-lying conformations have trans-configuration of the hydrogen atoms at nitrogen and C-17 (Figure 1).

For 1R the global minimum B is 2.3 kcal/mol lower in energy than the next lower A, separated by a barrier of ~4 kcal/mol. The third rotamer C is 4.4 kcal/mol higher than B. In case of the less potent isomer 1S, the two higher lying minima B and C are energetically more discriminated from the A minimum. A is 4.0 kcal/mol lower in energy than than 5.3 kcal/mol lower than B. The X-ray on the dibenzoate ester of 1R (Fig. 2) shows a $\tau 1$ value of 186° which is in good agreement for the calculated value (183°) for 1R.

Discussion and Conclusions

In the mouse tail-flick test, the N-CPM analog 5 is a pure agonist, about ten times as potent as morphine. Addition of an α-CH_3 group, making the C-17-position of the N-substituent chiral, does not change this activity profile but appears to negatively affect the relative receptor-binding affinity and agonist potency of one of the diastereomers, 1S. The 1R isomer has nearly equal agonist potency and μ-, δ-, and κ-receptor affinity as 5, while both the agonist activity and κ-affinity of the 1S isomer is reduced by a factor of 30. In general, the addition of a methyl group to C-17 does not produce much of a change in receptor selectivity. Furthermore, relative agonist activity is parallel to both μ- and κ-binding affinity. Thus, binding at either or both receptor could initiate the observed analgesic activity in the mouse.

The addition of a methyl group to C-17 of 5, also affects the conformational mobility of the N-cyclopropylmethyl substituent. In compound 5, there are two nearly equi-energy conformers A and B (Figure 1). Only one of these, B , remains low energy in the potent 1R isomer, while the other minimum, A , is lowest energy in the less active isomer. These results indicate that rotamer B is likely responsible for high-affinity binding at μ-, δ- and κ-receptors and for initiation of agonist activity. These results also suggest that there are similar requirements for binding of the N-substituent at δ and κ-receptors.

Observations of the mice during tail-flick analgesia testing indicated no Straub-tail response at any dose, and obvious diuresis at higher doses of 1R. In a preliminary test for addiction liability, 5 and 1R showed no precipitated withdrawal symptoms in the mouse-jump test of Saelens et al,(1971.) These observations are consistent with the κ-receptor affinity of 1R and 5. More definitive testing of 1R, both here and through the services of CPDD is in progress. However, the binding data does suggest that 1R and 5 have good affinity for μ and κ-receptors, and this dual mode of binding may explain the high analgesic potency of 1R apparently free of addiction liability.

Acknowledgements

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The Effects of Amphetamine-Like Designer Drugs on Monoaminergic Systems in Rat Brain

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ABSTRACT

The response of brain dopaminergic and serotonergic systems to the amphetamine-like designer drugs, 3,4-methylenedioxyamp amphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA), was investigated and compared to methamphetamine (METH). Like METH, single or multiple doses of either drug caused marked reductions in both tryptophan hydroxylase (TPH) activity and concentrations of 5-hydroxytryptamine (5HT) and 5-hydroxyindoleacetic acid (5HIAA) in several brain regions. The reduction in 5HT content corresponded to the depression of TPH activity in all regions examined.

In contrast to METH, which induced pronounced deficits in dopaminergic neuronal markers, repeated doses of MDA or MDMA did not alter striatal tyrosine hydroxylase (TH) activities or reduce striatal dopamine concentrations. A single dose of MDA or MDMA significantly elevated striatal dopamine content; however, after repeated drug administrations dopamine concentrations were comparable to control values.

The effects of MDA or MDMA administration in the rat brain are reminiscent of those elicited by p-chloroamphetamine, a presumed serotonergic neurotoxin.

INTRODUCTION

The amphetamine-like designer drugs, 3,4-dimethylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") have attracted recent attention by the public and the scientific community. Because of the potential toxicity of ecstasy, the DEA declared that MDMA is subject to Schedule I control as of July 1, 1985. Over the past fifteen years, we have described the neurochemical effects of toxic doses of methamphetamine (1971, 1973, 1976, 1980 and 1985). We now compare the effects of these amphetamine-like designer drugs on neurochemical parameters with those previously reported to occur with

methamphetamine (METH). We observed that single or multiple doses of MDA or MDMA produce neurochemical deficits in the serotonergic system which are similar to those induced by METH; in contrast, the response of dopaminergic pathways to these agents and METH are very different.

MATERIALS AND METHODS

Drug Administration

Male Sprague-Dawley rats, weighing 200-275 g, were housed 5 per cage and maintained at 26°C with a 12-hour alternating light-dark cycle. All drugs were dissolved in 0.9% saline and administered by subcutaneous injection. MDA and MDMA were hydrochloride salts of racemic mixtures; doses are expressed in terms of the free base. Subacute drug treatment consisted of 5 sequential doses at 6-hour intervals of either METH (15 mg/kg), MDA (10 mg/kg), or MDMA (10 mg/kg). Control animals received similar injections of saline vehicle alone.

Rats were sacrificed by decapitation 18 hours (subacute) or 3 hours (acute) after the last dose, and brains were immediately removed and placed on ice. The neostriata, hippocampi, and frontal cortex regions were removed bilaterally by dissection, and stored at -70°C until assayed. One of the paired striata, hippocampi, or cortex regions from each rat was assayed for TPH and TH (striata only) enzyme activities, while the other was used in the quantitation of and monoamine metabolite concentrations.

Enzyme Assays

All steps in the preparation of enzymes were performed at 0-5°. Tissues were weighed and homogenized (1:3) in 50 mM HEPES buffer, pH 7.4, containing 0.2% Triton X-100 and 5 mM dithiothreitol. Homogenates were centrifuged at 27,000 x g for 15 minutes. Duplicate 7.5-µl aliquots of the supernatant fraction from each sample were analyzed for TH activity by a tritium release assay (Nagatsu et al., 1964) utilizing ^{3}H-tyrosine as substrate. similar aliquots were assayed for TPH activity using a modified CO_2 -trapping procedure (Ichiyama et al., 1970; Sitaram and Lees, 1978); details of the assay are described by Hotchkiss et al. (1979).

Monoamine and Metabolite Assay

Tissue concentrations of dopamine (DA) and its primary metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and 5-hydroxytryptamine (5HT) and its major metabolite, 5-hydroxyindoleacetic acid (5HIAA), were measured by high-performance liquid chromatography (HPLC) with electrochemical detection. Tissues were weighed and homogenized in 0.3-0.5 ml mobile phase buffer containing 0.15 M monochloroacetic acid, 2.0 mM disodium EDTA, and 25 mg/l 1-octane sulfonic acid in 12.5% methanol at pH 2.9. After centrifugation at 4080 x g for 15

minutes the supernatant fractions were filtered through a 0.2-µm Microfilter system (Bioanalytical Systems, Inc West Lafayette, IN). Fifty-µ1 volumes were injected by a WISP™ automatic sample processor (Millipore Cor., Millford, MA) onto a 3-µm reverse Microsorb C_{18} column (Rainin Instrument Co., Woburn, MA). samples were run on a LC-154 liquid chromatograph equipped with a model LC-4B amperometric detector (Bioanalytical Systems, Inc.). The detector potential was set at +0.73 V. Monoamines and their metabolites were quantitated by measurement of peak heights and comparison with those of standards of known concentration prepared in mobile phase buffer.

RESULTS

Neostriatal TPH activity (Fig. 1) was dramatically depressed (to less than 25% of control) after subacute administration of

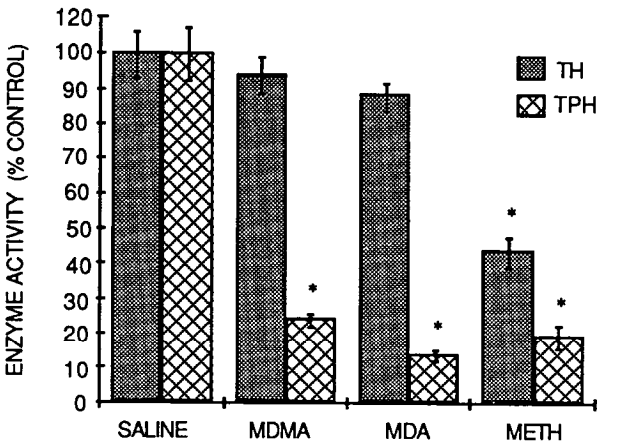

Fig. 1. Effect of subacute drug treatments on neostriatal tyrosine and tryptophan hydroxylase activities. Treatment consisted of s.c. injection of MDA (10 mg/kg), MDMA (10 mg/kg), or METH (15 mg/kg) every 6 hours for 5 doses. Rats were killed 18 hours after the last dose. Combined results from 2 experiments ($n \geq 12$) are presented as the means ± S.E.M., expressed as percent control. Control (saline) values (in nmol liberated/g tissue/h) were: TPH, 45.0; TH: 2645 nmol/g tissue/hr. *$p<0.001$ versus control, by the two-tailed Student's t test.

MDA (10 mg/kg), MDMA (10 mg/kg) or METH (15 mg/kg). Comparable decreases were observed in the neurotransmitter, 5-hydroxytryptamine (5HT) and its metabolite, 5-hydroxyindoleacetic acid (5HIAA) after all three amphetamine analogs (Fig. 2). Similar responses were observed in the hippocampus and frontal cortex regions (data not shown).

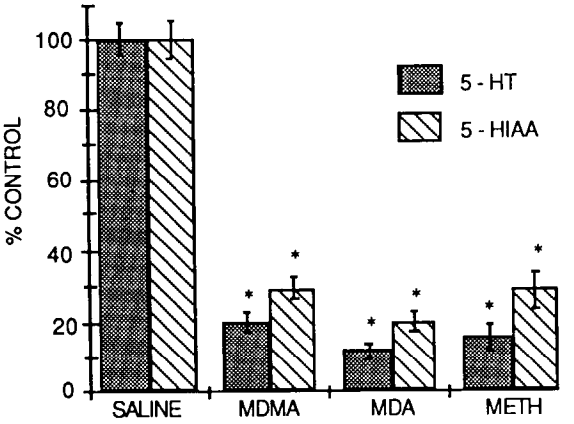

Fig. 2 Effect of subacute drug treatments on 5-hydroxytryptamine (5HT) and 5-hydroxyindoleacetic acid (5HIAA) concentrations. Results are the means ± S.E.M. from 2 experiments (n≥12), expressed as percent control. Experimental conditions are described in fig. 1. Control values (µg/g tissue) were as follows: 5HT, 0.469 and 5HIAA, 0.362.

The effects of each of the three drugs on the dopaminergic system were examined; there was a major difference observed in the response to MDA or MDMA compared to that observed after METH. The characteristic decrease in striatal TH activity was observed after METH (Fig. 1), as has been previously reported (Koda and Gibb, 1973), but no change was seen in TH activity after MDA nor MDMA. In contrast to METH, neither MDMA nor MDA significantly altered the concentrations of neostriatal dopamine but both drugs elevated HVA concentrations (Table 1) and its metabolites.

Table 1. Effect of subacute drug treatments on neostriatal dopamine and its metabolites

Treatment Group	Dopamine	DOPAC	HVA
Saline Control	8.2 ± .5	1.19 ± .12	.582 ± .032
MDMA 10 mg/kg	7.5 ± .4	1.20 ± .06	.673 ± .028*
MDA 10 mg/kg	8.0 ± .4	1.21 ± .07	.719 ± .026**
METH 15 mg/kg	2.1 ± .4 **	.47 ± .08 **	.293 ± .038**

Values represent µg/g tissue ± S.E.M.
n≥12, **p< 0.001, * p< 0.02 versus control, by the two-tailed Student's test.

When rats were administered one acute dose of MDA or MDMA, the TPH activity was reduced to less than 50% of control 3 h after receiving the drug. Concentrations of 5HT and 5HIAA were similarly depressed in all three brain regions. Both MDMA and MDA elevated neostriatal DA concentrations at 3 hr; MDMA, but not MDA, also elevated HVA concentrations after this one acute dose of the drug. MDA decreased DOPAC concentrations.

DISCUSSION

The present study provides evidence that both MDA and its N-methylated derivative, MDMA, are selectively toxic to serotonergic neurons in the rat brain. Ricaurte et al. (1985) recently reported MDA-induced neuronal damage in hippocampal and striatal regions of the rat brain, as well as decreases in concentrations of 5HT and 5HIAA and in the number of serotonin uptake sites. This selective effect of the 3,4-methylenedioxy derivatives of amphetamine and methamphetamine on the serotonergic system is in contradistinction to the effects of the parent compounds, amphetamine and methamphetamine, which compromise both the serotonergic and the dopaminergic system; neostriatal TH activity as well as dopamine, DOPAC and HVA are decreased by METH and amphetamine.

The selective effect of MDMA and MDA on serotonergic parameters is reminiscent of the response to p-chloroamphetamine by the serotonergic system, but no effect is observed on the dopaminergic system after administration of this halogenated amphetamine. The explanation for this selective response is currently under investigation.

In summary, administration of either MDA or MDMA caused a marked decrease in TPH activity in selected brain regions; there is a parallel decrease in the concentrations of 5HT and 5HIAA. No change in dopaminergic parameters was observed. The similarity to the effects of p-chloroamphetamine has interesting toxicologic and regulatory implications as well as serves as a focal point for studying the mechanism for this interesting response.

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Apparent Lesser: Physical Dependence Potential of Nor-Diazepam Compared to Diazepam in the Rat

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Diazepam is the most widely prescribed benzodiazepine anxiolytic-sedative-hypnotic, Its potential to induce chronic physical dependence is now well recognized in man (Owen and Tyrer 1983) and has been experimentally demonstrated in several animal species (Lukas and Griffiths 1982; McNicholas and Martin 1982; McNicholas et al., 1983; Gallaher et al., 1986). Although the nor-metabolite accumulates more extensively than the parent drug in man, little is known regarding the relative physical dependence potential of diazepam and nor-diazepam. Recently, McNicholas et al. (1985) described a spontaneous withdrawal syndrome for nor-diazepam chronically treated dogs that was about three times more severe than their historical standard, diazepam; however, only one chronic dose level was studied for each drug.

To more critically compare the relative physical dependence potential of diazepam and nor-diazepam, the following experimental strategy was developed. First, the rat was chosen because unlike man and dog, it eliminates diazepam and nor-diazeoam rapidly (t½= 1-3 hours) and nor-diazepam does not accumulate-(Caccia et al, 1980; Klotz 1979; Klotz et al., 1976; Friedman et al., 1986; Marcucci et al., 1970). Cocequently, it is the administered drug that is primarily responsible for dependence induction and the parent drug/metabolite comparison is not confounded. Secondly, to evaluate the degree of dependence, withdrawal was precipitated with the receptor antagonist Ro 15-1788. A comparison of dependence based on spontaneous withdrawal was abandoned because spontaneous withdrawal may be confounded by any differences in rates of drug elimination (Boisse and Okamoto 1978). Finally, a wide range of final chronic dose levels was studied to achieve a comprehensive and balanced comparison.

METHODS

Male Sprague-Dawley rats (350-575 gms, Charles River, Wilmington, MA) were used and pair housed in an environmentally controlled facility. Diazepam and nor-diazepam were suspended in 0.4% Agent K (Bio Serv) and injected intra-gastrically by gavage twice daily

(8 am, 6 pm); concurrent controls received isovolumic Agent K (5 ml/kg). Rats were treated bid with diazepam (40 mg/kg or 75 mg/kg) or nor-diazepam (75 mg/kg) for 35 days followed by Ro 15-1788 withdrawal precipitation 4 hours after the last AM dose. Following the first precipitation session, chronic treatments were continued at the same dose level for 4 more days and chronic doses were progressively increased in a stepwise manner (110, 150, 250 and 400 mg/kg) and rats sequentially precipitated at weekly intervals.

To test for dependence, rats were challenged with Ro 15-1788 four hours after the last benzodiazepine dose. The dose 25 mg/kg, i.p. was previously optimized to precipitate chlordiazepoxide dependence in the rat (Boisse et al., 1982; 1985). The method of withdrawal evaluation include operationa1 definitions for signs and each of their grades and inter-observer reliability has been reported (Ryan and Boisse 1983). Three or four independent trained observers rated signs just prior to (t=0) and at t=5 and the treatment. The intensity of individual signs (wd) and of the syndrome (total WD) was estimated from the average rating of all co-observers. To compensate for initial baseline differences in total WD score and to more sensitively quantify the reaction to Ro 15-1788 challenge, the initial WE score was subtracted from the WD score obtained after Ro 15-1788 and is called the delta (A) WD score.

Gross neurological testing included five different ladder and open-field tests with operationally defined grades (Ryan and Boisse 1983) which detected CNS depression. All grade points accrued for these tests were pooled to give a TDP or "total depression point" score.

RESULTS

Both chronic diazepam and nor-diazepam produced dependence in a dose-dependent fashion. Dose-response analysis for the WD criterion revealed non-parallel curves. The LDR curve calculated by least squares was more steep for diazepam (slope = 7.3 ± 0.5 SE) than for nor-diazepam (slope = 3.9 ± 0.7 SE); these slopes were significantly different ($P < .05$). The two curves intersected at the 150 mg/kg dose. For both drugs, maximum precipitated withdrawal was obtained with the highest final dose studied. For diazepam (250 mg/kg), the peak WD score was 16.2 (± 1.1 SE); for nor-diazepam (400 mg/kg), it was 15.8 (± 0.9 SE). The maximum peak WD score was not different for the two drugs, both drugs were significantly greater ($P < .001$) than vehicle control (9.8 ± 1.0 SE).

For the peak $WD criterion, maximum precipitated withdrawal was significantly greater (t-test, $P<.05$) for diazepam (9.5 a 1.2 SE) than for nor-diazepam (5.8 ± 1.3 SE) and both were significantly greater ($P < .05$) than chronic vehicle controls (2.2 ± 0.8 SE). The discrepancy in outcome for peak WD versus peak A WD criteria for dependence are largerly due to differences in initial base-

line WD scores before Ro 15-1788 administration. For nor-diazepam, the initial WD score (9.9 ± 0.9 SE) was significantly greater ($P < .05$) than for diazepam (6.7 ± 0.7 SE) or chronic vehicle control (7.8 ± 1.2 SE). The signs of spontaneous withdrawal for nor-diazepam were reduced motor activity (1.2 points) and augmented tactile evoked startle response (0.9 points). Reduced motor activity is both a withdrawal sign for WD score calculation and a sign of CNS depression for the TDP criterion. Nor-diazepam and diazepam treated rats both showed ataxia and significant TDP scores following the final chronic dose. Initial TDP scores were significantly reduced following antagonist injection.

Analysis of intensities of individual signs (wd's) for maximal syndromes (WD's) revealed 7 significant signs for diazepam, 9 signs for nor-diazepam. Both drugs showed curled claw, reduced spontaneous motor activity, increased struggle on handling, high step, and muscle hypertonus. Three signs were significantly more severe for diazepam than for nor-diazepam: curled claw, salivation and blanced ears. Ear twitches and diarrhea were present for nor-diazepam but absent for diazepam.

DISCUSSION

This study demonstrates that diazepam and nor-diazepam are capable of inducinq severe physical dependence in the rat. The failure of previous investigators to precipitate severe diazepam dependence in the rat (McNicholas and Martin 1982) apparently reflects inadequate dosing. For both diazepam and nor-diazepam, a high rate of drug delivery must be maintained to compensate for the rapid rate of elimination in order to achieve and maintain dependence inducing tissue levels in the rat.

The maximal precipitated withdrawal syndrome for diazepam and nor-diazepam was similar in severity based on the withdrawal directly observed (peak WD criterion). Overall, the signs expressed were similar except that curled claw, salivation and blanched ears were more pronounced for diazepam and ear twitches and diarrhea were present only for nor-diazepam.

The dose-response curve for precipitated withdrawal (WD criterion) was significantly more shallow for nor-diazepam than for diazepam. For the peak WD criterion, the LDR curve for nor-diazepam was not only more shallow, but reached a peak value that was about half that of diazepam. These results suggest that nor-diazepam may have a lesser apparent physical dependence potential than diazepam. Coupled with the observation of concurrent spontaneous withdrawal and CNS depression before Ro 15-1788 for nor-diazepam, these observations suggest that nor-diazepam might be behaving like a partial agonist for dependence induction. Alternatively, nor-diazepam and diazepam may differ in their affinities and/or intrinsic efficacies to activate benzodiazepine receptor sub-types.

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Chronic Nicotine Exposure: Studies on Water Intake, Body Weight, Blood Pressure and Behavior

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Introduction

There is accumulating evidence that physical dependence plays an important role in the continuation of the smoking habit, especially regarding nicotine intake (Pomerleau, et al., 1984, Russell, 1978). However, a suitable animal model for studying this phenomenon is lacking. In the hope of remedying this situation, we have been studying the effects of chronic exposure to nicotine in rats. In this study, we discuss our preliminary findings on water intake, body weight, blood pressure and behavior.

Methods

Adult male Sprague Dawley rats in the weight range 200-280g were used in these experiments. They were housed individually in wire mesh cages and had free access to food and water. The vivarium was temperature controlled (23 ± 2°C) with alternating light-dark cycles. The rats were allowed to acclimate to their environment for 1 week before serving as experimental subjects.

The method of Teiger (1974) was used with some modifications. Male rats were anesthetized and 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 in the peritoneal cavity. The cannula was attached to a flow-through swivel mechanism which allowed the animal to move about in the cage and to eat and drink normally. The swivel was connected to a syringe pump. When stabilized and recovered, they received continuous infusions of 8 ml of test solutions every 24 h for 6 days. Then, nicotine or saline was abruptly withdrawn and the animals were observed for 1/2 h at 6, 24, 48, 72 and 96 h after abrupt withdrawal.

In the experiments involving direct measurement of blood pressure, the descending aorta was exposed and cannulated according to the method of Weeks and Jones (1960). All statistical comparisons were made using the Mann-Whitney U-Test. Significant when $p = 0.05$ or less (Siegel , 1956).

(-)-Nicotine was purchased from Aldrich Chemical Company (Milwaukee, WI) and converted to the di 1 tartrate salt (Aceto et al., 1979). All nicotine doses were calculated as base. All drugs were dissolved in physiological sterile non-pyrogenic saline solution (Travenol Labs., Dearfield, IL).

Results

As shown in Fig. 1, nicotine, at 10 mg/kg/day, promptly and drastically curtailed water consumption during the first 24 h. Thereafter, water intake alternately rose and fell during the rest of the 6 day infusion period, suggesting that overriding mechanism(s) were activated. When nicotine was abruptly withdrawn, water intake rose dramatically and remained elevated throughout the withdrawal period. Mecamylamine at a dose of 5 mg/kg/day did not block nicotine's hypodipsic effect during the first 3 days. However, mecamylamine, alone and in combination with nicotine, significantly increased water consumption beginning on day 4.

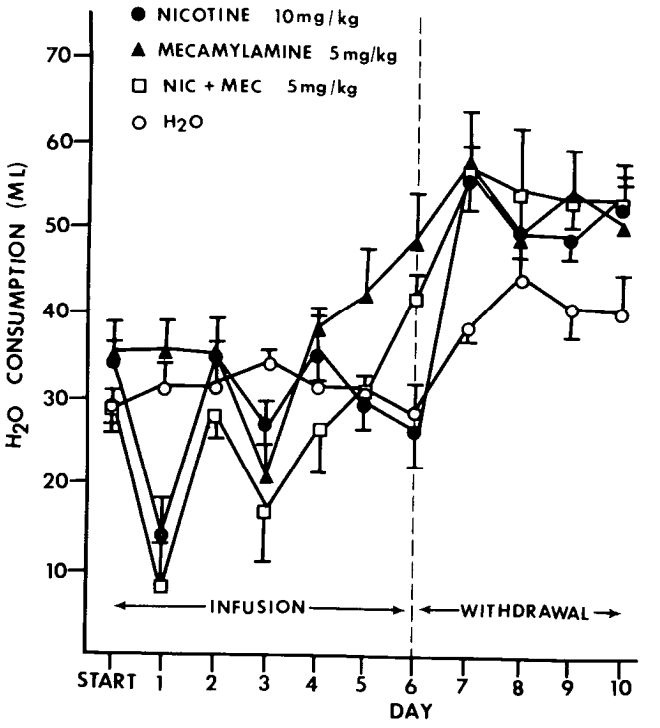

Fig. 1. The effects of nicotine (10 mg/kg/day); mecamylamine, (5 mg/kg/day); nicotine, (10 mg/kg/day); pluse mecamylamine (5 mg/kg/day), and vehicle (8 ml/day) on water consumption of rats during the infusion and abrupt withdrawal periods.

The body weights of all the animals receiving nicotine either alone or in combination with mecamylamine dropped significantly (see Fig. 2). Those receiving vehicle or mecamylamine gained weight throughout the infusion and withdrawal periods. These results suggest that mecamylamine did not antagonize nicotine. In turn, this argues against the involvement of cholinergic mechanisms.

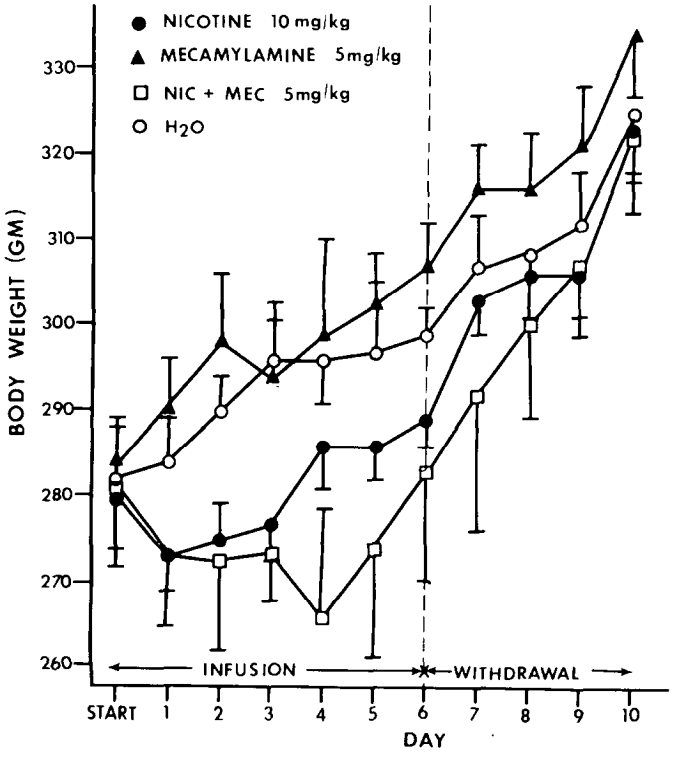

Fig. 2. The effects of nicotine (10 mg/kg/day); mecamylamine, (5 mg/kg/day); nicotine, (10 mg/kg/day) plus mecamylamine (5 mg/kg/day), and vehicle, (8 ml/day) on body weight during the infusion and abrupt withdrawal periods.

In Fig. 3 are illustrated the results of dose response studies. During the first 24 h, a suppression of drinking was obtained. Then, alternating increases and decreases in water intake occurred until day 4 when water consumption for the rats receiving 20.0 and 10.0 mg/kg/day rose dramatically during the infusion period instead of during the abrupt withdrawal of nicotine. At the lowest dose, the rise in water intake coincided with the abrupt withdrawal of nicotine. These results show that increased water consumption is not necessarily related to abrupt withdraw-

al. Water consumption remained significantly elevated during the withdrawal period at the highest dose. Another groups of rats receiving 10 mg/kg/day of nicotine showed no significant changes in blood pressure during infusion. However, on days 8, 9 and 10 during withdrawal, blood pressure was elevated significantly.

In Fig. 4, dose-related decreases in body weight are evident during the infusion. After abrupt withdrawal, the rats receiving the low dose showed significantly greater gains in body weight than the animals receiving vehicle or 10 mg/kg/day of nicotine. The rats receiving the highest dose of nicotine rapidly regained weight, so that by day 10 they weighed nearly as much as the vehicle controls.

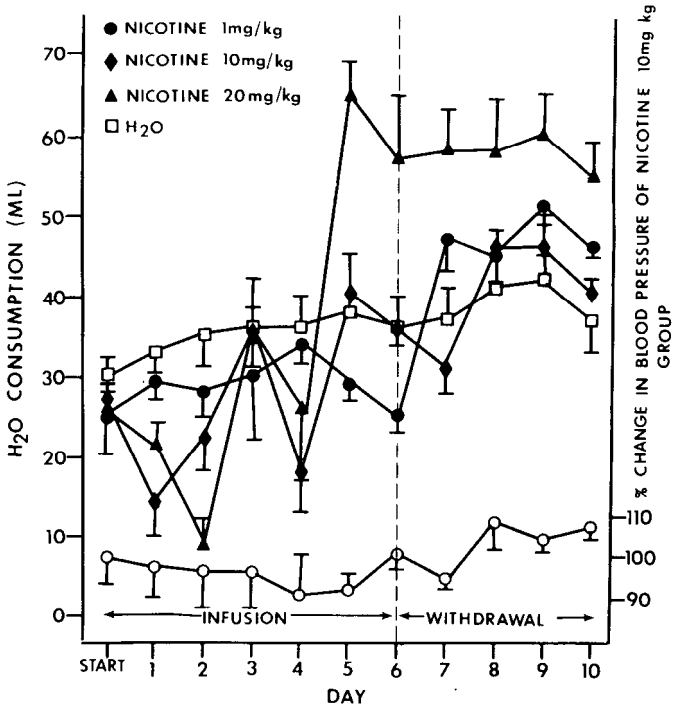

Fig. 3. Dose response studies of nicotine (1.0, 10.0 and 20.0 mg/kg/day) and vehicle. (8 ml/day) on water consumption and of nicotine (10 mg/kg/day) on blood pressure of rats during the infusion and abrupt withdrawal periods.

In all these experiments no behavioral withdrawal signs were seen. Instead, the animals were irritable when handled while receiving nicotine.

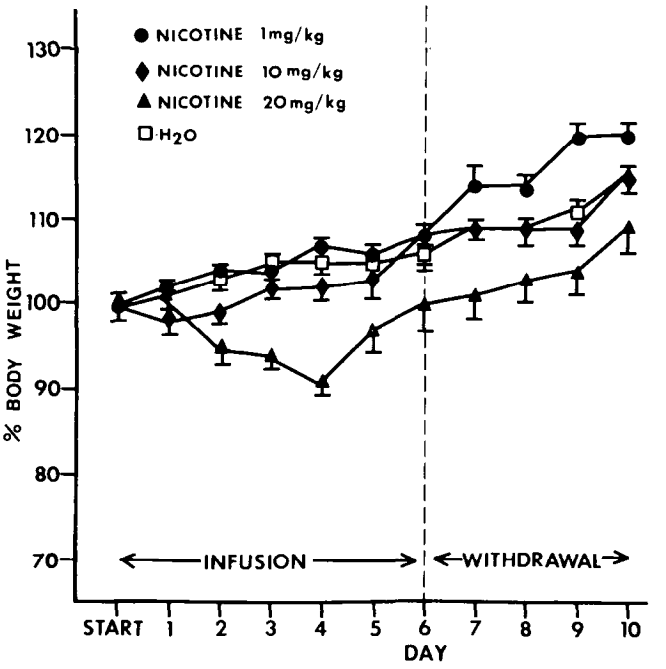

Fig. 4. Body weights of rats infused with nicotine (1.0, 10.0 and 20 mg/kg/day) or vehicle, (8 ml/day) during the infusion and abrupt withdrawal periods.

Discussion

Apparently, overriding mechanisms are triggered when drinking is severely restricted. The evidence indicates that this event is related more to the degree of suppression of drinking than to the abrupt withdrawal of nicotine and that little tolerance develops to these effects. Mecamylamine, the nicotine antagonist, neither prevented the body weight loss nor blocked the hypodipsic effect of nicotine during the first three days of infusion. Afterward, the interpretation of the results is confounded by the fact that the mecamylamine increased water intake per se. Although additional doses remain to be tested, the evidence at hand suggests that cholinergic mechanisms are not involved in either the body weight or water consumption changes.

A number of studies involving the chronic administration of nicotine have reported decreased fluid intake and loss of body

weight or failure to gain weight (Falkeborn et al., 1981, Clarke and Kumar, 1984, Wager-Srdar et al., 1984). However; the "rebound" phenomenon has not been noted, possibly due to procedural differences.

We believe that the results of this study, may have clinical relevance. It is known that smokers weigh less than comparable controls and that cessation of smoking usually results in weight gain (Schiffman, 1979, Wack and Rodin, 1982). Further, twenty percent of smokers indicated that control of body weight is an important reason for smoking (Little, 1964). Neither human nor animal studies have confirmed the inference that depression of weight or growth is explained by decreases in food (Wack and Rodin, 1982). Grunberg (1982) reported that nicotine administration to humans and rats was accompanied by decreased consumption of sweet, high caloric food. Since specific food consumption was not a factor in our experiments and since changes in drinking and body weight occurred within the first 24 h, our results suggest that H_2O regulation also plays a significant role in body weight changes.

Finally, Wenzel and Azmeh (1970) reported that withdrawal of chronically administered low doses of nicotine produced marked fluctuating rises in blood pressure of rats. Nicotine was administered via the drinking water for up to 14 weeks. In our studies, we showed a significant rise during withdrawal after only 6 days administration of nicotine. It is clear that chronically administered nicotine has pronounced effects on blood pressure and the implications are many. For example, it is possible that a heavy smoker and/or hypertensive who discontinues smoking may undergo a prolonged period of increased blood pressure. Obviously, more studies are indicated on this aspect of nicotine effects.

Acknowledgment

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Cocaine Inhibition of Locus Coeruleus Neurons

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The recreational use of cocaine has escalated dramatically over the past decade (Kozel and Adams 1984). In recent years, concern has been expressed over the larger doses being self-administered and the more widespread "destructive" use of the drug (Kozel and Adams 1984). Although cocaine is known to have both potent local anesthetic and sympathomimetic properties (Ritchie and Greene 1980), relatively little is known about the mechanisms that contribute to the central "stimulant" effects at the level of the single identified neuron. The modulatory action of cocaine on catecholamine neurotransmission (blocks catecholamine reuptake) is generally thought to be important in producing the central stimulant actions of cocaine (Gold and Verebey 1984). The present study was undertaken to determine the effects of systemically administered cocaine HCl on the electrical activity of single spontaneously firing noradrenergic neurons in the rat locus coeruleus.

METHODS

Male Sprague-Dawley rats (170-280 g) were anesthetized with urethane (1.25 g/Kg, i.p.), intubated following a tracheotomy, and allowed to breathe spontaneously. Body temperature was monitored by a rectal probe and maintained at 37 +/- 1°C. Animals were placed in a Kopf stereotaxic instrument for extracellular recordings. Glass micropipettes (Radnoti-quikfill) filled with 2M NaCl and saturated with fast green, tip diameter 1μ were stereotaxically directed toward the locus coeruleus through a bur hole 1.1 mm posterior to lambda and 1.1 mm lateral to midline. Noradrenergic LC neurons were generally encountered at a depth of 5.0-6.0 mm below the skull surface and identified by the following criteria: a) a characteristic positive-negative long duration (~2msec) waveform; b) the presence of cells of the mesencephalic nucleus of cranial nerve V (trigeminal), which could be activated by moving the lower mandible and were found just lateral to the LC recording area; c) acceleration of firing rate followed by inhibition when pressure was applied to the

contralateral hindpaw; d) location of cells below a zone of electrical silence corresponding to the fourth ventricle; e) a firing rate of 0.5-5.0 Hz and f) inhibition of firing rate produced by small (~ 10 µg/Kg) systemic doses of clonidine. Only one neuron per animal was studied. Drugs were administered through a catheter placed in the lateral tail vein (i.v.) or by the intraperitoneal (i.p.) route. Additional details of the electrophysiological recording procedure can be obtained from previous reports (Marwah and Aghajanian 1982; Marwah et al. 1983). Data analysis was accomplished using either the two-sample t-test or analysis of variance. A "P" value less than 0.05 was considered significant in statistical tests. Data is expressed as mean ± standard error.

