National Institute on Drug Abuse

90

Research

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Problems of Drug Dependence 1988

Proceedings of the 50th 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, 1988

Proceedings of the 50th Annual Scientific Meeting, The Committee on Problems of Drug Dependence, Inc.

Editor: Louis S. Harris, Ph.D.

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Foreword

In this volume the National Institute on Drug Abuse is publishing once again the scientific presentations from the annual scientific meeting of the Committee on Problems of Drug Dependenre. This 50th annual meeting of the CPDD was held in North Falmouth. Massachusetts, in June 1988. In the typical CPDD meeting, outstanding investigators from many disciplines present their current research on a wide range of topics related to substance abuse. The 1988 meeting was no exception. The topics ranged from the biochemistry of the neuron to the clinical treatment of drug dependent patients.

Members of the scientific community and other interested readers of the NIDA Research Monograph series will find this volume to be a valuable "state of the art" summary of research into the many factors involved in drug abuse.

John J. Boren, Ph.D.
Clinical and Behavioral Pharmacology Branch
Division of Clinical Research
National Institute on Drug Abuse

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AIDS and Intravenous Drug Abuse

C. Schuster and R. Pickens

The Acquired Immunodeficiency Syndrome (AIDS) is a fatal, infectious disease with no known cure. It is caused by the human immunodeficiency virus (HIV), which is transmitted by intimate sexual contact, exposure to infected blood, or from mother to child across the placenta or during delivery. At present there is no vaccine to prevent AIDS or HIV infection, and therapeutics are of only limited value in increasing life expectancy. Control of the virus is complicated by the fact that most infected individuals come from stigmatized groups--gay men and intravenous drug abusers--which has confounded the application of public health control measures with moralistic concerns. It has evoked in the public many of the same fears that were present in earlier epidemics, including bubonic plague and syphilis, and has presented a serious challenge to our social and biomedical research programs and medical care system (Institute of Medicine, 1988).

While it is a fatal disease, AIDS is entirely preventable. Prevention requires modification of the behaviors that expose an individual to the AIDS virus. However, the high-risk behaviors that are most frequently associated with HIV transmission--sexual behavior and intravenous drug abuse--are difficult to modify. Thus, curbing the spread of AIDS will be a difficult and complicated task, and a challenge to our generation.

This paper will review epidemiological and clinical data on the role of intravenous drug abuse in HIV infection and AIDS, and will describe the research and prevention programs established by the National Institute on Drug Abuse (NIDA) that are directed towards controlling this problem, These programs are part of a larger federal, state, and local effort designed to eliminate spread of HIV infection among drug abusers, and between drug abusers and their sexual partners and children.

HIV Infection

HIV infection is associated with a number of conditions, ranging from the production of antibodies only (with no clinical symptoms), to a compromise in immune function resulting in a wide range of fatal opportunistic infections or diseases (e.g., pneumocystis carinii, Kaposi's Sarcoma). While HIV infection is more important from a public health perspective, most public attention has been focused on the end-stage diseases of HIV infection, At present, therapeutics are of only limited benefit in the treatment of HIV-related diseases, and no vaccine for

preventing HIV infection or the development of clinical symptoms resulting from HIV infection has been found.

The incubation period between HIV infection and onset of clinical symptoms is variable, and may range from several months to several years. Over time, more and more HIV infected persons develop AIDS. Based on data from a cohort of homosexual men in San Francisco, 22 percent of seropositive subjects had developed AIDS after the first three years of the study, and, based on actuarial projections, half of the seropositives were expected to do so within six years, with another quarter developing an AIDS-related condition (Moss et al., 1988).

Epidemiology of AIDS

During May, 1988, the total number of reported AIDS cases in the U.S. reached 62,740. The rate of new AIDS cases has been increasing steadily since mid-1981 when the first cases were reported. By the end of 1992, the number of AIDS cases in the U.S. is expected to increase to a cumulative total of approximately 365,000, with approximately 263,000 deaths (Centers for Disease Control, 1988b).

Table 1 shows data from the AIDS Weekly Surveillance Report by the Centers for Disease Control for May 23, 1988. Of the 62,740 AIDS cases reported to that date, 91 percent involved males and 9 percent involved females. Among adult and adolescent males, the majority of AIDS cases were associated with HIV transmission by homosexual or bisexual contact. Intravenous drug use was the second most frequent mode of transmission (28 percent of AIDS cases), which included 7 percent reporting intravenous drug use and homosexual/ bisexual contact, and 21 percent reporting only intravenous drug use as risk factors. Among adult and adolescent females, the majority of AIDS cases were associated with being an intravenous drug user (54 percent). An additional number of AIDS cases (both male and female) is associated with being the sexual partner of an intravenous drug user (Centers for Disease Control, 1988a).

Of the total number of AIDS cases, 2 percent involved children under 13 years of age. For these children, most AIDS cases were associated with having a parent with AIDS or at risk for AIDS (77 percent). For the vast majority of these cases, the parent with or at risk for AIDS was either a mother who was an intravenous drug user, or the sexual partner of an intravenous drug user (Centers for Disease Control, 1988a).

Table 2 shows data from the same report for racial/ethnic groups. While AIDS cases have been reported for all racial/ethnic groups, the proportion of AIDS cases among U.S. blacks and Hispanics is substantially greater than their proportions in the U.S. general population. In an analysis of AIDS surveillance data from June 1, 1981-July 4, 1988, the Centers for Disease Control reported U.S. AIDS patients to be disproportionately black (26 percent) and Hispanic (13 percent), compared to proportions of these groups in the U.S. population (i.e., 12 percent black and 6 percent Hispanic), Among men with AIDS, 34 percent of blacks and 35 percent of Hispanics reported their only risk factor to be intravenous drug use or being the sex partner of a female intravenous drug user. Among females with AIDS, 74 percent of blacks and 80 percent of Hispanics reported only intravenous drug use or being the sex partner of a male intravenous drug user as a risk factor (Selik, Castro, and Pappaioanou, 1988).

TABLE 1[1]. AIDS CASES BY TRANSMISSION CATEGORIES (MAY 23, 1988)[2]

	MALES				FEMALES				TOTAL			
	Since Jan 1 Number	(%)	Cumulative Number	(%)	Since Jan 1 Number	(%)	Cumulative Number	(%)	Since Jan 1 Number	(%)	Cumulative Number	(%)
ADULTS/ADOLESCENTS												
Homosexual/Bisexual Male	6772	(62)	39001	(69)					6772	(56)	39001	(63)
Intravenous (IV) Drug Abuser	2283	(21)	8978	(16)	686	(54)	2546	(52)	2969	(24)	11524	(19)
Homosexual Male and IV Drug Abuser	800	(7)	4568	(8)					800	(7)	4568	(7)
Hemophilia/Coagulation Disorder	122	(1)	585	(1)	3	(0)	21	(0)	125	(1)	606	(1)
Heterosexual Cases[3]	206	(2)	1120	(2)	311	(25)	1415	(29)	517	(4)	2535	(4)
Transfusion, Blood/Components	227	(2)	990	(2)	137	(11)	537	(11)	364	(3)	1527	(2)
Undetermined[4]	485	(4)	1578	(3)	122	(10)	411	(8)	607	(5)	1989	(3)
SUBTOTAL [% of all cases]	10895	[90]	56820	[92]	1259	[10]	4930	[8]	12154	[100]	61750	[100]
CHILDREN[5]												
Hemophilia/Coagulation Disorder	14	(11)	53	(10)	1	(1)	3	(1)	15	(6)	56	(6)
Parent with/at risk of AIDS[6]	91	(68)	384	(71)	84	(85)	378	(84)	175	(75)	762	(77)
Transfusion, Blood/Components	23	(17)	84	(16)	10	(10)	50	(11)	33	(14)	134	(14)
Undetermined[4]	5	(4)	19	(4)	4	(4)	19	(4)	9	(4)	38	(4)
SUBTOTAL [% of all cases]	133	[57]	540	[55]	99	[43]	450	[45]	232	[100]	990	[100]
TOTAL [% of all cases]	11028	[89]	57360	[91]	1358	[11]	5380	[9]	12386	[100]	62740[7]	[100]

[1] These are provisional data from Centers for Disease Control (1988a); [2]Cases with more than one risk factor other than the combinations listed in the-tables or footnotes are tabulated only in the category listed first; [3] Includes 1518 persons (327 men, 1191 women) who have had heterosexual contact with a person with AIDS or at risk for AIDS and 1017 persons (793 men, 224 women) without other identified risks who were born in countries in which heterosexual transmission is believed to play a major role although precise means of transmission have not yet been fully defined; [4] Includes patients on whom risk information is incomplete (due to death, refusal to be interviewed or loss to follow-up), patients still under investigation, men reported only to have had heterosexual contact with a prostitute, and interviewed patients for whom no specific risk was identified; also includes one health-care worker who seroconverted to HIV and developed AIDS after documented needlestick to blood.; [5] Includes all patients under 13 years of age at time of diagnosis; [6] Epidemiologic data suggest transmission from an infected mother to her fetus or infant during the perinatal period; [7] Includes 6457 patients who meet only the 1987 revised surveillance definition for AIDS.

TABLE 2[1]. AIDS CASES BY RACIAL/ETHNIC GROUP (MAY 23, 1988)[2]

	WHITE NOT HISPANIC Cumulative Number	(%)	BLACK NOT HISPANIC Cumaltive Number	(%)	HISPANIC Cumulative Number	(%)	OTHER[8] IJNKNOWN Cumulative Number	(%)	TOTAL Cumulative Number	(%)
ADULTS/ADOLESCENTS										
Homosexual/Bisexual Male	28710	(78)	6010	(38)	3891	(44)	390	(68)	39001	(63)
Intravenous (IV) Drug Abuser	2229	(6)	5846	(37)	3392	(38)	57	(10)	11524	(19)
Homosexual Male and IV Drug Abuser	2796	(8)	1111	(7)	638	(7)	23	(4)	4568	(7)
Hemophilia/Coagulation Disorder	513	(1)	41	(0)	40	(0)	12	(2)	606	(1)
Heterosexual Cases[3]	458	(1)	1723	(11)	342	(4)	12	(2)	2535	(4)
Transfusion, Blood/Components	1136	(3)	234	(1)	119	(1)	38	(7)	1527	(2)
Undetermined[4]	747	(2)	793	(5)	406	(5)	43	(7)	1989	(3)
SUBTOTAL [% of all cases]	36589	[59]	15758	[26]	8828	[14]	575	[1]	61750	[100]
CHILDREN[5]										
Hemophilia/Coagulation Disorder	41	(18)	6	(1)	7	(3)	2	(20)	56	(6)
Parent with/at risk of AIDS[6]	111	(47)	464	(89)	180	(81)	7	(70)	762	(77)
Transfusion, Blood/Components	75	(32)	31	(6)	27	(12)	1	(10)	134	(14)
Undetermined[4]	7	(3)	23	(4)	8	(4)			38	(4)
SUBTOTAL [% of all cases]	234	[24]	524	[53]	222	[22]	10	[1]	990	[100]
TOTAL	36823	[59]	16282	[26]	9050	[14]	585	[1]	62740[7]	[100]

[1] These are provisional data from Centers for Disease Control (1988a); [2] Cases with more than one risk factor other than the combinations listed in the tables or footnotes are tabulated only in the category listed first; [3] Includes 1518 persons (327 men, 1191 women) who have had heterosexual contact with a person with AIDS or at risk for AIDS and 1017 persons (793 men, 224 women) without other identified risks who were born in countries in which heterosexual transmission is believed to play a major role although precise means of transmission have not yet been fully defined; [4] Includes patients on whom risk information is incomplete (due to death, refusal to be interviewed or loss to follow-up), patients still under investigation, men reported only to have had heterosexual contact with a prostitute, and interviewed patients for whom no specific risk was identified; also includes one health-care worker who seroconvened to HIV and developed AIDS after documented needlestick to blood.; [5] Includes all patients under 13 years of age at time of diagnosis; [6] Epidemiologic data suggest transmission from an infected mother to her fetus or infant during the perinatal period; [7] Includes 6457 patients who meet only the 1987 revised surveillance definition for AIDS; [8] Includes patients whose race/ethnicity is Asian/Pacific Islander (367 persons) and American Indian/Alaskan Native (64) persons.

AIDS is not limited to the U.S. but has been reported world-wide. In June 1988, the World Health Organization reported the cumulative AIDS cases by continent to be 11,753 for Africa; 74,862 for the Americas; 243 for Asia; 12,594 for Europe; and 958 for Oceania, Within Europe, the highest cumulative prevalence rates per million population are in Switzerland (76.1), France (75.7) and Denmark (57.3), compared to 286 per million population in the U.S. In Europe, AIDS cases associated with intravenous drug abuse have increased rapidly in recent years, particularly in Italy, Spain and France (World Health Organization, 1988).

HIV Infection in Intravenous Drug Abusers

While attention of the general public is focused on the end-stage diseases of AIDS, a more important measure of the extent of the health problem is prevalence of HIV infection. Given that many HIV-infected individuals will later develop AIDS, prevalence of HIV infection is an early indicator of the potential size of the future AIDS problem. The Center for Disease Control (1988b) currently estimates that 1.0 - 1.5 million individuals in the U.S. may be infected with HIV, with significant differences in infection rates for various regions of the country.

A number of studies have been conducted to determine HIV antibody rates among intravenous drug abusers. About 21 percent of the total intravenous drug abuser population in the U.S. is believed to be HIV infected at the present time. Most of these studies have involved opiate addicts in methadone detoxification or maintenance programs. Seropositivity rates range from 0 percent in many major U.S. cities to 60 percent in certain areas of New York, New Jersey, and Puerto Rico. Based on these data, a multiagency federal analysis team in 1987 estimated HIV seropositivity rate of 25 percent among an estimated 900,000 regular (at least weekly) intravenous drug users and 5 percent among an estimated 200,000 occasional intravenous drug users (Dondero *et al.,* 1987).

Table 3 shows current HIV seropositivity rates among intravenous drug abusers in various cities across the U.S. (Lange *et al.,* 1988; Battjes and Pickens, 1988a). Marked geographical differences in seropositivity rates are evident, with the highest rates in New York City (Harlem and Brooklyn) and in certain areas of New Jersey (Asbury Park). Other cities (Los Angeles, San Antonio, and Tampa) currently show very low HIV seropositivity rates, suggesting a window of opportunity for HIV prevention efforts.

TABLE 3
HIV SEROPOSITIVITY RATES AMONG INTRAVENOUS DRUG ABUSERS

	Lange *et. al.* 1988)	Battjes and Pickens (1988)
New York City	61%	60%
Asbury Park, New Jersey		43%
Baltimore, Maryland	29%	--
Trenton, New Jersey	--	12%
Denver, Colorado	5%	
Los Angeles/S. California		3%
San Antonio, Texas	2%	1%
Tampa, Florida	0%	--

HIV Transmission in Drug Abuse

Among intravenous drug abusers, HIV is transmitted primarily by the sharing of injection equipment ("needle sharing"), when a small amount of blood from an infected person is received by an uninfected person. Addicts frequently share needles, syringes, and other paraphernalia in the injection of illicit drugs, as such sharing has both practical and social significance (Battjes and Pickens, 1988c). Lacking sterile equipment, addicts often share syringes with other addicts to obtain a drug "high" or to avoid withdrawal symptoms. Since possession of syringes is illegal in many states, using another's equipment minimizes the chance of arrest. Sharing is also a deeply entrenched social ritual among intravenous drug abusers, reflecting companionship and trust. In a recent study across five U.S. cities, 93 percent of addicts in methadone treatment reported having shared injection equipment, with 26 percent of addicts who reported needle sharing indicating they shared on a daily basis for the past five years (Battjes and Pickens, 1988b).

HIV transmission between intravenous drug abusers and others is also possible by sexual contact. Approximately one-third of intravenous drug users with AIDS also have homosexual and/or bisexual behavior as a risk factor (see Table 1). Except for intravenous drug abuse, no other risk factor for AIDS was reported for the remaining two-thirds of the intravenous drug users with AIDS. As discussed previously, heterosexual transmission of HIV is closely associated with intravenous drug use. About 69 percent of U.S.-born AIDS cases attributed to heterosexual transmission involve sexual contact with an intravenous drug user (Chamberland, 1987). Because of its association with heterosexual transmission, many health authorities are concerned that intravenous drug abusers may be a major vector for the spread of HIV infection to the general population (Public Health Service, 1986). Of particular concern is the fact that many intravenous drug users are also prostitutes, which may which may increase the risk of sexual transmission of HIV.

While most attention has been focused on HIV transmission by intravenous drug use, it is important to recognize that HIV transmission may be associated with other. routes of drug administration as well. For example, prostitution may be necessary to afford "crack" cocaine. Regardless of the route of drug administration, whenever a person engages in promiscuous unprotected sex, the risk for HIV infection is increased (Haverkos and Edelman, 1988). Sex for drugs is reported to be common in "crack houses," where individuals' often engage in unprotected sexual activity (Friedman *et al,* 1988). Among intravenous drug abusers, changing sexual behavior may be difficult. In a recent study, whereas approximately 86 percent of intravenous drug abusers reported they had changed their drug-injection practices to reduce AIDS risk, only 14 percent reported they had started or increased their use of condoms (Battjes and Pickens, 1988b).

Children of intravenous drug abusers are susceptible to HIV infection by perinatal transmission. HIV may be transmitted between an infected mother and her child either prior to or during birth, or shortly thereafter. Approximately three-fourths of all perinatal AIDS cases have occurred in children of intravenous drug abusers (Rogers *et al.,* 1987).

NIDA's AIDS Program

NIDA is the lead federal institute for-drug abuse research. Its mission is to enhance knowledge that will reduce mortality and morbidity associated with illicit

drug use. To carry out its mission, NIDA supports extramural research and research training through funding of grants and contracts, conducts intramural research at the Addiction Research Center, and disseminates research findings to practitioners in the drug abuse field. NIDA also exerts a leadership role by establishing research priorities in line with scientific developments and national trends in drug abuse, and making policy recommendations based on knowledge derived from research findings. NIDA's activities in the AIDS area are reflective of these research and leadership functions.

Background and overview of the program. Experience suggests that information alone will not be sufficient for changing the behavior of intravenous drug abusers. It is well recognized that intravenous drug abusers do not significantly change their drug-use practices after receiving information about the harmful effects of illicit drugs, or even after a life-threatening experience caused by illicit drug use. Indeed, one recent study reported that the majority of intravenous drug abusers continued to share their injection equipment although over 90 percent knew the health consequences for HIV infection (Flynn *et al.,* 1987).

To be effective in controlling the spread of AIDS, most public health authorities believe that preventive interventions must include application of behavior-change strategies in addition to disseminating information about AIDS (Public Health Service, 1987). For intravenous drug abusers, this will involve eliminating behaviors that place a person at high risk for HIV infection (i.e., needle sharing and unprotected sexual activity). To assist in the development of such strategies, NIDA supports research that expands our knowledge base concerning preclinical, clinical, and epidemiological factors that are involved in intravenous and other types of drug abuse that are related to AIDS and HIV infection, investigates the role of abused drugs and drug-abuse practices that are causally related or serve as cofactors to AIDS and HIV infection, and develops and evaluates the effectiveness of behavior-change strategies for reducing the risks of AIDS and HIV infection.

As part of its leadership role, NIDA coordinates federal activities in the AIDS/drug abuse area by providing chairpersons for the Subgroup on Addiction and Behavior of the PHS Executive Task Force on AIDS and the Treatment Subcommittee of the National Drug Policy Board, maintains liaison with other public and private interest groups, holds scientific and policy conferences on topics related to AIDS and HIV infection, monitors incidence and prevalence of HIV infection in the U.S. drug abuse population, and develops strategies for controlling the spread of AIDS among intravenous drug abusers, their sexual partners and their children.

While a number of federal agencies are involved in AIDS prevention activities, NIDA's AIDS program is unique in that it focuses on drug abuse treatment as an AIDS prevention strategy. Among intravenous drug abusers, epidemiological studies show HIV infection is spread largely by needle sharing and promiscuous unprotected sexual behavior. Both of these behaviors are often maintained by drug dependence, a chronic biobehavioral disorder that is highly resistant to change without outside intervention. To overcome their dependence, most intravenous drug abusers require repeated and prolonged drug abuse treatment. Such treatment is important in the control of AIDS, for as long as intravenous drug abusers are drug dependent, they will continue to engage in behaviors that put them at risk for HIV infection (Hubbard *et. al.,* 1988). Unfortunately, drug abuse treatment currently lacks the capacity to deliver services to the large number of intravenous drug abusers who need treatment, as well as by deficiencies in

existing treatment approaches that contribute to unacceptably high rates of program dropout and relapse after treatment. Both issues are addressed in NIDA's AIDS program.

The NIDA AIDS program consists of six elements: (1) research to improve the effectiveness of drug abuse treatment; (2) basic AIDS research; (3) surveillance of HIV infection in intravenous drug abusers; (4) AIDS outreach to intravenous drug abusers not in treatment; (5) AIDS information/educational activities; and (6) enhancing drug abuse treatment capacity (see Table 4).