RESULTS

Intravenous cocaine inhibited single identified spontaneously firing noradrenergic LC neurons. The time course for the inhibitory effects of 0.25 and 2.0 mg/Kg of cocaine (C) on the spontaneous activity of LC neurons from two different animals is shown in Figure 1, panel A and panel B respectively. Prior administraton of physiological saline (S) did not elicit any discernable effect on either neuron.

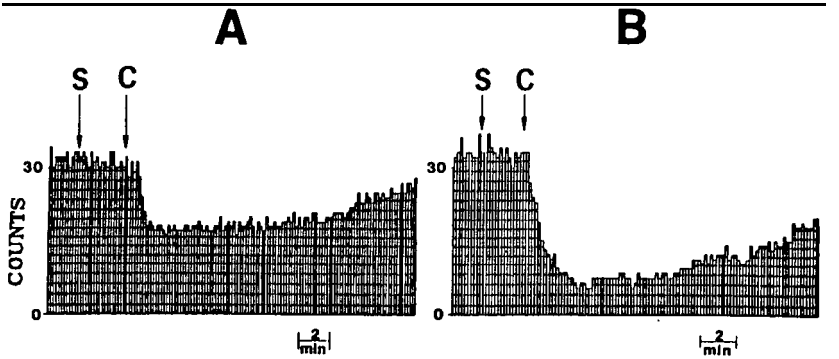

FIGURE 1

Histograms illustrating the effects of intravenous cocaine (C; 0.25mg/Kg-Panel A; 2mg/Kg-Panel B) or physiological saline (S, 0.2ml) administration on the activity of single LC neurons from two different animals. Each bar represents counts per 10 seconds.

The dose-dependent nature of the cocaine mediated inhibition of spontaneously firing LC neurons is depicted in Figure 2. The data is expressed as mean percent inhibition two minutes following cocaine injection as a function of the mean firing rate for a two-minute period prior to drug administration. All of the cocaine doses except the lowest, 0.0625 mg/Kg dose, produced inhibition of spontaneous LC firing rate significantly greater than that of saline controls $P < 0.01$.

Administration of procaine (1mg/Kg i.v.) to individual LC neurons (see Figure 3A) did not produce any discernable effect on firing rate or spike amplitude. The effects of intravenous

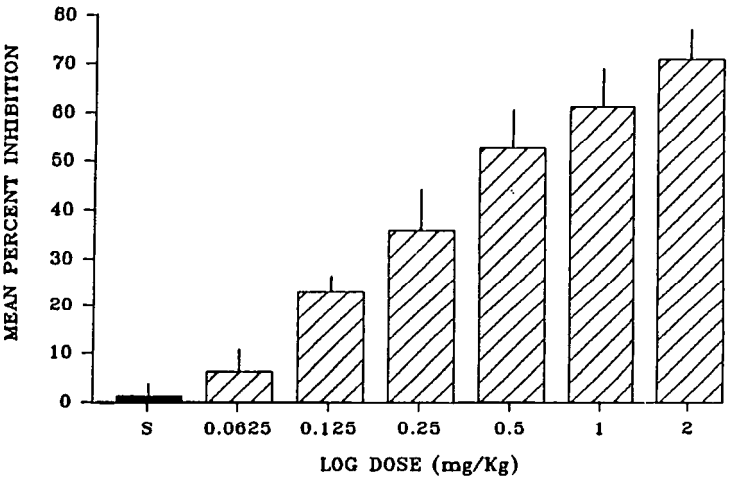

FIGURE 2

Bar graph depicting the dose-response relationship for the effects of intravenous cocaine or saline (S) on the activity of single LC neurons. Each bar represents mean percent inhibition two minutes after cocaine administration and was expressed as a function of the mean firing rate for 12 consecutive-ten second bins prior to drug administration.

procaine were also assessed by constructing a cumulative dose-response relationship (successive individual doses = 0.25, 0.25, 0.5, 1.0, and 2.0 mg/Kg). Procaine in cumulative doses of 0.25, 0.5, 1.0, 2.0, and 4.0 did not elicit any significant effects ($P \geq .20$) on LC neurons (n=4 animals) when compared to saline controls (see Table 1). Percent inhibition was calculated as stated above.

The activity of some of the slower firing (<1.5 Hz) LC neurons was sometimes completely suppressed by cocaine. Figure 3B shows a single slow spontaneously firing LC neuron that was completely inhibited by intravenous cocaine (1mg/Kg). Subsequent administration of the specific alpha-2-adrenoceptor antagonist, piperoxane (250 µg/Kg, i.v.) reversed the inhibitory effects of cocaine within one minute following administration. Another spontaneously firing LC neuron depicted in Figure 3C was also completely suppressed by intravenous cocaine (1mg/Kg). When pressure was applied to the contralateral hindpaw (toe pinch; tp) the cell was activated indicating its continued presence at the electrode tip. Subsequent administration of the specific opiate receptor antagonist, naloxone (1mg/Kg, i.v.) did not

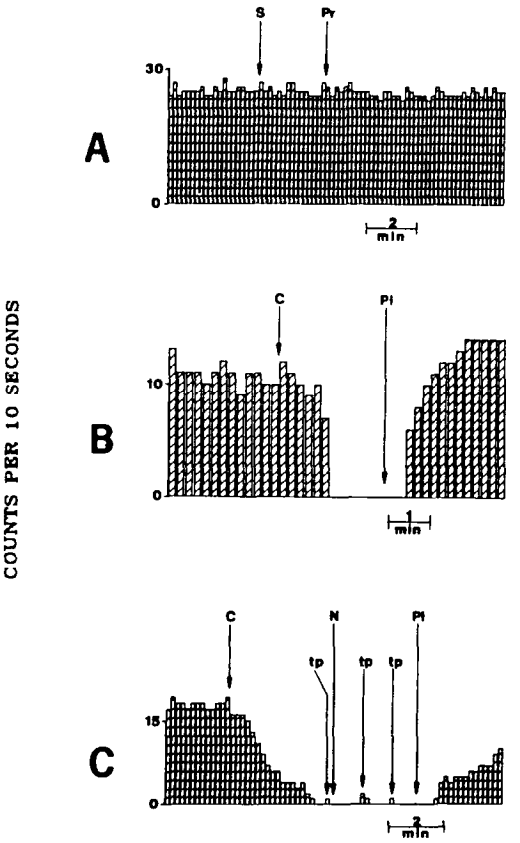

FIGURE 3

Histograms illustrating the effects of various treatments on the activity of single LC neurons from three different animals (A, B and C). Each bar represents counts per 10 seconds. Panel A: effects of intravenous saline (S, 0.2ml) and procaine (Pr, 1 mg/Kg). Panel B: effects of cocaine (C, 1 mg/Kg i.v.) and piperoxane (Pi, 250 µg/Kg i.v.). Panel C: effects of intravenous cocaine (C, 1 mg/Kg). pressure on the contralateral hindpaw (toe pinch; tp). naloxone (N, 1 mg/Kg i.v.) and piperoxane (Pi, 250 µg/Kg i.v.).

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reverse the inhibitory effects of cocaine on the LC neuron, eventhough pressure on the contralateral hindpaw could still accelerate the cell (tp x 2). The inhibitory effects of cocaine were, however, reversed by piperoxane (250 µg/Kg, i.v.).

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TABLE 1
EFFECT OF PROCAINE ON FIRING RATE OF LC NEURONS

TREATMENT					
SALINE	PROCAINE (mg/Kg)				
	0.25	0.5	1.0	2.0	4.0
1.2 *	-6.1	6.4	-6.2	-5.0	-6.4
± 2.5	10.3	6.7	5.3	3.6	19.0

* Mean percent inhibition ± standard error.

The response of LC neurons to cocaine (1mg/Kg) was significantly ($P<0.01$) attenuated when animals were pretreated with yohimbine (n=6; 5mg/Kg, i.p.) twenty minutes prior to cocaine (1 mg/Kg) challenge (data not shown). The mean percent inhibition of spontaneous activity by cocaine treatment in the presence (n=6) or absence (n=12) of yohimbine was 29.2 ± 4.3 percent and 61.3 ± 7.7 percent respectively.

DISCUSSION

Our data indicates-that noradrenergic neurons of the rat locus coeruleus are exquisitively sensitive to systemically administered cocaine. All of the spontaneously firing LC neurons receiving doses of cocaine greater than 0.0625 mg/Kg i.v. were inhibited (n=41). The inhibitory effect of cocaine on LC neurons was clearly dose dependent for the range of doses employed in this study (0.0625-2 mg/Kg, i.v.). The mechanism(s) by which cocaine inhibits LC neurons could encompass either a direct effect on the LC neurons or indirect effects mediated by other neurons impinging on the LC. Cardiovascular alterations (increase in mean arterial pressure) resulting from systemic cocaine administration were not temporally correlated with the changes observed in LC neuron firing rate (data not shown). The local anesthetic properties of cocaine also do not seem to contribute to the inhibition of spontaneous LC activity observed, since the structurally related local anesthetic, procaine, in doses up to 4 mg/Kg i.v. was without significant effect.

The existence of alpha-2-adrenergic and opiatergic neuroreceptors on noradrenergic LC neurons has been previously demonstrated (Bird and Kuhar 1977; Cedarbaum and Aghajanian 1976; Korf et al. 1974; Marwah and Aghajanian 1982). In the present study reversal of the effects of cocaine on LC neurons was observed with the specific alpha-2-adrenoceptor antagonist, piperoxane (i.v.), but not the specific opiate receptor antagonist, naloxone (i.v.). Furthermore, pretreatment with the specific alpha-2-adrenoceptor antagonist, yohimbine (i.p.) significantly attenuated the effects of cocaine on spontaneously

firing LC neurons. These results suggest that the inhibition of LC neurons by cocaine is mediated by alpha-2-adrenoceptors. At present it is unclear whether an interaction of cocaine with adrenoceptors may be mediated by a direct or an indirect mechanism. However, these results are not inconsistent with an increase in synaptic levels of catecholamines secondary to a blockade of catecholamine reuptake. Studies employing inhibitors of catecholamine synthesis and/or storage should clarify this issue. The source of catecholamines mediating the effects of cocaine may originate from the LC or other catecholaminergic neurons that impinge upon the LC.

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Opiate Receptor Mediated Regulation of the Immune Response *In Vivo*

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Evidence has accumulated over the years that the immune and neuroendocrine systems are interconnected at several levels, the sharing of specific receptors and their regulatory molecules being among the most notable. The presence of opiate receptors (Wybran et al., 1979; McDonough et al., 1980; Mehrisi and Mills, 1983; Johnson et al., 1982; Madden et al., 1982) and non-opiate like receptors (Hazum et al., 1979; Schweigerer et al., 1985) on leukocytes, coupled with the identification of endorphin-like substances being produced by these cells, places the endogenous opiates and the opiate receptor in an ideal position to be an internal regulator of the immune system and to form a portion of a larger neuroimmunoendocrine network. An increasing number of publications has described the effects of endogenous opiate peptides on parameters of immunocompetence and has been reviewed (Weber and Pert, 1984). These parameters include in vitro antibody (Ab) production, responsiveness to mitogenic stimulation, and natural killer (NK) cell activity.

Some of the early suggestions, however, that the immune system could be affected by opiates were based on studies of the immunologic status of heroin addicts (Louria et al., 1967; Sapira, 1968). The peripheral blood lymphocytes from addicts exhibited decreased proliferative responses to mitogens, though serum IgM levels were increased. Subsequent studies in mice injected repeatedly with morphine revealed a similar reduced ability to respond to concanavalin A, the effect being more pronounced in females than males (Brown et al., 1974). Furthermore, it has been shown recently that injection of rats with 30 to 50 mg/kg of morphine for four days caused suppression of natural killer cell activity and increased mortality in rats challenged with a syngeneic tumor (mammary adenocarcinoma). Suppressed NK cell activity could be observed three hours after a single injection subcutaneously or intracerebroventricularly; the morphine effect was reversed in each case by naltrexone (Shavit et al., 1985). These results suggested that opiates could be directly involved in altering

immune responsiveness, although indirect effects mediated through neuroendocrine pathways or other organ systems affected by repeated morphine administration are difficult to rule out.

MORPHINE SUPPRESSES ANTIBODY PRODUCTION IN VIVO

We have studied the effects of morphine administration on the production of antibody in Balb/c mice immunized with trinitrophenyl[40]-ovalbumin (TNP-OVA). Morphine was chronically infused into animals from an implanted source. Blood was collected from animals on day 6 following immunization. Serum Ab levels to trinitrophenyl-bovine serum albumin were determined using a solid phase enzyme-linked immunosorbent assay (ELISA). Administration of natural (-)-morphine suppressed the primary response (day 6) to TNP-OVA in vivo (Figure 1).

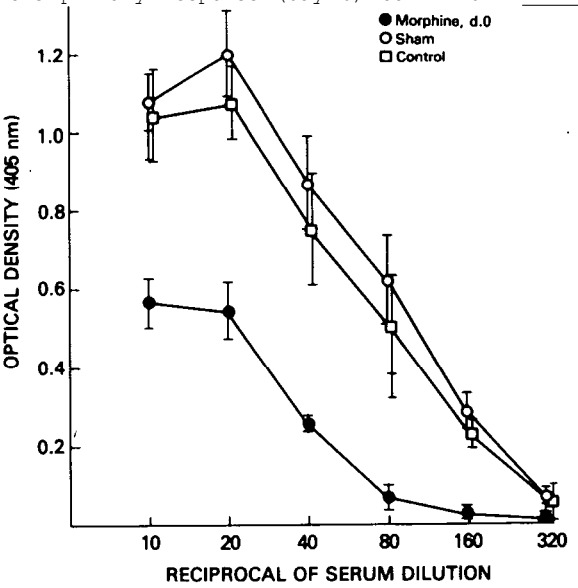

Figure 1. Morphine-induced suppression of antibody production in vivo. Mice implanted with a 75 mg morphine pellet and injected with 100 ug TNP-OVA on day 0 exhibit significant reduction by day 6 in serum antibody titers to TNP, as assayed by an ELISA procedure. Antibody levels in sham and control animals are equivalent, whereas morphine-implanted mice are significantly depressed (p 0.02). Data represent means ± standard error (SE) (n=4/group).

MORPHINE-INDUCED IMMUNOSUPPRESSION IS T-DEPENDENT

The generation of an Ab response to TNP-OVA requires the participation of B cells (Ab-producing cells), T cells (thymus-derived lymphocytes), and macrophages (antigen processing cells). The suppression of the Ab response to TNP-OVA could hypothetically be due to a direct or indirect effect on any or

all of these cell types. Trinitrophenol, covalently conjugated to Ficoll (a repeating polymer), stimulates the production of antibody without the requirement for accessory cells on T cells. Therefore, TNP-Ficoll is referred to as a T-independent antigen, as opposed to TNP-OVA, a T-dependent antigen. This distinction provides us with a tool through which we can begin to examine which cell or cells of the system are affected by morphine (Figure 2). Chronic morphine infusion has no effect on the Ab response to TNP-Ficoll, suggesting that opiates do not affect B cells directly but that the suppression is mediated directly or indirectly through T cells or macrophages.

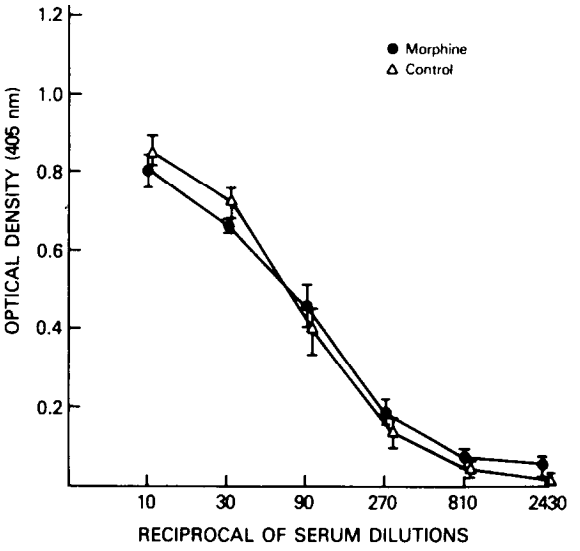

Figure 2. Lack of morphine-induced suppression in vivo to TNP-Ficoll. Mice immunized against 1.5 ug TNP-Ficoll fail to exhibit depressed serum antibody levels on day 6 in response to an implanted 75 mg morphine pellet. Data represent means ± SE (n=3/group).

NALOXONE REVERSAL OF MORPHINE-INDUCED IMMUNOSUPPRESSION

In order to determine whether the observed suppression of Ab production was caused via an interaction of morphine with the opiate receptor, we examined the effects of an opiate antagonist on the response. Simultaneous co-implantation of a (-)-naloxone pellet with the morphine pellet was sufficient to reverse the morphine-induced suppression of the primary Ab response to TNP-OVA (Figure 3). Naloxone alone had no effect when compared to a group of immunized controls.

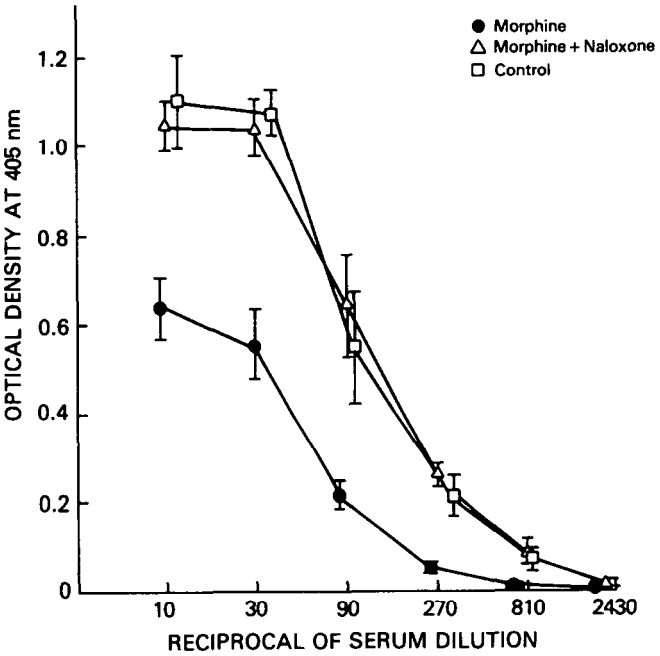

Figure 3. Naloxone reversal of morphine-induced antibody production in vivo. Mice immunized against 100 ug TNP-ovalbumin were implanted with 75 mg morphine or morphine plus 25 mg naloxone pellets. Serum taken on day 6 shows a significant reduction (p 0.01) of antibody production in the morphine-implanted mice, but the effect is reversed in the morphine-naloxone implanted animals (p 0.05). Data represent means ± SE (n=5/group).

IMMUNOSUPPRESSION INDUCED BY MORPHINE IS STEREOSPECIFIC

Further experiments were designed to compare the effects of (-)-morphine with (+)-morphine which does not bind to opiate receptors. When each compound was administered to different groups of mice, (-)-morphine demonstrated the expected suppression of Ab production, whereas the unnatural (+)-enantiomer of morphine had no significant effect (Figure 4). These experiments, coupled with naloxone antagonism of the effect, strongly suggest that morphine induces an in vivo immunosuppression by acting via the opiate receptor.

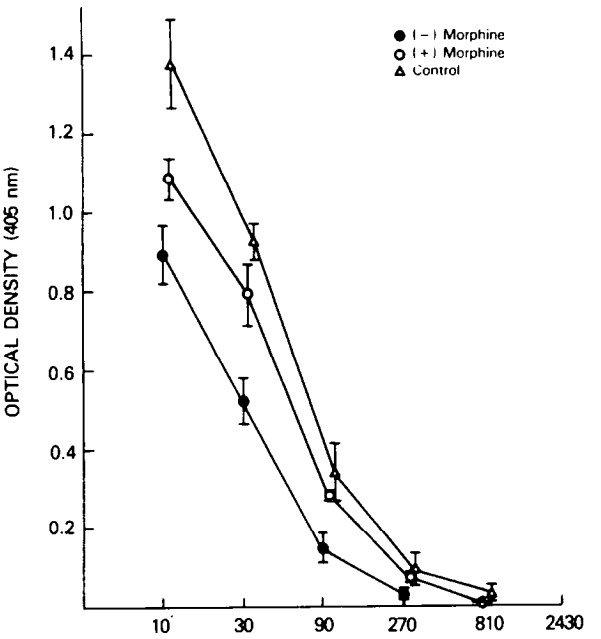

Figure 4. Stereospecificity of morphine-induced antibody production in vivo. Mice immunized against 100 ug TNP-ovalbumin were implanted with 75 mg (+)-morphine, the inactive enantiomer, or (-)-morphine. On day 6, sera were assayed for antibody levels. The (+)-morphine does not significantly reduce in vivo antibody production (p 0.051, where as (-)-morphine does (p 0.01). Data represent means ± SE (n3-4/group).

LYMPHOKINE ACTIVATED KILLER (LAK) CELL ACTIVITY IS SUPPRESSED IN MICE INJECTED WITH MORPHINE

Interleukin-2 (IL-21 is a T-lymphocyte derived, hormonal-like peptide (lymphokine) which stimulates the growth and differentiation of activated lymphocytes from several species in vitro and in vivo. Normal lymphocytes when incubated in IL-2 for several days can be induced to develop into lymphokine activated killer (LAK) cells (Grimm et al., 1982; Grimm and Rosenberg, 1984). These cells are capable of lysing tumor cell lines and fresh tumor targets in vitro and can be used in adoptive immunotherapy to cause regression of tumors in vivo (Mazumder and Rosenberg, 1984; Mule' et al., 1984).

We have investigated the cabability of LAK cells generated from Balb/cj mice injected with 50 mg/kg of morphine daily for 3 days to lyse a human tumor target (DAUDI) in a short-term chromium-51 (^{51}Cr) release assay. Table 1 shows the percentage of total ^{51}Cr released at various effector to target ratios by LAK cells generated from morphine-injected mice in comparison to saline-injected mice. These data show a decreased capacity of LAK cells generated from mice injected with morphine to lyse a tumor target. These preliminary data suggest that use of morphine in patients undergoing adoptive immunotherapy may be contraindicated.

TABLE I

Generation of LAK Cell Activity by Recombinant IL-2 From BALB/CJ Mice Injected with Morphine

	LAK Activity After IL-2 Activation (% lysis of DAUDI)		
Treatment	80:1	20:1	5:1
Morphine 50 mg/kg	36.7	15.5	-2.7
Saline	47.0	25.5	4.2

In summary, (-)-morphine induces an immunosuppression of an early event in the T-dependent, primary Ab response to TNP-OVA *in vivo*. This opiate-mediated immunosuppression is naloxone reversible and stereospecific, properties which suggest that it is mediated through the opiate receptor, which has been identified on cells of the immune system (see Madden et al., this meeting). These results imply that opiates might have direct influences on the immune system, though *in vivo* effects mediated through other organ systems affected by repeated morphine administration are difficult to rule out. The findings of Shavit et al., (1985) that ICV administration of morphine is more effective than a peripheral administration suggests that morphine may regulate the immune system indirectly by altering various neuroendocrine mediators of immunosuppression. Such neuroendocrine factors may also play a role in the suppression of Ab production and the inability to generate LAK cells that we observe. In any case, it seems likely that a network of cells in the brain, glands, and immune system communicate through shared receptors and regulatory molecules and that this neuroimmunoendocrine network has a major role in health and disease. It is applicable to the subject of this meeting that the study of opium alkaloids (Rice, 1985), the opiate receptor and endogenous opiate ligands has contributed greatly to this revelation.

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Novel Biochemical Determinants in the Preclinical Evaluation of Opiates

Fedor Medzihradsky

INTRODUCTION

Research in the field of opioid receptors has received a major impetus by the identification of ligands with high selectivity toward receptor types, e.g., mu (sufentanil, DAGO), kappa (U50,488, U69,593), and delta (DSLET, DPDPE). The use of such compounds has enhanced the classification of opioid receptors in brain membrane binding assays (Goldstein and James 1984) and in bioassays with smooth muscle preparations (Gillan and Kosterlitz 1982). We have initially established a procedure for the assay of opioid receptor binding in brain membranes, based on the competitive displacement of stereospecifically bound ^{3}H-etorphine under equilibrium conditions (Medzihradsky 1976). Using this assay, we have since 1977 determined the opioid receptor binding of about 600 compounds, tested within their preclinical evaluation as opiates (e.g., Medzihradsky 1978; Woods et al. 1986). In the course of these studies, we have correlated the receptor binding affinity in brain with the responses elicited in the guinea-pig ileum and mouse vas deferens preparations (Smith and Medzihradsky 1981, 1986), and with behavioral characteristics displayed in the rhesus monkey and pigeon (Woods et al. 1979). In addition to investigating equilibrium binding (Fischel and Medzihradsky 1981), we have studied the kinetics of ligand interaction with multiple opioid receptors (Fischel and Medzihradsky 1986), and have described a classificaiton of mu and kappa opiates on the basis of their differential sensitivity toward sodium in binding to opioid receptors (Medzihradsky et al. 1984). Recently, alkylation of the membrane preparation with the irreversible opioid antagonist β-CNA in the presence of receptor-selective opiates was used to investigate the specificity of ligand binding (James and Goldstein 1984).

However, none of the above outlined approaches provides information on the efficacy of ligand binding to opioid receptor. The assessment of ligand binding to receptor in brain membranes has to be complemented by a functional assay of effector response.

We have linked the occupancy of opioid receptor to the stimulation of GTPase in brain membranes (Barchfeld and Medzihradsky 1984; Clark and Medzihradsky 1985), and to the inhibition of adenylate cyclase in brain slices (Barchfeld et al. 1982). The assay of opioid receptor coupling to GTPase in isolated brain membranes can now be carried out under conditions of routine work, i.e., good precision, sensitivity and capacity. This functional assay distinguishes between opioid agonists and antagonists, and has revealed different characteristics of mu, kappa and delta opiates, particularly pronounced in brain membranes alkylated to provide specificity for one type of opioid receptor (Clark and Medzihradsky 1985, 1986; Clark et al. 1986).

METHODS

Membrane preparation (Fischel and Medzihradsky 1981). Membranes from rat cerebrum and regions of the monkey brain were isolated by differential centrifugation as described. Aliquots of the membrane suspension were kept frozen until use.

Receptor alkylation (Clark et al. 1986). Membranes were incubated at 25° with either β-FNA or Superfit, or both. Subsequently, the membranes were thoroughly washed by centrifugation and resuspension. Alternatively, the membranes were incubated with β-CNA in the presence of protecting concentrations of either sufentanil (mu receptor), U-50, 488 (kappa receptor), or DSLET (delta receptor).

Binding assay (Medzihradsky et al. 1984). The investigated opiates compete with ^{3}H-etorphine for bindlng to opioid receptor in brain membranes. After incubation at 25° to reach binding equilibrium, the suspension of brain membranes in the assay medium was quickly filtered and the bound radioactivity determined. The binding affinity of the tested compound is expressed as EC_{50} in displacing specifically bound ^{3}H-etorphine.

GTPase assay (Clark et al. 1986). The assay is based on the release of inorganic phosphate from (gamma-^{32}P)GTP in the presence of different concentrations of opiates. The released ^{32}P-phosphate was separated from nucleotides by adsorption on charcoal, and the radioactivity determined by liquid scintillation counting. The results are expressed as concentration of a compound to produce half-maximal stimulation of GTPase (K_S), and as maximal enzyme stimulation (S_{max}).

RESULTS AND DISCUSSION

The enhancement of brain GTPase by opiates (Table 1) fulfilled the criteria for mediation by opioid receptor. The stimulation was limited to agonists, and blocked by opioid antagonists (Barchfeld and Medzihradsky 1984; Clark and Medzihradsky 1986). The rank order of the K_S values for various opioid agonists

corresponded to that of their binding affinity to opioid receptor. Except in monkey midbrain (Table 1), kappa opiates stimulated brain GTPase to a lesser extent than mu and delta ligands of similar binding affinity. Various opioid antagonists (including naloxone, naltrexone, ICI 174,864 and MR 2266) did not induce coupling of opioid receptor to GTPase, but displayed selectivity in inhibiting the effect of agonists. E.g, ICI 174,864 blocked GTPase stimulation by DSLET and DPDPE, but not by sufentanil or bremazocine (Clark and Medzihradsky 1985).

TABLE 1

Stimulation of GTPase in monkey brain

Opiate	Brain region	Stimulation of GTPase activity K_S (μM)	S_{max} (%)
	frontal cortex		
Levorphanol		20	33
Bremazocine		24	
DSLET		4	13
	striatum		
Levorphanol		27	21
Bremazocine		21	13
DSLET		5	26
	midbrain		
Levorphanol		25	30
Bremazocine		24	31
DSLET		5	23

In order to study the coupling of individual opioid receptor types to GTPase, brain membranes were alkylated by two different approaches. Both direct and protective alkylation conveyed specificity of brain membranes toward mu, delta or kappa opioid receptors (Table 2). Direct alkylation with β-FNA abolished GTPase stimulation by mu but not delta, kappa or nonselective agonists. On the other hand, alkylation with Superfit prevented the coupling of the delta but not mu or kappa opioid receptor to GTPase. Finally, successive alkylation with Superfit and β-FNA rendered the brain membranes specific for the kappa receptor. Alkylation of brain membranes with the irreversible nonselective opioid antagonist β-CNA abolished GTPase stimulation by mu, delta and kappa agonists. However, protective alkylation with β-CNA in the presence of an excess of mu, delta or kappa ligands provided specificity in the coupling of the corresponding opioid receptor to brain GTPase (Table 2). The advantage of protective

alkylation is the versatility and simplicity of receptor resolution. Of course, the method depends on the availability of highly selective protective opioid ligands. The present disadvantage of the approach is the high cost of β-CNA.

TABLE 2

Stimulation of GTPase in alkylated rat brain membranes

Membrane treatment	Opiate	Stimulation of GTPase activity S_{max} (%)
No treatment		
	Sufentanil	22
	DSLET	26
	Bremazocine	13
	Etorphine	29
Direct alkylation		
β-FNA (blocks mu receptor)	Sufentanil	0
	DSLET	27
	Bremazocine	8
	Etorphine	20
Superfit (blocks delta receptor)	Sufentanil	16
	DSLET	0
	Bremazocine	12
	Etorphine	19
β-FNA and Superfit (provides kappa specifically)	Sufentanil	0
	DSLET	0
	Bremazocine	10
	Etorphine	9
Protective alkylation		
β-CNA (blocks mu, kappa and delta receptors)	Sufentanil	0
	DSLET	0
	Bremazocine	0
β-CNA and Sufentanil (provides mu specificity)	Sufentanil	22
	DSLET	0
	Bremazocine	0
β-CNA and DSLET (or DPDPE) (provides delta specificity)	Sufentanil	0
	DSLET	26
	Bremazocine	0

TABLE 3

Evaluation of compounds on the basis of opioid receptor binding and receptor-effector coupling

A. Binding	B. Coupling
PHASE 1	PHASE 1
Compound	Compound
Nonalkylated brain membranes:	Nonalkylated brain membranes:
displacement of specific ^{3}H-etorphine binding	stimulation of low-K_m brain GTPase
Results: Opioid character; receptor binding affinity	Results: Functional consequence of receptor occupancy; agonist/antagonist character
If the compound is identified as opiate, it enters Phase 2	If the compound stimulates GTPase, it enters Phase 2
PHASE 2	PHASE 2
Compound	Compound
Receptor alkylation	Receptor alkylation
μ-membranes: κ-membranes: δ-membranes:	μ-membranes: κ-membranes: δ-membranes:
displacement of specific ^{3}H-etorphine binding	stimulation of low-k_m brain GTPase
Results: Selectivity and affinity in binding to specific types of opioid receptors	Results: Selectivity in receptor-effector coupling; efficacy of ligand binding to opioid receptor types

The successful resolution of multiple opioid receptors by the implemented alkylations was underlined by the results obtained with the nonselective opiate etorphine: the sum of GTPase stimulation due to its mu, delta and kappa receptor binding components (determined in the corresponding alkylated membranes) equaled maximal enzyme stimulation by etorphine in nonalkylated membranes (Table 2). The described direct and protective alkylations can routinely be carried out in batch operation, and the obtained membranes conveniently kept frozen until use. Except for decreased concentrations of the alkylators (Clark and Medzihradsky 1986), identical protocols are followed to treat brain membranes used in studies on ligand binding to individual opioid receptor types.

Considering the apparently low efficacy of opioid receptor binding, i.e., the existence of spare receptors (Clark and Medzihradsky 1986) the testing of novel opiates in binding assays is incomplele. The availability of a routinely applicable functional assay of receptor-effector coupling (Table 1) and brain membranes with high selectivity for the mu, delta or kappa receptor (Table 2), should now benefit the preclinical evaluation of opiates (Table 3). The two phases of the new experimental schemes provide for initial screening, i.e., the identification of the tested compound as an opiate, and subsequent determination of binding selectivity, affinity and efficacy, all with the use of just one radiolabeled ligand.

While the assessment of pure antagonists on the basis of their lacking stimulation of GTPase is unequivocal, the quantitative responses of mixed agonists-antagonists in the effector assay have yet to be determined. The described alkylations have been successfully applied to membranes from monkey brain (Medzihradsky et al., unpublished observations). The use of this tissue in the biochemical evaluation should further enhance the correlation with behavioral and physiological findings, and thus generate a comprehensive profile of the tested compound.

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Synthesis and Biochemical Characterization of Two Novel Irreversible Ligands Specific for "Peripheral" Benzodiazepine Receptors

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Central benzodiazepine (BDZ) receptors were first described by Squires and Braestrup (1977) and Mohler and Okada (1977) by the saturable, high affinity, and stereospecific binding of [^{3}H]diazepam. Numerous studies have described the pharmacological, biochemical and physical properties of these receptors in detail (see Haefly, et al., 1985 for review). Currently, ligands for BDZ receptors can be classified as agonists, antagonists, and inverse agonists.

A second type of BDZ receptor was first described in peripheral tissues (Squires and Braestrup 1977) and in transformed cells of neural origin (Syapin and Skolnick 1979) and later in the CNS (shoemaker, et al 1983). These sites have high affinity for [^{3}H]diazepam but low affinity for other BDZs such as clonazepam, Ro 15-1788, and the β-carbolines. The physiological function of these "peripheral" receptors remains elusive and their pharmacological relevance is currently being studied (Shoemaker, et al., 1983; Le Fur, et al., 1983). Two ligands, Ro 5-4864 (4'-Cl-diazepam) and PK 11195 (1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecarboxamide), demonstrate high affinity for these "peripheral" binding sites and define the sites by showing saturability and selectivity over the central type BDZ sites, at which these ligands are virtually inert (Le Fur, et al., 1983). Our goal is to clarify the mechanism of action of these specific "peripheral" type BDZ ligands and to define the pharmacological and physiological roles of these sites in the brain and the periphery. Furthermore it is of interest to determine the relationship of the "peripheral" type to the "central" type BDZ receptors on a molecular basis and to discern whether an association exists with the abuse of centrally active BDZs.

Irazepine, prepared several years ago, (Rice, et al., 1979) is an ethylisothiocyanato-derivative of flurazepam, another BDZ agonist with anxiolytic properties. Irazepine was found to be a potent, irreversible, ligand for the central BDZ sites. Consequently, the ethylisothiocyanato-derivatives of Ro

5-4864; AHN 086 and PK 11195; AHN 070 were prepared. These are the first potent, irreversible ligands specific for "peripheral" BDZ receptors.

CHEMISTRY

The synthesis of AHN 086 (1), 1-(2-isothiocyanatoethyl-7-chloro-1,3-dihydro-5-(4-chlorophenyl)-2H-1,4-benzodiazepine-2-one, is shown in Figure 1. It was prepared in three steps from nor-R0 5-4864 (3) (a generous gift from Dr. Karl Weber, Boehringer-Ingelheim).