Improving effectiveness of drug abuse treatment. Improving treatment effectiveness is being addressed through NIDA's regular research and demonstration research programs. At present, most intravenous drug abusers are heroin addicts, but a significant number are believed to be using cocaine alone or in combination with heroin. Drug abuse treatment needs to be responsive to multiple drug use by clients, as well as trends in drug use by clients. Research is encouraged that will: (1) overcome deficiencies in existing treatment approaches that contribute to poor program performance, illicit drug use during treatment, dropout from treatment, and relapse to illicit drug use following treatment; (2) lead to the development of new and improved methods for the treatment of intravenous drug abuse; and (3) have practical clinical utility (i.e., are practical to implement in treatment settings) (Ruback and Innes, 1988). Both behavioral and pharmacological strategies are needed, including attempts to mainstream drug abuse treatment into the primary health care system to address the multiple medical problems associated with intravenous drug abuse.

Basic AIDS research. Support is provided for a wide range of activities to improve our understanding of factors related to the spread of HIV infection among intravenous drug abusers. Of interest are studies of: (1) the epidemiology and natural history of HIV infection among intravenous drug abusers; (2) the role of abused drugs as possible cofactors to AIDS and HIV infection; (3) the role of drug-use practices in the spread of AIDS and HIV infection, including types of intravenous and non-intravenous drugs used, needle-sharing practices, etc.; (4) basic behavioral and genetic factors that contribute to the development of intravenous drug use; (5) strategies for the elimination of intravenous drug use; (6) prevention of intravenous drug use; (7) the development and evaluation of behavior-change strategies for controlling the spread of AIDS and HIV infection, including studies of basic behavioral processes underlying risk-taking behavior, and (8) effects of drugs of abuse and pharmacological agents used in the treatment of drug abuse on immune function.

Surveillance activities. NIDA maintains a monitoring system for determining prevalence of HIV infection among intravenous drug abusers. At present, the system operates in seven U.S. cities to sample intravenous drug abusers on admission to methadone detoxification and maintenance programs IO determine rates and trends in HIV infection. The system also collects data on sexual and drug-use practices that may be related to infection. After a sufficient number of rounds of testing have been completed, NIDA will begin reporting on trends in HIV infection among intravenous drug abusers in different geographical regions of the U.S.

AIDS outreach activities: One major component of NIDA's AIDS activities is the community outreach demonstration program. This large-scale program is designed to disseminate information obtained from AIDS and drug-abuse research to intravenous drug abusers and others working in the drug abuse field. The

TABLE 4
ELEMENTS OF NIDA'S AIDS PROGRAM

The focus of NIDA's AIDS Program is to reduce transmission of HIV infection among intravenous drug abusers, and between intravenous drug abusers and their sexual partners and children. It consists of six elements:

I. Improving Effectiveness of Drug Abuse Treatment

- A. Improving existing treatment approaches
- B. Developing new treatment approaches

II. Basic AIDS Research

- A. Epidemiology/cofactor studies
- B. Evaluating behavior-change strategies
- C. Immunological studies

111. Survellance Activities

- A. Monitoring system
- B. Nature and extent of intravenous drug use

IV. Outreach Activities

- A. Comprehensive Outreach Program
- B. Targeted Outreach Program
    1. Data collection
    2. Information dissemination
    3. Seroprevalence determination
    4. Outreach model evaluation

V. Information/Education Activities

- A. Treatment staff training
- B. Public education

VI. Enhancing Drug Abuse Treatment Capacity

- A. Increasing number of treatment slots
- B. Increasing treatment personnel
- C. Improving quality of treatment

information concerns facts about the nature of AIDS, the risk factors for becoming infected, and the effectiveness of AIDS risk-reduction measures. The program is directed towards intravenous drug abusers who are not in treatment. The primary message is for addicts to stop using drugs and get into treatment to overcome their dependency. However, if they are unwilling or unable to do so, they should avoid needle sharing and adopt other AIDS risk-reduction measures.

By FY 1989, the program will be contacting approximately 40,000 at-risk people in 53 U.S. cities. The program focuses on major cities where intravenous drug abuse is the most prevalent, and therefore the risk for HIV infection is the highest. The program employs indigenous outreach workers to reach intravenous drug users who are not in treatment and their sexual partners who may or may not themselves be intravenous drug abusers. Outreach is accomplished on the street, in jails, in emergency rooms, in housing projects, in health clinics, in "copping" areas, etc. The information disseminated is in both English and Spanish language versions.

This program also collects information on HIV seropositive rates, drug-use practices, health problems, sexual practices, etc., of intravenous drug abusers not in treatment. Since most of our data about intravenous drug abusers is based on addicts in treatment, this program will provide invaluable data about a previously hidden population -- addicts not in treatment -- about whom we know little.

The program will also allow the test of the effectiveness of various models for disseminating information to clients, as well as, evaluate the effectiveness of various intervention characteristics (i.e., effectiveness of low vs. high intensity interventions, effectiveness of outreach to sexual partner directly *or* via intravenous drug abuser). Finally, by reinterviewing clients at six month intervals, it will be possible to evaluate the overall effectiveness of the project in terms of what types of risk-reduction strategies have been adopted, changes that have occurred in HIV seropositivity rates, etc.

AIDS information/education activities. This program disseminates information based on research findings to drug-abuse authorities, treatment personnel, and the general public. Training and technical assistance is provided to drug-abuse authorities and treatment personnel so they may deal more effectively with the psychological and medical problems of HIV infection and AIDS. Drug-abuse counselors are provided with recent information on strategies to use in counseling HIV seropositive and seronegative clients, clients with AIDS, and their sexual partners. Public education is accomplished by videos, audios, booklets, and posters about AIDS and intravenous drug abuse. NIDA also maintains a hotline telephone service (1-800-662-HELP), which provides information on the availability of treatment services.

Enhancement of drug abuse treatment. Increasing capacity for treatment of intravenous drug abuse is being addressed as part of NIDA's leadership activity in the drug abuse field. At present, treatment capacity of 148,000 treatment slots is not sufficient to handle the estimated 1.2 million intravenous drug abusers who need treatment. Rather substantial increases in treatment capacity are needed, along with training of new clinical personnel to staff the expanded capacity, training of outreach workers to encourage more intravenous drug abusers to enter treatment, and establishing a data system for more accurately identifying types of clients entering treatment, program characteristics, etc. These issues are being addressed as part of PHS planning for control of the AIDS epidemic.

Drug Abuse and AIDS

The problem of intravenous drug abuse has been with us for a long time. However, it has recently assumed an even greater public health significance because of its association with HIV infection. This is not only because of the rapid spread of HIV among intravenous drug abusers, but also because of the potential for spread of the virus from intravenous drug abusers to the general heterosexual population. There is also the tragedy of perinatal AIDS, which is also closely associated with intravenous drug abuse.

We must hope that AIDS prevention efforts will have a major impact on spread of this deadly virus. Unfortunately, an AIDS vaccine is still in the distant future. In the intervening period, it is essential that we do whatever is possible to initiate behavior change in the populations at risk. Among intravenous drug abusers, the primary problem will be getting them to stop sharing injection equipment. Drug abuse treatment will play an important role in accomplishing this goal. Treatment must be available for intravenous drug abusers who desire it, and more intravenous drug abusers than are currently on treatment waiting lists must be encouraged to enter treatment. However, to be optimally effective as an AIDS prevention strategy, the effectiveness of currently-available treatment needs to be improved and new treatment approaches developed.

For those intravenous drug abusers who are unwilling or unable to come into treatment, humanitarian alternatives must be available to allow them to minimize their risk of infection. Equally important will be preventing the transmission of HIV from intravenous drug abusers to their sexual partners. For those who will not practice sexual abstinence, the evidence is clear that barrier contraception can effectively minimize the chances of spread of HIV from one individual to his or her sexual partner. Finally, we must urge women who are themselves intravenous drug abusers or are the sexual partners of intravenous drug abusers to be tested for HIV infection before becoming pregnant.

Control of AIDS will be a difficult public health problem. In the drug abuse field, our effort will require a focus on treatment and prevention of intravenous drug abuse, as well as on transmission of HIV itself. This will require knowledge of the basic factors underlying intravenous drug abuse, as well as improved strategies for treating and preventing intravenous drug abuse. By providing the means for accomplishing these goals, drug abuse research will play an important rote in controlling the AIDS epidemic.

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AUTHORS

Charles R. Schuster, Ph.D., Director
National Institute on Drug Abuse
5600 Fishers Lane
Rockville, MD 20857

Roy W, Pickens, Ph.D.
Associate Director for AIDS
National Institute on Drug Abuse
5600 Fishers Lane
Rockville, MD 20857

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Introduction of Nathan B. Eddy Memorial Award

E. Way

It is indeed a great pleasure for me to have the privilege of introducing a good personal friend as this year's recipient of the Nathan B. Eddy Award. Professor Albert Herz, Director of Neuropharmacology and the Max Planck Institute for Psychiatry in Munich has been the leader of an extraordinarily active and productive group for a quarter of a century. The contributions from his laboratory display broad and profound interests in opioid mechanisms and range from investigations at the molecular level on simple cellular models to complex social behavioral studies on animal colonies. Time does not permit me to detail these discoveries but I shall provide some prime examples.

His early detailed studies correlating the physical properties of opiates with pharmacologic efficacy facilitated the identification of their sites of antinoceptive action in the brain and significantly enhanced understanding of the role of disposition factors in opiate action. A valuable tool for characterizing various opiate receptors was provided by studies of the cross-tolerant properties of opioid agonists in simple excised tissue preparations. Elegant electrophysiological investigations from his laboratory have stimulated greater emphasis towards determining the role of ion fluxes in opiate action and led to a better understanding of the mechanisms involved in neurotransmission.

More recently, significant contributions have been made in establishing the functional role of endogenous opioid peptides in the regulation of endocrine action; the studies focused on the modulation of the secretion of hypothalamic releasing factors by opioidergic mechanisms. Experiments on isolated rat hypothalami showed that inhibitory influence of opioids on LHRH release depends on the presence of gonadal steroids and that corticotropin-releasing factor (CRF) induces the release of ß-endorphin and dynorphin by inhibition of the gonadotropin-releasing hormone.

A molecular approach on the influence of recurrent stress upon the biosynthetic activity of pituitary cells secreting proopiomelanocortin (POMC) or prolactin indicated that the biosynthetic activity of corticotrophs and lactorophs is differentially modulated under chronic stress. Chronic, intermittent electrical foot-shock caused an increase in mRNA levels encoding for POMC in the

anterior pituitary. The effects of GABA on gene expression of POMC were found to be differently regulated in the pituitary lobes; POMC mRNA being inhibited in the neurointermediate lobe and unaffected in the anterior pituitary.

Other studies indicate that both Ca^{++} and cAMP are involved in the regulation of gene expression of POMC. In contrast, studies on the regulation of proenkephalin A gene expression in primary cultures of bovine adrenal chromaffin cells suggest that membrane depolarization plays an important role. It represents a mode by which substances acting directly on Na^{+} or Ca^{++} channels may modulate the regulation of proenkephalin A mRNA biosynthesis and opioid peptide production.

Studies on pain mechanisms revealed that the spinal dynorphin system plays a special role in chronic pain and seems to be hyperactive under these conditions. Chronic arthritic rats showed a remarkable increase in the content of dynorphin in the lumbosacral spinal cord. The changes paralleled the time course of the arthritis and were accompanied by a relative decrease in r-receptors and an increase in u-receptors in this area.

Conditioning experiments for the study of the rewarding and aversive properties of opioids in rats showed that conditioned place preference is stereospecfic and is produced by μ-opioid receptor active isomers. The aversive effects of certain κ-opioid receptor ligands were shown to correlate with their dysphoric and psychotomimetic effects in humans.

In studies on receptors and coupling mechanisms, it was observed that chronic opiate treatment induced receptor uncoupling from its effector. This is a highly significant event and provides insight as to the molecular events involved in opiate tolerance.

Light microscopic authoradiography studies on the distribution of opioid binding sites in rat brain with ^{3}H-bremazocine revealed a distribution of ς-ςsites different from that of μ- and δ- receptors with levels highest in particular diencephalic nuclei. By means of target size analysis, the molecular mass of opioid receptors was determined in various tissue and cell preparations; the data indicated that an additional membrane component of 40-44Da kDa is necessary for high-affinity opioid binding.

Herz's laboratory was the first to make the monoclonal antibody against enkephalin that help enormously in the study of opioid peptide actions in the brain. They prepared the idiotypic antibody of ß-endorphin which behaved like an opioid agonist and more recently other monoclonal antiidiotypic opioid receptor antibodies. I hope this cursory presentation suffices an indication of the broad scope and in-depth research carried out in his laboratory.

Dr. Herz is not only a well-known research scientist but is also universally recognized as a distinguished teacher and scientific statesman. It is indeed a tribute that there are a number of other highly established and respected colleagues in the opioid field who collaborated with or trained under him. He holds or has held important offices in several organizations. He has worked actively to organize outstanding meetings and symposia for many scientific organizations. He truly is an eloquent spokesman for drug abuse research.

On the basis of his tireless service and lasting creative contributions to this field, I can think of no person more deserving of the Nathan B. Eddy Award than Professor Albert Herz.

AFFILLIATION:

E. Leong Way, Department of Pharmacology, University of California, San Francisco, CA 94143

Bidirectional Effects of Opioids in Motivational Processes and the Involvement of D_1 Dopamine Receptors

A. Herz

ABSTRACT

Exogenous and endogenous opioids significantly affect motivational processes: depending on the particular opioid receptor type with which they interact opposite effects are induced: activation of μ and δ opioid receptors is rewarding whereas activation of κ opioid receptors induces aversive effects. Antagonism of μ-opioid receptors also induces aversion, suggesting the existence of a tonically active opioidergic reward pathway. There is evidence that ß-endorphin pathways arising from the hypothalamus play an important role in this respect. Opioid-induced reward as well as aversion seem to be mediated by the mesolimbic dopamine system, reward by increased, aversion by decreased transmission at D_1 dopamine receptors.

Opioids and other drugs of abuse exert marked effects on mood and motivational processes. In humans they produce euphoria and drug seeking behavior. The repeated administration of opioids results in the development of tolerance and dependence. Although such latter actions are important for the maintenance of drug addiction once established, it is apparent that they are not causal factors. Rather there is now substantial evidence that it is the ability of a drug toactivate endogenous reward pathways which determine its potential for abuse and the addiction process which may subsequently ensue. In addition, as will be shown, there is indication that opioids activating κ-receptors may produce aversive states. Furthermore, the same neural system which mediates the reinforcing or motivational effects of opioids may also underlie such effects of other drugs of abuse as well as those of natural rewards.

MULTIPLICITY OF OPIOID RECEPTORS AND LIGANDS

Soon after the initial detection of the opioid receptors and endogenous opioid peptides, the enkephalins, several observations pointed to a multiplicity of opioid receptors. Presently the occurrence of at least 3 opioid receptor types (μ, δ, κ) is generally accepted (Höllt 1986). These different receptors are the targets of a considerable series of different opioid peptides which are split

TABLE 1:

Opioid Receptor-Ligand Relationship

Receptor Type	Endogenous Ligand	Exogenous Ligand	Antagonist
μ	(Morphine-like Alkaloid?) ß-Endorphin?	Morphine DAGO	Naloxone (low dosage)
δ	Enkephalins	DPDPE	ICI 174864
κ	Dynorphin	U-50,488H U-69593	Nor-Binaltorphimine
(ε)	ß-Endorphin		(ß-Endorphin$_{1-27}$)

off from three precursor molecules by enzymatic processing. Proopiomelanocortin (POMC) is the precursor molecule from which ß-endorphin (ß-EP) is derived; methionine-enkephalin (met-enkephalin) and leucine-enkephalin (leu-enkephalin) as well as various larger enkephalin containing peptides are derived from proenkephalin A whereas proenkephalin B (or prodynorphin) represents the precursor for dynorphin and related peptides. This heterogeneity raises questions concerning the relationship between the various opioid peptides and receptor types (Paterson et al., 1984) (Table 1). Morphine exhibits high affinity and selectivity for the μ-receptor. At present, however, there is no endogenous opioid peptide known which shows selectivity for this receptor type. The enkephalins exhibit some selectivity for δ-receptors and are therefore considered as possible endogenous ligands of these receptors. Prodynorphin-derived peptides bind with high affinity and selectivity to κ-receptors and probably represent their adogenous ligands for this receptor type. In recent years, peptides (and alkaloids) have been synthesized which exhibit high affinity and selectivity for the various receptor types; in addition, antagonists with high selectivity for the various receptors have been developed. These synthetic compounds have aided greatly the identification of the receptor types underlying the abuse potential of opioids. It should be emphasized, however, that, although the affinity of ligand for the various receptor types is a major factor in determining the receptor through which a compound acts, the availability (i.e. concentration) of a ligand at a given synapse and the presence of other receptor types there will also determine the receptors to which a ligand binds in-vivo to exert its biological effects.

A great deal of work has performed in the last decade to analyse opioid actions in respect to the receptors (and ligands) involved (zukin and Zukin 1984; Watson et al., 1984). These studies revealed in many cases a complex pattern. Thus, it is now apparent

that many opioid effects can not be attributed to the activation of a single receptor type and several receptor types may be involved in a particular pharmacological or physiological response. Nevertheless some characteristic pharmacological profiles can be attributed to the activation of the various receptor types. Whereas in the case of μ- and δ-receptors this profile shows many similarities, the activation of K-receptors results in a rather different spectrum of pharmacological actions: The lack of respiratory depression, of gastrointestinal activity and of antidiuretic action is particularly remarkable. Although continuous application of K-receptor ligands also produces tolerance, the withdrawal symptomatology was found to be different and relatively mild in comparison to withdrawal from morphine-like compounds. Thus the pharmacological Spectrum of K-receptor agonists seems to offer some promising aspects for their medical use, e.g. as analgesics. In view of that, the analysis of their motivational properties seems to merit particular interest.

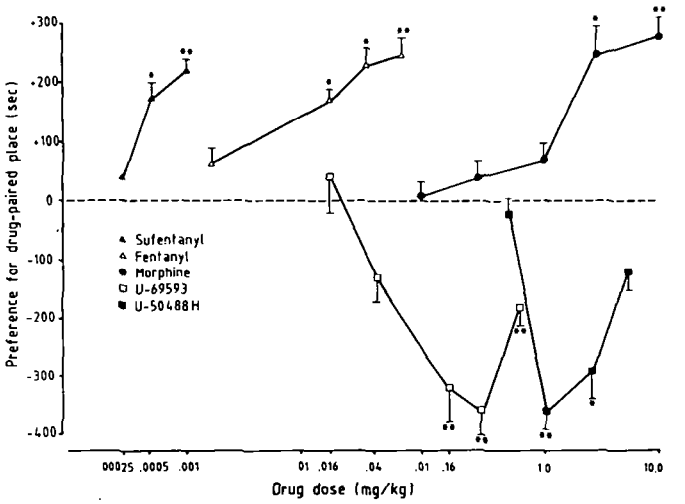
CONDITIONED PLACE PREFERENCE VS. AVERSION

A variety of animal models including self-administration and intracranial self-stimulation have been used to determine the motivational effects of drugs of abuse. In these procedures, administration of a drug or rewarding stimulus such as electrical brain stimulation is contingent upon the performance of a specific bahavioural test, i.e. lever pressing. Data derived in this way provide a measure of primary reinforcement processes. In the experiments discussed here an alternative approach has been used to characterize drug-induced motivational effects, that is to say, place preference conditioning. In this procedure, the association which develops between the presentation of a drug and a previous neutral stimulus is examined. This procedure has been used to confirm the reinforcing effects of a variety of psychoactive drugs. Importantly, this technique permits the detection of both rewarding and aversive drug-induced motivaional states.

Place preference conditioning has provided the first demonstration that opioids have reinforcing or aversive effects depending on the receptor type with which they interact (Mucha and Herz 1985). Administration of the μ-receptor agonists morphine, fentanyl or sufentanyl resulted in dose-related preferences for the drug-associated place, confirming that these drugs function as reinforcers in the drug-naive animal. Furthermore the potency of these agonists in eliciting these effects parallels their differing affinity to l-receptors, indicating that the activation of this specific receptor type, underlies their reinforcing properties. In contrast to p-agonists, the selective κ-agonists U-50, 488H and U-69593 produced pronounced conditioned place aversion. Here too, the potencies of these ligands in producing this effect correlates wall with differences in their binding affinities to κ-receptors.

By employing the intracerebroventricular (IVC) route of administration it has also been possible to examine the effects of those opioids, i.e. peptides, which do not readily penetrate the blood brain barrier after systemic application. ICV administration of the specific δ-agonist DPDPE produced marked preference for the drug-

FIGURE 1: Place Conditioning produced by μ- and κ-opioid agonists.



Place conditioning produced by the μ-opioid agonists morphine, fentanyl and sufentanyl and the κ-opioid agonists U-50, 488H and U-69593. Ordinate: mean difference (sec) between time spent in drug- and vehicle-paired. sides of the test box. Abscissa: drug dose. Points above zero indicateplacepreference, points below a place aversion. Each point represents the mean conditioning score + S.E.M. of 8-10 rats. Asterisks denote significant place conditioning (Wilcoxon) test: *p < 0.05; **p < 0.01).

associated place. Pretreatment with the δ-agonist ICI 174,864 which in itself lacked reinforcing or aversive effects antagonized the DPDPE-induced preference, but it did not affect morphine-induced preference Shippenberg et al., 1987). Thus it is apparent that the reinforcing properties of opioids may result from an activation of either μ- or δ-receptors and such agonists of either receptor type Will have marked potential for abuse. In contrast κ-receptor agonists will induce aversive states.