Racemic 1-(2-chlorophenyl)-N-ethylisothiocyanato-N-(1-methyl-propyl)-3-isoquinolinecarboxamide, AHN 070 (2a) was prepared in four steps from PK 11209 (11) (a generous gift from Dr. G. Le Fur, Pharmuka Laboratories shown in Figure 2. The (+)(2b) and (-)(2c) stereoisomers of AHN 070 were prepared in the same manner starting with the commercially available (+) and (-) set-butylamine, respectively. The chemical structures and purity of AHN 086, (±), (+), and (-) AHN 070 and all intermediates were fully characterized by thin layer chromatography, melting point, infrared spectroscopy, nuclear magnetic resonance spectroscopy, mass spectroscopy, combustion analysis, and where applicable, optical rotation.

BIOCHEMICAL METHODOLOGY

Male Sprague Dawley rats (140-200g) (Taconic Farms, Germantown, NY) were killed by decapitation. The kidneys or whole brains were decapsulated, and homogenized in twenty volumes of ice-cold buffer as specified in a Brinkmann Polytron (setting 6-7 sec). This tissue homogenate was centrifuged at 23,000 x g for twenty minutes (4°C) and the resulting pellet resuspended in 100-2000 volumes of buffer, for the kidneys and in 50-200 volumes for the brains. Radioligand binding to this tissue suspension wasassayed in a volume of 1 ml (2 ml for Scatchard analysis of [^{3}H]PK 11195 binding) consisting of: 0.25 ml of tissue suspension, 0.1 ml radioligand [^{3}H]Ro 5-4864 Sp. Act. 78.9 Ci/mmol) or [^{3}H]PK 11195 (Sp. Act. 85 Ci/mmol) (New England Nuclear, Boston, MA) diluted in assay buffer, drugs, and/or buffer to final volume. Nonspecific binding was defined using Ro 5-4864 or PK 11195 (final concentration, 10 µM) for [^{3}H]Ro 5-4864 and [^{3}H]PK 11195, respectively. Incubations were performed at 0-4°C and terminated by rapid filtration over Whatman GF/B filters, with three washes (5 ml) of assay buffer using a Brandel M-24R filtering manifold (Brandel Instruments, Gaithersburg, MD). Filters were preincubated with 0.1% polgethyleneimine in distilled water for Scatchard analysis of [^{3}H]PK 11195 binding. Protein was determined using the Miller modification (Miller, 1959) of the Lowry (Lowry, et al., 1951) technique.

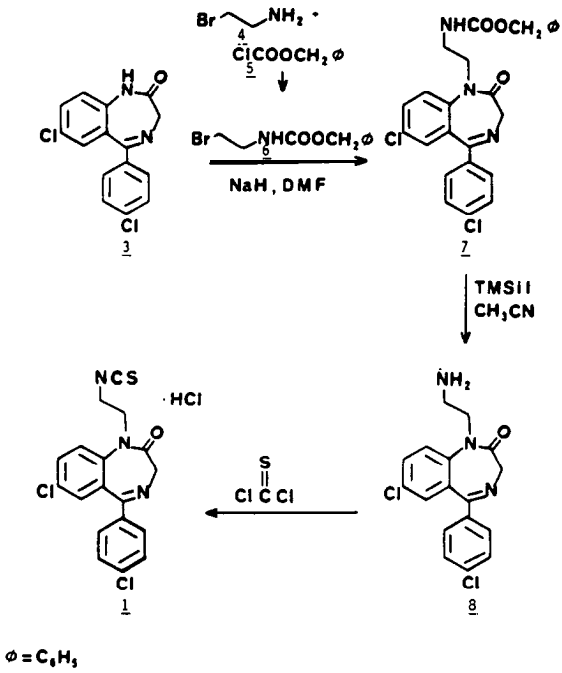

FIGURE 1: Synthesis of AHN086

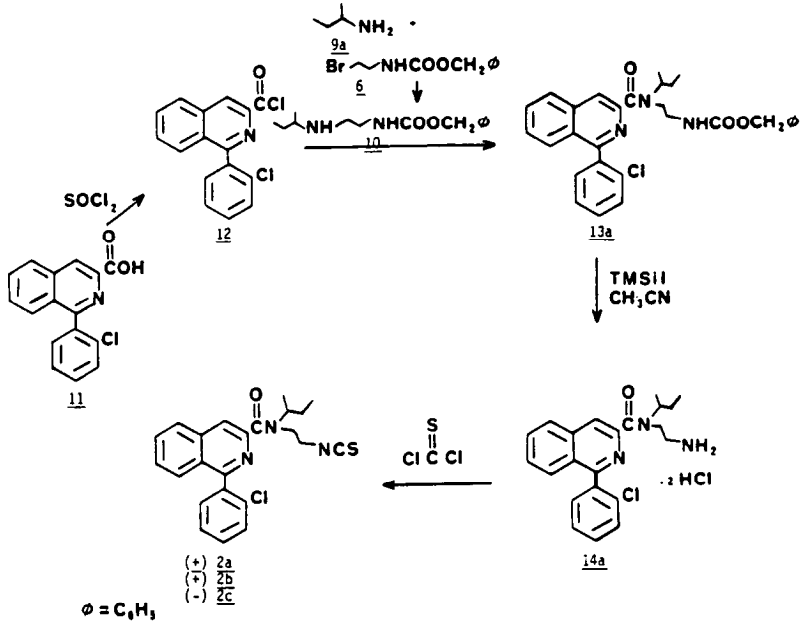

FIGURE 2: Synthesis of ARN070

RESULTS AND DISCUSSION

The IC_{50} values for AHN 086 and AHN 070 to inhibit [^{3}H]Ro 5-4864 binding in the brain and kidney (2.1 ± 0.2 nM and 1.2 0.1 nM, respectively for AHN 086) and (1.8 ± 0.3 nM and 1.43 ± 0.1 nM, respectively for AHN 070) were estimated by extrapolating the observed IC_{50} at different tissue concentrations to an infinite tissue dilution {Figure 3). The IC_{50} values of AHN 086 and AHN 070 to inhibit [^{3}H]PK 11195 binding were identical to the values obtained for [^{3}H]Ro 5-4864.

Scatchard analysis of [^{3}H]Ro 5-4864 binding to the "peripheral" BDZ binding sites in kidney membranes, after preincubation with AHN 086 or AHN 070 followed by extensive washing, revealed a concentration dependent decrease in the $Bmax_3$ 10 between 10 nM and 1 µM, Table 1). In contrast, only the K_D of [^{3}H] PK 11195 was effected by up to 100 nM of AHN 086 (Table 2). No specific binding of either radioligand was detectable after preincubation of the membranes with 1 µM AHN 086 or AHN 070 that could be displaced by 10 µM PK 11195.

The irreversible nature of AHN 086 and AHN 070 binding to "perfpheral" BDZ receptors was demonstrated by plotting binding of [^{3}H]Ro 5-4864 versus receptor concentration at near saturating concentrations of radioligand (4). In both cases, the x-axis intercept is significantly different from the origin, which is strong evidence for an irreversible blockade of receptors (Rice, et al., 1979). Furthermore, the effects of AHN 086 and AHN 070 on the Bmax of [^{3}H]Ro 5-4864 persisted through extensive washing of tissues. In contrast, addition of 10 nM Ro 5-4864 elicited an inhibition of radioligand binding that could be reversed by washing to approximately 98% of control values.

A slight stereoselectivity of the (+) and (-) stereoisomers of AHN 070 could be observed with the (-) isomer being 2.5-fold more potent in displacing [^{3}H]PK 11195 as well as [^{3}H]Ro 5-4864. Therefore both AHN 086, a structural analog of Ro 5-4864, and AHN 070, a structural analog of PK 11195, are high affinity ligands that bind specifically and irreversibly to the "peripheral" BDZ binding sites in brain and kidney. It is hoped that these two ligands will aid in the purification, isolation and determination of the amino acid sequence and subsequently the fractionation of the acylated receptor to confirm the identity of the amino acid which becomes covalently bonded. Ultimately this will prove useful for the determination of the physiological function of these receptors and an improved understanding of their relationship on a molecular basis to the central BDZ receptors.

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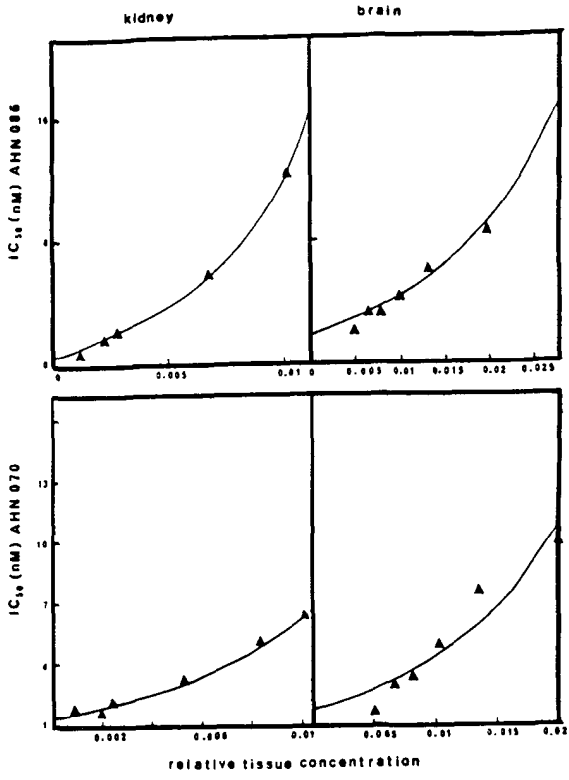

FIGURE 3: IC_{50} values for AHN086 and AHN070

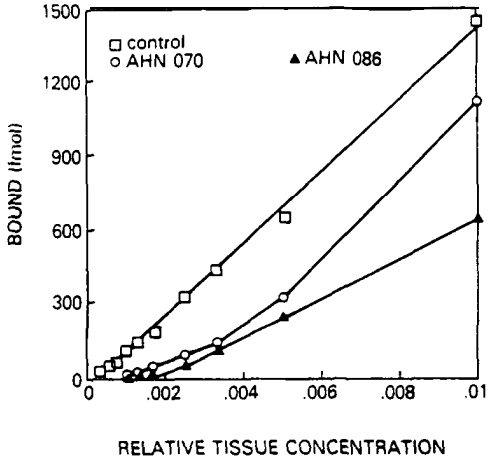

FIGURE 4: Irreversibility of AMN070 and AXN086

TABLE 1:

Effects of AHN 086 and AHN 070 on [^{3}H] RO 5-4864 binding to PBS: Scatchard Analysis

AHN 086	K_D [a]	B_{MAX} [b]	%Control	n
0	1.23 ± 0.39	5659 ± 554	100	7
10 nM	0.98 ± 0.35	4096 ± 336*	72	6
100 nM	2.99 ± 0.72*	3100 ± 294*	55	4
AHN 070				
10 nM	1.43 ± 0.55	4600 ± 547	81	7
100 nM	3.63 ± 0.77*	3901 ± 429*	69	6

TABLE 2:

Effects of AHN 086 and ANH 070 on [^{3}H] PK 11195 binding to PBS: Scatchard Analysis

AHN 086	K_D [a]	B_{MAX} [b]	n
0	0.72 ± 0.10	5988 ± 1342	6
10	0.64 ± 0.03	6343 ± 1266	5
100	1.39 ± 0.25*	8049 ± 2728	5
AHN 070			
10	0.94 ± 0.21	6853 ± 1096	4
100	2.05 ± 0.50*	6586 ± 961	4

Kidney membranes prepared in 50 mM potassium phosphate buffer (pH 7.0) were incubated at a dilution of 1:250 (w/v) with increasing concentrations of AHN 086 or AHN 070. Scatchard analyses were performed on these membranes after four washes in 1:250 dilutions. At least eight ligand concentrations (in triplicate) were used for each determination. Values represent X ± S.E.M. with the number of determinations as indicated. a: in nM, b: in fmol/mg protein, *$p<0.001$ compared to the absence of AHN 086 or AHN 070.

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Developmental Toxicity of Prenatal Delta-9-Tetrahydrocannabinol: Effects of Maternal Nutrition, Offspring Growth, and Behavior

Donald E. Hutchings, Stephen Brake, Brian Morgan, Elizabeth Lasalle, and Thomas M. Shi

Animal studies of the neurobehavioral effects following prenatal exposure to delta-9-tetrahydrocannabinol (THC) have often yielded ambiguous results. A major problem shared by many of these studies is that developmental toxicity may not have resulted from direct drug effects on the embyro and fetus, but rather, were secondary to THC-induced maternal toxicity (Abel, 1980). For example, one acute effect of THC administration in rats is a substantial inhibition of food and water intake with consequent maternal undernutrition and dehydration. Also, THC can disrupt normal maternal care at parturition and inhibit milk production and let-down, all with possible negative consequences for the neurobehavioral development of the offspring (for discussion, see Hutchings, 1985).

The present study reports on the effects of THC on maternal and fetal toxicity, and on the growth and behavior of the offspring. Two dose-levels were administered during the last two weeks of gestation. To control for the effects of reduced food and water intake among THC exposed dams, a pair-fed control group was included. In addition, to obviate possible postnatal effects of being reared by a drug-treated dam. all experimental and control litters were reared by surrogate dams. The present study included a total of 73 treated and control dams and their 932 offspring.

METHOD

Beginning on Day 8 of gestation, either 15 or 50 mg/kg of THC suspended in sesame oil was administered to two groups of gravid dams once daily by gastric intubation (THC-15; THC-50). Both dose-level groups received daily drug administration through Day 22 of gestation, approximately 24 hr prior to expected parturition. One control group received the vehicle and was pair-fed to the food and water intake of the 50 mg/kg THC group (PF). A nontreated control group was left undisturbed throughout pregnancy (NT). Within 1-5 hrs of birth, all treated and control offspring were sexed and weighed and litters culled when

necessary to 10 pups; litters containing fewer than 8 pups were also sexed and weighed but excluded from further testing. Offspring were then fostered to normal mothers of the same strain that had delivered approximately 24 hrs earlier. All experimental and control mothers were then sacrificed to determine the number of implantation sites.

Litters were randomly assigned to either activity or nipple-attachment testing; none of the litters were tested on both measures. Activity level testing was carried out using a 6-channel electronic activity monitor with 6 remote sensors. Eight litters each of treated and control pups were tested. Treated and control litters were tested for 60 minutes at 3-day intervals from Day 2 through 32 days of age (day of birth was designated Day 0). A detailed description of the equipment and test procedure has been described previously (Hutchings et al, 1980). Individual body weights were measured and recorded.

Offspring from 10 THC-15, 10 THC-50, 9 PF, and 9 NT litters were tested for nipple attachment on days 2, 5, 8, 11 and 14 of age. (For a detailed description of the method, see Brake et al, in press). Approximately 15 min prior to the beginning of testing, a "test dam" was anesthetized with an intraperitoneal injection of 50 mg/ml sodium pentobarbital. She was then placed on her side in a small plastic arena, with the ipsilateral fore and hindlimbs gently taped to the wall to expose all the nipples. The liter to be tested was removed from the foster dam and placed in an incubator maintained at 33-35 C. for five min prior to the beginning of testing. Then, 2-3 littermates were tested for a five min session. Pups were placed by hand in the arena perpendicular to the dam's flank, with their snout in contact with the ventrum, but not a nipple. If, during testing, pups moved away from the ventrum or positioned themselves laterally for more than 10 sec, they were re-started with their snout to the ventrum. Data collected included the latency to attach to a nipple and proportion of animals failing to attach.

RESULTS

ANOVA or repeated-measures ANOVA, and Scheffe post hoc tests were performed on all measures. To analyze offspring effects, the litter served as the unit of analysis. (All comparisons characterized below as "significant" denote a probability of less than .02). There were no maternal deaths among any of the THC treated or control groups. However, among the dams exposed to THC, dose-related toxicity was observed. Among the THC-50 dams, there was a substantial reduction in food and water intake, particularly on the first several days after the initiation of drug treatment. After drug administration of gestation Day 8, both food and water intake was reduced 75-80% during the next 24 hr. Over the following 3-4 days, tolerance to the THC induced inhibition of food and water intake developed and both food and water intake recovered to approximately 80-85% of that

displayed by the NT dams. Table 1 shows that the PF, THC-15 and THC-50 dams failed to gain as much body weight from conception to term compared with the NT dams, a difference that was highly significant.

Another major toxic effect observed among the THC treated dams was in maternal care at parturition. Thirty-five percent (7/20) of the THC-15 and 45% (9.19) of the THC-50 failed to completely remove and consume the placental and extra-placental membranes. This occurred in only 16% (1.17) of the PF dams and in none of the NT.

Table 1 further shows that mean implantation sites were identical across all groups, whereas resorptions were slightly higher for the THC treated groups. Percentages of perinatal mortality (i.e., non-viable offspring observed at birth) was less than 1% for the NT and PF groups but 3-4% for the THC groups. Total perinatal mortality was calculated by combining both perinatal death and resorptions and dividing by total implantation sites. Thus, the total proportion of offspring mortality for both THC groups was approximately double that of either control groups.

Overall, birthweights of the males were higher than the females. However, the PF, THC-15, and THC-50 groups had significantly lower birthweights compared with the NT. In addition, there was a dose-related increase in the proportion of viable male offspring at birth.

Though there were no differences in body weight between treated and control offspring at 32 days of age, rate of postnatal growth varied as a function of pair-feeding and dose. Bodyweight of the PF control group caught up to the NT controls by Day 2 of life. The THC-15 group showed a slow rate of growth during the first five days followed by a more rapid rate, thus catching up to both control groups by day 11. By comparison, growth rates were significantly slower over the first 5 days in the THC-50 group than in the other groups. Further, the THC-50 group never showed a rapid catch-up in growth, although their weights gradually approached those of the other groups by Day 32.

THC-50 and PF control pups displayed significantly longer latencies to attach to a nipple. In addition, a significantly higher proportion of pups from both of these groups failed to attach during testing. The mean activity data for the THC-treated and control litters failed to reveal any differences among any of the groups throughout testing.

The only measure in the study to show a negative dose-response was postnatal mortality. Among the THC-15 group, 6% of the males and 4% of the females died after Day 1 of life. Of the THC-50 group, the proportions were 1% among both the males and females. There were no deaths among either control groups.

DISCUSSION

The present study, by virtue of the inclusion of a pair-fed control and the use of surrogate fostering, clearly distinguishes primary developmental toxic effects of THC from secondary effects mediated by maternal undernutrition. Offspring mortality did not differ between the NT and PF controls but was substantially higher among the THC groups, indicating that THC produces embryotoxicity independent of maternal undernutrition. In addition, there was a dose-related increase in the sex ratio of live male to female offspring. A similar increase in sex ratio favoring males has also been reported for rats exposed both prior to mating and during pregnancy to marijuana smoke (Fried and Charlebois, 1979) as well as in a human study of pregnant marijuana smokers (Tennes et al, 1985). In the present study, exposure to THC occurred after conception and implantation, suggesting that female conceptuses have a greater susceptibility to THC embryolethality.

THC was also found to have dose-related effects on rate of growth independent of maternal undernutrition; whereas the bodyweights of the PF controls caught up to those of the NT group within two days, the bodyweights of the THC-50 group were significantly less than those of the NT group throughout most of the study. By comparison, the THC-15 group showed inhibited growth only during the first five days followed by a growth spurt, so that they caught up to the controls by Day 11 of life. These dose-related effects on growth may be mediated either by the persistence of pharmacologically active amounts of the compound in neonatal tissue and/or inhibition of a hormonally regulated pathway affecting growth.

THC and maternal nutrition, however, were confounded with respect to nipple-attachment measures. THC-15 and NT litters did not differ in their latency to attach while the THC-50 litters took significantly longer and failed to attach more often. However, similar results were obtained for the PF control litters. These results suggest that the impaired nipple-attachment observed among the high-dose offspring was not a primary effect of THC. Rather, these effects may be secondary to the significant reduction in food and water intake produced among the THC-50 dams. Offspring activity level, however, was unaffected by either nutrition or THC.

Considering that the THC nutritional deficit produced in the dams was most severe during early organogenesis, when the CNS is particularly susceptible to damage (e.g., see Morgan and Winick, 1985), the effects on nipple attachment are not all surprising. Moreover, the failure to observe behavioral effects on either measure for THC alone, agrees with human observations of neonates. Although Fried (1982) found infants of mothers who smoked marijuana during pregnancy to be somewhat irritable for about a month after birth, longterm follow-up to school age

failed to reveal any persistent behavioral or cognitive deficits. In a similar study, Tennes et al (1985) did not observe neonatal irritability and, like Fried, found no persistent neurobehavioral impairment during the first year of life. Thus, both well-controlled animal studies and human clinical studies have yet to indicate that exposure to marijuana or THC during pregnancy produces significant neurobehavioral impairment in the offspring. However, the embryotoxic properties of THC, with possible selective effects on females, its postnatal growth inhibiting effects, as well as reported effects on male reproductive physiology and performance (e.g. Dalterio et al, 1984) leave little doubt of its potent developmental toxicity.

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TABLE 1

MATERNAL & OFFSPRING EFFECTS

	NT	PF	THC-15	THC-50
LITTERS	17	17	20	19
MEAN MATERNAL WT GAIN	185.5g	135.1g	129.2g	122.8g
MEAN IMPLICATION SITES	14	14	14	14
% RESORPTIONS	5.41%	3.38%	6.30%	7.71%
% PERINATAL MORTALITY	0.65%	0.39%	3.78%	4.58%
TOTAL OFFSPRING MORTALITY	6.06%	3.77%	10.07%	12.28%
NUMBER BORN LIVE				
MALE	104 (47%)	107 (48%)	137 (54%)	132 (57%)
FEMALE	119 (53%)	115 (52%)	117 (46%)	101 (43%)
MEAN LITTER SIZE	13	13	13	12
MEAN BIRTH-WEIGHT				
MALE	7.20g	6.80g	6.65g	6.58g
FEMALE	6.83g	6.36g	6.30g	6.20g

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Biological Evaluation of Compounds For Their Physical Dependence Potential and Abuse Liability. X. Drug Testing Programs of The Committee on Problems of Drug Dependence, Inc. (1986)

Arthur E. Jacobson

The Drug Testing Program of the Committee on Problems of Drug Dependence (CPDD) was established to evaluate compounds for their physical dependence potential and abuse liability. Drug testing, as a public service, represents one of the main purposes of the CPDD. This work has, in the past, led to our ability to forewarn the submitters of the compounds, and the public, about a drug's potential for abuse. The data which are obtained from this program also benefit the CPDD and scientific research in other ways. It serves as a reservoir of information which the CPDD uses to enable it to enact another of its purposes, to act in an advisory capacity, providing scientific information to academic, governmental and industrial groups, on request. The gathered data have, further, led to basic research into areas which would, otherwise, have remained unexplored. Several papers have been published on this work (e.g. Woods et al., 1983; Aceto et al., 1985; Lessor et al., 1986). The Drug Testing Program of the CPDD has historically been involved with opioid-like drugs and, also, is presently obtaining base-line data as a prerequisite to expansion to the testing of stimulants and depressants.

CONSTITUENCY OF THE DRUG TESTING PROGRAM

The Drug Testing Program on opioid-like compounds has served three distinct audiences: 1) university researchers who seek rodent antinociceptive and, perhaps, initial monkey studies (single dose suppression and precipitated abstinence) for the discernment of the relationship between the molecular structure of their synthesized molecules and biological activity, for publication purposes and for leads to further research in the area; 2) pharmaceutical firms that generally wish to determine the physical dependence potential and abuse liability of their compounds before further work, or for transmission of these data to the FDA to satisfy regulations before marketing; 3) governmental organizations, such as the DEA, or the WHO, who desire sufficient data for scheduling purposes, nationally and/or internationally.

This paper serves, mainly, as an introduction to the work, and summary of the results, of the scientists (see below) involved with the Drug Testing Program. The opioid-like compounds are classified in this paper in accord with classical opioid structural types, so that the changes which are made in these classes can be more easily observed and compared from year to year (Jacobson 1986, and previous years).

OPIOID AND STIMULANT/DEPRESSANT TESTING LABORATORIES

There are three groups concerned with the evaluation of compounds as opioids: the Medical College of Virginia (Drs. M.D. Aceto, L.S. Harris, E.L. May, E.R. Bowman, R.L. Balster, and P.M. Beardsley), the University of Michigan (Drs. J.H. Woods, F. Medzihradsky, C.B. Smith, G.D. Winger, and D.E. Gmerek), and NIDDK, NIH (Dr. A.E. Jacobson and M. Mattson). The complete work of the MCV and UM groups is published in this monograph (Aceto et al. 1987; Woods et al. 1987), and should be consulted for the detailed information on the evaluated compounds.

Five groups have been involved with the evaluation of the stimulant and depressant classes of compounds: the University of Chicago (Drs. C. Johanson and R. Schuster), the Medical College of Virginia (Drs. Patrick, Yutrzenka, and Harris), the Johns Hopkins University (Drs. R. Griffiths, N. Ator, R. Lamb, and J. Brady), NIDDK, NIH (Dr. A. Jacobson, M. Mattson), and NIDA (Dr. E. Cone). Dr. J. Woods (University of Michigan) has served to coordinate this program.

SUBMISSION PROCEDURES

The procedure which is followed in both programs is similar. Compounds are obtained by, or submitted to, Dr. A. E. Jacobson (NIH, NIDDK) who runs initial mouse antinociceptive tests on opioid-like compounds, or biochemical assays on benzodiazepines. The compounds are logged into a computerized system and distributed to the various groups for evaluation. With the opioids, the Medical College of Virginia group evaluates the compounds in various mouse assays for their antinociceptive and narcotic antagonist activity, as well as in single dose suppression assays in monkeys. Precipitated withdrawal studies, primary physical dependence and, occasionally, self-administration data are obtained in the rhesus monkey. Further, rat infusion methodology is used is paradigms similar to single-dose suppression, and primary physical dependence studies in the monkey. Biochemical evaluation, electrically stimulated mouse vas deferens preparations, most of the self-administration data and all of the drug discrimination data are obtained from the University of Michigan, as well as some single-dose suppression and precipitated withdrawal studies in the monkey and, occasionally, primary physical dependence in the monkey. Reports are submitted to Dr. A. E. Jacobson at NIH. Dr. Jacobson receives written release of the obtained data from the submitter of the compound, draws the chemical structures of the released compounds, and sends these to the appropriate laboratory for inclusion in the Annual Report from the Medical College of

Virginia and the University of Michigan. The Annual Reports, as well as a compilation and summary of the data from these reports, are published as part of the Proceedings of the CPDD in the NIDA Monograph, from MCV, UM, and NIH.

The stimulant/depressant classes of drugs are handled similarly. Distribution of a drug is to the Medical College of Virginia for estimate of potency in rodent tests, to the Addiction Research Center, NIDA (Dr. E. Cone) for solubility and stability studies, and then to the University of Chicago for study in pigeons and monkeys (e.g. self-administration and drug discrimination), and to the Johns Hopkins University for study in baboons. Reports are submitted back to Dr. Jacobson and the released data are incorporated in the Proceedings of the CPDD in the NIDA Monograph. During this next year, the compounds which will be evaluated will be chosen from compounds requested by the WHO, or from drugs to be added to our reference standards list, e.g. deprenyl (or its levo enantiomer, eldepryl, an antiparkinsonian agent), tranylcypromine (an anti-depressant), ethchlorvinyl (a sedative-hypnotic), meprobamate (a sedative), methaqualone, Ro 15-1788, or substances submitted by industry.

The requirements for submission of compounds for evaluation under the auspices of the CPDD have been previously discussed (Jacobson, 1981).

CLASSES OF OPIOID-LIKE COMPOUNDS, AND SPECIFIC COMPOUNDS OF INTEREST AMONG THE EXAMINED OPIOIDS AND STIMULANTS/DEPRESSANTS

The evaluated compounds are listed in 12 tables. Ten 4,5-epoxymorphinans are listed in tables 1 & 2. Six morphinans and a phenylmorphan are shown in table 3. There are 23 of the 6,7-benzomorphans in tables 4 - 6. Table 7 contains eight methadone-like compounds, and table 8 contains eight pethidine-like compounds and two compounds based on the fentanyl structure. The remainder of the tables (9 - 12) concerns 24 compounds which could not be classified by classical opioid terminology. These are the miscellaneous compounds.

Opioid-like Drugs Submitted By A Governmental Unit ("Street" or "Designer" Drugs)-

Four compounds were submitted by the DEA, through NIDA. Two of them are extremely potent analogs of fentanyl, the cis and trans 3-methyl fentanyl (NIH 10456 & 10457, table 8). The more potent cis compound was found to be 1000 to 2000 times more potent than morphine in mouse antinociceptive assays (hot plate, PPQ, tail flick), and was 1000 times more potent than morphine in the single dose suppression assay in monkeys. Its affinity to opioid receptors in rat brain membranes and in the mouse vas deferens was in reasonable accord with the in vivo data. The somewhat less potent trans compound was 600 times More potent than morphine in suppressing abstinence in the single dose suppression study. Two far less potent pethidine-like compounds, NIH 10460 and 10461

(table 8) also completely suppress abstinence in single dose suppression studies and were as potent as, or more potent than morphine in the various antinociceptive studies in rodents. Priority was given to NIDA and DEA for this work and the evaluation was accomplished expeditiously.

Two Compounds From Pharmaceutical Industry -

1) Meptazinol

More extensive work was done with a compound which had been originally evaluated 15 years ago. Meptazinol (NIH 8683, table 9), which has a structure reminiscent of pethidine, with a T-membered nitrogen ring, was evaluated by morphine substitution and primary physical dependence in 1971. In single dose substitution studies it did not substitute for morphine; it precipitated withdrawal in withdrawn monkeys, thus apparently acting as a weak narcotic antagonist. Cur SDS and precipitated withdrawal contemporary data are in complete accord with the formerly obtained data. The new primary physical dependence study indicated that the compound was, essentially, morphine-like from the induced weight loss and withdrawal syndrome produced on abrupt withdrawal. The original primary physical dependence study showed mild physiological dependence following abrupt withdrawal. Further data have been obtained from antinociceptive studies in rodents (meptazinol is about 1/2 to 1/5 as potent as morphine), from in vitro experiments in binding to rat brain membranes (ca. 1/150 the affinity of morphine), mouse vas deferens (it did not suppress the twitch at any concentration used, nor reverse the action of morphine on the twitch), self-injection (rates maintained by meptazinol were slightly above those maintained by saline at all doses, but the results were variable), etorphine discrimination (monkeys showed etorphine-appropriate responding which was suppressed by preadministration of WIN 4441 (quadazocine)), and ethylketazocine-discrimination (variable results in two independent studies). Meptazinol clearly shows some opioid-like activity and some narcotic antagonist activity in these several assays. It would appear to be a mixed agonist-antagonist with mu, and perhaps kappa, opioid receptor mediated effects when evaluated in vivo in rhesus monkeys.

2) Nalmefene

At one of our previous Annual Meetings, Key Pharmaceutical Company asked for the opinion of the CPDD on the data obtained by the Drug Testing Program of the CPDD on nalmefene (17-cyclopropylmethyl-4,5-epoxy-3,14-dihydroxy-7-methylenemorphinan, NIH 10365). The evaluative effort was given to a sub-committee, but has not, as yet, been reported to the CPDD. Cur data have been obtained from the opioid working groups and compiled by Dr. Harris (MCV). The data obtained with the compound indicates that it is a potent, pure narcotic antagonist. It does not substitute for morphine in the SDS test in monkeys. It has ten times the potency of naloxone in precipitating withdrawal in non-withdrawn monkeys, and it has a longer duration of action than naloxone. It was not self-

administered and it was without morphine like effects in a primary physical dependence study, in rhesus monkeys. In the latter assay the drug showed toxicity at the high doses used. The compound has no dependence potential that can be ascertained in our assays. Key Pharmaceutical is planning to submit an NDA on this drug for the management of narcotic overdosage.

Opioid-like Compounds From The University Constituency - Effect of Chiral Center on Biological Activity - "Spiro-epoxides"

Two C-6 Spiro-epoxides based on the 4,5-epoxymorphinans (NIH 10366 and 10367, table 2) were examined and were found to be of interest from a stereochemical point of view. Our tests clearly indicate the remarkable ability of the opioid receptor to differentiate between enantiomers at a somewhat remote chiral center. NIH 10366 is a very potent antinociceptive agent, about 100 times more potent than morphine. Its isomeric relative, NIH 10367 is inactive as an antinociceptive, although it appears to have retained reasonable affinity for the opioid receptors. A somewhat larger ring attached as a Spiro group to the C-6 position, as seen in NIH 10322 and 10324 (table 2), was much less effective for the retention of narcotic antagonist or antinociceptive properties. The N-cyclopropylmethyl derivative, NIH 10322, was found to be considerably less potent than its molecular parent, naltrexone, as a narcotic antagonist and showed mixed agonist-antagonist properties. The Spiro attachment at C-6, as such, appears to have little apparent effect on in vivo activity, in contrast with the effect of the chiral center a small distance away, and the effect of the size and nature of the ring at C-6. These rather complex molecules can be contrasted with the molecularly simple 6-oxomorphinan, NIH 10331 (table 3). That morphinan does not have even a phenolic hydroxyl group and, yet, is three to six times more potent than morphine in mouse antinociceptive assays. The lack of the phenolic hydroxyl in NIH 10331 may have militated against the acquisition of narcotic antagonist activity. Generally, N-cyclopropylmethyl substituted morphinans are potent antagonists; the NIH 10331, however, does not have antagonist properties.

Stimulant Submitted By Governmental - MDMA

Lastly, a stimulant of great interest to the DEA and NIDA was evaluated by various groups associated with the stimulant/depressant Drug Testing Program. The drug, (±)-3,4-methylenedioxymethamphetamine (MDMA) was purported to be a highly useful psychotherapeutic agent by psychotherapists on the west coast. It became a “street” drug and was controlled by the DEA under its new emergency procedures. The compound was examined by self-administration (i.v.) in cocaine-trained rhesus monkeys, and rodent infusion, by Drs. Beardsley, Balster and Harris (MCV) (Beardsley et al., 1986). Three separate studies were carried out by Dr. Johanson (University of Chicago). In those studies, MDMA substituted for d-amphetamine in monkeys (i.v., and i.g.) and in pigeons (i.m.) trained to discriminate d-amphetamine from saline (Evans and Johanson, 1986; Kamien et al., 1986). In a self-injection study (i.v.) in baboons by Drs. Lamb and Griffiths (1986)

(Johns Hopkins University), all three baboons substituted MDMA for cocaine; the number of injections and response rates maintained by MDMA were less than those maintained under baseline conditions with cocaine. The results obtained from the involved groups demonstrated that MDMA could serve as a reinforcer in various species and, taken together with other preclinical behavioral studies, suggested a potential for recreational use of MDMA by humans (Beardsley et al., 1986).

DRUG TESTING PROGRAM STATISTICS

An arbitrary time period of 5/1 through 4/30 has been used to relate and compare the intake and output of the Drug Testing Program and statistical data have been compiled so that comparisons can be made over an 8 year time span. The mean, and standard deviation of the mean, of measures of interest have been obtained from the previous seven years, and observations from this year, the eighth year in which data have been compiled in this fashion, have been compared with those data.

The number of compounds which are sent from NIDDK, NIH, to MCV and UM for evaluation as opioid-like drugs, under the auspices of the CPDD, shows considerable annual fluctuation. Thus, the mean of that number is 93; the standard deviation is 30. During the current year, I sent MCV and UM 63 compounds, the low end of the deviation of the mean, a statistically insignificant deviation but, perhaps, somewhat meaningful. Although the number of compounds was essentially the same as those sent in two of the previous seven years (1979 and 1983), it must be noted that UM and MCV received a considerably higher number of compounds during the other five years.