Place aversion is induced not only by κ-receptor ligands but also by the universal opioid receptor antagonist naloxone (Mucha and Iversen 1984). The same effect is obtained by the specific μ-receptor antagonist [D-Tic[1]] CTAP. These findings suggest the existence of a tonically active endogenous μ-opioidergic reward system, the disruption of which results in aversive states. An enhancement in the motivational effect of naloxone is observed in animals which are tolerant and physically dependent on morphine; this finding is consistent with the aversive state produced by precipitated withdrawal. As the δ-receptor antagonist ICI 174,864 did not induce

aversion, it may be suggested that the δ-receptor-mediated enkephalin system is not tonically active. The specific κ-receptor antagonistnor-binaltorphimine caused some place preference, but the effect was of borderline significance, thus excluding definitive statements about the tonic activity of the dynorphin system in motivational processes (Bals-Kubik, in preparation).

THE CEREBRAL ß-ENDORPHIN SYSTEM

The question arises as to the identity and location of the endogenous opioid systems involved in reward and aversive states. Concerning the reward system, the cerebral ß-endorphin (ß-EP) pathways originating in the nucleus arcuatus of the mediobasal hypothalmus (MBH) seems to be relevant. ß-EP is self-administered rats and monkeys and produces conditioned place preference in rats after ICV administration (Van Ree et al., 1979; Amalric et al., 1987). A functional role of endogenous ß-EP is indicated by experiments in which place conditioning was performed in rats with bilateral radiofrequency lesions of the MBH. In these animals, ß-EP content in the hypothalamus and the major projection sites were largely diminished and the aversive effects of naloxone were found to be markedly reduced (but not completely abolished). The place preference induced by morphine and place aversion induced by U-50, 488H were, however, not affected (Mucha et al., 1985). The attenuation of the aversive effects of naloxone is most easily explained by a diminution of the release of ß-EP with the consequence of a decrease in the tonic activity of an endogenous opioidergic reward pathway mediatedby ß-EP.

In line with the assumption of a role of ß-EP in reinforcement processes are results obtained with a metabolite of ß-EP, ß-EP_{1-27} (Bals-Kubik et al., 1988). Place conditioning experiments in rats showed that ß-EP_{1-27} dose-dependently antagonized the reinforcing effects of ß-EP. It also antagonized the place preference induced by selective μ(DAGO) and δ-agonists (DPDPE), but not the aversion induced by κ-agonists and the place preference induced by the psychostimulant D-amphetamine. This antagonistic activity of ß-EP_{1-27} appears tobeof functional significance since this metabolite of ß-EP is present in various brain structures at concentrations considerably higher than that of ß-EP (Zacharian and Smyth 1979).

There are further indications for a role of endogenous opioid peptides, in particular ß-EP, in affective behaviours. The phenomenon of stress-induced analgesia and its at least partial blockade by naloxone indicates that endogenous opioid peptides are released in stressful situations. There is indication that opioid peptides play a key role also in positively motivated rewarding situations. Thus, non-food-deprived rats expecting or receiving highly desirable food as candy or chocolate milk exhibited an increase in nociceptive thresholds which could be antagonized by naloxone (Dum et al., 1984). In the hypothalmus of such rats ß-EP levels were significantly reduced (while dynorphin levels were unchanged). A decrease in opioid receptor binding sites in hypothalmus, but not in other brain areas was also observed (Dum and Herz 1983). These and other results indicate a release of ß-EP in the hypothalamus in response to palatable food and indicate that opioid peptides are released in the

brain not only in stressful but also in rewarding situations. Furthermore, it is most likely that ß-EP plays an important role in this emotional response to (Dum and Herz 1987).

EXPERIENCE IN HUMANS

The motivational properties of morphine and other μ-opioid receptor ligands, being the base of their high abuse potential in humans, need no further discussion. Respective data concerning δ-opioid receptor ligands are presently not available. Concerning κ-opioid receptor ligands a study in human volunteers in which psychiatric rating scales were used to evaluate emotional and conceptual; experiences showed that a benzomorphane derivative with preferential κ-receptor agonist activity (Mr 2033) elicited dose-dependent dysphoric and psychotomimetic effects which were readily antagonized by naloxone (Pfeiffer et al., 1986). This result indicates that not only σ-receptors but also κ-opioid receptors mediate psychotomimesis. Similar effects reported for agonists-antagonists with benzomorphane structure may also be related to activation of κ-opioid receptors by these drugs. It is suggested that such dysphoric-psychotomimetic effects are equivalent to the aversion observed in the place conditioning experiments in rats.

As described above, not only κ-opioid receptor agonists but also naloxone (preferentially acting as antagonist at μ-receptors) and the pure μ-receptor antagonist [D-Tic[1]] CTAP produce aversive effects in rodents. There are also several reports describing aversive effects of naloxone in opioid-naive humans. However, taken as a whole, the aversive symptomatology seems to be rather mild and in some cases was not seen at all (Grevert and Goldstein 1978; Downs and Woods 1976). In monkeys, naloxone was found to function as a negative reinforcer. The reason for such species differences is not quite clear. It maybe that in the humans occupation of opioid receptors with endogenous ligands in the test situation (= basal tone) is lower than that in rats, mice and monkeys.

LINKS BETWEEN OPIOIDS AND DOPAMINE IN MOTIVATIONAL PROCESSES

Several findings, e.g. the attenuation of the reinforcing effects produced by electrical brain stimualtion by dopamine (DA) receptor antagonists have suggested the involvement of dopaminergic systems in processes of reinforcement (Wise 1987). However, if dopaminergic neurons are a critical component in tonically active pathways subserving reward, then the blockade of DA receptors or the inhibition of DA release should, as observed with naloxone, induce aversive states. Furthermore, if the general DA hypothesis of reward is correct such manipulations should also abolish the reinforcing effects of all types of drugs of abuse. Until recently, however, such effects have not been demonstrtated. Thus, the DA antagonists pimozide, haloperidol and α-flupentixol are ineffective in producing place and taste aversion and do not function as negative reinforcers in other paradigms.

The identification of two distinct DA receptor types (D_1,D_2) and the availability of antagonists selective for each of them made it

possible to reevaluate the dopamine hypothesis of reward and the involvement of D_1 - vs. D_2 -receptors in the mediation of reinforcing and aversive states. Acute administration of the selective D_1 -antagonist SCH-23390 caused clear conditioned place aversions whereas the D_2 antagonists sulpiride and spiperone as well the mixed D_1 /D_2 antagonist haloperidol lacked such effects (Shippenberg and Herz 1987b, 1988). These data support the hypothesis that there is a tonic activation of D_1- but not of D_2-receptors, the disruption of which results in aversive states,

Chronic infusion of the D_1 -receptor antagonist SCH-23390 - but not infusion of D_2-receptor antagonists - during the conditioning period completely abolished the reinforcing effect of morphine; the aversive effects of the selective K-agonist U-69593 as well as naloxone were also prevented by this treatment, whereas D_2-antagonists were without effect. Thus, it appears that D_1 -receptors, but not D_2-receptors, are critical for the expression of the reinforcing as well as the aversive effects of opioids (Shippenberg and Herz 1988) .

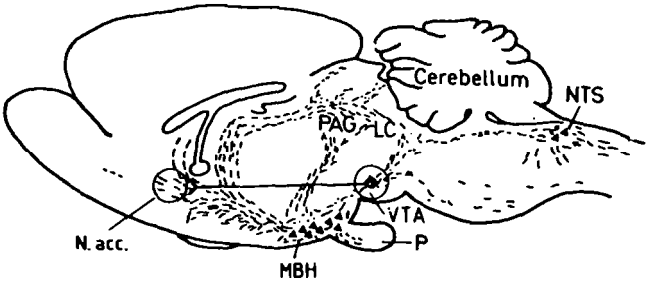
BRAIN DOPAMINE REWARD PATHWAY SUBSERVING OPIOID REWARD

The use of microinjection techniques has permitted the identification of the specific brain regions involved in the reinforcing properties of opioids. Both self-administration and place conditioning studies revealed that the injection of morphine or enkephalin analogs into several brain areas including the lateral hypothalmus, septum, ventral tegmental area (VTA) and nucleus accumbens are reinforcing (wise and Bozarth 1982). Furthermore, doses required to produce this effect are lowest in the VTA, suggesting that this site may be of critical importance. The question whether or not the sane brain regions underlying reinforcement are also involved in mediating the aversive effects of opioids is presently under investigation; preliminary data pint to a particular role of the nucleus accumbens in this regard.

The VTA and the nucleus accumbens, areas critically involved in the reinforcing and/or aversive actions of opioids, represent key components of the mesolimbic DA system; the DA-perikarya are located in the VTA, the nucleus accumbens represents the terminal field of fibres arising from there. Several lines of evidence suggest that an interaction between opioid- and DA-system occurs in the VTA: ß-EP fibres originating in the MBH innervate the VTA and the presence of D_1- and D_2 -receptors therein is well documented. ß-EP containing fibres as well as opioid receptors are widely distributed in the nucleus accumbens. Several findings indicate an increased dopamimergic neurotransmission in the mesolimbic system under the action of morphine and an increased release of DA in the nucleus accumbens by morphine has been recently directly measured in vivo by microdialysis (DiChiara and Imperato 1988).

Recent data also indicate that the aversive effects of the K-opioid receptor ligands are related to changes (diminution) in the activity of the mesolimbic pathway: D_1 -receptor blockade during the conditioning period inhibited not only the rewarding but also the

FIGURE 2: Cerebral ß-EP and mesolimbic System.



Schematic representation of the cerebral ß-EP system and its putative connections with DA pathway ascending from the midbrain (VTA). The major portion of the cerebral ß-EP system arises from perikarya located in the medio-basal hypothalmus (MBH) and projects from there to many partis of the brain, in particular the diencephalon and midbrain. (A minor part of the ß-EP fibres originates in the nucleus tractus solitarius NTS). Thus fibres make connections with the DA system originating in the ventral tegmental area (VTA) and ascend to mesolimbic areas including the nucleus accumbens (N. acc.) . P = pituitary.

aversive opioid effects; microinjection of specific K-opioid receptors ligands were highly effective in inducing aversion when injected into the nucleus accumbens (Shippenberg and Bals-Kubik, unpublished); finally, in vivo microdialysis experiments showed that U-50, 488H reduced DA release in nucleus accumbens (DiChiara and Imperato 1988).

Table 2 summarizes these results. It includes also the psychostimulants D-amphetamine and cocaine. Amphetamine also increases DA release, whereas cocaine increases dopaminergic transmission by other mechanisms. The final result is an activation of D_1 receptors, giving rise for a general theory on the common mechanisms of action of addictive drugs (Wise and Bozarth 1987). On the other side there is indication that decrease in D_1 receptor activity induces aversion.

ACKNOWLEDGEMENT

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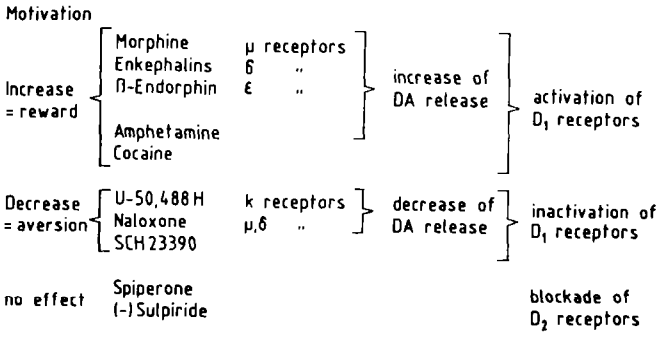
TABLE 2:

Dopaminergic Transmission-Relationship



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AFFILIATION

Department of Neuropharmacology, Max-Planck-Institut fur Psychiatrie, Am Klopferspitz 18a, D-8033 Planegg-Martinsried, Federal Republic of Germany.

Effects of 6-Hydroxydopamine Lesions of the Nucleus Accumbens on a Concurrent Schedule of Food, Water and Cocaine Self-Administration

S. I. Dworkin, G. Guerin, S. M. Dworkin and J. Smith

Male Fisher rats were trained on a fixed-ratio 1, fixed-ratio 9, concurrent chain schedule of food, water and intravenous cocaine self-administration. Food and water presentations were contingent upon responding on separate levers that were continuously available. Cocaine infusions were delivered contingent on responding on a third lever that was available for 6 continuous hours (9:00 AM - 3:00 PM). A dose-effect curve for cocaine (.08 - .83 mg/infusion) was determined before and after a 6-OHDA lesion of the nucleus accumbens.

Responding was well maintained by the schedule contingencies. Increasing the dose of cocaine resulted in an inverted U shaped curve for responding maintained by cocaine infusions. The 0.17 mg/infusion maintained the highest rate of responding on the lever which resulted in cocaine infusions. Responding on both the levers that produced food and water deliveries was not significantly altered by changes in the cocaine dose. The neurotoxin lesion resulted in a significant decrease in cocaine self-administration at both the 0.17 and 0.33 mg/infusion dose. Total daily responding on both the food and water lever was not altered by the neurotoxin lesion. However, the hourly patterns of responding on both the food and water lever was modified by the lesion. 6-OHDA lesions can produce a selective, dose-specific decrease in cocaine self-administration while not altering daily food or water intake. Dopaminergic innervations of the nucleus accumbens appear to be more involved in modulating the reinforcing efficacy of cocaine compared to the regulation of food or water intake.

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AFFILIATION

Dept. of Psych., LSUMC, Shreveport, LA 7113-3932

Chronic Cocaine Exposure During Gestation Results in Neurobehavioral Deficits Evident Early in Ontogeny

L. Spear, N. Frambes, C. Kirstein, J. Bell, L. Rogers, V. Yoottanasumpun and N. Spear

Initial studies were conducted to investigate the appopriateness of the doses and route of administration chosen for use [10, 20 or 40 mg/kg/3cc cocaine HCl given subcutaneously daily from gestational day (E) 8-20]. In exp. 1, in which the behavior or the dams was time sampled at .5, 1, 2 and 4 hrs. post-injection on E8, 12, 16 and 20, dams given 10 mg/kg cocaine (C10) exhibited significantly less sniffing at 2 and 4 hrs. post-injection than C20 and C40 dams, whereas the later groups did not differ from each other behaviorally. In exp. 2 in which cocaine levels were assessed after sacrifice at .5 or 2 hrs. post-injection on E20, plasma and brain cocaine levels were significantly greater in C40 than C20 dams and fetuses at both sacrifice periods. Plasma cocaine levels in the dams ranged from about 1000-2500 ng/ml, above those reported in human users. Cocaine readily entered fetal plasma and brain, with brain/plasma cocaine ratios in fetuses exceeding those of the dams.

Offspring of Sprague-Dawley dams exposed to this treatment protocol were examine during the early postnatal period in two studies. Control groups included offspring of dams pair-fed to the 40 mg/kg cocaine dams and dams on ad lib feeding; in exp. 2, litters were surrogate fostered at birth. No alterations were observed in litter size or sex ratios, pup body weight at birth or day 21 (P21) or reflex/physical development. Cocaine offspring exhibited impaired conditioning of an odor/milk association at P7 and an odor/footshock association at P18, but no alterations in learning/retention of an odor/footshock association at P7-8. Cocaine infants exhibited less wall climbing behavior, a decreased sensitivity to apomorphine and an increased sensitivity to haloperidol, data consistent with an attenuation in dopaminergic (DA) activity. At P21, no alterations in DA levels or turnover were observed; preliminary data suggest a possible elevation in DA levels in cocaine offspring at P1.

AFFILIATION: Department of Psychology and Centers for Developmental Psychobiology & Neurobehavioral Sciences, SUNY-Binghamton, NY 13901.

Differential Effects of the Manipulation of Serotonin Systems on Intravenous Amphetamine and Cocaine Self-administration in Rats

L. Porrino, L. Sharpe, M. Ritz, N. Goodman, S. Goldberg and M. Kuhar

Previous studies (c.f, Yu et al. 1986) have shown that significant reductions in intravenous self-administration (IVSA) of amphetamine (AMPH) in rats can be obtained by increasing the activity of serotonin (5-HT) system, The purpose of the present study was to investigate the effects of pharmacological manipulation of 5-HT system on IVSA of cocaine (COC) in rats. Rats with chronic indwelling venous catheters were trained to self-administer COC (1 mg/kg/inf) on an FR10 schedule in daily 4 hr sessions. After stable rates of responding were established, fluoxetine (2, 5, 5, or 10 mg/kg ip), a 5-HT reuptake blocker, or cinanserin (3, 10, or 17.5 mg/kg sc), a 5-HT receptor antagonist, was injected 30 minutes prior to IVSA test sessions. Neither fluoxetine nor cinanserin pretreatment at any dose tested significantly altered IVSA of COC. Following completion of COC testing, AMPH (.125 mg/kg/inf) was substituted for COC. After stable rates of IVSA of AMPH were obtained, the effects of fluoxetine and cinanserin were tested. Confirming previous studies, fluoxetine significantly reduced IVSA of AMPH by 25-50% of baseline rates. Cinanserin pretreatment also reduced IVSA of AMPH, although this effect was less pronounced (3-18%). Pharmacological manipulation of 5-HT systems without effects on IVSA of COC, significantly alters IVSA of AMPH by rats. These findings may have bearing on the possible use of fluoxetine in the treatment of stimulant abuse in lumans and also indicate that treatment of COC and AMPH abuse may require different therapeutic regimens.

Yu, D.S., Smith, F.L., Smith, D.G., and Lyness, W.H. Fluoxetine-induced attenuation of amphetamine self-adminstration in rat. Life Sci. 39:1383-1388, 1986.

AFFILIATION

Addiction Research Center, NIDA, PO Box 5180, Baltimore, MD 21224

Evaluation of Reinforcing Properties of Amantadine and its Effect on Cocaine Self-injection in Baboons

C. Sannerud and R. Griffiths

The dramatic increase in cocaine abuse in the United States has led to the search for pharmacological approaches to treatment intervention. Recently, there have been preliminary clinical reports that amantidine (100 mg b.i.d.), an agent with indirect dopamine agonist properties and without some of the side effects reported for dopamine agonist treatments, has been used with some success to treat cocaine dependence (Tennant and Sagherian, 1987).

The ability of amantadine to maintain self-injection behavior and to alter self-administration of cocaine was examined in 3 baboons using a standard intravenous cocaine self-injection procedure. Responding was maintained under a FR 80- or 160-response schedule of intravenous cocaine delivery (0.32 mg/kg/injection). Each drug injection was followed by a 3 hour time-out allowing a maximum of 8 injections per day. Vehicle or amantadine doses were substituted for cocaine for a period of 15 or more days. Evaluation of a wide range of amantadine doses (0.21 - 32 mg/kg/injection) showed that this compound did not maintain self-administration behavior above vehicle control levels. In another experiment using the cocaine self-injection baseline in 4 baboons, amantadine (10 or 32 mg/kg/day) was administered via a chronic intravenous infusion. Cocaine self-injection behavior was maintained and re-initiated during chronic amantadine exposure, suggesting that the reinforcing efficacy of cocaine was not modified by chronic amantadine administration. The high dose of amantadine (32 mg/kg) in combination with an intermediate dose of cocaine (0.32 mg/kg) produced a disruption in cocaine self-injection accompanied by behavioral agitation and decreased food intake, suggesting psychomotor stimulant toxicity. Although this study provides no evidence to support the preliminary observations that amantadine may be useful in treating cocaine abuse, the data do suggest that amantadine may potentiate some behavioral effects of cocaine.

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AFFILIATION: Dept. of Psychiatry and Behavioral Sciences, Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Cocaine-Induced Changes in the Neurotensin Systems of the Basal Ganglia

G. Hanson, P. Smiley, L. Bush and J. Gibb

It is known that administration of cocaine enhances the activity of dopaminergic pathways in the basal ganglia, an effect which contributes to cocaine-induced increases in motor activity and changes in mental states; however, the responses to cocaine by related nondopaminergic pathways have not been well studied. The present research examined the effects of cocaine on extrapyramidal projections which employ the neuropeptide, neurotensin (NT), as their transmitter. These NT systems were evaluated because of their close association with nigral-striatal dopamine neurons and the possibility that NT systems play a role in psychotic disorders such as schizophrenia. We assessed the response of striatal and nigral NT afferent projections by measuring the NT content of these tissues following cocaine administrations.

One hour after multiple cocaine injections (5 doses at 6 h intervals; 30 mg/kg/dose, i.p.) the levels of striatal and nigral neurotensin-like immunoreactivity (NTLI) dramatically increased to 200-250% of control. Striatal changes were maximal at 1-8 h but no longer detectable after 48 h. The maximal nigral effect was observed 24 h following cocaine administration (NTLI content was 450% of control) and diminished by 48 h. Following a single cocaine dose, increases in NTLI concentrations were measured in the substantia nigra but not in striatal tissue. Combinations of cocaine with selective D-1 (SCH 23390) and D-2 (sulpiride) antagonists demonstrated that both DA receptor subtypes were involved in the cocaine-mediated changes in the NT systems: D-1 receptors had a greater role in the striatum while in nigral tissue D-1 and D-2 receptors appeared to contribute equally. Comparable administrations of the related drug, methamphetamine, caused similar increases in striatal and nigral NTLI levels. The methamphetamine-induced striatal and nigral changes appeared to be mediated entirely by D-1 receptors with little or no D-2 receptor participation. Finally, treatments with blockers of the DA uptake carrier complex, such as GBR 12909 and amfonelic acid, produced cocaine-like increases in striatal and nigral NTLI content, suggesting that cocaine influenced the NT systems by its action at this uptake site. These findings demonstrate that the striatal and nigral NT systems are substantially altered by cocaine treatment; however, the significance of these dramatic changes in NTLI content remain to be elucidated. (Supported by USPHS grants DA 00869 and DA 04222).