The reports generated from the data on compounds which have been released for publication can be observed in this monograph (Aceto et al., 1987; Woods et al., 1987). There are about 90 of those reports, consistent with the calculated mean of 95. There is, generally, only a peripheral relationship between these first two statistical items. The data generated from the compounds sent to MCV and UM this year will appear over the next three, or more, years, due to our allowance of that time period before automatic publication, and the time necessary to complete work on compounds which appear scientifically, commercially and/or governmentally significant. Thus, it can be noted in the reports that we are still gathering information on, for example, relatively ancient kappa receptor-selective compounds such as ketocyclazocine and ethylketocyclazocine. Kappa-selective compounds are, of course, of considerable scientific interest and, perhaps, commercial importance.

The sources of the compounds included in this monograph are varied, as usual. About 13% of these compounds came from industrial groups. This percentage has remained fairly constant for the past two or three years, but is appreciably different from earlier years when about 50% of our compounds came from industry. Universities provided ca. 59% of our samples this year, and NIH another 18%.

Thus, over three-quarters of our reports were generated on compounds from non-commercial sources. We have, also, been receiving a significantly increased number of compounds for evaluation from the DEA, through NIDA. Presumably, these governmental agencies are interested in obtaining data on compounds illicitly synthesized, the "designer drugs", with the hope of adding them to the DEA list of proscribed substances.

ABBREVIATIONS USED IN TAELES 1 - 12.

ED50 OR AD5Q: Antinociceotive assay (ED50, sc injection except where noted, mice) [Confidence limits are listed in the MCV and UM reports (Aceto et al. 1987; Woods et al. 1987)]: HP = hot plate; N = Nilsen; PPQ = phenylquinone; TF = tail flick; TEA = tail flick antagonism vs. morphine. These assays are done at MCV, except for the HP and N which are done at NIDDK, NIH.

I = inactive, without a reasonable dose-response relationship, or insufficiently active for statistical analysis.

EC50 Determinations:
These assays are done at UM. RBH = binding affinity, in the presence of 150mM NaCl, to rat cerebrum membrane preparations, in nM (parenthesized number is the +sodium/-sodium [+Na/-Na] ratio). EC50 was determined by displacement of 0.5nM 3H-etorphine. The EC50 of morphine, for comparison = 23.6 (1.69). NE = no effect.
NOTE: The present EC50 data cannot be directly compared with those from my previous reports (Jacobson 1983, and previous years) in which -Na values were quoted. However, the previously stated numbers can be recalculated for comparison with those which will be utilized this year and in the future, through the use of the +Na/-Na ratio.

GPI = electrically stimulated guinea pig ileum EC50, rounded to one significant figure, in nM except where noted. (E = exponential, e.g. 7E-9 M = 7 x 10-g M, where -9 is an exponent [7E-2 M = 7 x 10-2 M = 0.07 M] (parenthesized numbers are maximum percent inhibition at EC50); [bracketed letters: A = antagonized by 10-7M naltrexone; NA = not antagonized by naltrexone; SA = slight antagonism; NE = no effect on inhibition of twitch]. NOTE: The GPI assay has been phased out of the normal routine. These data will not be obtained as part of the general assays. The VD assay will continue.

VD = electrically stimulated mouse vas deferens EC50 values, rounded to one significant figure. Agonist activity stated as E = x10 M, thus: 7E-2 = 0.07 M (parenthesized numbers are maximum percent inhibition at EC50); [bracketed letters: A = antagonized by 10-7 M naltrexone; NA = not antagonized by naltrexone; NE = no effect on inhibition of twitch; SA = slight antagonism by naltrexone]. Compounds which suppress the twitch and are not antagonized by naltrexone (noted herein) or UM 979 [NIH 8859, (-)-5,9-alpha dimethyl-2-(3-furylmethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorphan] 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). (The effect of UM 979 is not noted in

this report, but see the UM report (Woods et al. 1987) for these data). Compounds which bind with reasonable affinity in the rat brain homogenate assay, suppress the twitch in the VD, but are not blocked by narcotic antagonists may have antagonist properties, also. This is experimentally determinable by observing their antagonism to morphine's suppression of the twitch in the VD preparation (for these data see Woods et al. 1987).

Data From Monkey Colonies:
These data are from either MCV or UM. SDS = single dose suppression: NS = no suppression; CS = complete suppression; PS = partial suppression. (Parenthesized numbers = dose range studied, in mg/kg; if CS, then dose at which CS was observed is noted in the parentheses). Potency comparison with morphine [M] may be stated, in brackets.
NW = studies in non-withdrawn monkeys. PW = precipitated withdrawal at dose levels, in mg/kg, indicated in parentheses &/or comparison with naloxone [N], in brackets; NP = no precipitation; SP = slight precipitation.

Other Studies (OTHER):
RI = rat infusion (from MCV): NS = no suppression; CS = complete suppression; PS = partial suppression.
PPD = primary physical dependence.
SA = self-administration (from UM): NE = no effect; High =codeine-like; IN - intermediate between saline and codeine; SE = slight effect.
Normal monkeys: M-like = morphine-like effect.
DD = drug discrimination (from UM).

Previous Reports (PR):
A column which relates the titled year (e.g. Problems of Drug Dependence 1986 - the titled year is 1986) in which previous work on a compound has been reported. Note that the year of actual publication generally occurs one year after the titled year. These data are published in the annual compilations of "Problems of Drug Dependence". The data which have been published in previous reports are shown by a "PR" in the appropriate column (e.g. a PR in the SDS column would indicate that the SDS work was done in the year, or one of the years, cited in the PR column).

NOTE: The numbers used in the tables may be rounded. For precise values, and details of the procedures, see the MCV and UM reports in these Proceedings (Aceto et al. 1987; Woods et al. 1987).

Abbreviations for structural formulae: CPM=cyclopropylmethyl; C B M=cyclobutylmethyl; Me =methyl; Et=ethyl; Pr=propyl.

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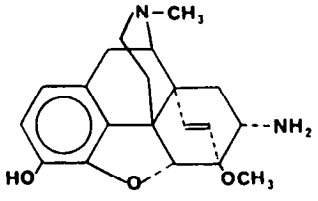
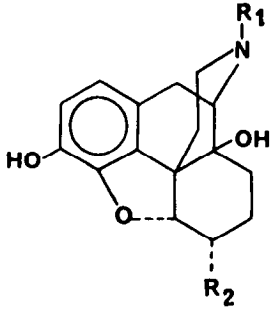
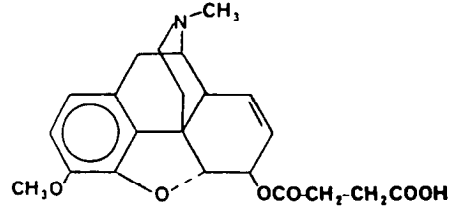
TABLE 1 - 4,5-EPOXYMORPHINANS[a]



10093



10274: NALBUPHINE, R_1=CBM, R_2=..OH
10357: CYCLOFOXY, R_1=CPM, R_2=F
10365: NALMEFENE, R_1=CPM, R_2= =CH

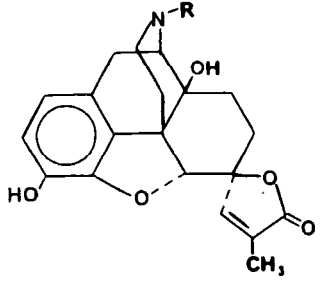


10384

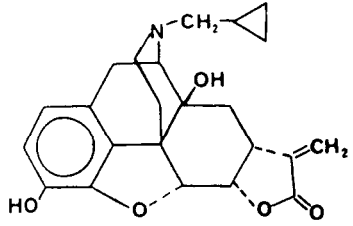
COMPOUND #'S			ED50 OR AD50				IN VITRO		MONKEY STUDIES		PR
NIH#	MCV#	UM#	HP	PPQ	TF	TFA	RBH	VD	SDS	NW	
10093	4438	-	I	-	I[b]	-	-	-	-	-	-
10274 8359	4385	686	PR	0.26	PR	PR	-	-	PS(10,20)[c]	PR	1968, 1984
10357	4433	-	I	I	I	0.003	-	-	NS(0.001,0.01)	PW(1xN)	-
10365[d]	4426	10365	I	PR	PR	PR	PR	PR	PR	PR	1985
10384	4448	10384	I	PR	PR	PR	19	5E-6(98)[A]	PR	-	1985

a) See text for explanation of column headings.
b) Intravenous route of administration.
c) SDS (1984); SDS & NW (1968 - as NIH 8359).
d) Other assays - PPD (ND), SA (1985).

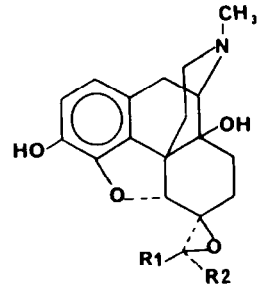
TABLE 2 - 4,5-EPOXYMORPHINANS (CONTINUED)[a]



10322: R=CPM
10324: R=Me



10363

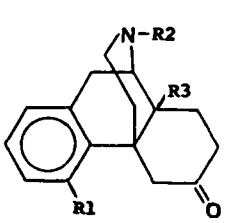


10366: R_1=H, R_2=CO_2Et
10367: R_1=CO_2Et, R_2=H

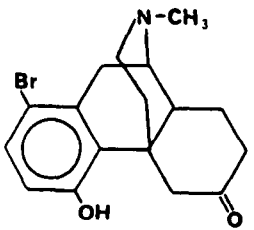
COMPOUND #'S			ED50 OR AD50				IN VITRO		MONKEY STUDIES	
NIH#	MCV#	UM#	HP	PPQ	TF	TFA	RBH	VD	SDS	NW
10322	4404	-	I	11.7	I	1.6	-	-	-	-
10324	4408	-	3.5	2.4	18.6	I	-	-	-	-
10363	4425	10363	I	-	I	0.01	IS[b]	IS[b]	NS(0.005,0.2)	PW(1xN)
10366	4423	10366	I	I	I	I	40.5	8E-7(94)[A]	NS(0.03-1.0)	-
10367	4424	10367	0.08	0.02	0.1	I	15.4	1E-7(96)[A]	CS(1.0)	-

a) See text for explanation of column headings.
b) Insufficiently soluble for assays.

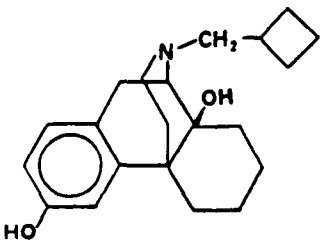
TABLE 3 - MORPHINANS AND PHENYLHORPRANS[a]



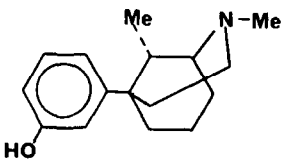
9931: R1=OMe, R2=CPM, R3=H
9975: R1=OMe, R2=ALLYL, R3=OH
10331: R1=R3=H, R2=CPM
10371: R1=R3=OMe, R2=H



9935



10275: (-)-BUTORPHANOL
8791



9955

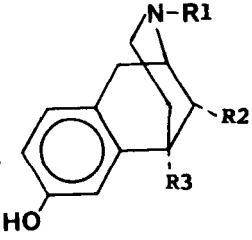
COMPOUND #'S			ED50 OR AD50					IN VITRO		MONKEY STUDIES		PR
NIH#	MCV#	UM#	HP	N	PPO	TF	TFA	RBH	VD	SDS	NW	
9931	4280	1313	21.7	PR	PR	PR	PR	23.9	4E-7(39)[A][b]	-	PR	1982
9935	-	9935	14.8	-	-	-	-	913(1.99)	NE	-	-	-
9955	4275	9955	9.3	I	PR	PR	PR	292(0.82)	NE	PR	PR	1982, 1983
9975	4296	1347	I	PR	PR	PR	PR	194(0.54)	4E-9(40)[NA][c]	PR	-	1982
10275 8791	4386	941	0.78	0.09	15.0	I	I	-	-	NS(0.05,0.5)	PW(1.5-9)[d]	1973, 1980, 1984
10331	4465	-	0.4	-	0.2	0.9	I	-	-	-	-	-
10371	4466	-	I	-	0.3	3.9	I	-	-	-	-	-

a) See text for explanation of column headings.
b) Antagonism occurs with equimolar concentration of naltrexone.
c) NIH 9975 antagonizes the effects of morphine in the VD assay.
d) Incomplete withdrawal (atypical antagonist); SA (1980).

TABLE 4 - 6,7-BENZOMORPHANS[a]

7569: (-)-METAZOCINE, R1=R2=R3=Me
7571: (+)-METAZOCINE, R1=R2=R3=Me

7981: (±)-CYCLAZOCINE, R1=CPM, R2=R3=Me
10449: (+)-CYCLAZOCINE, R1=CPM, R2=R3=Me
10450: (-)-CYCLAZOCINE, R1=CPM, R2=R3=Me



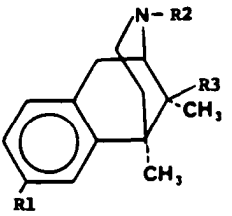
8374: (+), R1=R2=Me, R3=n-Pr
10249: R1=CH_2-  , R2=R3=Me

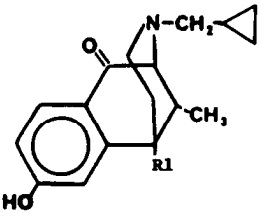
10157: R1=PHENETHYL, R2=$(CH_2)_2CH(CH_3)_2$, R3=Me
10158: R1=n-Pr, R2=$(CH_2)_2CH(CH_3)_2$, R3=Me

COMPOUND #'S			ED50 OR AD50					IN VITRO		PR
NIH#	MCV#	UM#	HP	N	PPQ	TF	TFA	RBH	VD	
7569	4501[b]	743	0.62	0.5	0.3	0.81	I	-	-	1960, 1969, 1984
7571	4431	744[c]	I	-	-	I	I	I	I	1960, 1969, 1984
7981	4510	407	1.5	0.1	0.05	I	0.03	-	-	1962 (SDS)
8374	4480	696	12.3	-	3.1	I	21.5	-	-	1968 (SDS), 1985
10157	4349	10157	I	-	PR	PR	PR	1230	NE	1984
10158	4346	10158	I	-	PR	PR	PR	145	NE	1984 (SDS, NW)
10249	4388	10249	I	-	18.1	I	I	-	-	1984 (SDS)
10449	4511	-	-	-	I	-	-	-	-	-
10450	4512	-	-	-	0.014	-	-	-	-	-

a) See text for explanation of column headings.
b) Monkey SDS: NS(1.0-4.0), NW (1969); RI: SDS-nearly CS, PPD-definite PD.
c) SA (1984); SDS (1960, 1969).

TABLE 5 - 6,7-BENZOMORPHANS (CONTINUED)[a]



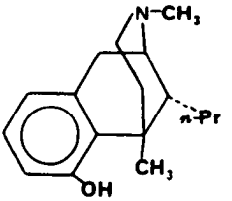


9625: R1=OH, R2=Me, R3=$(CH_2)_2CO(CH_2)_2CH(CH_3)_2$

10165: (8848): (±)-EKC, R1=Et
10452: (-)-EKC, R1=Et

10253: R1=R3=H, R2=C=NH NH_2-C_6H_5

10346: (8847): (±)-KC, R1=Me
10451: (-)-KC, R1=Me

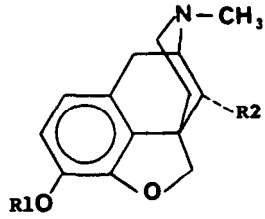


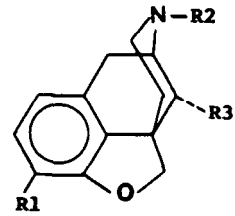
10416

COMPOUND #'S			ED50 OR AD50				PR
NIH#	MCV#	UM#	HP	N	PPQ	TF	
9625	4176	1401[b]	1.1	-	PR	PR	1979 THRU 1983
10165 8848	4348	975	0.09	0.05	0.4	I	1983, 1985[c]
10253	4389	-	I	15.6	I	I	-
10346 8847	4414	974	0.65	0.1	I	PR	1985 (SDS), 1974 (SDS)
10416	4477	-	I	3.9	I	I	-
10451	4513	-	-	0.06	-	-	-
10452	4515	-	-	0.005		-	-

a) See text for explanation of column headings.
b) In Vitro - RBH: 1.96, VD: 23-8(93) [Al; PPD (1981,1982); SDS (1979,198O).
c) RBH, VD, SDS (1983); SDS, NW (1985).

TABLE 6 - 6,7-BENZOMORPHANS (CONTINUED)[a]



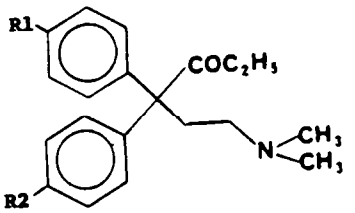


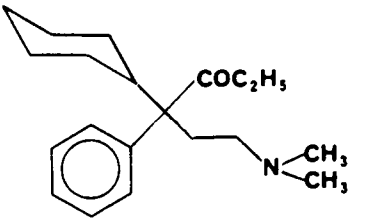
10348: R1=Me, R2=$(CH_2)_2CO_2Et$
10349: R1=Me, R2=$(CH_2)_3OH$
10368: R1=Me, R2=n-Pr
10369: R1=H, R2=n-Pr
10370: R1-COMe, R2=n-Pr
10379: R1=OH, R2=ALLYL, R3=n-Pr
10380: R1=OH, R2=PHENETHYL, R3=n-Pr
10413: R1=H, R2=Me, R3=n-Pr

COMPOUND #'S			ED50 OR AD50				IN VITRO	
NIH#	MCV#	UM#	HP	PPQ	TF	TFA	RBH	VD
10348	4418	-	I	I	I	I	-	-
10349	4419	-	I	I	I	I	-	-
10368	4434	10368	I	0.8	8.1	I	2.8 uM	NE
10369	4435	10369	1.8	0.1	0.4	I	-	-
10370	4436	10370	I	0.2	0.7	I	133	9E-8(81)[A]
10379	4455	-	I	I	I	0.4	-	-
10380	4456	-	1.7	0.2	1.4	I	-	-
10413	4476	-	I	2.9	20.7	I	-	-

a) See text for explanation of column headings.

TABLE 7 - METHADONE-LIKE COMPOUNDS[a]





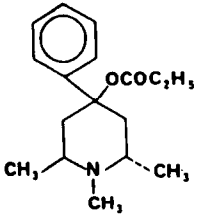
10393

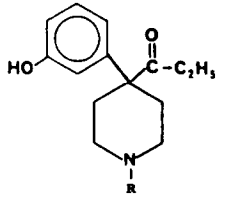
10385: R1=R2=H
10386: R1=H, R2=F
10387: R1=H, R2=Cl
10388: R1=H, R2=Br
10389: R1=H, R2=OMe
10391: R1=H, R2=Me
10396: R1=R2=F

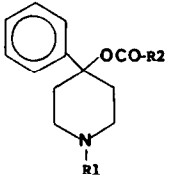
COMPOUND #'S			ED50 OR AD50			
NIH#	MCV#	UM#	HP	PPQ	TF	TFA
10385	4457	-	2.4	1.0	5.1	I
10386	4458	-	I	9.2	30.0	I
10387	4459	-	I	I	I	I
10388	4460	-	I	I	I	I
10389	4467	I	I	I	I	I
10391	4464	-	-	1.4	3.4	I
10393	4462	-	I	13.7	I	7.7
10396	4463	-	I	I	I	I

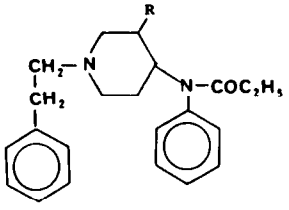
a) See text for explanation of column headings.

TABLE 8 - PETHIDINES **AND** FENTANYLS[a]









10432 10440: R=$(CH_2)_2CO_2Et$ 10460: R1=$(CH_2)_2C_6H_5$,R2=Me 10456: R=cis Me

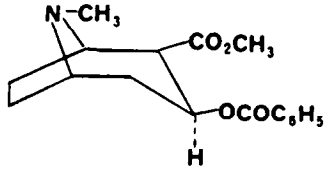
10433: (+) 10453: R=$(CH_2)_3CO_2Et$ 10461: R1=Me, R2=Et 10457: R=trans Me

10434: (-) 10454: R=CH_2CO_2Et

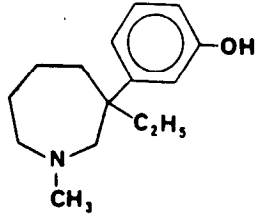
COMPOUND #'S			ED50 OR AD50				IN VITRO		MONKEYS
NIH#	MCV#	UM#	HP	PPO	TF	TFA	RBH	VD	SDS
10432	4493	-	1.5	0.1	2.7	I	-	-	-
10433	4494	-	1.1	0.2	0.7	I	-	-	-
10434	4495	-	I	3.0	11.0	I	-	-	-
10440	4503	-	I	I	I	I	235	NE	NS(1,10)
10453	4519	10453	I	I	I	I	440	NE[b]	-
10454	4520	10454	I	I	I	I	1.7uM	NE[b]	-
10456	4522	-	0.0005	0.0002	0.003	I	5.8	6E-10[A]	CS[1000xM]
10457	4523	-	0.009	0.002	0.01	I	24	7E-9[A]	CS[600xM]
10460	4527	10460	0.74	0.04	0.83	I	160	3E-9(53)[A]	CS(0.25,1)
10461	4528	10461	0.9	0.48	0.7	I	1100	NE	CS(10xM)

a) text for explanation of column headings.
b) Antagonized sulfentanil's agonist activity.

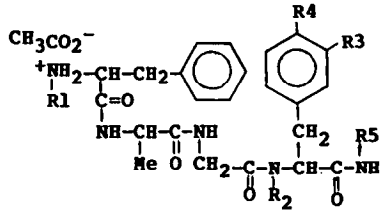
TABLE 9 - MISCELLANEOUS[a]



8211: COCAINE



8683: MEPTAZINOL



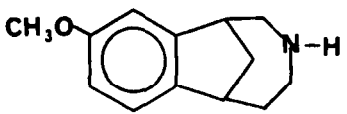
9947: R1=R2=R3=H, R4=F, R5= 

9948: R1=R4=R5=H, R2=Et, R3=Br
9949: R1=R4=R5=H, R2=Et, R3=F
9950: R1=Me, R2=CPM, R3=R5=H, R4=F

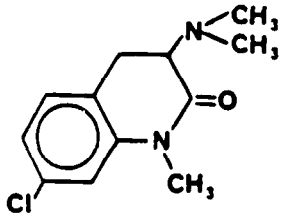
COMPOUND #'S			ED50 OR AD50				IN VITRO			PR
NIH#	MCV#	UM#	HP	PPQ	TF	TFA	RBH	GPI	VD	
8211	4256[b]	-	-	-	-	-	-	-	-	-
8683[c]	4403	888	5.3	1.6	12.8	I	-	-	-	1973, 1984
9947	4271	9947	2.1	PR	PR	PR	0.53(1.28)	5E-10(65)[A]	3E-11(100)[NA][d]	1982 (SDS)
9948	4272	9948	3.3	PR	PR	PR	1.23(1.0)	1E-8(37)[A]	3E-10(100)[A]	1982 (SDS)
9949	4273	9949	0.7	PR	PR	PR	1.5	9E-12(45)[A]	7E-11(100)[A]	1982 (SDS)
9950	4274	9950	2.1	PR	PR	PR	0.33	1E-10(37)[A]	1E-9(100)[A]	1982 (SDS)

a) text for explanation of column headings.
b) Other tests - RI: PPD (no overt effect on weight; less spontaneous activity before abrupt withdrawal).
c) Monkeys - SDS: NW - PW(0.005xN). Other tests - RI: SDS - NS, PPD (morphine-like); SA - high; DD - EKC-like etorphine-like; SDS PPD (1973); SDS, NW (1984).
d) Antagonized by ICI 174864, a specific delta receptor antagonist.

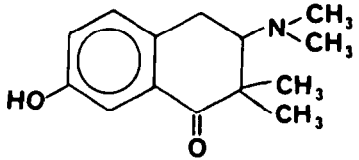
TABLE 10 - MISCELLANEOUS (CONTINUED)[a]



10036



10333
10334: (-)
10335: (+)

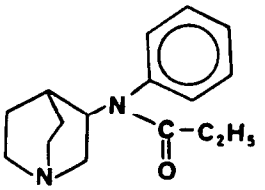


10372

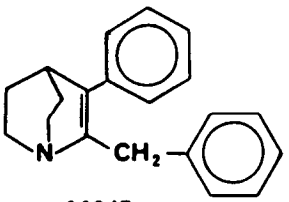
COMPOUND #'S			ED50 OR AD50				IN VITRO		MONKEY STUDIES	
NIH#	MCV#	UM#	HP	PPQ	TF	TFA	RBH	VD	SDS	NW
10036[b]	-	1399	1.8	-	-	-	PR	PR	NS(1.0-3.0)	-
10333	4409	I	I	10.1	I	I	>100 uM	1E-5(39)[NA]	NS(1-16)	NP(4-32)[c]
10334	4410	I	I	25.9	I	I	>100 uM	6E-5(35)[NA]	NS(2-32)	-
10335	4411	10335	I	3.0	I	I	>6 uM	1E-4(65)[NA]	NS(1-32)	-
10372	4437	-	I	9.9	6.2	I	-	-	PS(1.5-12)	-

a) See text for explanation of column headings.
b) Previous report - 1985
c) Some withdrawal signs noted.

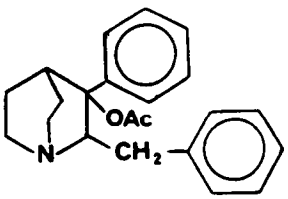
TABLE 11 - MISCELLANEOUS (CONTINUED)[a]



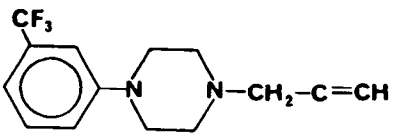
10258



10347



10362

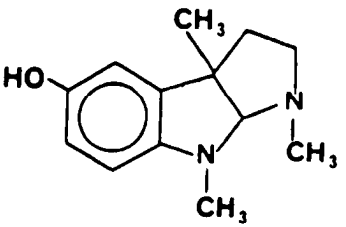


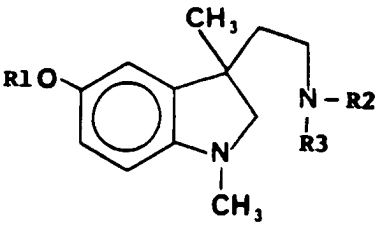
10319

COMPOUND #'S			ED50 OR AD50				MONKEYS
NIH#	MCV#	UM#	HP	PPQ	TF	TFA	SDS
10258	4390	-	I	4.6	I	I	-
10319 10455	4400	10319	I	5.8	I	I	CS(20);NS(1-32)[b,c,d]
10347	4417	-	I	I	I	I	-
10362	4422	-	I	I	I	I	-

a) See text for explanation of column headings.
b) Used cumulative doses for assay at UM; MCV - CS(8) (repeat), UM - NS(1-32)(repeat).
c) RBH - NE; MVD - NE; SA - NE; DD - NE.
d) Previous report - 1985(RBH, VD).

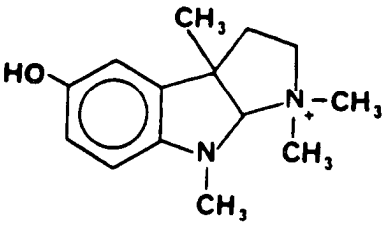
TABLE 12 - MISCELLANEOUS (CONTINUED)[a]

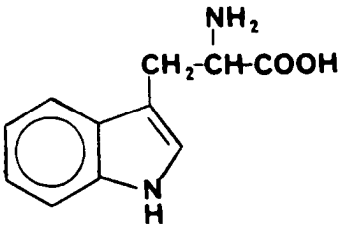




10398: (-)-ESEROLINE, R=OH
10421: (-)-ESERINE, R=OCONHMe
10439: (+)-ESEROLINE, R=OH

10408: R1=R2=Me, R3=H
10409: R1=R2=R3=Me
10423: R1=H, R2=R3=Me
10425: R1-R2=H, R3=Me





10422

10429: L-TRYPTOPHAN

COMPOUND #'S			ED50 OR AD50			
NIH#	MCV#	UM#	HP	PPQ	TF	TFA
10398	4481	-	1.6	0.7	2.3	I
10408	4482	-	I	2.9	I	I
10409	4483	-	I	I	I	I
10421	4484	-	I	0.05	0.06	I
10422	4485	-	I	I	I	I
10423	4486	-	I	I	I	I
10439	4504	-	I	2.3	I	I
10425	4488	-	I	1.7	I	I
10429	4475	-	-	-	-	-

a) See text for explanation of column headings.

Dependence Studies of New Compounds in the Rhesus Monkey, Rat and Mouse (1986)

M. D. Aceto, E. R. Bowman, L. S. Harris, and E. L. May

All the drugs, except, cocaine, (+)- and (-)-metazocine, (+)-cyclazocine, (+)-cyclazocine, (-)-cyclazocine, (+)-ketocyclazocine, (-)-ketocyclazocine, and (-)-ethylketocyclazocine were supplied by Dr. Arthur Jacobson, Medicinal Chemistry Section, NIADDK, NIH under the auspices of the Committee on Problems of Drug Dependence, Inc. The chemical structures of the test compounds were unknown to us when they were originally submitted.

For the most part, the procedures described by Seevers and his colleagues (1936, 1963) and Deneau (1956) regarding the facilities and training of the monkeys were used and a brief description follows. The monkeys were injected with 3 mg/kg s.c. of morphine sulfate every 6 hr for at least 90 days before being used. This dose schedule was reported by Seevers and Deneau (1963) to produce maximal physical dependence.

Modified procedures for the precipitated withdrawal (PPt-W) and single-dose suppression (SDS) tests were reported by Aceto and co-workers (1977 and 1978). The PPt-W test was initiated by the injection of a test drug 2½ hr after an injection of morphine and the animals were observed for signs of withdrawal. The SDS test was started approximately 15 hr after the last dose of morphine at which time the animals were showing withdrawal signs. The onset and duration of action of the test drug were noted. In both tests, a vehicle control and an appropriate positive control (naloxone·HCl 0.05 mg/kg or morphine sulfate, 3.0 mg/kg) along with 3 different treatments (doses) of a test compound were randomly allocated to the 5 monkeys of a group. Occasionally 4 monkeys comprised a group and 2 doses of test compound were studied. Usually, 3 or 4 groups per compound were used. All drugs were given subcutaneously (1 ml/kg) or intravenously (1-2 ml) and the vehicle used is indicated for each compound. The observer was "blind" with regard to the treatment given. A minimal 2-week washout and recuperation period between tests was allowed. In the primary physical dependence (PPD) tests, the animals of a group received the drug every 4-6 hr for 30-50 days. They were placed in abrupt withdrawal and challenged with naloxone periodically, then observed for signs of physical dependence. All potency estimates are rough approximations only.

The rat-infusion method was reported by Teiger (1974) and certain modifications are indicated as follows. Semi-restrained, male, Sprague-Dawley rats were medicated by continuous infusion through indwelling intraperitoneal cannulas for 6 days with a drug. Rats were anesthetized after which each was fitted with a specially prepared cannula which was passed subcutaneoulsy 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 hr.

In the substitution for morphine (SM) test, the animals first received morphine (50 mg/kg/24 hr on the first day, 100 mg/kg/24 hr on the second day, and 200 mg/kg/24 hr 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 ½ hr at 24,48,72 and/or 96 hr after stopping the infusion of morphine.

In the primary physical dependence (PPD) study, the rats received test compound for 6 days and then were placed in abrupt withdrawal and observed as above. Occasionally a drug was given along with morphine.

Table I

Comparative Data-ED50, mg/kg s.c. (95% C.L.) of Selected Standards in 3 Mouse Agonist-Antagonist Tests

Drug	Tail-Flick Test	Tail-Flick Antagonism Test	Phenylquinone Test
Pentazocine	15% at 10.0	18 (12.4-26)	1.65
Cyclazocine	17% at 1.O[a]	0.03 (0.02-.78)	0.011 (0.0046-0.03)
Nalorphine*HCl	None at 10.0	2.6 (0.69-9.75)	0.6 (0.025-1.44)
Naloxone*HCl	None at 10.0	0.035 (0.010-0.93)	No Activity
Naltrexone •HCl	None at 10.0	0.007 (0.002-0.02)	No Activity
Morphine Sulfate	5.8 (5.7-5.9)	----	0.23 (0.20-0.25)

[a]Mice were ataxic at 3.0 and 10.0 mg/kg but no further increase in reaction time was seen.

Three mouse tests were used in our laboratory to provide a preliminary estimate of the potency and profile of activity of each test compound. The tests were the tail-flick agonist (TF)

and the morphine antagonist (TF vs M) tests and the phenylquinone (PPQ) test (Dewey et al., 1970; Dewey and Harris, 1971). Reference-standard data for these tests are shown in Table 1. In addition, Dr. Jacobson provided us with estimated starting doses. These doses were based on results obtained from the mouse-hot plate (HP) (Eddy and Leimbach, 1953; Jacobson and May, 1965; Atwell and Jacobson, 1978) and Nilsen (N) (Perrine et al., 1972) tests from his laboratory. Reference data for these tests are shown in Table 2.

Table 2

Comparative Data (ED50 mg/kg s.c.) [95% S.E.] from the Hot Plate and Nilsen Test

Compound	Hot Plate Test	Nilsen Test
	Subcutaneous	Subcutaneous
	Oral	Oral
Morphine Sulfate	0.98(0.83-1.1)	1.3(1.0-1.7)
	6.3(4.7-8.3)	8.3(6.0-11.4)
Codeine Phosphate	6.8(4.5-10.2)	7.4(4.9-11.0)
	13.5(9.7-18.7)	14.7(9.2-23.3)
Levorphanol Tartrate	0.2(0.1-0.3)	0.2(0.16-0.3)
	-	2.5(1.7-3.7)
Meperidines·HCl	5.3(4.0-7.1)	-
	-	-
(-)-Metazocines·HBr	0.6(0.5-0.9)	0.5(0.3-0.7)
	10.6(8.0-14.1)	26.0(21.0-33.0)
Dihydromorphinone·HCl	0.19(0.15-0.25)	0.2(0.15-0.3)
	0.9(0.7-1.2)	1.8(1.5-2.1)
Nalorphine·HCl	9.9(5.7-2.1)	23.0(16.2-32.7)
	-	-
Cyclazocine	1.5(1.1-2.1)	0.1(0.07-0.16)
	-	-
Pentazocine	9.3(6.7-12.8)	6.5(4.4-8.8)
	-	-
Chlorpromazine·HCl	1.1(0.9-1.5)	-
	-	-

Naloxone·HCl and Naltrexone·HCl, no dose response.
Phenobarbital, Amobarbital, Valium, Oxazepam, Flurazepam, Mepromate and Mescaline are inactive on the hot plate test.

Acknowledgements

This study was supported by a contract (#271-85-8101) from the National Institute on Drug Abuse, Dr. Khursheed Asghar, Contract Officer.