AFFILIATION

Department of Pharmacology and Toxicology, College of Pharmacy, University of Utah, Salt Lake City, UT 84112.

Pharmacologic Aspects of Cocaine Rush

K. Kumor, M. Sherer, C. Muntaner, J. Jaffe and R. Herning

Intravenous users [N=60] of cocaine reported that a subjective effect, termed rush, which appears shortly after I.V. injection is important in their drug seeking and self-administration behavior confirming the reports of other investigators [Spotts and Shontz, 1976; Seecof and Tennant, 1986].

We measured rush using two methods of self-report, an interval scale and a computerized bar graph. Rush was measured during five separate studies of cocaine tolerance and drug interactions: 1. after two sequential injections of cocaine 30 mg given at an interval of 70 or 180 min [Kumor et al, 1987], 2. during steady state cocaine concentrations obtained after loading injections of 40-80 mg of cocaine [Kumor et al, 1988], 3. with haloperidol 8 mg pretreatment 20 min prior to a cocaine 40 mg challenge [Sherer et al, 19871, 4. with bromocriptine 2.5 and 5 mg pretreatment 60 min prior to cocaine 40 mg challenges [Kumor et al, 19881 and 5. with nifedipine 10 mg pretreatment given prior to cocaine 20 to 40 mg challenges.

The results of these studies showed that rush can be measured reliably and has a distinct pattern of pharmacologic responses from other subjective effects including self reported "good feelings". After repeated injections of cocaine given at an interval of 70 min only rush showed tolerance, all other subjective scales scores were unchanged in magnitude and duration. Similarly during the cocaine infusion studies the only subjective scale scores unaffected by the presence of maintained cocaine plasma concentrations was rush. Rush scale scores segregated independently from other subjective scale scores during the haloperidol, bromocriptine and nifedipine studies. Rush did not covary with peripheral cardiovascular responses and thus is not a sensory appreciation of pulse and blood pressure.

AFFILIATION

National Institute on Drug Abuse, Addiction Research Center, 4940 Eastern Ave., Baltimore, Md. 21224

Prolactin and Luteinizing Hormone Pulse Frequency Analysis in Cocaine Abusers

J. Mendelson, N. Mello, S. Teoh and J. Ellingboe

Numerous experimental animal studies have shown that cocaine blocks reuptake of dopamine at neural receptor sites. Recent studies have shown that cocaine binding sites associated with dopamine uptake may also be receptors which mediate the reinforcing properties of cocaine. There is also recent evidence that chronic cocaine administration to experimental animals produces degeneration of central dopaminergic neurons and reduction in dopamine concentration in the brain. Prolactin secretion is mediated by dopaminergic inhibitory control and hyperprolactinemia has been found in men and women who abuse cocaine. Eight men ages 24 to 26 who reported a chronic history of cocaine abuse from 1 to 4 years provided informed consent for participation in this study. All had normal physical examinations, blood chemistry and hemogram studies. Each subject was studied 12 to 24 hours following intranasal or freebase use of cocaine in doses ranging from 1/2 to 2 gm. Urine drug screening carried out at the initiation of each study confirmed cocaine use by presence of cocaine metabolites. An indwelling catheter was inserted into the subject's antecubital vein and blood samples were collected every 10 minutes over 6 hours for analysis of prolactin and luteinizing hormone (LH) by radioimmunoassay procedures. Pulse frequency analysis of prolactin and luteinizing hormone levels were carried out by Pulsar Program developed by Merriam and Wachter. Eight age matched control subjects participated in an identical study of pulse frequency anaysis of prolactin and luteinizing hormone. None of the control subjects had any abnormality of prolactin or luteinizing hormone secretory function. In contrast, four of the eight cocaine users had clinically significant hyperprolactinemia (> 25 ng/ml). The cocaine users had significantly greater mean plasma prolactin levels as well as prolactin pulse amplitude ($P < 0.2$ and $P < 0.3$) than control subjects. The cocaine users with hyperprolactinemia had significantly higher mean plasma prolactin levels, prolactin pulse amplitude and prolactin pulse peak length ($P < 0.1$) than control subjects. However, cocaine users with high prolactin levels had significantly fewer prolactin pulse peaks ($P < 0.1$) than controls. Cocaine users with high prolactin levels also had significantly lower mean LH levels ($P < 0.1$) and significantly lower LH pulse amplitude ($P < 0.2$) than cocaine users with normal prolactin levels. Thus hyperprolactinemia in cocaine users was associated with suppression of LH secretory activity.

AFFILIATION

Alcohol and Drug Abuse Research Center, McLean Hospital-Harvard Medical School, 115 Mill Street, Belmont, Massachusetts 02178

MDMA Biological Disposition in Man: MDA is a Biotransformation Product

J. Alrazi and K. Verebey

MDMA, a psychoactive stimulant, is a schedule I controlled substance since July 1, 1985. Prior to its scheduling by the DEA, MDMA was used by psychiatrists in their practices. They felt that MDMA in doses of 100-160 mg was different from other hallucinogenic amphetamines. In their opinion, MDMA was an effective psychotropic "catalyst" that promoted trust and confidence between patient and therapist. The decision of the DEA to place MDMA into schedule I was based on the structural similarities of MDMA to MDA and other phenethylamines which are known to be addictive and have no current medical use in the United States. Additionally, MDA, a close structural congener to MDMA, was shown to destroy serotonergic nerve terminals in animals.

A healthy 40 year old male volunteer, weighing 140 lbs., ingested a single 50 mg oral dose of MDMA HCl. Blood samples were collected from 30 minutes to 24 hours at regular intervals. Fractional urine was collected from 0 to 72 hours. Saliva, perspiration and stool samples were also collected. MDMA and MDA were found in plasma and urine samples. The presence of MDA, a metabolite of MDMA, occurs through n-demethylation.

In plasma, MDMA peaked 2 hrs after the dose at a level of 105.6 ng/ml and declined monoexponentially to 5.1 ng/ml by 24 hrs. The calculated half-life of MDMA was 7.6 hrs. Unchanged MDMA was the major urinary excretion product. In 72 hrs a total of 36 mg (72%) of the 50 mg dose was recovered from the urine as MDMA and MDA. The missing 28% of the dose may have biotransformed in other metabolites.

Recently, MDA has been identified as a neurotoxic substance, selectively destroying serotonergic nerve terminals in rat brain. The findings in this study, that the biotransformation of MDMA in man results in the formation of MDA, should be a warning to the future legal or illicit use of MDMA by humans.

AFFILIATION:

Psychiatric Diagnostic Laboratories of America, Inc., South Plainfield, NJ 07080 and New York Sate Division of Substance Abuse Testing and Research, Brooklyn, NY 11217

Behavioral and Cardiovascular Effects of Alcohol and d-Amphetamine Combinations in Normal Volunteers

S. Higgins, M. Capeless, J. Hughes, W. Bickel and M. Belinson

Polydrug use in stimulant abusers is prevalent, yet much remains to be learned concerning the behavioral and physiological effects of such combinations. The extant literature on this topic in humans is mostly limited to the effects of stimulant-alcohol combinations on psychomotor behavior. Most of these studies fail to examine effects of the compounds alone as well as in combination or to include more than one dose of stimulant.

The present study examined the behavioral and cardiovascular effects of orally administered alcohol (0,28,56 g/70 kg) and d-amphetamine elixir (0,12.5,25 mg/70 kg) in eight normal volunteers (5 M & 3 F). Each subject was exposed once to each dose of d-amphetamine and alcohol alone and in combination across 9 sessions.

Behavioral and physiological observations were obtained every 30 min for 1 hr before and 4 hrs after drug administration. Cardiac effects were assessed via continuous holter-monitor recording throughout the 5 hr session.

d-Amphetamine and alcohol administered alone generally produced orderly dose-related behavioral and cardiac effects. The effects of combining these compounds, however, differed across measures and cannot be characterized as either "antagonistic" or "additive" without reference to particular drug doses and dependent variables. On the Digit Symbol Substitution Task (DSST), for example, the low dose of ethanol increased rates of responding above placebo levels, whereas the high ethanol dose decreased response rates; both active doses of d-amphetamine increased rates of responding. When the low ethanol dose was combined with either active dose of d-amphetamine, response rates were further increased above levels &served when the compounds were administered alone (i.e., additive effects). Combining the high ethanol dose with either active dose of d-amphetamine reversed the rate-suppressing effects of the high ethanol dose (i.e., antagonistic effects). With heart rate, as another example, the active doses of ethanol and d-amphetamine increased rates when administered alone, and combining ethanol and d-amphetamine produced larger increases in heart rate than were observed when the compounds were adminis-

tered alone (i.e., additive effects).

As measured by the DSST, we found no evidence that the dose combinations we examined increased behavioral toxicity. The dose-dependent, additive effects of ethanol on cardiac output observed in this study suggests that the combined use of these compounds may increase cardiac risk. Overall, these results further illustrate how the effects of drug combinations are dependent on dose and measure and clearly are not predictable solely on the basis of the pharmacological classes of the constituent compounds.

AUTHORS

Stephen T. Higgins, Ph.D., John R. Hughes, M.D., Warren K. Bickel, Ph.D., (Departments of Psychiatry & Psychology), Mark Capeless, M.D., (Department of Medicine), Maureen Belinson, R.N., (Department of Psychology), University of Vermont, Burlington, Vermont 05401.