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SUMMARY OF COMPOUNDS TESTED

COMPOUND # NIH	MCV	CHEMICAL CLASS OR GENERIC NAME	MOUSE TF	MOUSE TFvsM	MOUSE PPQ	MOUSE HP	MOUSE N	RAT SM	RAT PPD	MONKEY SDS	MONKEY PPt-W	MONKEY PPD
7569	4501, 4430	(-)-metazocine	+	+	+	+	+	+	+			
7571	4502, 4431	(+)-metazocine	+	+	+	+	+					
7981	4510	(±)-cyclazocine	+	+	+[a,b]	+	+					
8211	4526	cocaine							+			
8374	4480	6.7-benzomorphan	+	+	+	+	+					
8683	4403	meptazinol	+			+		+	+	+		+
10093	4438	endoethenotetrahydrooripavine		+[c]								
10124	4336	nalorphine		+[c]								
10165	4348	(±)-ethylketocyclazocine	+	+	+[a,b]	+						
10249	4388	6,7-benzomorphan	+	+	+	+						
10253	4389	6,7-benzomorphan	+	+	+	+						
10258	4390	quinuclidine	+	+	+	+						
10274 (8359)	4385	nalbuphine	+	+	+					+		
10275	4386	butorphanol	+	+	+	+		+	+			+

COMPOUND # NIH	MCV	CHEMICAL CLASS OR GENERIC NAME	MOUSE TF,	TFvsM,	PPQ,	HP,	N	RAT SM,	PPD	MONKEY SDS,	PPt-W,	PPD
10319 (10455)	4400, 4521	piperazine[d]	+	+	+	+				+		
10322	4404	14-hydroxydihydromorphinone	+	+	+	+	+					
10324	4408	14-hydroxydihydromorphinone	+	+	+	+						
10331	4465	6-morphinone (6-oxomorphinan)	+	+	+	+						
10333	4409	dihydrocarbostyril	+	+	+	+				+	+	
10334	4410	dihydrocarbostyril	+	+	+	+				+		
10335	4411	dihydrocarbostyril	+	+	+	+				+		
10346	4414	(±)-ketocyclazocine	+		+[a,b]	+						
10347	4417	quinuclidine	+	+	+	+						
10348	4418	methyleneoxy-6,7-benzomorphan	+	+	+	+						
10349	4419	methyleneoxy-6,7-benzomorphan	+	+	+	+						
10357	4433	14-hydroxydihydromorphinone	+	+	+	+				+	+	
10362	4422	quinuclidine	+	+	+	+						
10363	4425	14-hydroxydihydromorphinone	+	+	+	+				+	+	

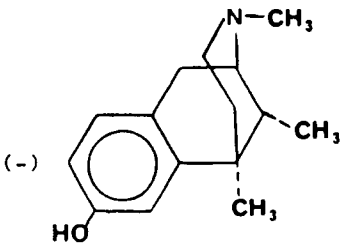
COMPOUND # NIH	MCV	CHEMICAL CLASS OR GENERIC NAME	MOUSE TF,	TFvsM,	PPQ,	HP, N	RAT SM, PPD	MONKEY SDS,	PPt-W,	PPD
10365	4426	nalmefene	+	+	+	+				+
10366	4423	14-hydroxydihydromorphinone	+	+	+	+				
10367	4424	14-hydroxydihydromorphinone	+	+	+	+				
10368	4434	methyleneoxy-6,7-benzomorphan	+	+	+	+				
10369	4435	methyleneoxy-6,7-benzomorphan	+	+	+	+				
10370	4436	methyleneoxy-6,7-benzomorphan	+	+	+	+				
10371	4466	4,14-dimethoxymorphinan-6-one	+	+	+	+				
10372	4437	tetrahydronaphthalene	+	+	+	+		+		
10379	4455	methyleneoxy-6,7-benzomorphan	+	+	+	+				
10380	4456	methyleneoxy-6,7-benzomorphan	+	+	+					
10385 (2820)	4457	diphenylhexan-3-one	+	+	+	+				
10386	4458	diphenylhexan-3-one	+	+	+	+				
10387	4459	diphenylhexan-3-one	+	+	+	+				
10388	4460	diphenylhexan-3-one	+	+	+	+				
10389	4467	diphenylhexan-3-one	+	+	+	+				

COMPOUND #		CHEMICAL CLASS	MOUSE					RAT	MONKEY	
NIH	MCV	OR GENERIC NAME	TF,	TFvsM,	PPQ,	HP,	N	SM, PPD	SDS,	PPt-W, PPD
10391	4464	diphenylhexan-3-one	+	+	+					
10393	4462	cyclohexylphenylhexan-3-one	+	+	+	+				
10396	4463	diphenylhexan-3-one	+	+	+	+				
10398	4481	(-)-eseroline	+	+	+	+				
10408	4482	5-methoxyindoline	+	·I·	+	+				
10409	4483	5-methoxyindoline	+	+	+	+				
10413	4476	methyleneoxy-6,7-benzomorphan	+	+	+	+				
10416	4477	6,7-benzomorphan								
10421	4484	(-)-eserine (physostigmine)	+	+	+	+				
10422	4485	(-)-eseroline	+	+	+	+				
10423	4486	5-hydroxyindoline	+	+	+	+				
10425	4488	5-hydroxyindoline	+	+	+	+				
10429	4475	4-tryptophan		+						+
10432	4493	4-phenylpiperidine	+	+	+	+				
10433	4494	4-phenylpiperidine	+	+	+	+				

COMPOUND #		CHEMICAL CLASS	MOUSE				RAT	MONKEY	
NIH	MCV	OR GENERIC NAME	TF,	TFvsM,	PPQ,	HP, N	SM, PPD	SDS,	PPt-W, PPD
10434	4495	4-phenylpiperidine	+	+	+	+			
10439	4504	(+)-eseroline	+	+	+	+			
10440	4503	4-phenylpiperidine	+	+	+				
10449	4511	(+)-cyclazocine			+				
10450	4512	(-)-cyclazocine			+[a,b]				
10451	4513	(-)-ketocyclazocine			+[a,b]				
10452	4515	(-)-ethylketocyclazocine			+[a,b]				
10453	4519	4-phenylpiperidine	+	+	+	+			
10454	4520	4-phenylpiperidine	+	+	+	+			
10455	10319	piperazine[d]	+	+	+	+		+	
10456	4522	4-anilinopiperidine	+	+	+	+		+	
10457	4523	4-anilinopiperidine	+	+	+	+		+	
10460	4527	4-anilinopiperidine	+	+	+	+		+	
10461	4528	4-anilinopiperidine	+	+	+	+		+	

[a]Naloxone AD50 vs. ED80, [b]Yohimbine AD50 vs. ED80, [c]Special i.v. study, [d]Submitted twice under different code numbers

NIH 7569, MCV 4430, 4501, UM 743 (-)-2'-Hydroxy-2,5,9a-trimethyl-6,7-benzomorphan hydrobromide [(-)-metazocine]



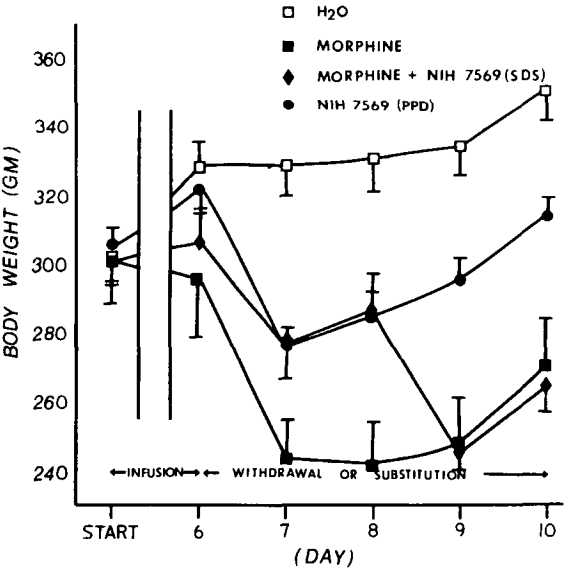
MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF- 0.81 (0.33 - 1.98)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.3 (0.1 - 0.91)
4) HP - 0.62 (0.45 - 0.91)
5) N - 0.40 (0.32 - 0.67)

Rodent data reported previously

RAT INFUSION
(SM and PPD)

As shown in the figure and table (-)-metazocine nearly substituted for morphine. Note that when it was withdrawn at the end of day 8, the rats promptly went into complete withdrawal. When given alone for 6 days, it produced physical dependence as evidenced by the loss of body weight and the development of typical behavioral withdrawal signs. Control vehicle and morphine-treated rats behaved as expected.



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NIH 7569, MCV 4430, 4501, UM 743 (-)-2'-Hydroxy-2,5,9a-trimethyl-6,7-benzomorphan hydrobromide [(-)-metazocine]

(cont...)

Substitution for morphine and primary physical dependence studies in the rat with (-)-metazocine

Treatment	Hour after termination of infusion			
	24	48	72	96
H_2O Control[a]				
N=4	2.3	1.0	0.8	0.5
Morphine Infusion[b]				
N=4	7.5	6.5	5.5	2.0
Morphine Infusion	0.8	1.5	5.0	1.8
plus (-)-Metazocine[c]	0.20	0.50	0.02	0.34
substitution (SDS)				
N=4				
(-)-Metasocine				
Infusion[d] (PPD)	8.6	5.2	1.4	2.6
N=5	0.008	0.03	0.06	0.09

Mean number of withdrawal signs (hypersensitivity, squeaking, aggression, wet-dog shakes, rubbing and chewing) noted during 1/2-hr observation periods at specified intervals and calculated probability values (one-tailed Mann-Whitney U-test) when compared with H_2O controls.

[a]8ml/24 hr

[b]50 mg/kg/24 hr on day 1, 100 mg/kg/24 hr on day 2; 200 mg/kg/24 hr on days 3-6;

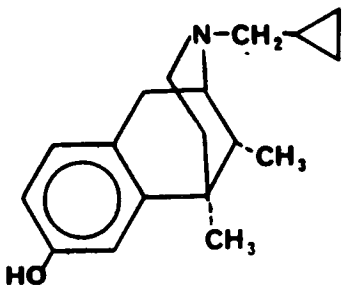
[c]50 mg/kg/24 hr days 7-8

[d]25 mg/kg/24 hr day 1; 50 mg/kg/24 hr on day 2, 24 mg/kg/24 hr on days 3-6.

NIH 7571, MCV 4431, 4502. UM 744 (+)-2'Hydroxy-2,5,9a-trimethyl-6,7-benzomorphan hydrobromide [(+)-Metazocine]

	MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)
See optical isomer NIH 7569	1) TF- Inactive at 1.0, 10.0 and 30.0 2) TF vs. M - 0% at 15; 23% at 20.0; 56% at 30.0; 68% at 60.0 3) PPQ - 4) HP - Inactive at 20.0 5) N - Inactive at 20.0

NIH 7981, MCV 4510 (±)-2-Cyclopropylmethyl-5,9a-dimethyl-2'-hydroxy-6,7-benzomorphan [(±)-Cyclazocine]

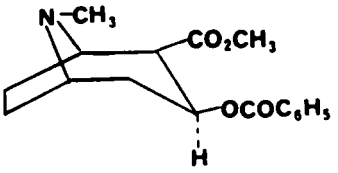


MOUSE DATA ED50 or AD50 (95% C.L.) mg/kg s.c.)

1) TF- 17% at 10.0[a]
2) TF vs. M - 0.03 (0.2-0.78)
3) PPQ - 0.05 (0.03 - 0.11)[b,c]
4) HP - 1.5 (0.7 - 0.16)
5) N - 0.11 (0.07 - 016)

Reported previously
Naloxone AD50 vs ED80 of NIH 7981 = 0.3 (0.09 - 0.85)
[c]Yohimbine inactive vs ED80 of NIH 7981

NIH 8211, MCV 4526 Cocaine hydrochloride



Rat Infusion (PPD)

When given to rats continuously either at a dose of 50 mg/kg/day for 6 days, or at a dose regimen of 10 mg/kg/day on days 1-3 and 100 mg/kg/day on days 4-6. cocaine did not significantly affect either body weight, water consumption or overt behavior during drug administration and during abrupt withdrawal in sharp contract with morphine (see Fig. and Table). However, at both dose regimens the animals showed significantly less spontaneous activity in the photocell activity cages just before the initiation of abrupt withdrawal of drug on day 6, but not during abrupt withdrawal.

NIH 8211, MCV 4526 Cocaine hydrochloride

(cont....)

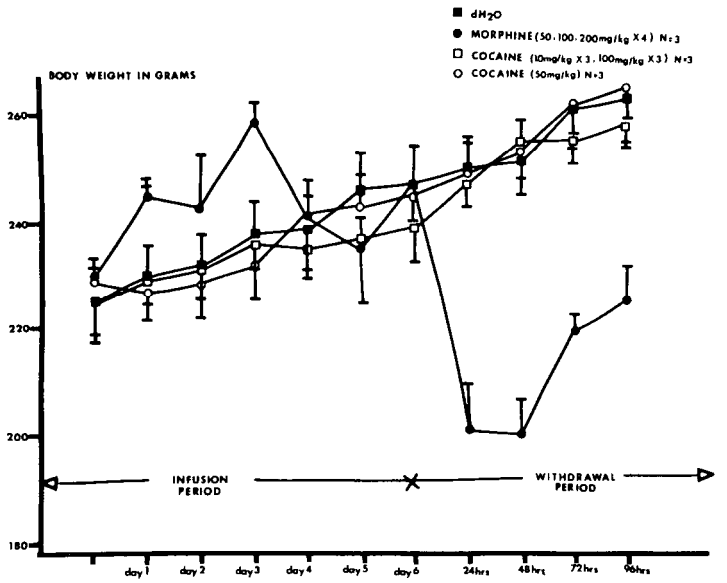


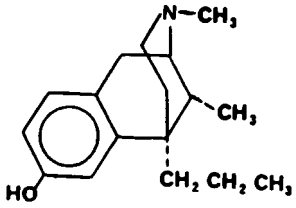
Table (Cocaine)

Means[a] of Withdrawal Signs[b] of Morphine-Treated and Cocaine-Treated Rats Compared With H_2O Controls

	HOURS IN WITHDRAWAL				
Treatment	0	24	48	72	96
H_2O Controls[c]	0.5	0	0	0	0
Cocaine[d]	1.3	0	1.0	0	0.3
Cocaine[e]	4.0	0	0	0	0
Morphine Controls[f]	1.2	5.3[a]	13.3[a]	13.7[a]	14.0[a]

a Significant at $p < 0.05$, one-tailed test (Mann Whitney U-test)
b Hypersensitivity, squeaking, aggression, wet-dog shakes, rubbing, chewing
c N=4, 7ml/24 hr
d N=4, 50 mg/kg/day (days 1-6)
e N=2, 10 mg/kg/day (days 1-3), 100 mg/kg/day (days 4-6)
f N=3, 50 mg/kg 1st day, 100 mg/kg 2nd day, 200 mg/kg days 3-5. H_2O days 7-10

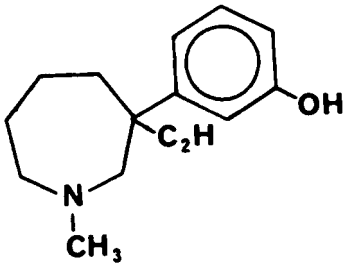
NIH 8374, MCV 4480, UM 696 (+)-2,9α-Dimethyl-2'-hydroxy-5-propyl-6,7-benzomorphan hydrochloride



MOUSE DATA ED or AD50 (95% C-L.)
(mg/kg s.c.)

1) TF- Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 21.5 (13.4 - 34.5)
3) PPQ - 3.1 (0.9 - 10.7)
   HP - 12.3 (10.0 - 15.2)

NIH 8683, MVC 4403, UM 888 m-(3-Ethyl-1-methylhexahydro-1H-azepine-3-yl) phenol hydrochloride (Meptazinol hydrochloride)



MOUSE DATA ED or AD50 (95% C.L.)
(mg/kg i.v.)

1) TF - 12.8 (5.8 - 28.3)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 1.6 (0.5 - 5.1)
4) HP - 5.3 (4.0 - 7.1)

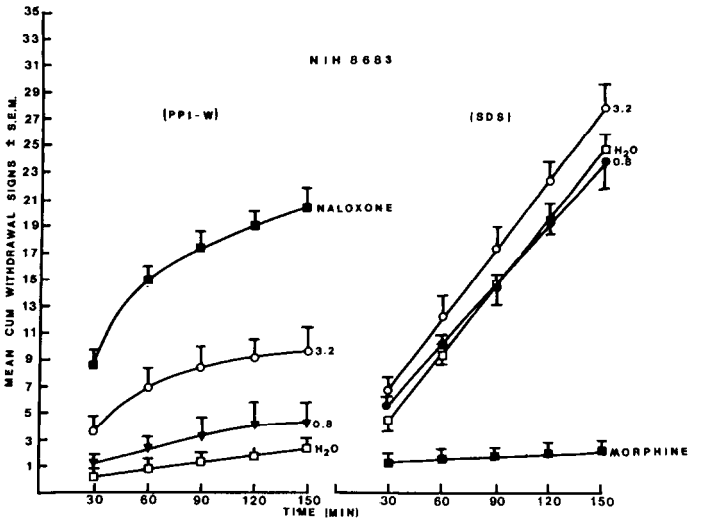
MONKEY DATA

A. (SDS) $\frac{\text{\# Animals}}{\text{Dose (mg/kg s.c.)}}$, $\frac{3}{3.2}$, $\frac{3}{0.8}$, $\frac{2^a}{0.2}$,

$\frac{3\text{(Morphine)}}{3.0}$, $\frac{3(H_2O)}{1\ ml/kg}$

As shown in the figure, NIH 8683 did not substitute for morphine in the dose range studied. Tremors were noted at the 2 higher doses.

NIH 8683, MVC 4403, UM 888 m-(3-Ethyl-1-methylhexahydro-1H-azepine-3-yl) phenol hydrochloride (Meptazinol hydrochloride)



B. (PPt-W)

# Animals / Dose (mg/kg s.c.)	2^a / 6.4	4 / 3.2	3 / 0.8
2^a / 0.2	4 (Naloxone) / 0.05	4 (H_2O) / 1 ml/kg	

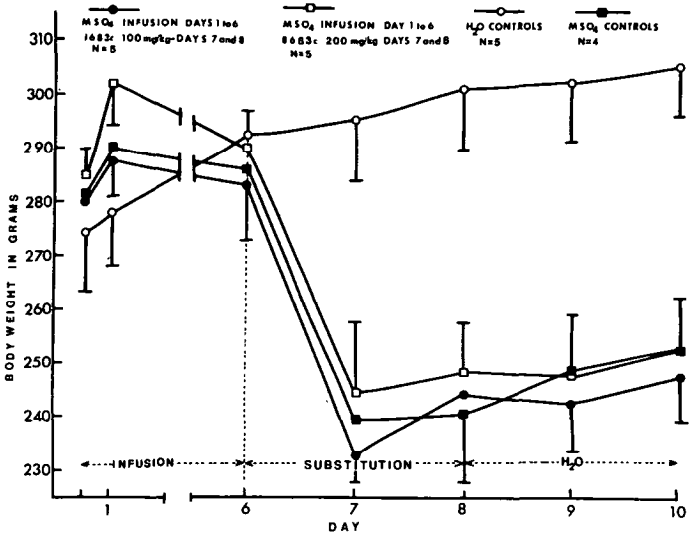
NIH 8683 precipitated some withdrawal signs at the highest dose. Although the drug acted promptly, it had a short duration of action (approx. 1 hr, see figure).

[a]Not plotted, N=2.

RAT DATA

A. Substitution for morphine study: As shown in the first figure at doses of 100 or 200 mg/kg/24 hr, NIH 8683 did not substitute for morphine. The animals receiving both dose regimens lost as much body weight as the morphine controls. In addition, as shown in Table 1, the animals receiving the lower dose also manifested opioid-like withdrawal signs. At the highest dose, although the means at 48 and 72 hr appear large and approach statistical significance when compared to the water controls, most of the withdrawal signs were generated by only 2 rats. It is also possible that higher doses might have substituted.

FIGURE 1

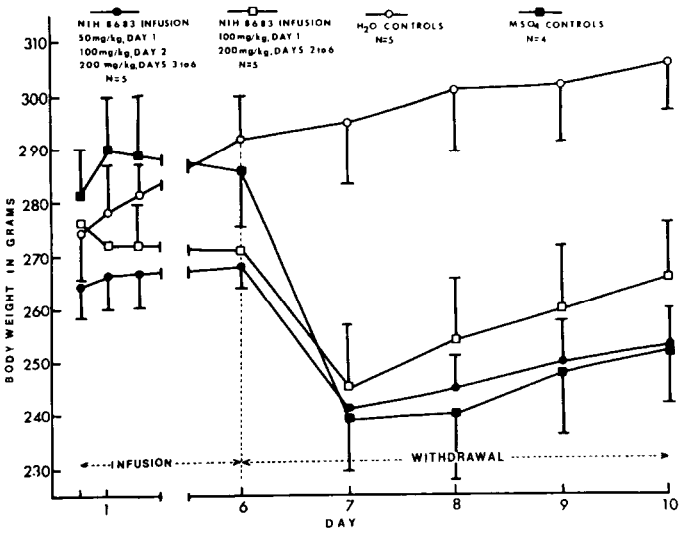


B. Primary Physical Dependence Study (PPD): In the second figure and table are shown the results of the PPD study. NIH dependence study. At both dose schedules, the drug produced physical dependence indistinguishable from that observed in the morphine controls as evidenced by loss of body weight and development of withdrawal syndrome after NIH 8683 was abruptly withdrawn. We recently reconfirmed these results using the lower dose regimen.

NIH 8683, MVC 4403, M 888 m-(3-Ethyl-1-methylhexahydro-1H-azepine-3-yl) phenol hydrocfhloride (Meptazinol hydrochloride)

(cont....)

FIGURE 2



General Conclusions and Recommendations

NIH 8683 (racemate) is showing an unusual profile of activity. In the mouse, the compound has an antinociceptive profile of activity similar to that of morphine. In the morphine-dependent rhesus monkey, it appears to be a weak morphine antagonist. Finally, in the rat, the drug does not substitute for morphine but produces morphine-like physical dependence. This compound is related structurally to certain N-methyl benzomorphans and homobenzomorphans. Extensive studies of the optical isomers of these N-methyl derivatives revealed a remarkable separation of agonist-antagonist properties among several species. Similar studies of the optical isomers of NIH 8683 are recommended. In addition, PPD studies of the racemate in rhesus monkeys are urged.

NIH 8683 TABLE 1

MEAN WITHDRAWAL SCORES[1] AND PROBABILITY VALUES[2] CALCULATED DURING 1 HOUR OBSERVATION PERIODS AT SPECIFIED INTERVALS FOR COMPARIONS BETWEEN H_2O ONLY GROUP AND NIH 8683 OR MORPHINE CONTROLS IN A SUBSTITUTION FOR MORPHINE STUDY IN CONTINUOUSLY-INFUSED RATS

Treatment/24 hr	Hours in Withdrawal			
	24	48	72	96
H_2O Only N=5	x = 0.8	x = 1.2	x = 1.4	x = 1.4
Morphine Sulfate Infusion[3]	x = 9.3 p = 0.008	x = 12.3 p = 0.008	x = 5.0 p = 0.016	x = 4.0 p = 0.076
Morphine Infusion[4] 8683C Substition	x = 6.4 p = 0.016	x = 12.4 p = 0.048	x = 13.2 p = 0.004	x = 6.4 p = 0.012
Morphine Infusoin[5] 8683C Substitution	x = 10.6 p = 0.004	x = 8.0 p = 0.155	x = 10.4 p = 0.133	x = 8.0 p = 0.274

[1]Hypersensitivity, 1 squealing, aggression, wet-dog shakes, rubbing and chewing
[2]One-tailed test (Mann-Whitney U-Test)
[3]50 mg/kg - day 1, 100 mg/kg - day 2, 200 mg/kg - days 3-6, N=4
[4]100 mg/kg - days 7 and 8, N-5
[5]200 mg/kg - days 7 and 8, N=5

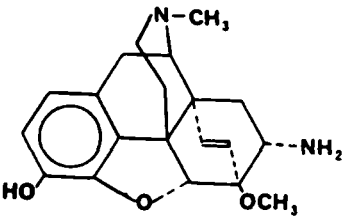
NIH 8683 TABLE 2

MEAN WITHDRAWAL SCORES[1] AND PROBABILITY VALUES[2] CALCULATED DURING ½ HOUR OBSERVATION PERIODS AT SPECIFIED INTERVALS FOR COMPARIONS BETWEEN H_2O ONLY GROUP AND NIH 8683 OR MORPHINE CONTROLS IN A PRIMARY PHYSICAL DEPENDENCE STUDY IN CONTINUOUSLY-INFUSED RATS

Treatment/24 hr	Hours in Withdrawal			
	24	48	72	96
H_2O Only[3] N=5	x = 0.8	x = 1.2	x = 1.4	x = 1.4
Morphine Sulfate Infusion[4]	x = 9.3 p = 0.008	x = 12.3 p = 0.008	x = 5.0 p = 0.016	x = 4.0 p = 0.076
NIH 8683C Infusion[5]	x = 11.8 p = 0.016	x = 7.0 p = 0 .075	x = 4.4 p = 0.0274	x = 3.6 p = 0.0461
NIH 8683C Infusion[6]	x = 13.2 p = 0.004	x = 1 1.8 p = 0 .004	x = 2.8 p = 0.111	x = 2.0 p = 0.383

[1]Hypersensitivity, squealing, aggressor, wet-dog shakes, rubbing and chewing
[2]One-tailed test (Mann-Whitney U-Test)
[3]Studies shown in Tables 1 and 2 for NIH 8683 were done at same time. H_2O and morphine controls are the same and are listed each time to facilitate comparisons
[4]550 mg/kg - day 1, 100 mg/kg - day 2, 200 mg/kg - days 3-6, N-4
[5]650 mg/kg - day 1, 100 mg/kg - day 2, 200 mg/kg - days 3-6, N-5
100 mg/kg - day 1, 200 mg/kg - days 2-6, N=5

NIH 10093, MCV 4438 7α-Amino-6,14-endoethenotetrahydrooripavine



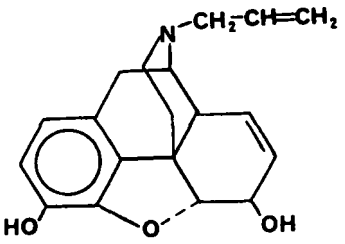
MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg i.v.)

1) TF -
2) TF vs. M - Inactive at 0.1, 1.0, 10.0 and 20.0[a]
3) PPQ -
4) HP-

[a]Vehicle - lactic acid plus H_2O

Special i.v. study

NIH 10124, MCV 4436 Nalorphine hydrochloride

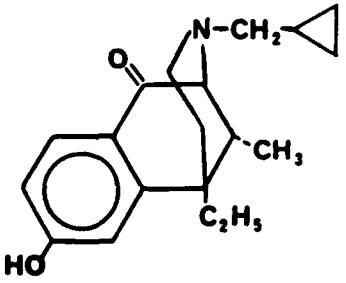


MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg i.v.)

1) TF -
2) TF vs. M - 0.8 (0.3 - 2.0)
3) PPQ -
4) H P -

Special i.v. study

NIH 10165, 8848, MCV 4348, UM975 (±)-2-Cyclopropylmethyl-5-ethyl-8-oxo-9α-methyl-6,7-benaomorphan methanesulfonate (Ethylketocyclazocine)



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

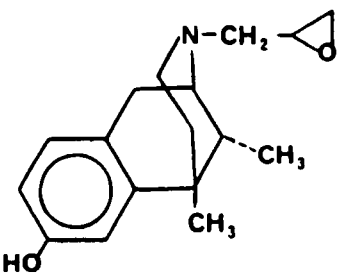
1) TF - Inactive at 0.4 (0.1 - 1.0)[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.05 (0.03 - 0.10)[b,c]
4) HP - 0.09 (0.07 - 0.120)[a]

[a]Reported previously

[b]Naloxone AD50 vs ED80 of NIH 8858 = 0.23 (0.12 - 0.46)

[c]Yohimbine inactive vs ED80 of NIH 8848 at 0.1, 1.0, 10.0 and 20.0

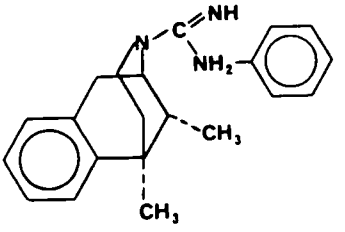
NIH 10249, MCV 4388 N-(2,3-Epoxypropyl)normetazocine



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF- Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 9% at 1.0, 14% at 10.0 and 30% at 30.0
3) PPQ - 18.1 (11.7 - 28.0)
4) HP - Inactive at 20.0

NIH 10253, MCV 4389 N-(N-Phenylguanidino)-5,9-dimethyl-6,7-benzomorphan hydrochloride



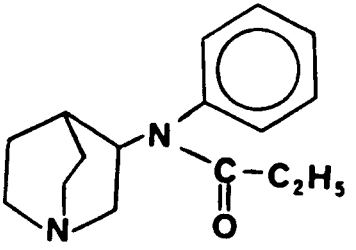
MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF- Inactive at 1.0, 10.0 and 30.0
2) TF vs. M- 0% at 0.1, 24% at
3) 1.0, 0% at 10.0 and 2% at 30.0
PPQ - 15.6 (6.4 - 38.2)[a]
4) HP - Inactive at 50.0

Vehicle (assays 1,2,3) Tween 80 + EtOH + H_2O

[a]1 of 6 died of convulsions at 60.0 Ataxia also noted

NIH 10258, MCV 4390 3-(N-Propionyl)anilinoquinuclidine hydrochloride

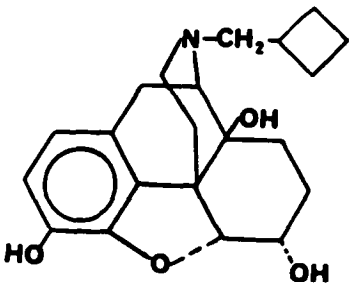


MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

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
3) PPQ - 4.6 (1.6 - 13.6)
4) HP - Inactive at 50.0, 40% at 100.0

[a]Stimulation at 30.0

NIH 10274, 8359, MCV 4385, UM 686 Nalbuphine hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

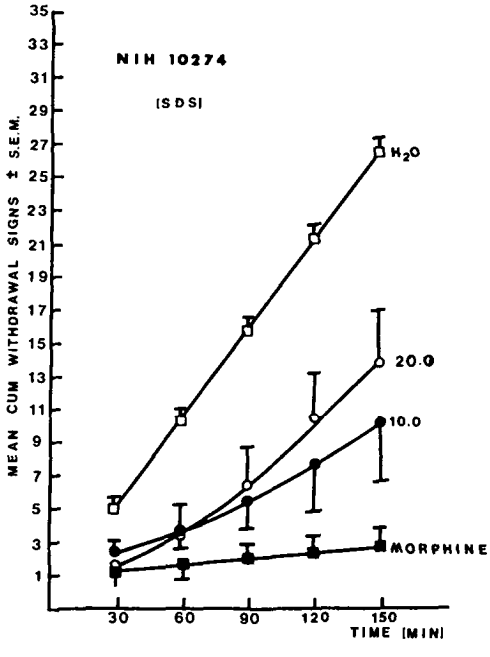
1) TF - 10% at 1.0, 22% at 10.0, and 15% at 30.0
2) TF vs. M - 20% at 1.0, 69% at 3.0, 63% at 10.0 and 57% at 30.0
3) PPQ - 0.26 (0.09 - 0.81)
4) HP-

Rodent data reported previously.

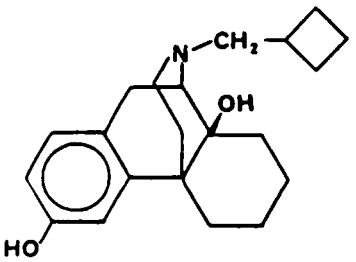
MONKEY DATA SDS # Animals, Dose (mg/kg s.c.) 3, 20.0 3, 10.0 3 (Morphine), 3.0

3 (H_2O), 1ml/kg

NIH 8359 partly suppressed withdrawal in withdrawn morphine-dependent monkeys at the 2 higher doses. The drug has a quick onset but a shorter duration of action than morphine (see figure).



NIH 10275, 8791, MCV 4386, UM 941 Butorphanol Tartrate



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

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 - a) Inactive at 1.0, 10.0 and 30.0
   b) 15.0 (5.9 - 38.0)[a]
4) HP - 0.78 (0.6 - 1.0)
5) N - 0.09 (0.06 - 0.4)

[a]Repeated all other rodent data reported previously.

MONKEY DATA

A. (SDS) # Animals / Dose (mg/kg s.c.) 3 / 0.5, 3 / 0.05

3 / 3.0 (Morphine), 3 / 1 ml/kg (H_2O)

The drug did not substitute for morphine and may have exacerbated withdrawal, (see figure).

B. (PPt-W) # Animals / Dose (mg/kg s.c.) 1[a] / 9.0, 3 / 6.0, 3 / 3.0

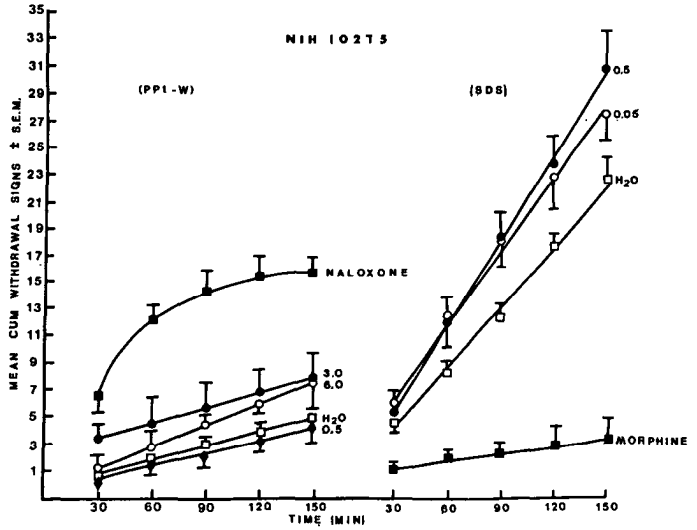
2[a] / 1.5, 3 / 0.5, 3 / 0.05 (Naloxone), 3 / 1 ml/kg (H_2O)

NIH 10275 did not precipitate a full withdrawal syndrome up to the convulsive dose of 9.0 mg/kg. Some withdrawal signs were noted namely, salivation, tremors, contact avoidance, pacing, drowsiness and wet-dog shakes. Most of these signs were observed at the 6.0 mg/kg dose. Only one monkey in the entire study retched, vomitted, and vocalized when its abdomen was palpated. Onset of action was rapid but duration was shorter than naloxone (90 min). This drug appears to be an atypical antagonist.

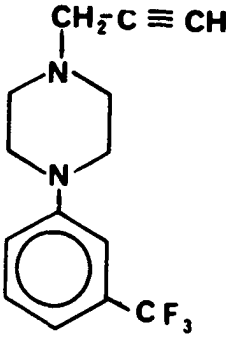
[a] Not plotted, N less than 3.

NIH 10275, 8791, MCV 4386, UM 941 Butorphanol Tartrate

(cont)....



NIH 10319, 10455, MCV 4400, 4521 N-Propargyl-N'-(3-trifluoro-methylphenyl)piperazine hydrochloride



MOUSE DATA ED or AD50 (95% C.L.) (mg/kg s.c.)

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 - 5.8 (1.5 - 22.0)
4) HP - 50% at 15. 50% at 20.0

MONKEY DATA
(SDS)

# Animals	3 ,	4 ,	4 ,
Dose (mg/kg s.c.)	20.0	10.0	2.5

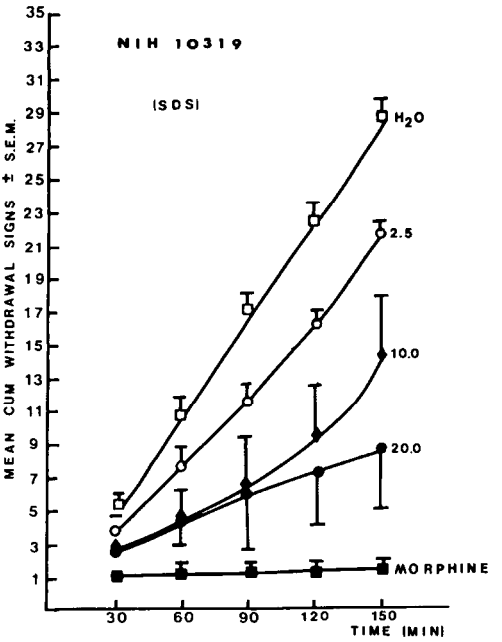
4(Morphine), 3.0

4(H_2O), 1 ml/kg

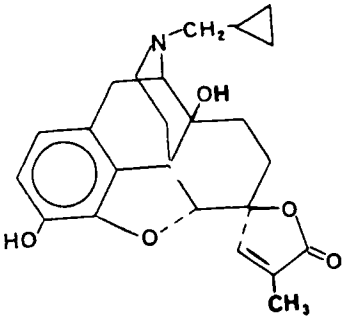
NIH 10319, 10455, MCV 4521, 4400 N-Propargyl-N'-(3-trifluoro-methylphenyl)piperazine hydrochloride

(cont)....

NIH 10319 produced a dose-related suppression of withdrawal signs. At the highest dose, it completely suppressed withdrawal in 2 of 3 animals. Onset of action is rapid and duration of action is shorter than that of morphine.



NIH 10322, MCV 4404 6α-(2-Carboxy-1-propenyl)-naltrex-6β-ol-γ-lactone

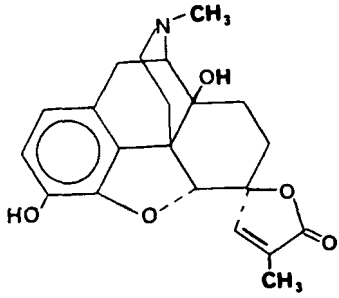


MOUSE DATA ED50 or AD50 (95% C.L.) mg/kg s.c.)

1) TF- Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 1.6 (0.9 - 2.7)[a]
3) PPQ - 11.7 (6.5 - 22.0)[a]
4) HP- 50% at 20.0
5) N - 50% at 20.0

[a]Vehicle - Tween80 + H_2O

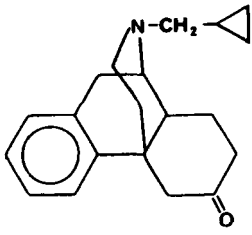
NIH 10324, MCV 4408 6α-(2-Carboxy-1-propenyl)-oxymorph-6β-ol-γ-lactone)



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF- 18.6 (12.5 - 27.8)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 2.4 (1.1 - 5.3)
4) HP - 3.5 (2.4 - 5.3)

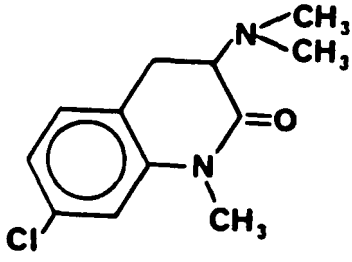
NIH 10331, MCV 4465 (-)-N-Cyclopropylmethylmorphinan-6-one salicylate



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - 0.9 (0.4 - 2.3)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.2 (0.07 - 0.3)
4) HP - 0.4 (0.3 - 0.6)

NIH 10333, MCV 4409 (+)-7-Chloro-3-dimethylamino-1-methyl-3,4-dihydrocarbostyril hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

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 - 10.1 (5.3 - 19.3)
4) HP- 40% at 50 mg/kg, 60% at 100 mg/kg

NIH 10333, MCV 4409 (+)-7-Chloro-3-dimethylamino-1-methyl-3,4-dihydrocarbostyril hydrochloride

(cont....)

MONKEY DATA

A. (SDS)	# Animals	3	2	2
	Dose (mg/kg s.c.)	16.0	4.0	1.0

3(Morphine)	3(H_2O)
3.0	1 ml/kg

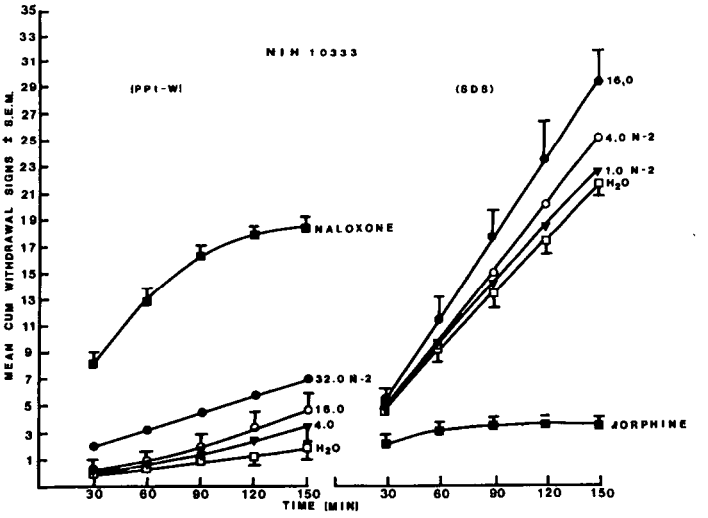
As shown in the figure, this drug did not substitute for morphine at 1.0 - 16.0 mg/kg and may have exacerbated withdrawal at the highest dose.

B. (PPt-W)	# Animals	2[a]	3	3
	Dose (mg/kg s.c.)	32.0	16.0	4.0

3(Naloxone)	3(H_2O)
0.05	1 ml/kg

This compound produced some withdrawal signs designated, pacing, drowsiness, tremors, wet-dog shakes, and one animal retched at the highest dose. The drug did not precipitate a full withdrawal syndrome. In fact, at the highest dose, it also produced ataxia and jaw sag in one animal (see figure).

[a]Not plotted N less than 3



NIH 10334, MCV 4410 (-)-7-Chloro-3-dimethylamino-1-methyl-3,4-dihydrocarbostyril hydrochloride

MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

See racemate NIH 10333

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 - 25.9 (11.9 - 56.3)
4) HP - 30% at 50 mg/kg

MONKEY DATA (SDS)	# Animals Dose (mg/kg s.c.)	3, 32.0	3, 8.0	2, 2.0
3(Morphine), 1 ml/kg	3(Morphine), 3.0			

NIH 10334 did not substitute for morphine in the dose range 2.0 - 32.0 mg/kg. At the highest dose, some ataxia, jaw sag and salivation was noted.

NIH 10335, MCV 4411 (+)-7-Chloro-3-dimethylamino-1-methyl-3,4-dihydrocarbostyril hydrochloride

MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

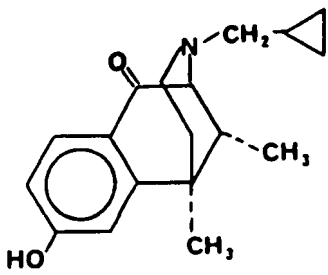
see racemate NIH 10333

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 - 3.0 (1.2 - 7.0)
4) HP - 0% at 20 or 50 mg/kg

MONKEY DATA (SDS)	# Animals Dose (mg/kg s.c.)	2, 32.0	3, 16.0	2, 4.0
3 (H_2O) 1 ml/kg	3(Morphine) 3.0			

In the dose range of 1.0 - 32.0 mg/kg, NIH 10335 did not substitute for morphine.

NIH 10346, 8847, MCV 4414, UM 974 (±)-Ketocyclasocine



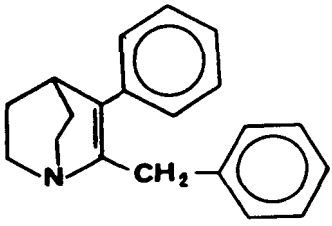
MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 0.5, 5.0 and 20.0
2) TF vs. M-
3) PPQ - 0.1 (0.08 - 0.2)[a,b]
4) H P - a) 0.42 (0.32 - 0.56)
b) 0.65 (0.50 - 0.84)

[a]Naloxone AD50 vs ED80 of NIH 8847 is 0.5 (0.2 - 1.4)

[b]Yohimbine AD50 vs ED80 of NIH 8847 is inactive at 0.1, 1.0, 10.0 and 20.0

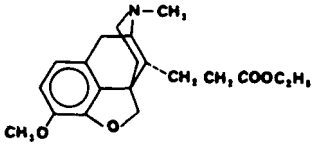
NIH 10347, MCV 4417 2-Benzyl-3-phenyl-$\Delta^{2,3}$-quinuclidine hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

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 - Inactive at 1.0, 10.0 and 30.0
4) HP - Inactive up to 50.0, convulsions

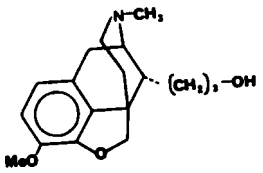
NIH 10348, MCV 4418 (-)-9α-(2-Carbethoxyethyl)-2'-methoxy-2-methyl-5,1'-methyleneoxy-6,7-benzomorphan hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

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 - 0% at 1.0, 26% at 10.0, 43% at 30.0 and 54% at 60.0
4) HP- Inactive up to 50.0,

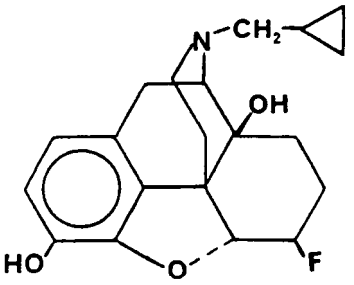
NIH 10349, MCV 4419 (-)-9α-(3-hydroxypropyl)-2'-methoxy-2-methyl-5,1'-methyleneoxy-6,7-benzomorphan hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF- Inactive at 1.0, 10.0 20% at 30.0
2) TF vs. M - 22% at 0.1, 35% at 1.0, 35% at 30.0 and 20% at 60.0
3) PPQ - Inactive at 1.0 and 30.0, 23% at 10.0
4) HP - Inactive at 5.0 and 20.0

NIH 10357, MCV 4433 17-Cyclopropylmethyl-3,14-dihydroxy-4,5 α-epoxy-68-fluoromorphinan hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0, 10.0, and 30.0
2) TF vs. M - 0.003 (0.001 - 0.008)
3) PPQ - 16% at 0.5, 34% at 1.0, 61% at 10.0, 47% at 20.0 and 58% at 30.0
4) HP - Inactive

MONKEY DATA

A. (SDS)

# Animals	3,	3,
Dose (mg/kg s.c.)	0.01	0.001

3(Morphine), 3.0 3(H_2O) 1 ml/kg

This compound did not substitute for morphine, instead, it appeared to exacerbate withdrawal at the lowest dose (see figure).

B. (PPt-W)

# Animals	1[a],	3,	3,
Dose (mg/kg s.c.)	0.001	0.01	0.05

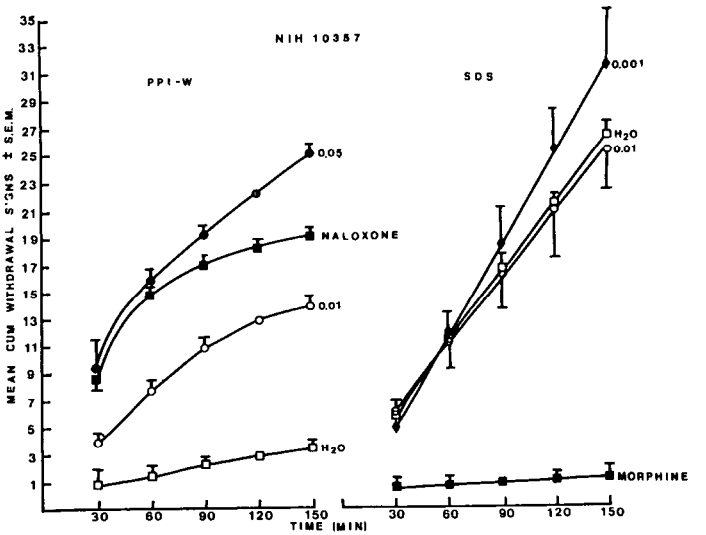
3(Naloxone), 0.05 3(H_2O) 1 ml/kg

NIH 10357, MCV, 4433 17-Cyclopropylmethyl-3,14-dihydroxy-4,5 α-epoxy-6 β-fluoromorphinan hydrochloride

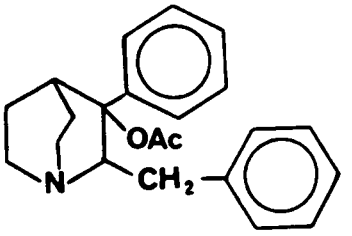
(cont)....

As illustrated in the figure, NIH 10357 promptly precipitated withdrawal. The action was dose related. The drug appears to be similiar to naloxone.

[a]Not plotted in Figure, N=1.



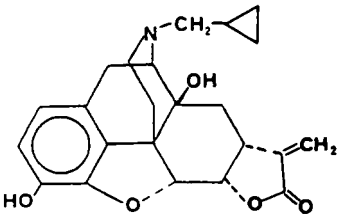
NIH 10362, MCV 4422 2-Benzyl-3-phenyl-3-acetoxyquinuclidine hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.)

1) TF- Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 34% at 1.0, 2% at 10.0 and 23% at 30.0
3) PPQ - Inactive at 1.0 and 10.0, 14% at 30.0
4) HP- Inactive at 5.0 and 20.0

NIH 10363, MCV 4425 7α-(1-Carboxyvinyl)-6α-naltrexal lactone



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF[a] - Inactive at 1.0, 10.0 and 30.0
2) TF vs. Ma - 0.01 (0.03 - 0.03)
3) PPQ -
4) HP - Inactive at 20.0

[a]Vehicle - Tween 80 + H_2O

MONKEY DATA

A. (SDS)	# Animals	3,	3,
	Dose (mg/kg s.c.)	0.02	0.005

3 (Morphine),	4 (Tween 80 + H_2O)
3.0	1 ml/kg

As shown in the figure, a dose of 0.02 mg/kg exacerbated withdrawal, whereas the 0.005 dose was inactive. Morphine substituted completely.

B. PPt-W - 2 hr after morphine (nonwithdrawn animals)

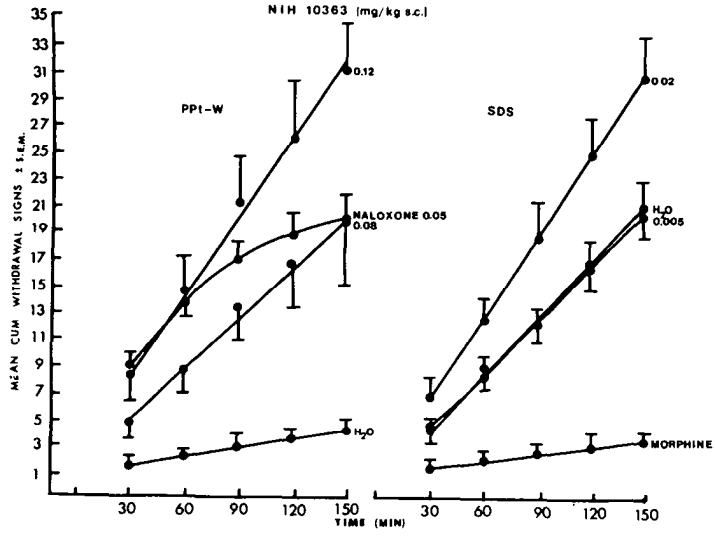
# Animals	3 ,	4 ,	(Morphine),
Dose (mg/kg s.c.)	0.12	0.08	3.0

4 (Tween 80 + H_2O)
1 ml/kg

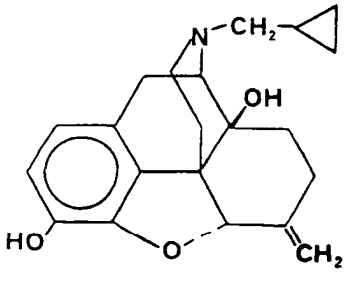
NIH 10363 promptly precipitated withdrawal at doses of 0.08 and 0.12 mg/kg. The data for the 0.02 mg.kg dose was not plotted because N=1. The action resembles that of naloxone.

NIH 10363, MCV 4425 7α-(1-Carboxyvinyl)-6α-naltrexal lactone

(cont....)



NIH 10365, MCV 4422 17-Cyclopropylmethyl-3,14-dihydroxy-4,5-epoxy-7-methylenemorphinan hydrochloride (Nalmefene)



MOUSE DATA ED50 (95% C.L.)

1) TF - Inactive at 1.0, 10.0 and 30.0

2) TF vs M - 0.001 (0.002 - 0.004)

3) PPQ - Inactive at 0.003, 0.03, 1.0, 10.0 and 30.0

4) HP - Inactive

MONKEY DATA

(PPD)

The experimental details and summary *are* presented in the accompanying table. Three experimentally naive rhesus monkeys (2 males and 1 female) weighing 2.1 - 2.8 kg at the start of the study were injected as indicated below and observed for ½ hr once a day (a.m.) shortly after drug was administered.

TABLE 1

Summary of a primary physical dependence study in rhesus monkeys with NIH 10365.

Day	Dose (mg/kg) 4-6 times a day	Comments
1	0.1[a]	Although restlessness and fighting were the main signs noted during the first 10 days, these signs are not necessarily drug effects and may simply reflect normal behavior while being observed. Drowsiness was noted in all the animals on day 11. In addition, slowing was noted in all three subjects on day 12. Salivation and scratching were the only signs observed on days 13 and 14. On day 15, one monkey developed severe diarrhea and was treated accordingly. Some slowing, restlessness and drowsiness were noted thru day 16.
2	0.2[a]	
3	0.4a	
4	0.8[a]	
5	1.6[a]	
6-8	1.6[b]	
9-10	3.2[a]	
11-12	6.4[a]	
13-16	6.4[a]	
17 day	Abrupt Withdrawal	Thirteen to fourteen hrs into abrupt withdrawal, the signs designated as extreme restlessness (2/3)[c], wet-dog shakes (2/3) and vocalization (2/3) were seen.
18-22	6.4[b]	Throughout the rest of study, the main signs observed were restlessness. By day 25, 1/3 was slow and 3/3 were slow on days 26-29. On day 29, one monkey had a swollen scrotum and moved about very slowly.
23-25	9.6[b]	
26-29	6.4[b]	

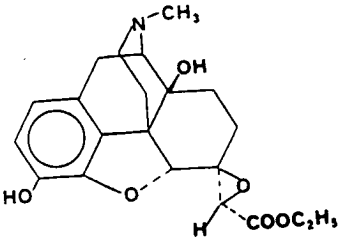
TABLE 1

(. . . .cont)

30 day	Abrupt Withdrawal and Naloxone Challenge	Severe slowing in 1 monkey and some restlessness (2/3) and drowsiness (3/3) were noted. Nineteen hrs after abrupt withdrawal of NIH 10365, a naloxone challenge of 1.0 mg/kg s.c. to 2 monkeys did not produce any remarkable signs. During the afternoon, one male animal expired, and the following day the other male was found dead. A gross autopsy was performed by a university veterinarian. Fluid retention in the thoracic and abdominal cavities and gastrointestinal tract was observed. Some hemorraghic areas were observed on the lungs of both animals.
	Conclusion	NIH 10365 does not seem to have a significant opioid physical dependence liability However, chronic and high doses of the drug apparently cause fluid retention, and male monkeys seem to be more susceptible.

[a] 6 times a day at 6 a.m., 9 a.m., noon, 3 p.m., 6 p.m. and midnight.
[b] 4 times a day at 6 a.m., noon, 6 p.m. and midnight.
[c] Ratios refer to the number of animals showing a sign over the total number of subjects.

NIH 10366, MCV 4423 E-6-Carboethoxymethylene-6β-oxido-oxymorphone

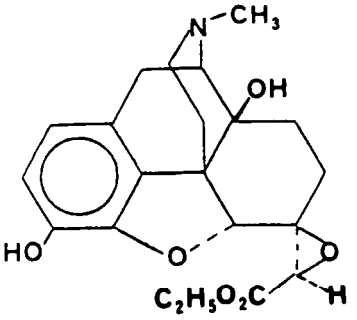


MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 0-29% from 0.1 to 30.0
3) PPQ - O-23% at 1.0, 10.0 and 30.0
4) HP - Inactive at 5.0 and 20.0

Vehicle - Tween 80 + H_2O

NIH 10367, MCV 4424 Z-6-Carboethoxymethylene-6B-oxido-oxymorphone acetate

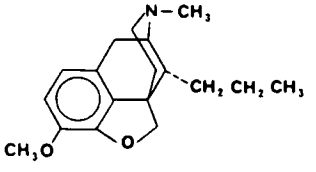


MOUSE DATA ED50 or AD50 (95% C.L.) mg/kg s.c.)

1) TF - 0.1 (0.04 - 0.3)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.02 (0.09-0.05)
4) HP - 0.08 (0.06 - 0.1)

Vehicle - Tween 80 + H_2O

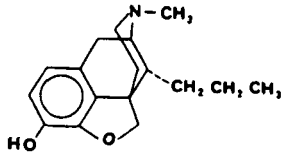
NIH 10368, MCV 4434 (-)-2'-Methoxy-2-methyl-5,1'-methyleneoxy-9α-propyl-6,7-benzomorphan hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - 8.1 (2.3 - 28.2)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.8 (0.3 - 2.8)
4) HP- Inactive up to 20.0

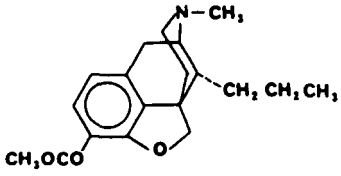
NIH 10369, MCV 4435 (-)-2'-Hydroxy-2-methyl-5,1'-methyleneoxy-9α-propyl-6,7-benzomorphan hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - 0.4 (0.2 - 0.8)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.1 (0.06 - 0.2)
4) HP - 1.8 (1.3 - 2.6)

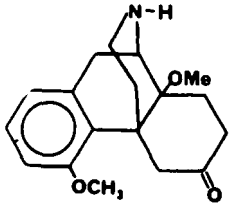
NIH 10370, MCV 4436 (-)-2'-Acetoxy-2-methyl-5,1'-methyleneoxy-9α-propyl-6,7-benzomorphan hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - 0.7 (0.5 - 1.0)
2) TF vs. - Inactive at 1.0, 10.0 and 30.0
3) PPO - 0.2 (0.1 - 0.3)
4) HP - 30% 5.0, 20% at 20.0

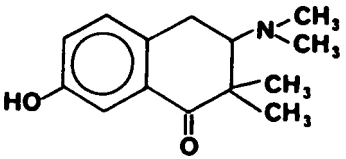
NIH 10371, MCV 4466 (-)-4,14-Dimethoxymorphinan-6-one hydrobromide



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF- 3.9 (1.5 - 10.3)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.3 (0.1 - 0.8)
4) HP - Inactive up to 20.0, 10% at 20.0

NIH 10372, MCV 4437 2,2-Dimethyl-3-Dimethylamino-7-hydroxy-1-tetralone hydrobromide



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF- 6.2 (2.2 - 17.6)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 9.9 (5.7 - 17.3)
4) HP- Inactive at 5.0 and 20.0

Rodent data reported previously

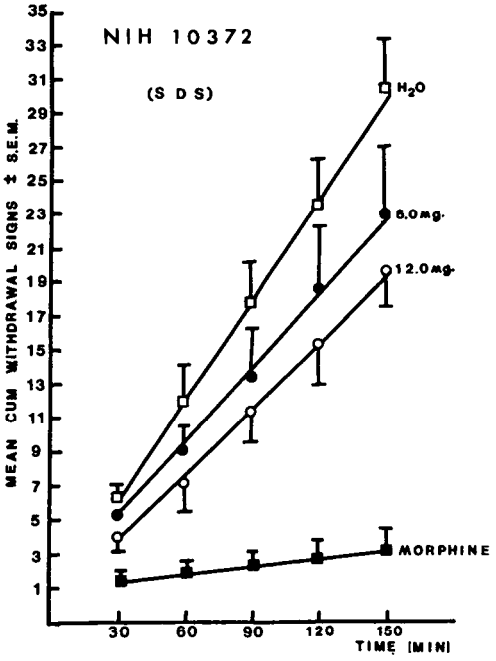
MONKEY DATA

A. (SDS) # Animals / Dose (mg/kg s.c.) 1[a] / 1.5, 4 / 6.0, 3 / 12.0

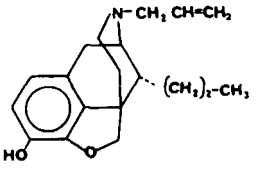
4 (Morphine) / 3.0 4 (H_2O) / 1 ml/kg

NIH 10372 produced a dose-related reduction in withdrawal signs at 1.5-12.0 mg/kg (see figure). However, the drug did not totally suppress the withdrawal signs. Lack of sufficient compound precluded further testing at higher doses. Two animals receiving the highest dose died approximately 3 weeks after receiving drug. Gross autopsy did not reveal any abnormalities.

[a]Not plotted, N=1.



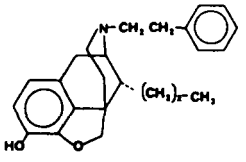
NIH 10379, MCV 4455 (-)-2-Allyl-2'-hydroxy-5,1'methyleneoxy-9α-propyl-6,7-benzomorphan hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 0.4 (0.1 - 1.0)
3) PPQ - 17% at 1.0 and 10.0, 40% at 30.0
4) HP - 20% at 20.0

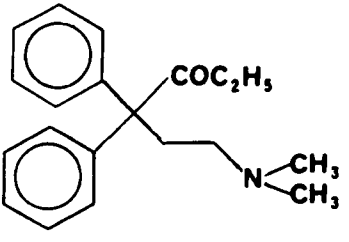
NIH 10380, MCV 4456 (-)-2'-Hydroxy-5,1'methyleneoxy-2-phenethyl-9α-propyl-6,7-benzomorphan hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - 1.4 (0.9 - 2.4)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.2 (0.1 - 0.4)
4) HP -

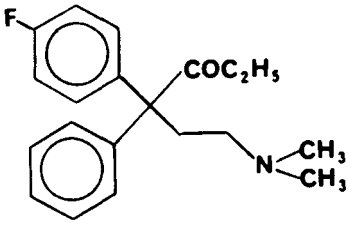
NIH 10385, MCV 4457, 2820, UM 118 6-Dimethylamino-4,4-diphenyl-hexan-3-one hydrochloride



MOUSE DATA ED50 or AD5O (95% C.L.) (mg/kg s.c.)

1) TF - 5.1 (3.1 - 8.4)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 1.0 (0.3 - 3.0)
4) HP - 2.4 (1.7 - 3.3)

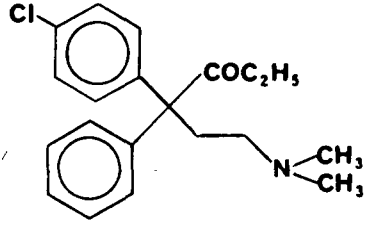
NIH 10386, MCV 4458 6-Dimethylamino-4-(4-fluorophenyl)-4-phenyl-hexan-3-one hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - 30.0 (19.5 - 48.9)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 9.2 (5.3 - 15.8)
4) HP - Inactive at 5.0 and 20.0

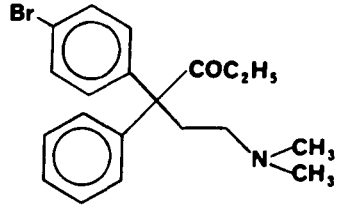
NIH 10387, MCV 4459 6-Dimethylamino-4-(4-chlorophenyl)-4-phenyl-hexan-3-one hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

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 - 11% at 1.0, 17% at 10.0 and 14% at 30.0
4) HP - Inactive at 5.0 and 20.0

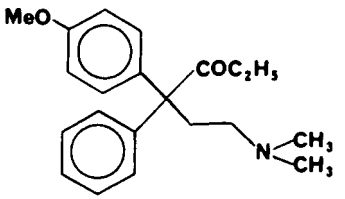
NIH 10388, MCV 4460 6-Dimethylamino-4-(4-bromophenyl)-4-phenyl-hexan-3-one hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) mg/kg s.c.)

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 - 23% at 1.0, 19% at 10.0 and 16% at 30.0
4) HP - Inactive at at 5.0 and 20.0

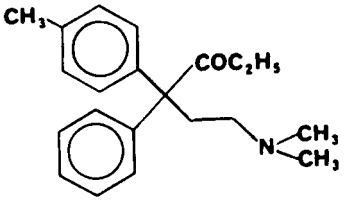
NIH 10389, MCV 4467 6-Dimethylamino-4-(4-methoxyphenyl)-4-phenyl-hexan-3-one hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

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 - 19% at 1.0, 11% at 10.0 and 36% at 30.0
4) HP - Inactive at 5.0 and 20.0

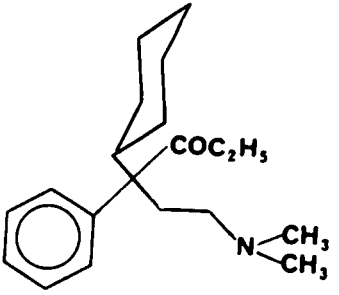
NIH 10391, MCV 4464 6-Dimethylamino-4-(4-methylphenyl)-4-phenyl-hexan-3-one hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - 3.4 (1.7 - 7.1)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 1.4 (0.7 - 2.8)
4) HP -

NIH 10393, MCV 4462 6-Dimethylamino-4-cyclohexyl-4-phenylhexan-3-one hydrochloride



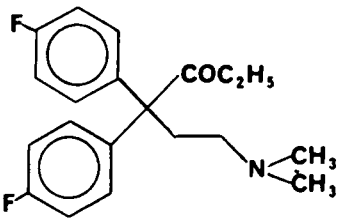
MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF- Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 7.7 (3.0 - 20.2)
3) PPQ - 13.7 (12.5 - 15.1)
4) HP - Inactive at 5.0 and 20.0[a]

[a]Walked backward in circles

172-682 0 - 87 - 15

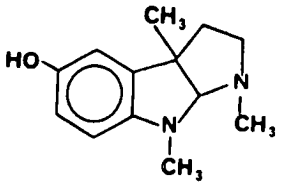
NIH 10396, MCV 4463 6-Dimethylamino-4,4-(4,4'-difluorodiphenyl-hexan-3-one hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 3% at 1.0, 23% at . 10.0 and 2% at 30.0
3) PPQ - Inactive at 1.0 and 10.0, 43% at 30.0
4) HP - Inactive at 5.0 and 20.0

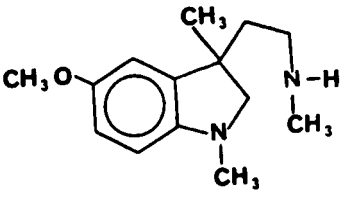
NIH 10398;MCV 4481 (-)-Eseroline



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - 2.3 (1.0 - 5.3)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.7 (0.3 - 1.7)
4) HP - 1.6 (1.1 - 2.2)

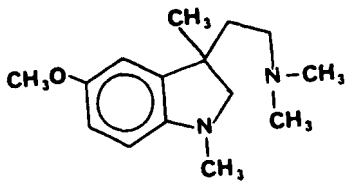
NIH 10408, MCV 4482 (3S)-1,3-Dimethyl-3-(2-methylaminoethyl)-5-methoxyindoline oxalate



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 18% at 0.3, 58% at 1.0, 31% at 3.0, 20% at 10.0 and 40% at 30.0
3) PPQ - 2.9 (0.9 - 9.3)
4) HP - Inactive at 5.0 and 20.0

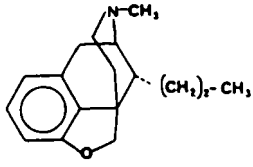
NIH 10409, MCV 4483 (3S)-1,3-Dimethyl-3-(2-dimethylaminoethyl)-5-methoxyindoline oxalate



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - Inactive 1.0, at 10.0 and 30.0
2) TF vs. M - 0% at 1.0, 54% at 3.0. 30% at 10.0 and 0% at 30.0
3) PPQ - 17% at 1.0, 66% at 3.0, 43% at 10.0 and 51% at 30.0
4) HP - Inactive at 5.0 and 20.0

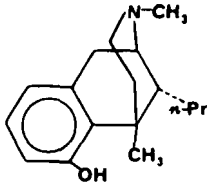
NIH 10413, MCV 4476 (-)-2-Methyl-5,1' -methyleneoxy-9α-propyl-6,7-benzomorphan hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF 20.7 (14.3 - 30.1)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 2.9 (1.3 - 6.3)
4) HP- Inactive at 5.0 and 20.0

NIH 10416 MCV 4477 (-)-2,5-Dimethyl-1'-hydroxy-9α-propyl-6,7-benzomorphan hydrochloride

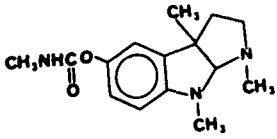


MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

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 - 3.9 (1.8 - 8.7)
4) HP - Inactive at 5.0 and 20.0[b]

[a]Convulsions and lethality at 30.[0]
[b]Tremors and convulsions

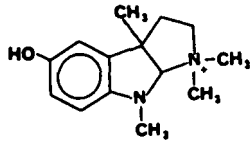
NIH 10421 MCV 4484 (-)-Eserine (Physostigmine)



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.) Vehicle (Lactic acid and H_2O)

1) TF - 0.06 (0.05 - 0.08)
2) TF vs. M - Inactive at 0.5 and 1.0
3) PPQ - 0.05 (0.02 - 0.16) 1/6 died at 0.25 mg/kg
4) HP - Toxic at 0.5

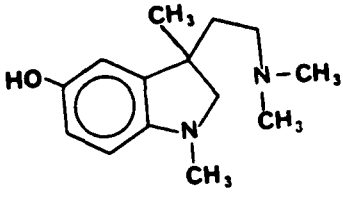
NIH 10422 MCV 4485 (-)-Eseroline methylmethosulfate



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 11% at 1.0, 32% at 10.0 and 26% at 30.0
3) PPQ - 33% at 0.001, 47% at 0.01, 67% at 0.1, 55% at 1.0, 43% at 10.0 and 11% at 30.0
4) HP - Inactive at 5.0 and 20.0

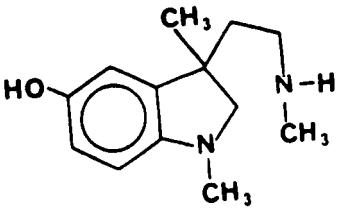
NIH 10423 MCV 4486 (3S)-1,3-Dimethyl-3-(2-dimethylaminoethyl)-5-hydroxyindoline hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 0.1, 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 0.1, 10.0 and 30.0
3) PPQ - 9% at .O.1, 17% at 1.0, 29% at 10.0 and 11% at 30.0
4) HP- Inactive at 5.0 and 20.0

NIH 10425 MCV 4488 (3S)-1,3-Dimethyl-3-(2-methylaminoethyl)-5-hydroxyindoline hydrochloride

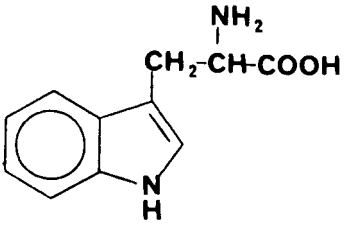


MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 0.1, 1.0, 10.0 and 18% at 20.0
2) TF vs. M - Inactive at 0.1, 1.0, 10.0 and 16% at 30.0
3) PPQ - 1.7 (0.1 - 27.7)
4) HP - Inactive at 5.0 and 20.0

TLC indicates major impurities

NIH 10429 MCV 4475 L-Tryptophan tartrate



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF -
2) TF vs. M - 0% at 1.0, 26% at 10.0 and 30.0, 6% at 40.0
3) PPQ -
4) HP -

Vehicle - Tween 80 + H_2O

MONKEY DATA (SDS)	# Animals Dose (mg/kg s.c.)	1[a], 32.0	3, 64.0	3, 128.0

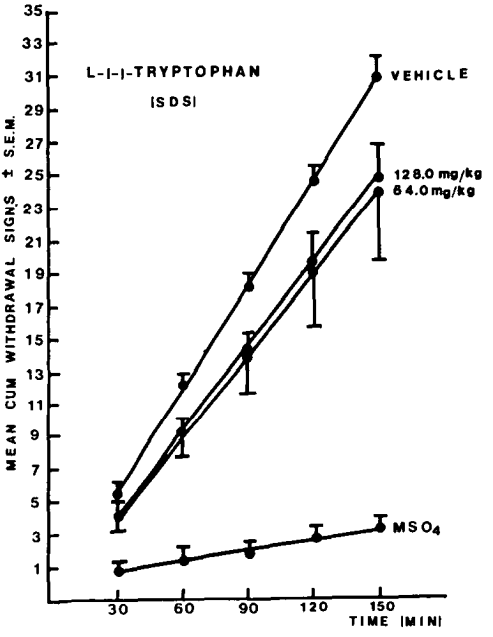
3(H_2O+Tween 80) 1 ml/kg	3(Morphine) 3.0

As shown in the accompanying figure, tryptophan neither substituted for morphine nor exacerbated withdrawal.

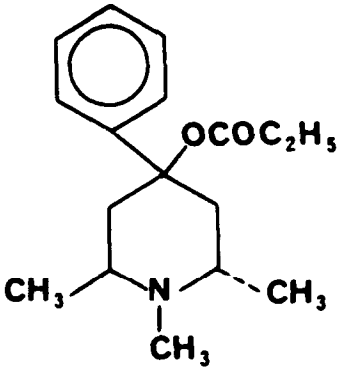
[a]This dose not plotted, N=1.

NIH 10429 MCV 4475 L-Tryptophan tartrate

(cont....)



NIH 10432, MCV 4493 (±)-4-Phenyl-4-propionoxy-1,2,6-trimethyl-piperidine hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - 2.7 (1.0 - 7.5)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.1 (0.03 - 0.5)
4) HP - 1.5 (1.1 - 2.1)

NIH 10433, MCV 4494 (+)-4-Phenyl-4-propionoxy-1,2,6-trimethyl-piperidine hydrochloride

	MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)
See NIH 10432	1) TF- 0.7 (0.2 - 2.0) 2) TF vs. M - 0% at 1.0, 12% at 10.0 and 56% 30.0 3) PPQ - 0.2 (0.07 - 0.7) 4) HP - 1.1 (0.9 - 1.3)

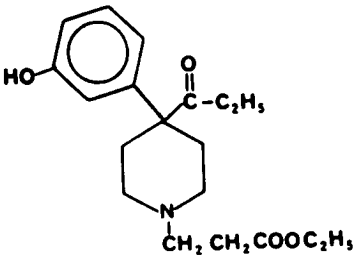
NIH 10434, MCV 4495 (-)-4-Phenyl-4-propionoxy-1,2,6-trimethyl-piperidine hydrochloride

	MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)
See NIH 10432	1) TF - 11.0 (6.8 - 17.8) 2) TF vs. M - 0% at 0.1, 14% at 0.5, 8% at 1.0, 10% at 5.0, 12% at 10.0 and 0% at 25.0 3) PPQ - 3.0 (1.9 - 4.7) 4) HP - Inactive at 5.0 to 20.0

NIH 10439, MCV 4504 (+)-Eseroline sulfate

	MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)
See optical isomer NIH 10422	1) TF - 0% at 1.0, 14% at 10.0 and 13% at 30.0 2) TF vs. M - Inactive at 0.1, 1.0, 10.0 and 30.0 3) PPQ - 2.3 (0.5 - 9.6) 4) HP - Inactive at 5.0 to 20.0 TLC indicated major impurities

NIH 10440, MCV 4503 N-(2-Carbethoxyethyl)-N-norketobemidone hydrobromide



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 0.1, 1.0, 10.0 and 30.0
2) TF vs. M - 10% at 0.01, 31% at 0.1, 0% at 1.0, 0% at 10.0 and 10% at 30.0
3) PPQ - Inactive at 0.1, 1.0, 10.0 and 30:0

NIH 10449, MCV 4511 (+)-Cyclazocine

See racemate NIH 7981

MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF -
2) TF vs. M -
3) PPQ - Inactive at 0.1 and 1.0
4) HP -

NIH 10450, MCV 4512 (-)-Cyclazocine

See racemate NIH 7981

MOUSE DATA ED50 or AD50 (95% C-L.) (mg/kg s.c.)

1) TF -
2) TF vs. M -
3) PPQ - 0 .014 (0.0045 - 0.046)[a,b]
4) HP -

[a]Naloxone AD50 vs ED80 of (-)-cyclazocine = 0.7 (0.2 - 2.3)

[b]Yohimbine inactive vs ED80 of (-)-cyclazocine as an antagonist at 0.1, 1.0 and 10.0

NIH 10451, MCV 4513 (-)-Ketocyclazocine

See racemate NIH 10346

MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF -
2) TF vs. M -
3) PPQ - 0.06 (0.02 - 0.2)[a,b]
4) HP -

[a]Naloxone AD50 vs ED80 of (-)-ketocyclazocine = 0.15 (0.06 - 0.35)

[b]Yohimbine inactive vs ED80 of (-)-ketocyclazocine at 1.0, 10.0 and 20.0

NIH 10452, MCV 4515 (-)-Ethylketocyclazocine

See racemate NIH 10165

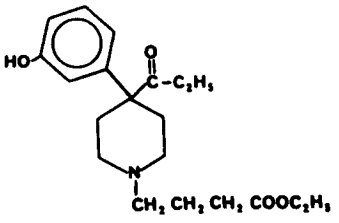
MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF -
2) TF vs. M -
3) PPQ - 0.005 (0.002 - 0.011)[a,b]
4) HP -

[a]Naloxone AD50 vs ED80 of (-)-ethylketocyclazocine ED80 = 0.9 (0.2 - 3.7)

[b]Yohimbine inactive as an antagonist vs ED80 of (-)-ethylketocyclazocine at 10.0

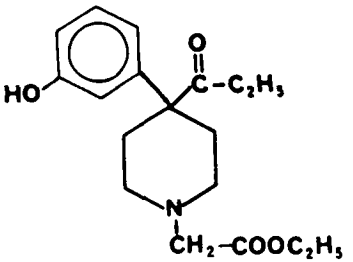
NIH 10453, MCV 4519 N-(Carboethoxypropyl)-N-norketobemidone oxalate



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF -
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ -
4) HP -

NIH 10454, MCV 4520 N-(Carboethoxymethyl)-N-norketobemidone oxalate



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

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 - 17% at 0.1, 20% at 1.0, 43% at 10.0 and 40% at 30.0
4) HP - Inactive at 20.0

NIH 10455[a], 10319, MCV 4521, 4400 N-propargyl-N'-(3-trifluromethylphenyl)piperazine hydrochloride

See NIH 10319

MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

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 - 3.3 (0.34 - 32.54)
4) HP - 50% at 15, 50% at 20.0
5) N -

MONKEY DATA (SDS)	# Animals / Dose (mg.kg s.c.)	3 / 8.0	3 / 2.0

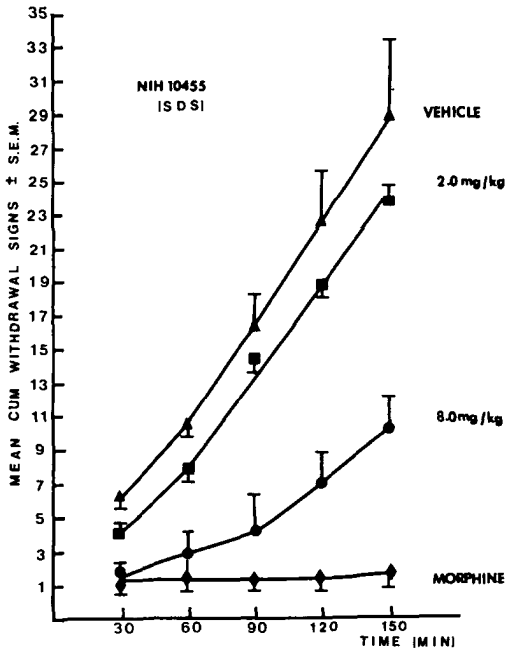
3(Morphine) / 3.0	3(H_2O) / 1 ml/kg

At the highest dose, NIH 10455 substituted quickly, completely but briefly for morphine. At the peak effect, the drug is approximately 1/3 as potent as morphine (see figure). Some eyelid ptosis and slowing were also observed at the highest dose.

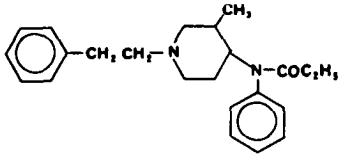
[a]This compound was submitted twice under different code numbers and rodent and monkey studies were conducted each time in blind studies.

NIH 10455[a], 10319, MCV 4521, 4400 N-propargyl-N'-(3-trifluro-methylphenyl)piperazine hydrochloride

(cont)....



NIH 10456, MCV 4522 (±)-cis-3-Methylfentanyl hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

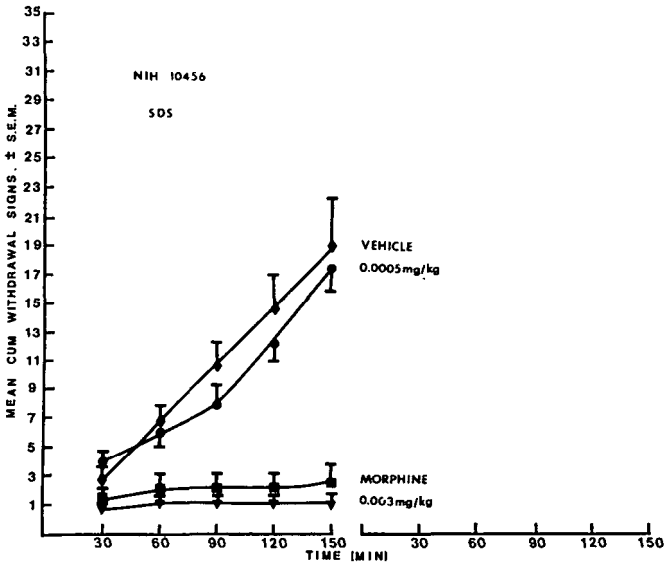
1) TF - 0.002 (0.001 - 0.006)
2) TF vs. M Inactive at 0.0001, 0.01 and 0.1[a]
3) PPQ - 0.0001 (0.00008 - 0.00003)
4) HP - 0.0005 (0.0003 - 0.0007)

MONKEY DATA (SDS)	# Animals / Dose (mg/kg s.c.)	3 / 0.003	3 / 0.0005
3(Morphine) / 3.0	3(H_2O) / 1 ml/kg		

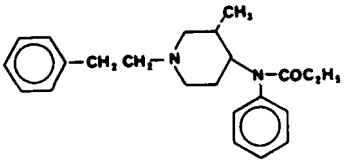
At the highest dose, NIH 10456 substituted completely for morphine (see Figure). In addition, the animals developed body sag and scratched themselves frequently. These signs are usually seen when narcotics are given to non-tolerant monkeys. The drug acted promptly and its duration of action was at least 2 1/2 hr. Its potency is estimated to be 1000 x that of morphine.

NIH 10456, MCV 4522 (±)-cis-3-Methylfentanyl hydrochloride oxalate

(cont)....



NIH 10457, MCV 4523 (±)-trans-3-Methylfentanyl hydrochloride



MOUSE DATA ED50 or AD50 (95% C.L.) mg/kg s.c.)

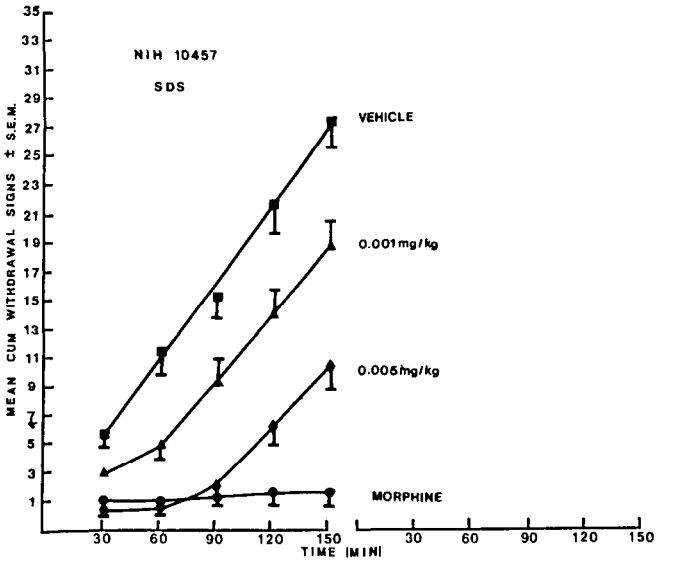
1) TF- 0.01 (0.005 - 0.03)
2) TF vs. M - Inactive at 0.005, 0.05 and 0.5
3) PPQ - 0.002 (0.001 - 0.003)
4) HP - 0.009 (0.006 - 0.013)

MONKEY DATA (SDS)	# Animals			
	Dose (mg/kg s.c.)	3 , 0.003	3 , 0.0005	
3(Morphine), 3.0	3(H_2O) 1 ml/kg			

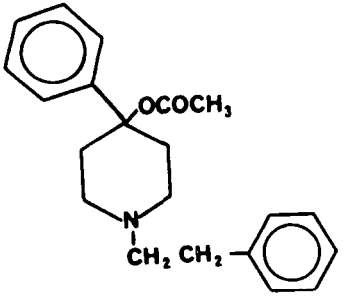
Dose-related suppression of withdrawal signs was observed (see figure). At the highest dose, the drug substituted completely for morphine. The drug acted promptly but its duration of action was relatively short (about 90-120 min) compared with morphine. Potency estimate is 600 x morphine at peak effect.

NIH 10457, MCV 4523 (+)-trans-3-Methylfentanyl hydrochloride

(cont)....



NIH 10460, MCV 4527 1-(2-Phenylethyl)-4-phenyl-4-acetoxypiperidine (PEPAP)



MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF- 0.83 (0.35 - 1.97)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.04 (0.01 - 0.16)
4) HP- 0.74 (0.53 - 1.04)

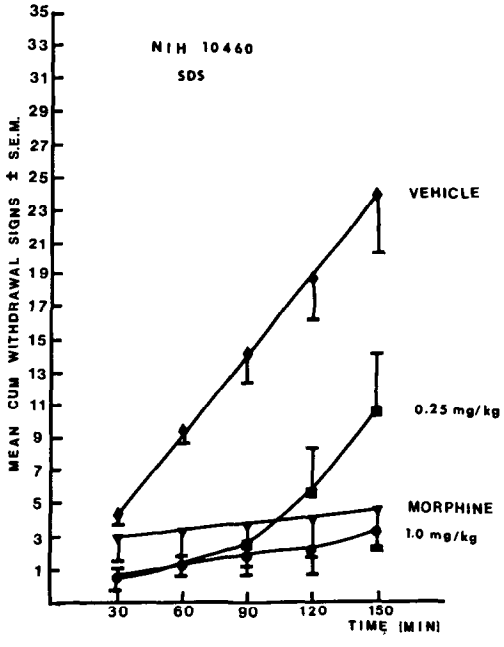
MONKEY DATA (SDS)	# Animals		
Dose (mg/kg s.c,)	4 , 1.0	3 , 0.25	
3(Morphine), 3.0	3(H_2O) 1 ml/kg		

NIH 10460 substituted completely for morphine. The drug acted promptly and its duration of action was about 2 hr (see figure). Its potency is 10 x morphine. Body sag and frequent scratching were seen at the highest dose. In the preliminary study in which the animal received a cummulative dose of 3.0 mg/kg in 1/2 hr,

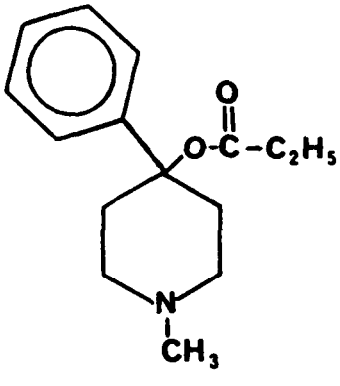
NIH 10460, MCV 4527 1-(2-Phenylethyl)-4-phenyl-4-acetoxypiperidine (PEPAP)

(cont)....

the animal was ataxic and did not require the usual injection of morphine at noon.



NIH 10461, MCV 4528 1-Methyl-4-phenyl-4-propionoxypiperidine hydrochloride (MPPP)

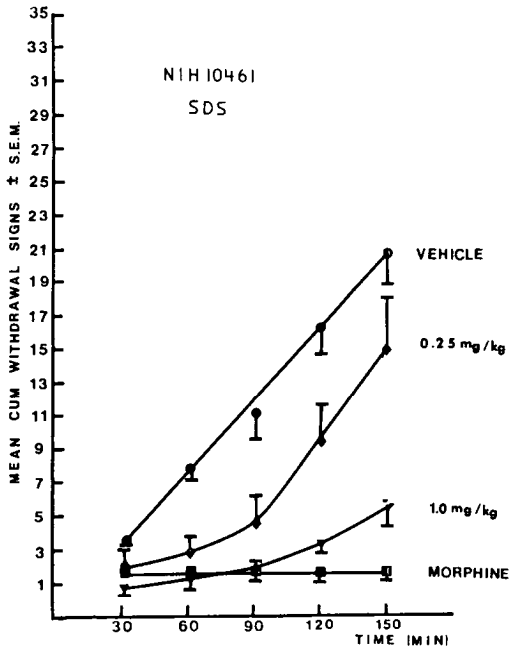


MOUSE DATA ED50 or AD50 (95% C.L.) (mg/kg s.c.)

1) TF - 0.70 (0.34 - 1.43)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.18 (0.01 - 0.23)
4) H P -

NIH 10461, MCV 4528 1-Methyl-4-phenyl-4-propionoxypiperidine hydrochloride (MPPP)

(cont)....



MONKEY DATA (SDS)	# Animals	4	3
	Dose (mg/kg s.c.)	1.0	0.25

3 (Morphine)	3 (H_2O)
3.0	1 ml/kg

NIH 10461 promptly substituted completely for morphine but its duration of action was only 60-90 min (see figure). Scratching was observed at both doses. At its peak effect the drug is approximately 10 times more potent than morphine. In the preliminary study, after a cummulative dose of 3.0 mg/kg in 1/2 hr, ataxia, slowing, scratching and eyelid ptosis were observed. A naloxone challenge (0.04 mg/kg) to this animal precipitated withdrawal. A bloody ejaculate was noted. However, the urine stream was clear.

Evaluation of New Compounds for Opioid Activity: 1986 Annual Report

James H. Woods, Charles B. Smith, Fedor Medzihradsky, Gail D. Winger, and Debra E. Gmerek

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, Medicinal Chemistry Section, NIAKDD, National Institutes of Health, Bethesda, MD. The drugs, which come originally from pharmaceutical companies, universities, and government laboratories, are submitted to Dr. Jacobson, who performs 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 mouse-analgesia data is released to the evaluating laboratory, and the submitter is requested to release the chemical structure within three years.

DRUG DISCRIMINATION IN RHESUS MONKEYS

We currently use two groups of monkeys to test the discriminative effects of submitted drugs. One of these groups are trained to discriminate the administration of the kappa agonist ethylketazocine (EKC). The other groups are trained to discriminate the mu agonists, codeine or etorphine.

The procedures used with the EKC-trained monkeys have been described in 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 equipped 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 trial. The left lever is designated correct if they were given a sham

injection before the start of the trial. Each trial 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 earned 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 trials. During a training session, if EKC is given, it is given on the penultimate trial of that session. Responding on the drug-appropriate lever is reinforced during that trial and on the subsequent, final trial of the day. These last two trials may be preceded by from zero to four sham trials on a training day. A training session of six sham trials is also scheduled from time to time.

With this type of multiple discrete trial training, the animals can be tested with a cumulative dosing procedure. On a test session, the first trial is preceded by an injection of saline, and prior to subsequent trials, 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 trials 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. Each 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 trial of a training session must be on the injection appropriate lever, either sham or EKC.

The procedure for the codeine-trained (or etorphine-trained) monkeys is similar, but not identical. For simplicity, only the codeine training procedure is described here. These animals are also trained and tested in a discrete, multiple-trial paradigm. The main difference between the codeine procedure and the EKC procedure is that the codeine monkeys are on a fixed-ratio 20 schedule rather than a fixed-ratio 100 schedule and they receive a single pellet for correct responses. They can earn as many as 10 pellets during the five minute, food-availability period of each trial, but each pellet is earned by making 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 40 total responses prior to earning the first food pellet of each trial. Tests of the discriminative effects of submitted drugs in the codeine-trained monkeys are also done using a cumulative dosing procedure with dosing criteria identical to those used in the EKC-trained monkeys.

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).

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 was illuminated, the 30th response produced a five-sec intravenous drug injection accompanied by another light that is illuminated during drug delivery. After each injection, a ten-min timeout condition was 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 occur first. Other details of the procedure and initial findings with a variety of narcotics are given in previous reports (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 were obtained for each monkey. A saline substitution was conducted before and after the series of observations on a test drug; the control rates of codeine-reinforced responding were 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 SPECIFIC ^{3}H-ETORPHINE BINDING

Details of the binding assay have been described previously (Woods et al., 1979; Medzihradsky et al., 1984). Briefly, aliquots of a membrane preparation from rat cerebrum were 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 was 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 H-etorphine was determined from log-probit plots of the data. It should be noted that since April 1982 the concentration of ^{3}H-etorphine in the binding assay was reduced from 3.0 nM to 0.5 nM, a concentration approaching the K_D of the radiolabeled opioid. This change was implemented in order to let the determined EC50 approximate the true K_j of a given drug. However, due to the different concentrations of the radiolabeled ligand the EC50 determined since April, 1982 are lower than those obtained previously. For the purpose of reference, Table II contains EC50 values of representative opiates determined in binding assays using 0.5 nM ^{3}H-etorphine. Unless specifically noted in the Report, it should be assumed that 0.5 nM etorphine was used in the binding assays.

INHIBITION OF TWITCH IN ELECTRICALLY-DRIVEN GUINEA PIG ILEUM AND MOUSE VAS DEFERENS PREPARATIONS.

Currently, submitted drugs are evaluated only on the mouse vas deferens preparation. See previous Annual Reports by us to the Committee for details of procedure used in the assessment of some of the drugs reported here. For details, see accompanying article (Smith, C.B. this volume).

TABLE I

MOUSE ANALGESIA. Before submission to The University of Michigan, all compounds are evaluated for analgesic activity by Dr. Arthur E. Jacobson. Shown below are comparative data (ED 50mg/kg) (95% Confidence Interval) from Hot Plate and Nilsen[a] assays. umol/kg

Compound	HOT PLATE (sc/mg/kg)	HOT PLATE (oral, mg/kg)	NILSEN (sc, mg/kg)	NILSEN (oral, mg/kg)
NIH #	(sc, umol/kg)	(oral, umol/kg)	(sc. umol/kg),	(oral, umol/kg)
Morphine sulfate NIH 0001, 9929	0.98 (0.83-1.1)	6.3 (4.7-8.3)	1.3 (1.0-1.7)	8.3 (6.0-11.4)
	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 NIH 0002	6.8 (4.5-10.2)	13.5 (9.7-18.7)	7.4 (4.9-11.0)	14.7 (9.2-23.3)
	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 NIH 4590	0.2 (0.1-0.3)	-	0.2 (0.16-0.3)	2.5 (1.7-3.7)
	0.5 (0.2-0.7)	-	0.5 (0.4-0.7)	6.2 (4.2-9.1)
Meperidine.HCl NIH 5221	5.3 (4.0-7.1)	-	-	-
	18.7 (14.1-25.0)	-	-	-
(-)-Metazocine.HBr NIH 7569	0.6 (0.5-0.9)	10.6 (8.0-14.1)	0.5 (0.3-0.7)	26.0 (21.0-33.0)
	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 I 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.HCl	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 (23.5-44.9)	-	22.8 (15.4-30.9)	-
Naltrexone.HCl			No dose response	
NIH 85Ø3				
Naloxone.HCl			No dose response	
NIH 789Ø				

No antinociceptive activity in hot plate assay: Phenobarbital, amobarbital, diazepam, meprobamate, mescaline, oxazepam, flurazepam.

Chloropromazine.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).

TABLE II

EC50 of representative opioids in displacing the specific binding of 0.5 nM ^{3}H-etorphine in a membrane preparation from rat cerebrum

Compound	EC50 (nM) -NaCl	EC50 (nM) +NaCl	+Na/-Na
UM 911*	14.6	28.3	1.94
Morphine	14.0	23.6	1.69
Dextrorphan	6180	9820	1.59
UM 1071R*	1.14	1.55	1.36
Ketazocine	10.7	14.1	1.32
Ethylketazocine	5.22	6.60	1.26
(-)SKF 10047	4.09	3.93	0.96
Etorphine	0.47	0.37	0.79
(-)Cyclazocine	0.85	0.53	0.63
Naltrexone	1.43	0.63	0.44

NOTE: Binding data for these and other compounds, determined in binding assays using 3.0 nM ^{3}H-etorphine, are included in the 1978 and 1981 ANNUAL REPORTS.

*2-(3-methylfurfuryl)-2'-hydroxy-5,9a-dimethyl-6,7-benzomorphon methane sulfanate.

** IR-5R-9R-2"R-5,9-dimethyl-2'-hydroxy-2-tetrahydrofurfuryl-6,7- benzomorphan hydrochloride

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 III. Also shown are dates of Annual Reports in which results are reported of earlier tests on those compounds conducted at Michigan.

TABLE III

SUMMARY OF TESTS PERFORMED

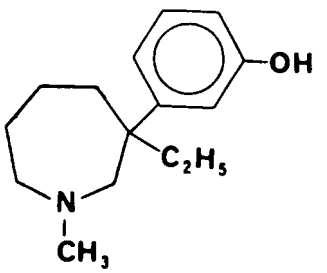
UM	NIH	MCV	CHEMICAL CLASS AND/OR GENERIC NAME	SDS	NW	N	SA	GPI	MVD	BIND	PDS
0888	8683		Meptazinol	1973	1973	-	+	-	+	+	1973
1401	9625	4176	Benzomorphan	-	-	-	-	-	+	_	1982
1313	9931		Morphinan	-	1982	-	-	-	+	+	-
	9935		Morphinan	-	-	-	-	+	-	+	-
	9947	4271	Peptide	-	-	-	-	+	+	+	-
	9948	4272	Peptide	-	-	-	-	+	+	+	-
	9949	4273	Peptide	-	-	-	-	=	=	+	-
	9950	4274	Peptide	-	-	-	-	=	=	+	-
	9955	4275	Phenylmorphan	-	-	-	-	-	+	+	-
1347	9975	4296	Morphinan	1982	-	-	-	-	+	+	-
1399	10036		Benzazocine	+	-	-	-	-	1985	1985	-
	10157	4349	Benzomorphan	+	-	-	-	-	1985	1985	-
	10158	4346	Benzomorphan	-	-	-	-	-	+	+	-
	10319	4440	Phenylpiperazine	-	-	-	-	1985	1985	-	-
	10333	4409	Dihydrocarbosytril	-	-	-	-	-	+	-	-
	10334	4410	Dihydrocarbosytril	-	-	-	-	-	+	+	-
	10335	4411	Dihydrocarbosytril	-	-	-	-	-	+	+	-
	10363	4425	Oxymorphone	-	-	-	-	-	+	+	-
	10366	4423	Oxymorphone	+	-	-	-	-	+	+	-
	10367	4424	Oxymorphone	+	-	-	-	-	+	+	-
	10368	4434	Benzomorphan	-	-	-	-	-	+	+	-
	10369	4435	Benzomorphan	-	-	-	-	-	+	+	-
	10370	4436	Benzomorphan	-	-	-	-	-	+	+	-

TABLE III (continued)

SUMMARY OF TESTS PERFORMED

UM	NIH	MCV	CHEMICAL CLASS AND/OR GENERIC NAME	SDS	NW	N	SA	GPI	MVD	BIND	PDS
	10384	4448	Codeine	-	-	-	-	-	+	+	-
	10440	4503	Phenylpiperidine	-	-	-	-	-	+	+	+
	10453	4519	Phenylpiperidine	-	-	-	-	-	+	+	-
	10454	4520	Phenylpiperidine	-	-	-	-	-	+	+	-
	10456	4522	Methylfentanyl	-	-	-	-	+	+	-	-
	10457	4523	Methylfentanyl	-	-	-	-	-	+	+	-
	10460	4527	Phenylpiperidine	-	-	-	-	-	+	+	-
	10461	4528	Phenylpiperidine	-	-	-	-	-	+	+	-

NIH 8683 3-(3-Ethylhexahydro-1-methyl-1 H-azepin-3-yl)phenol hydrochloride (Meptazinol hydrochloride)



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 5.3 (4.0 - 7.1)

This is an update of our previous report and includes a summary of data obtained to date.

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1600 nanoM in the presence of 150 milliM NaCl.

MOUSE VAS DEFERENS PREPARATION

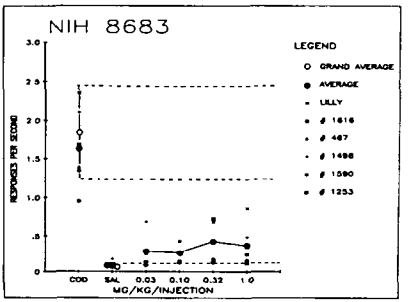
NIH 8683 was studied on the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-8} M to 3 x 10^{-5} M. The EC50 for this drug was not determinable since it did not suppress the twitch at any concentration. In an equimolar concentration NIH 8683 did not reverse the inhibitory action of a maximally effective concentration of morphine (10 microM).

SELF-INJECTION STUDY

Self-administration of NIH 8683 was evaluated in seven rhesus monkeys trained to respond for 0.32 mg/kg/inj codeine (Woods, J.H., Drug and Alcohol Dependence, 1980, 8:233-230). Data from one animal was discarded due to its high rates of saline-maintained responding. Doses of 0.03, 0.10, 0.32 and 1.0 mg/kg/inj NIH 8683 were substituted for codeine during single 130 min sessions. Each dose was tested twice in at least four of the six subjects. Rates maintained by NIH 8683 were slightly above those maintained by saline at all doses. This is illustrated in the accompanying figure, which shows the data from the individual monkeys who are identified by number or by name. There was more variability in the rates of responding maintained by NIH 8683 than is generally the case in this type of test. For this reason, the typical inverted-U shaped function relating dose per injection to rate of responding did not appear with NIH 8683.

NIH 8683 3-(3-Ethylhexahydro-1-methyl-1 H-azepin-3-yl)phenol hydrochloride (Meptazinol hydrochloride)

...(continued)



CODEINE DISCRIMINATION STUDY

Cumulative doses of from 0.1 to 5.6 mg/kg NIH 8683 were administered to two rhesus monkeys trained to discriminate 0.00032 mg/kg etorphine from saline. The procedure has been described (Bertalmio et al., J. Pharmacol. Meth., 7:289-299, 1982). NIH 8683 was tested four times in each monkey. One monkey indicated that NIH 8683 had stimulus properties in common with etorphine on all four occasions, at a dose of 1.0, 1.8 or 3.2. mg/kg. The second monkey indicated that NIH 8683 had stimulus properties in common with etorphine on two of the four occasions, and made the majority of his responses on the etorphine-appropriate lever (65%) on a third occasion. This monkey also showed etorphine-appropriate responding at doses of 1.0 mg/kg or above. Neither monkey responded on the etorphine-appropriate lever with cumulative administration of NIH 8683 following presession administration of 1.0 mg/kg/Win 44441 (quadazocine). Response rates were suppressed by doses of NIH 8683 of 3.2 or 5.6 mg/kg, and this was not modified by prior administration of quadazocine.

ETHYLKETAZOCINE-DISCRIMINATION STUDY

Four monkeys trained to discriminate 0.0032 or 0.0056 mg/kg EKC from sham injections were given increasing doses of NIH 8683 is a procedure identical to that used to evaluate the discriminative effects of etorphine. NIH 8683 produced EKC-appropriate responding in each of the four monkeys at doses of 0.1, 1.0, 3.2 or 10 mg/kg. Response rates were quite suppressed at 10 mg/kg in all monkeys. The evaluation of NIH

NIH 8683 3-(3-Ethylhexahydro-1-methyl-1H-azepin-3-yl)phenol hydrochlride (Meptazinol hydrochloride)

....(continued)

8683 was replicated in two of these monkeys and, on the second occasion, EKC-appropriate responding did not develop.

MORPHINE-DEPENDENT MONKEYS

Doses of 3.0, 6.0 and 12.0 mg/kg were administered to morphine-dependent rhesus monkeys. Increasing doses led to increased severity of the withdrawal signs. (Reported previously.)

PRIMARY DEPENDENCE STUDY

Doses of 8 and then 16 mg/kg NIH 8683 were administered every six hours to rhesus monkeys. Doses of 24 and 32 mg/kg every six hours produced convulsions, as did 16 mg/kg every four hours. Both 8 and 16 mg/kg every six hours produced signs of mild CNS depression mixed with signs of mild CNS stimulation. Chronic administration of 16 mg/kg every six hours resulted in mild physiological dependence demonstrated by withdrawal signs of grade 0-1 on day 42 following nalorphine administration, and withdrawal signs of grade 1-2 on day 33 following naloxone administration. Abrupt withdrawal of NIH 8683 on day 48 resulted in withdrawal signs of grade 0-1. (Reported previously.)

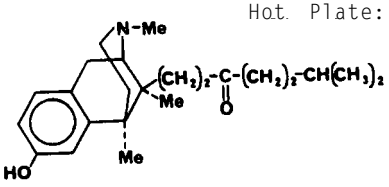
SUMMARY

NIH 8683 displaced etorphine only at very high concentrations. It was neither an opioid agonist nor an antagonist in the mouse vas deferens at the concentrations evaluated.

NIH 8683 did have opioid activity in vivo however. It produced a quadazocine-reversible generalization to etorphine in two rhesus monkeys, a mild degree of opioid-like physiological dependence, and had a weak reinforcing effect. It also generalized to EKC in four rhesus monkeys on initial evaluation, but not on repeat evaluation in two of these animals. It is most unusual to have a drug that produced both EKC and etorphine-like discriminative effects.

NIH 8683 was a clear opioid antagonist in monkeys physiologically dependent to morphine. It was approximately 1/40th the potency of nalorphine in this regard.

NIH 9625 1-[2α,,6α,,11S)-(±)-1-(1,2,3,4,5,6-hexahydro-8-hydroxy-3,6,11-trimethyl-2,6,methano-3-benzazocin-11-yl]-6-methyl-3-heptanone methanesulfonate



MOUSE ANALGESIA, ED50,
Hot Plate: 1.1 (0.8-1.3)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1.96 nanoM in presence of 150 milliM NaCl

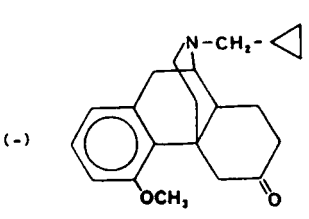
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.94×10^{-8}	93.0 ± 6.3%
After naltrexone	2.60×10^{-7}	92.7 ± 2.0%
With equimolar concentration of naltrexone	Reversal	
Equimolar concentration with morphine	No antagonism	

SUMMARY

NIH 9625 is a morphine-like agonist without antagonistic activity. It is both equipotent and equi-effective when compared with morphine. It is more potent than morphine in the binding assay.

NIH 9931 (-)-N-Cyclopropylmethyl-4-methoxymorphinan-6-one



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 21.7 (17.9-26.3)
Assay: 0% at 10, 40% at 20

NIH 9931 (-)-N-Cyclopropylmethyl-4-methoxymorphinan-6-one

....(continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 23.9 nanoM in presence of 150 milliM NaCl.

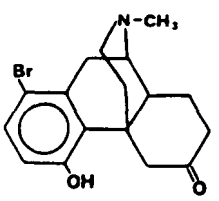
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	3.55×10^{-7}	38.8 ± 4.5%
After naltrexone	3.81×10^{-8}	10.6 ± 0.6%
With equimolar concentration of naltrexone	Reversal	
Equimolar concentration with morphine	Partial reversal	

SUMMARY

NIH 9931 appears to be a complex opiate agonist-antagonist upon the isolated mouse vas deferens preparation. It is significantly more potent in the binding assay than in the mouse vas deferens.

NIH 9935 1-Bromo-4-hydroxy-6-keto-N-methylmorphinan hydrobromide



MOUSE ANALGESIA,
Hot Plate: 14.8 (11.3-19.3)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 459 nanoM in presence of 150 milliM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 9935 did not alter the twitch of the mouse vas deferens preparation at any concentration.

NIH 9935 1-Bromo-4-hydroxy-6-keto-N-methylmorphinan hydrobromide

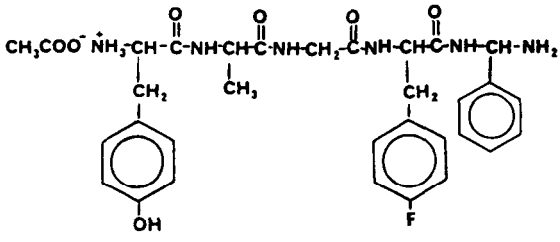
. . . .(continued)

SUMMARY

NIH 9935 possesses weak opioid actions in either preparation.

NIH 9947 L-Tyrosyl-D-alanylglycyl-L-4-fluorophenylalanyl-L-phenylglycinamide acetate

MOUSE ANALGESIA,
Hot Plate: 2.1 (1.5-3.0)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 0.53 nanoM in presence of 150 milliM NaCl.

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	Inhibitory EC50 (M)	Maximum Response
Drug alone	5.32×10^{-10}	64.8 ± 9.2%
After naltrexone	6.72×10^{-8}	43.8 ± 10.3%
After UM 979	2.39×10^{-9}	78.6 ± 11.1%

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.61×10^{-11}	100%
After naltrexone	3.94×10^{-11}	99.2 ± 0.8%
After UM 979	2.07×10^{-11}	100%
After ICI-174-864	3.51×10^{-9}	95.5%

NIH 9947
nylglycinamide acetate

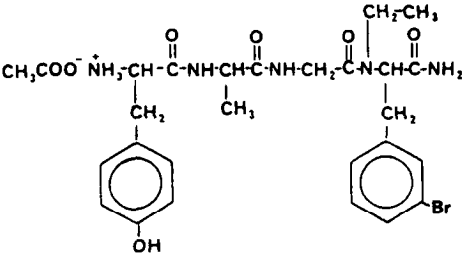
. . . .(continued)

SUMMARY

NIH 9947 is morphine-like upon the guinea pig ileum and is more sensitive to antagonism by naltrexone than by UM 979. NIH 9947 is a very potent delta receptor agonist upon the mouse vas deferens which is antagonized by ICI-174864, but not by UM 979 and only slightly by naltrexone. It is also a very potent displacer of etorphine.

NIH 9948 L-Tyrosyl-D-alanylglycyl-N-α-ethyl-1-m-bromophenylalanine amide acetate

MOUSE ANALGESIA,
Hot Plate: 3.3 (2.3-4.9)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1.23 nanoM in presence of 150 milliM NaCl.

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.06×10^{-8}	36.7 ± 4.5%
After naltrexone	5.72×10^{-8}	67.1 ± 6.4%
After UM 979	4.05×10^{-9}	46.8 ± 9.0%

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.47×10^{-10}	100%
After naltrexone	4.42×10^{-9}	100%
After UM 979	5.24×10^{-10}	100%

NIH 9948 L-Tyrosyl-D-alanylglycyl-N-a-ethyl-1- m -bromopheny-lalanine amide acetate

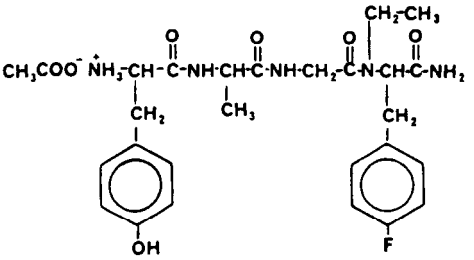
. . . .(continued)

SUMMARY

NIH 9948 is morphine-like upon both the guinea pig ileum and the mouse vas deferens. It is much more potent than morphine upon the vas deferens, and much less efficacious than morphine upon the ileum. It is a potent displacer of etorphine in the binding assay; consistent with actions on the smooth muscle preparation.

NIH 9949 N-α-Methyl-L-tyrosyl-D-alanylglycyl-N-α-ethyl-L-p-fluorophenylalanine amide acetate

MOUSE ANALGESIA,
Hot Plate: 0.07 (0.50-0.97)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1.5 nanoM in presence of 150 milliM NaCl.

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	Inhibitory EC50 (M)	Maximum Response
Drug alone	8.94×10^{-12}	44.9 ± 5.6%
After naltrexone	2.59×10^{-8}	70.6 ± 2.7%
After UM 979	2.50×10^{-9}	61.4 ± 1.8%

NIH 9949 N-α-Methyl-L-tyrosyl-D-alanylglycyl-N-α-ethyl-L-p-fluorophenylalanine amide acetate

. . . .(continued)

MOUSE VAS DEFERENS PREPARATION

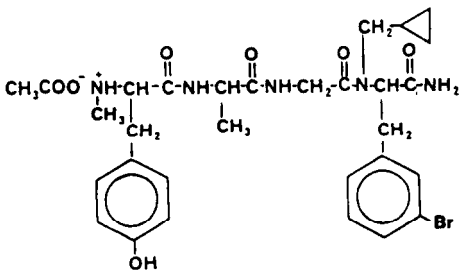
	Inhibitory EC50 (M)	Maximum Response
Drug alone	6.66×10^{-11}	100%
After naltrexone	5.96×10^{-9}	100%
After UM 979	3.51×10^{-10}	100%

SUMMARY

NIH 9949 is morphine-like upon both the guinea pig ileum and the mouse vas deferens. It is much more potent than morphine upon each of the preparations. It displaced ^{3}H-etorphine in rat brain membranes with high potency.

NIH 9950 N-α-Methyl-L-tyrosyl-D-alanylglycyl-N-α-cyclopropylmethyl-L-m-bromophenylalanine amide acetate

MOUSE ANALGESIA,
Hot Plate: 2.1(1.1-4.2)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 0.33 nanoM in presence of 150 milliM NaCl.

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	Inhibitory EC50 (M)	Maximum Response
Drug alone	9.58×10^{-11}	36.8 ± 9.7%
After naltrexone	1.75×10^{-8}	51.6 ± 8.3%
After UM 979	1.35×10^{-9}	46.2 ± 13.9%

172-682 0 - 87 - 16

NIH 9950 N-α-Methyl-L-tyrosyl-D-alanylglycyl-N- α-cyclopropyl-methyl-L-m-bromophenylalanine amide acetate

....(continued)

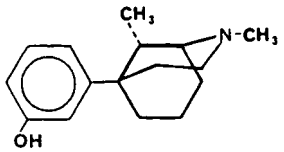
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.03×10^{-9}	100%
After naltrexone	3.79×10^{-8}	100%
After UM 979	7.43×10^{-10}	100%

SUMMARY

NIH 9950 is morphine-like upon both the guinea pig ileum and the mouse vas deferens. It is more potent than morphine in each of the three preparations and is markedly more potent in the binding assay.

NIH 9955 (±)-2,9α-Dimethyl-5-(m-hydroxyphenyl)morpian hydrochloride



MOUSE ANALGESIA,
Hot Plate: 10.0 (6.0-16.8*
Inactive

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 292 nanoM in presence of 150 milliM NaCl

MOUSE VAS DEFERENS PREPARATION

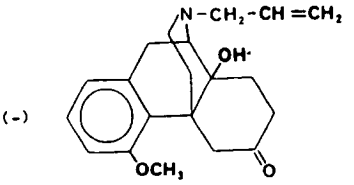
NIH 9955 caused no inhibition of the twitch either with control preparations or in the presence of naltrexone, 10^{-7} M. At a concentration of 3×10^{-5} M, NIH 9955 caused an increase in the magnitude of the twitch.

SUMMARY

This compound may have some opioid activity based only on the findings from the binding assay. This was not corraborated by the findings from the mouse vas deferens.

NIH 9975 (-)-N-Allyl-14-hydroxy-4-methoxymorphinan-6-one

MOUSE ANALGESIA,
Hot Plate: 0% at 20, 20% at 50



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 194 nanoM in the presence of 150 milliM NaCl.

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

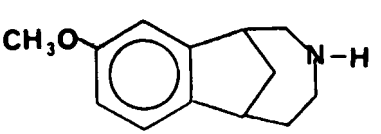
	Inhibitory EC50 (nM)	Maximum Response
Drug alone	3.98 ± 1.03	40.0 ± 3.2%
After naltrexone	3.81 ± 0.75	39.5 ± 3.1%
With equimolar concentration of naltrexone	Slight reversal	
Equimolar concentration with morphine	Partial reversal	

SUMMARY

The findings from both preparations indicate opioid activity. The compound may not have strong agonist actions; based on the relatively low potency in the binding assay and lack of activity in the mouse vas deferens.

NIH 10036 1,2,3,4,5,6-Hexahydro-9-methoxy-1,6-methano-3-benzazocine oxalate

MOUSE ANALGESIA,
Hot Plate: 1.8 (1.1-2.7)



NIH 10036 1,2,3,4,5,6-Hexahydro-9-methoxy-1,6-methano-3-benzazocine oxalate

. . . .(continued)

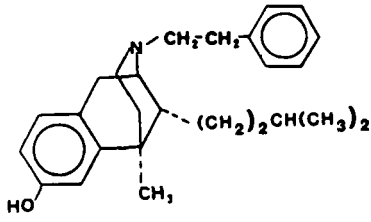
See 1985 Annual Report for in vitro studies.

SINGLE-DOSE SUPPRESSION STUDY

NIH 10036 was given cumulatively to 14-hr withdrawn morphine-dependent monkeys (n=5) in doses of 1.0, 3.0 and 1.9 mg/kg. There was a possible slight exacerbation of withdrawal observed at 3.0 mg/kg, otherwise, no effects at the other doses.

NIH 10157 2'-Hydroxy-5-methyl-9α-(3-methyl)butyl-2-phenethyl-6,7-benzomorphan oxalate

MOUSE ANALGESIA,
Hot Plate: 50% at 50

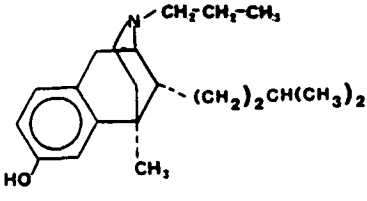


See 1985 Annual Report for in vitro studies.

SINGLE-DOSE SUPPRESSION STUDY

NIH 10157 was given cumulatively to 14-hr withdrawn morphine-dependent rhesus monkeys (n=3) at doses of 5.6 and 10.0 mg/kg. At these doses, NIH 10157 failed to alter withdrawal signs.

NIH 10158 2'-Hydroxy-5-methyl-9α-(3-methyl)butyl-2-propyl-6,7-benzomorphan oxalate



MOUSE ANALGESIA,
Hot Plate: No dose response

NIH 10158 2'-Hydroxy-5-methyl-9α-(3-methyl)butyl-2-propyl-6,7-benzomorphan oxalate

. . . .(continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 165 nanoM in presence of 150 milliM NaCl.

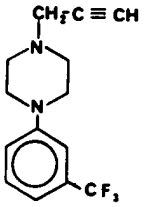
MOUSE VAS DEFERENS PREPARATION

NIH 10158 did not inhibit the contraction of this preparation at any concentration. Concentrations of 10^{-6} M and higher increased markedly the magnitude of the twitch. In a concentration of 10^{-7} M, NIH 10158 did not reverse the inhibition produced by an equimolar concentration of morphine sulfate.

SUMMARY

NIH 10158 is devoid of opiate agonistic or antagonistic actions upon the isolated mouse vas deferens preparation. This compound is quite unusual in having a moderately high affinity for the etorphine binding site without having significant opioid activity in the mouse vas deferens.

NIH 10319 N-Propargyl-N'(3-trifluoromethylphenyl)piperazine.HCl



MOUSE ANALGESIA,
Hot Plate: 60% at 100

See 1985 Annual Report for in vitro studies.

SINGLE-DOSE SUPPRESSION STUDY

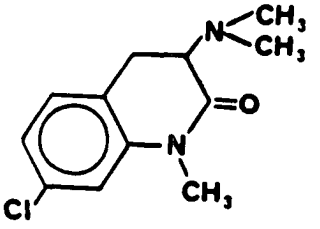
NIH 10319 was given cumulatively to 14-hr withdrawn morphine-dependent rhesus monkeys (n=6) at the following doses: 1.0, 3.2, 10, 18, 32. No effect was observed until 1 hr following 32 mg/kg NIH 10319. At this point there was mydriosis, slight muscle relaxation and decreased abdominal defense reactions to palpitation. However, the monkeys continued to sit on the floor of their cages, showed irritability towards each other and pronounced apprehension

NIH 10319 N-Propargyl-N'(3-trifluoromethylphenyl)piperazine.HCl

. . .(continued)

towards handling. NIH 10319 does not suppress withdrawal in a morphine like manner.

NIH 10333 (±)-7-Chloro-3-dimethylamino-1-methyl-3,4-dihyrocarbostyril hydrochloride.



MOUSE ANALGESIA,
Hot Plate: Inactive (60% at 100)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 100 microM in the presence of 150 milliM NaCl (18% displacement at 100 microM).

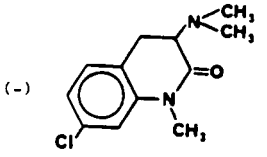
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.25×10^{-5}	39.3 ± 8.5%
After naltrexone	1.89×10^{-5}	42.3 ± 3.3
With equimolar concentration of naltrexone	No reversal	
Equimolar concentration of morphine	No reversal	

SUMMARY

NIH 10333 is devoid of significant opiate agonist or antagonist activity upon the isolated mouse vas deferens preparation. It also failed to displace etorphine at a significant potency.

NIH 10334 (-)-7-Chloro-3-dimethylamino-1-methyl-3,4-dihyrdocarbostyril hydrochloride



MOUSE ANALGESIA,
Hot Plate: Inactive (30% at 50)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 10 microM in the presence of NaCl (20% displacement at 6 microM).

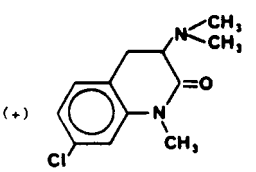
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	5.47×10^{-5}	34.6 ± 6.7%
After naltrexone	5.29×10^{-5}	30.6 ± 5.0%
With equimolar concentration of naltrexone	No reversal	
Equimolar concentration with morphine	No reversal	

SUMMARY

NIH 10334 is devoid of opiate agonistic or antagonistic activity upon the isolated mouse vas deferens preparation nor did it possess significant potency in displacing etorphine in the binding assay.

NIH 10335 (±)-7-Chloro-3-dimethylamino-1-methyl-3,4-dihydrocarbostyril hydrochloride



MOUSE ANALGESIA,
Hot Plate: Inactive (to 50)

NIH 10335 (±)-7-Chloro-3-dimethylamino-1-methyl-3,4-dihydrocarbostyril hydrochloride

. . . .(continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 6 microM in the presence of NaCl.

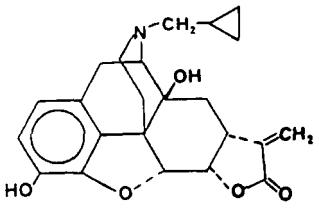
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.38 x 10-^{4}	64.8 ± 3.3%
After naltrexone	1.36 x 10-^{4}	58.2 ± 4.2%
With equimolar concentration of naltrexone	No reversal	
Equimolar concentration with morphine	No reversal	

SUMMARY

NIH 10335 was without significant opioid activity in either of the in vitro assays.

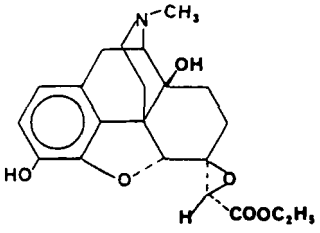
NIH 10363 7α-(1-Carboxyvinyl)-6 α-naltrexal lactone



MOUSE ANALGESIA,
Hot Plate: Inactive (at 20)

Using H_2O, ethanol, dimethyl sulfoxide and diluted HCl (pH 3), NIH 10383 was found to be insoluble in both in vitro assays.

NIH 10366 β-oxido-oxymorphone



MOUSE ANALGESIA,
Hot Plate: Inactive (to 20)

NIH 10366 E-6-Carboethoxymethylene-6B-oxido-oxymorphone

. . . .(continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

IC50 of 40.5 nanoM in presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	8.11×10^{-7}	94.3 ± 11.9%
After naltrexone	2.11×10^{-6}	91.8 ± 6.7%
With equimolar concentration of naltrexone	Partial reversal	
Equimolar concentration with morphine	Increased inhibitory action	

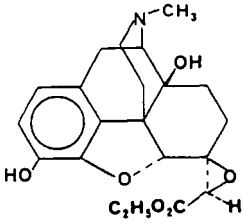
ASSESSMENT IN WITHDRAWN, MORPHINE-DEPENDENT RHESUS MONKEYS

NIH 10366 was given cumulatively (0.03, 0.1, 0.3, 1.0 mg/kg) to withdrawn morphine-dependent monkeys (n=3). There was no observable effect.

SUMMARY

The in vitro findings suggest that NIH 10366 is a morphine-like agonist with a comparable potency. At the doses examined in vivo (too small), morphine did not suppress withdrawal.

NIH 10367 Z-6-Carboethoxymethylene-6B-oxido-oxymorphone acetate



MOUSE ANALGESIA,
Hot Plate: 0.08 (0.06-0.10)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

IC50 of 15.4 nanoM in presence of NaCl.

NIH 10367 Z-6-Carboethoxymethylene-6B-oxido-oxymorphone acetate

. . . .(continued)

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.23×10^{-7}	96.1 ± 9.5%
After naltrexone	2.68×10^{-6}	95.9 ± 11.1%
With equimolar concentration of naltrexone	Complete reversal	
Equimolar concentration with morphine	Increased inhibitory effect	

ASSESSMENT IN WITHDRAWN, MORPHINE-DEPENDENT RHESUS MONKEYS

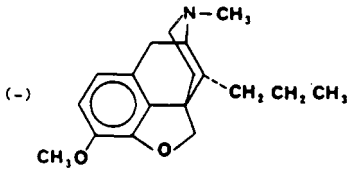
NIH 10367 completely suppressed the intermediately severe withdrawal produced by 14-hrs of deprivation. It was more potent than morphine in this regard, and it has a short duration of action (2 hrs at a dose of 1 mg/kg).

SUMMARY

NIH 10367 is a morphine-like agonist with greater potency in the dependent monkey and in the binding assay, but approximately the same potency as morphine in the vas deferens preparation.

NIH 10368 (-)-2'-Methoxy-2-methyl-5,1'-methyleneoxy-9α-propyl-6,7-benzomorphan hydrochloride

MOUSE ANALGESIA,
Hot Plate: Inactive



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 2.8 microM in presence of NaCl

NIH 10368 (-)-2'-Methoxy-2-methyl-5,1'-methyleneoxy-9a-propyl-6,7-benzomorphan hydrochloride

...(continued)

MOUSE VAS DEFERENS PREPARATION

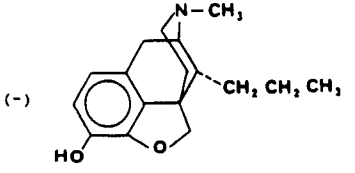
NIH 10368 did not suppress the twitch at any concentration. At a concentration of 10^{-5} M this drug increased markedly the NIH magnitude of the twitch. NIH 10368 at a concentration of 10^{-6} did not block responses to morphine. NIH 10368 at a concentration of 10^{-5} M did not reverse the inhibitory effect of an equivalent concentration of morphine.

SUMMARY

NIH 10368 is devoid of opiate agonistic or antagonistic activity upon the isolated mouse vas deferens preparation. In addition, it displaced etorphine with only very low affinity.

NIH 10369 (-)-2'-Hydroxy-2-methyl-5,1'-methyleneoxy-9α-propyl-6,7-benzomorphan hydrochloride

MOUSE ANALGESIA,
Hot Plate: 1.8 (1.3-2.6)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 39 nanoM in presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	3.50×10^{-7}	77.1 ± 8.0%
After naltrexone	3.32×10^{-6}	43.4 ± 8.1%
With equimolar concentration of naltrexone	Marked reversal	
Equimolar concentration with morphine	Did not alter effect	

NIH 10369 (-)-2'-Hydroxy-2-methyl-5,1'-methyleneoxy-9α-propyl-6,7-benzomorphan hydrochloride

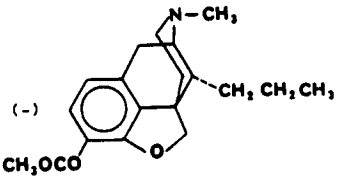
. . . .(continued)

SUMMARY

NIH 10369 has significant opiate activity in both preparations.

NIH 10370 (-)-2'-Acetoxy-2-methyl-5,1'-methyleneoxy-9α-propyl-6,7-benzomorphan hydrochloride

MOUSE ANALGESIA,
Hot Plate: Inactive (20% at 20)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 133 nanoM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

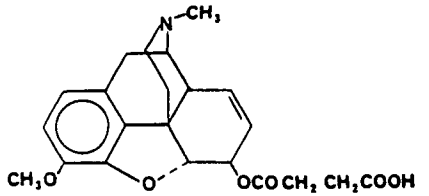
	Inhibitory EC50 (M)	Maximum Response
Drug alone	8.52×10^{-8}	80.6 ± 4.0%
After naltrexone	4.22×10^{-7}	61.6 ± 2.8%
With equimolar concentration of naltrexone	Marked reversal	
Equimolar concentration with morphine	Did not alter effect	

SUMMARY

NIH 10370 is a morphine-like "mu" opiate agonist upon the isolated mouse vas deferens preparation. Its potency in the binding assay is consistent with its action on the vas deferens.

NIH 10384 Codeine succinate

MOUSE ANALGESIA,
Hot Plate: Inactive (to 20)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

IC50 of 19 microM in the presence of NaCl.

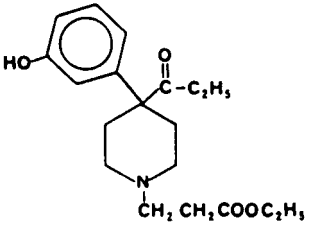
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	4.51×10^{-6}	98.2 ± 0.9%
After naltrexone	8.09×10^{-5}	86.9 ± 3.2%
With equimolar concentration of naltrexone	Reversal	
Equimolar concentration with morphine	Did not alter effect	

SUMMARY

NIH 10440 N-(2-Carbethoxyethyl)-N-norketobemidone hydrobromide

MOUSE ANALGESIA,
Hot Plate: Inactive (to 20)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

IC50 of 235 nanoM in the presence of NaCl.

NIH 10440 N-(2-Carbethoxyethyl)-N-norketobemidone hydrobromide

. . . .(continued)

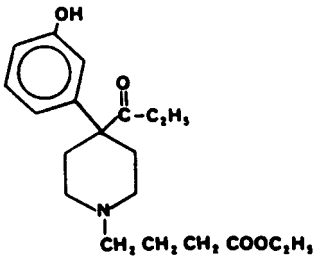
MOUSE VAS DEFERENS PREPARATION

NIH 10440 did not inhibit the twitch of the vas deferens at any concentration studied. Concentrations of 1 microM to 10 microM caused increases in the magnitude of the twitch above basal levels. In the presence of naltrexone, 100 nanoM, there was no appreciable differences in the responses of this preparation to NIH 10440. NIH 10440, 100 nanoM, did not alter responses to concentrations of morphine from 10 nanoM to 10 microM. IN the presence of a maximally effective concentration of morphine (10 microM) an equimolar concentration of NIH 10440 produced the same increase in the magnitude of the twitch as seen in control experiments.

SUMMARY

NIH 10440 appears to be devoid of opioid activity on the mouse vas deferens preparation and has a very low potency in the binding assay. It is unlikely that this compound has significant opiate activity.

NIH 10453 N-(Carboethoxypropyl)-N-norketobemidone oxalate



MOUSE ANALGESIA,
Hot Plate: Inactive (to 20)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 440 nanoM in the presence of NaCl.
MOUSE VAS DEFERENS PREPARATION

In concentrations of 10^{-10} M to 10^{-4} M NIH 10454 produced no significant inhibition of the twitch. At a concentration of 10^{-7} M, it caused a 7-fold shift to the right in the sufentanil concentration-effect curve. The data suggest that NIH 10454 is an opiate antagonist approximately one-third as potent as naltrexone.

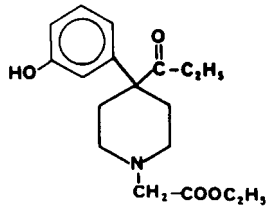
NIH 10453 N-(Carboethoxypropyl)-N-norketobemidone oxalate

. . . .(continued)

SUMMARY

The binding data on NIH 10453 suggest that the compound will be significantly less potent than naloxone and naltrexone. The relative potency of NIH 10453 cannot be determined with the current information on the mouse vas deferens.

NIH 10454 N-Carbethoxy-methyl-N-norketobemidone oxalate



MOUSE ANALGESIA,
Hot Plate: Inactive (16% at 20)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1.7 microM in the presence of NaCl.

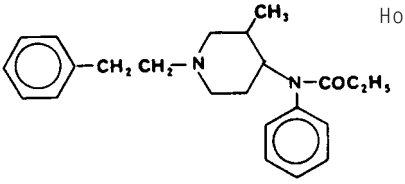
MOUSE VAS DEFERENS PREPARATION

In concentrations of 10^{-10} M to 10^{-4} M NIH 10454 produced no significant inhibition of the twitch. At a concentration of 10^{-7} M, it caused a 7-fold shift to the right in the sufentanil concentration-effect curve. The data suggest that NIH 10454 is an opiate antagonist approximately one-third as potent as naltrexone.

SUMMARY

This compound has lower affinity for the opiate binding site than standards (e.g., naloxone, naltrexone), but appears to have antagonist activity in the vas deferens. Its relative potency in the latter preparation cannot be determined with the current protocol.

NIH 10456 (±)-cis-3-Methylfentanyl hydrochloride



MOUSE ANALGESIA,
Hot Plate: 0.0005 (0.0003-0.0007)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 5.8 nanoM in the presence of NaCl.

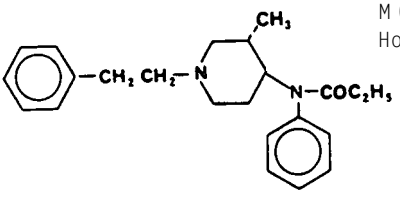
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (nanoM)	Maximum Response
Drug alone	0.62 ± 0.16	100%
After naltrexone	9.75 ± 38.5	100%
With ICI 174,864	1.72 ± 0.66	100%
With equimolar concentration of naltrexone	Complete reversal	
Equimolar concentration with ICI 174,864	Slight rightward shift	

SUMMARY

Both preparations demonstrate that NIH 10456 has potent opioid activity. In the vas deferens, NIH 10456 may have activity at both mu and delta receptors. We have not as yet enough experience with the current protocol to know if this is an unusual finding.

NIH 10457 (±)-trans-3-Methylfentanyl hydrochloride



MOUSE ANALGESIA,
Hot Plate: 0.009 (0.006-0.013)

NIH 10457 (±)-trans-3-Methylfentanyl hydrochloride

....(continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 24 nanoM in the presence of NaCl.

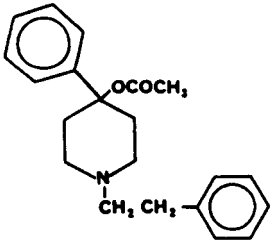
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (nanoM)	Maximum Response
Drug alone	69.2 ± 66.1	100%
After naltrexone	733.0 ± 203.0	100%
With equimolar concentration of naltrexone	Complete reversal	
Equimolar concentration with ICI 174,864	No shift	

SUMMARY

The assays demonstrate NIH 10457 to be a potent opioid. Its action upon the vas deferens appears to be quite complex; this is a novel pattern of activity upon this preparation.

NIH 10460 1-(2-Phenylethyl)-4-phenyl-4-acetoxypiperidine (PEPAP)



MOUSE ANALGESIA,
Hot Plate: 0.74 (0.53-1.04)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 160 nanoM in the presence of NaCl.

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NIH 10460 1-(2-Phenylethyl)-4-phenyl-4-acetoxypiperidine (PEPAP)

....(continued)

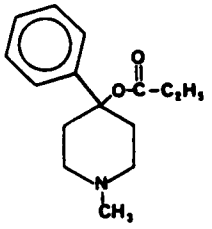
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	3×10^{-9}	52.8%
After naltrexone	6×10	15.8%
With equimolar cncentration of naltrexone	Complete reversal	
Equimolar concentration with sufentanil	Slight reversal	

SUMMARY

On the mouse vas deferens preparation, NIH 10460 appears to be an opiate agonist/antagonist more potent but less efficacious than morphine. The slight reversal of sufentanil suggests some antagonist activity. The potency estimate of 10460 in both preparations suggest that it is less potent than morphine.

NIH 10461 1-Methyl-4-phenyl-4-propionoxypiperidine hydrochloride (MPPP)



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: 0.90 (0.66-1.23)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1110 nanoM in the presence of NaCl.

NIH 10461 1-Methyl-4-phenyl-4-propionoxypiperidine hydrochloride (MPPP)

....(continued)

MOUSE VAS DEFERENS PREPARATION

NIH 10461 did not significantly inhibit the twitch at any concentration studied. NIH 10461, 100 nanoM, did not shift the sufentanil concentration-effect curve. NIH 10461, 10 microM, did not reverse the inhibitory action of a maximally effective concentration of sufentanil.

SUMMARY

The binding assay suggests that NIH 10461 might have opioid activity at high concentrations. This was not confirmed in the mouse vas deferens. Therefore, the compound is not likely to have significant opioid activity.

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Subject Index

In order to simplify the Index, page numbers have been added under NIH headings. The subject subheadings along with page numbers can still be found under the chemical name.

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See Pentazocine

MCV 4336 (NIH 2105; NIH 10124; UM 1411)

See Nalorphine

MCV 4348 [NIH 8848; NIH 10165; UM 975; (±)-2-Cyclopropylmethyl-5-ethyl-8-oxo-9 α-methyl-6,7-benzomorphan methane sulfonate]

See Ethylketocyclazocine

MCV 4349 (NIH 10157)

See 2'-Hydroxy-5-methyl-9 α-(3-methyl)butyl-2-phenethyl-6,7-benzomorphan

MCV 4385 (NIH 8359; NIH 10274; UM 686)

See Nalbuphine hydrochloride

MCV 4386 (NIH 8791; NIH 10275; UM 941)

See Butorphanol tartrate

MCV 4388 (NIH 10249)

See N-(2,3-Epoxypropyl)normetazocine

MCV 4389 (NIH 10253)

See N-(N-Phenylguanidino)-5,9-dimethyl-6,7-benzomorphan hydrochloride

MCV 4390 (NIH 10258)

See 3-(N-Propionyl)anilinoquinuclidine hydrochloride

MCV 4403 [NIH 8683; UM 888; *m*-(3-Ethyl-l-methylhexahydro-lH-azepine-3-yl)phenol hydrochloride]

See Meptazinol hydrochloride

MCV 4404 (NIH 10322)

See 6α-(2-Carboxy-1-propcnyl)-naltrex-6 β-o1-γ-lactone

MCV 4408 (NIH 10324)

See 6α-(2-Carboxy-l-propcnyl)-oxymorph-6 β-ol-y-lactone

MCV 4409 (NIH 10333)

See (±)-7-Chloro-3-dimetylamino-l-methyl-3,4-dihydrocarbostyri1 hydrochloride

MCV 4410 (NIH 10334)

See (-)-7-Chloro-3-dimethylamino-l-methyl-3,4-dihydrocarbostyri1 hydrochloride

MCV 4411 (NIH 10335)

See (+)-7-Chloro-3-dimethylamino-l-methyl-3,4-dihydrocarbostyril hydrochloride

MCV 4414 (NIH 8847; NIH 10364; UM 974)

See (±)-Ketocyclazocine

MCV 4417 (NIH 10347)

See 2-Benzyl-3-phenyl-$\Delta^{2,3}$-quinuclidine hydrochloride

MCV 4418 (NIH 10348)

See (-)-9 α-(2-Carbethoxyethyl)-2'-methoxy-2-methyl-5,1'-methylencoxy-6,7-benzomorphan hydrochloride

MCV 4419 (NIH 10349)

See (-)-9 α-(3-Hydroxypropyl)-2'-methoxy-2-methyl-5,1'-methylencoxya-6,7-benzomorphan hydrochloride

MCV 4422 (NIH 10362)

See 2-Benzyl-3-phenyl-3-acetoxyquinuclidine hydrochloride

MCV 4423 (NIH 10366)

See E-6-Carbocthoxymethylene-6 β-oxido-oxymorphone

MCV 4424 (NIH 10367)

See Z-6-Carbethoxymethylene-6 β-oxido-oxymorphone acetate

MCV 4425 (NIH 10363)

See 7α-(1-Carboxyvinyl)-6 α-naltrexal lactone

MCV 4426 (NIH 10365; 17-cyclopropylmethyl-3,14-dihydroxy-4,5-epoxy-7-methylenemorphinan hydrochloride)

See Nalmefene

MCV 4430 [NIH 7569; MCV 4501; UM 743; (-)-2'-Hydroxy-2,5,9α-trimethyl-6,7-benzomorphan hydrobremide]

See (-)-Metazocine

MCV 4431 [NIH 7571; MCV 4502; UM 744; (+)-2'-Hydroxy-2,5,9α-trimethyl-6,7-benzomolphan hydrobromide]

See (+)-Metazocine

MCV 4433 (NIH 10357)

See 17-Cyclopropylmethyl-3,14-dihydroxy-4,5 α-epoxy-6ß-fluoromorphinan hydrochloride

MCV 4434 (NIH 10368)

See (-)-2'-Methoxy-2-methyl-5,1'-methyleneoxy-9α-propyl-6,7-benzomorphan hydrochloride

MCV 4435 (NIH 10369)

See (-)-2'-Hydroxy-2-methyl-5,1'-methyleneoxy-9α-propyl-6,7-benzomorphan hydrochloride

MCV 4436 (NIH 10370)

See (-)-2'-Acetoxy-2-methyl-5,1'-methyleneoxy-9 α-propyl-6,7-benzomorphan hydrochloride

MCV 4437 (NIH 10372)

See 2,2-Dimethyl-3-dimethylamino-7-hydroxy-1-tetralone hydrobromide

MCV 4438 (NIH 10093)

See 7α-Amino-6,14-endoethenotetrahydrooripavine, 412, 380

MCV 4457 (NIH 10385; MCV 2820; UM 118)

See 6-Dimethylamino-4,14-diphenylhexan-3-one hydrochloride

MCV 4458 (NIH 10386)

See 6-Dimethylamino-C(4-fluorophenyl)-4-phenylhexan-3-one hydrochloride

MCV 4459 (NIH 10387)

See 6-Dimethylamino-4-(4-chlorophenyl)-4-phenylhexan-3-one hydrochloride

MCV 4460 (NIH 10388)

See 6-Dimethylamino-4-(4-bromophenyl)-4-phenylhexan-3-one hydrochloride

MCV 4462 (NIH 10393)

See 6-Dimethylamino-4-cyclohexyl-4-phenylhexan-3-one hydrochloride

MCV 4463 (NIH 10396)

See 6-Dimethylamino-4,4-(4,4'-difluoro)diphenylhexan-3-one hydrochloride

MCV 44604 (NIH 10391)

See 6-Dimethylamino-4-(4-methylphenyl)-4-phenylhexan-3-one hydrochloride

MCV 4467 (NIH 10389)

See 6-Dimethylamino-4-(4-methoxyphenyl)-4-phenylhexan-3-one hydrochloride

MCV 4475 (NIH 10429)

See L-Tryptophan tartrate

MCV 4448 (NIH 10384; 6-Succinylcodeine)
See Codeine succinate

MCV 4449 (NIH 7890; UM 426)
See Naloxone

MCV 4455 (NIH 10379)
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MCV 4494 (NIH 10433)
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