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A Pharmacological Analysis of the Discriminative Stimulus Properties of d-Amphetamine in Rhesus Monkeys

J. Kamien and W. Woolverton

Rhesus monkeys (N=4) were trained to discriminate g-amphetamine (AMPH; 0.67 or 1.33 µmol/kg, i.v.) from saline in a two lever, food-reinforced drug discrimination paradigm. After acquisition of the discrimination (average=127 sessions), the monkeys were tested with a series of compounds selected to characterize pharmacologically the discrimination. AMPH (0.08-2.6 µmol/kg), cocaine (0.06-1.0 µmol/kg; N=4), the dopamine (DA) uptake inhibitor bupropion (0.25-2.0 µmol/kg; N=2), and the norepinephrine (NE) uptake nisoxetine (1.0-16 µmol/kg; N=4) produced a dose-related increase in the percent of responses that occurred on the AMPH-appropriate lever during test sessions in all animals tested. For all other drugs tested, individual differences were noted. Compounds with primarily D2 DA receptor activity, including apomorphine (0.06-1.0 µmol/kg), piribedil (0.25-8.0 µmol/kg; N=4), bromocriptine (0.12-1.0 µmol/kg; N=4) and propylbutyldopamine (0.25-4.0 µmol/kg; N=3) occasioned AMPH-appropriate responding in 1 or 2 monkeys. Similarly, the DI agonist SKF 38393 (0.5-64 µmol/kg; N=4) and pentobarbital (4.0-32 µmol/kg; N=4) substituted for amphetamine in only 1 monkey. In tests for antagonism, the D1 antagonist SCH 23390 (SCH; 0.015-0.03 µmol/kg; N=2) completely blocked the discriminative stimulus effects of AMPH in a dose-related manner in both monkeys tested. On the other hand, the, D2 antagonists pimozide (0.015-0.12 µmol/kg; N=4) and raclopride (0.015-0.12 µmol/kg; N=2), and the NE antagonists prazosin (0.32-2.6 µmol/kg; N=3) and phentolamine (3.15-12.6 µmol/kg; N=2) caused only occasional and inconsistent reductions in AMPH-appropriate responding. Thus, compounds that inhibit DA and/or NE uptake occasioned the most consistent AMPH-like responding and a D1 antagonist provided the most consistent antagonism. The data suggest a prominent role for DA and D1 DA receptors in the mediation of the discriminative stimulus properties of AMPH in rhesus monkeys, and raise the possibility that NE is involved as well.

AFFILIATION

Drug Abuse Research Center, Departments of Psychiatry and Pharmacological and Physiological Sciences, Pritzker School of Medicine, The University of Chicago, Chicago, IL 60637 USA

The Nicotine Withdrawal Syndrome: Nicotine Absence or Caffeine Excess?

D. Sachs and N. Benowitz

The nicotine withdrawal syndrome is thought to occur when serum nicotine level abruptly falls after an individual stops smoking. We came upon data which suggest that caffeine may also contribute to withdrawal. A 57 y/o female who had successfully stopped smoking 3 yr earlier, but was still using nicotine polacrilex, underwent a double-blind, randomized, placebo-substitution trial using 0, 1, and 2 mg nicotine polacrilex doses in 1 wk time blocks. Blood sampling and psychometric measurements were obtained between 5:00-6:00 PM on 5 sequential Fridays. In addition, the patient ranked withdrawal symptoms each evening at home using a 0 (none) to 4 (severe) Likert Scale.

During the 2 wk she used placebo medication (0 or 1 mg), her total tobacco withdrawal symptoms increased over 5-fold ($p<0.01$). Anxiety, increased eating, and insomnia, in particular increased significantly, while restlessness showed a substantial increasing trend. Her weight also increased 3.5 lbs, from 125.5±1.29 (±SD) to 129.0±0.09 lbs ($p<0.05$). Heart rate appeared to decrease as well by 9 beats/min, from 67.0±6.6 to 58.0±2.8 (p=NS). Also, mean serum nicotine was 2.0±1.3 (placebo) vs 9.7±2.4 ng/ml (2mg) ($P<0.005$). Likewise, serum cotinine was significantly decreased during placebo usage: 91.0±127.3 vs 275.5±37.8 ng/ml ($p<0.025$). Serum caffeine, however, increased significantly during the 2 placebo weeks: 12.6±3.5 (placebo) vs 4.5±0.6 mg/L (2mg) ($p<0.001$).

Insomnia, anxiety, and restlessness can be caused by caffeine, alone. Thus, the tobacco withdrawal syndrome may be caused by both nicotine deprivation and caffeine excess. Moreover, controling caffeine level during smoking treatment might improve treatment success by further minimizing withdrawal symptoms.

DAVID P.L. SACHS, M.D., Director, Palo Alto Center for Pulmonary Disease Prevention, Palo Alto, CA; NEAL L. BENOWITZ, M.D., Chief, Division of Clinical Pharmacology and Experimental Therapeutics, San Francisco General Hospital, San Francisco, CA.

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The Effects of Chronic Caffeine Exposure on the Reinforcing Properties of Caffeine

S. Evans and R. Griffiths

The present study was designed to directly investigate whether the reinforcing properties of caffeine could be enhanced by chronic exposure to caffeine. Thirty-two normal healthy subjects who were daily, moderate consumers of caffeine-containing foods (average of 300 - 400 mg caffeine per day) participated. Throughout the study subjects were required to abstain totally from all sources of dietary caffeine. Subjects were stratified into two groups based on several factors including caffeine preference: preference for caffeine over placebo was assessed using a caffeine vs placebo choice procedure. Subsequently. subjects were assigned to receive either caffeine (300 mg t.i.d.) or placebo (placebo t.i.d.) for 18 consecutive days. AI the end of this time subjects were again exposed to a preference choice procedure similar to that in the beginning of the study.

On initial exposure, caffeine significantly increased subjective measures such as tension-anxiety, active. and jittery compared to placebo. Surprisingly. during the terminal portion of the chronic phase there were no significant differences between the two groups with the exception of two subjective measures (liking and stomachache). This suggests that rapid and complete tolerance developed to the subjective effects of caffeine. However, in spite of maintaining subjects in the chronic caffeine group on 900 mg each day, this chronic exposure did not affect choice. The percent selection of caffeine over placebo in both groups remained relatively unchanged after chronic caffeine or placebo (43% and 38%, respectivley). It is not clear why a greater proportion of the chronic caffeine group did not choose caffeine. It is possible that a longer period of placebo exposure may have been necessary in order for more subject to come into contact with the adverse effects of caffeine withdrawal. hereby increasing caffeine choice. However, when caffeine choosers were compared to those subjects who chose placebo. irrespective of dosing condition, a wide range of subjective measures were significant indicating that choosers responded to caffeine and/or placebo differently than nonchoosers. Three types of results differentiated between caffeine choosers and nonchoosers: 1) caffeine choosers showed greater effects than nonchoosers to the positive effects of caffeine; 2) caffeine choosers showed greater effects than nonchoosers to the adverse effects of placebo: 3) nonchoosers showed greater effects than choosers to the adverse effects of caffeine.

AFFILIATION

The Johns Hopkins University, School of Medicine
Baltimore, Maryland 21224

Endogenous Opioids and Reinforcement: Role of Multiple Opioid Receptor Types and Dopamine

T. Shippenberg, R. Bals-Kubik and A. Herz

A prominent behavioiral effect of opioid agonists is their ability to function as positive reinforcers, an effect which underlies their marked potential for abuse. The neurochemical substrates and site of action of opioids, however, in producing these effects remain ill-defined. Accordingly, the present study addressed these issues by use of the conditioned place preference paradigm; an animal model which permits the detection of both reinforcing and aversive drug-induced motivational (MOT) states.

Intracerebroventricular administration of selective μ-(DAGO, morphine) or δ-agonists (DPDPE) to rats produced dose-related preferences for the drug-associated place. In contrast, κ-opioid agonists (U-50488H, dynorphin: 66A-078) or opioid antagonists (naloxone, CTAP) produced conditioned place aversions. Mapping studies revealed that the ventral tegmental area (VTA) and its major projection site, the nucleus accumben (NAC) are critical sites of action of opioids in producing these effects. Thus, injections of μ-agonists into the VTA were reinforcing whereas injections Of K-agonists or opiod antagonists intpo the NAC were aversive. Animal involvement of the mesolimbic dopaminergic (DA) system in mediating the MOT effects of opioids is suggested by the findings that 6-OHDA lesions of the NAC abolish tthe place preferences and aversions produced by, respectively, μ- and κ-opioid receptor agonists. Furthermore, the ability of DA-1 (SCH-23390) but not DA-2 (sulpiride, spiperone) receptor antagonists to abolish opioid-induced place coinditioning suggest that the DA-1 receptor is critical for the expression of both these effects.

In summary, place conditioning studies demonstrate the involvement of both μ- and δ-receptors in mediating the reinforcing effects of opioids and suggest that in the case of μ-agonists such effects result from a stimulation of DA release in the NAC and the activation of DA-1 receptors. In contrast, the activation of κ-opioid receptors or the inactivation of μ-receptors results in aversive states. In view of data indicating a κ-agonist induced inhibition of NAC DA release, it is suggested that this action and a subsequent decrease in DA-1 receptor activity underlies the aversive effects of opioid agonists.

AFFILIATION

Max-Planck Institute for Psychiatry, Martinsried, Republic of Germany

Some Behavioral and Biochemical Features of Rat Pups Exposed to Methadone or Buprenorphine *In Utero*

J. Olley, G. Tiong and A. Cowan

Opiate-dependent women of childbearing age may be on methadone maintenance programmes. The partial u-agonist buprenorphine, is also of potential interest because of its pharmacokinetic and pharmacodynamic properties. Virgin female Long Evans rats (165-220g) were given daily subcutaneous injections with doses of opioid increasing for 5 days up to the maintenance dose which was continued until parturition. Treated females were mated on day 8 and were killed within 24 h of parturition after their litter had been fostered. Litters were culled to 6 male pups (day 5), weaned (day 18) and indicators of the status of their endogenous opioid systems were examined (days 24/25). Untreated (U) pups were compared with pups exposed to vehicle, 10% methanol in saline (V); methadone, 4 or 8 mg/kg (M4 or M8 respectively); buprenorphine 0.5, 1.0 or 2.0 mg/kg (80.5, B1 or B2 respectively).

Litter size was reduced after exposure in utero to M4 or B2. Significant mortality occurred after buprenorphine, usually within 48 h of birth, however, normal growth ensued if the first day of fostering was successful.

The latency of the tail flick response (50°C) at three weeks was markedly reduced after buprenorphine exposure. There were minimal signs of tolerance to morphine and dependence on opioid in the M8 group only.

Brain enkephalin levels of V, M8 and B2 pups were measured by RIA. Significant decreases in enkephalins were observed in the striatum of M8 pups. These studies indicated that neither methadone nor buprenorphine treatment of mothers was devoid of effects on endogenous opioid mechanisms in rats up to three weeks after birth. (Significance is indicated by $p<0.05$).

This work was supported by the Felton Bequests Committee.

AFFILIATION

Department of Pharmacology, Monash University, Clayton, 3168. Australia. *Visiting Fellow.

Pharmacological Characterization of the Tail Withdrawal Response as a Measure of Analgesia in Rhesus Monkeys

C. France and J. Woods

The latency for rhesus monkeys to remove their tails from warm water has been suggested to be a reliable measure of analgesia (e.g., Dykstra and Woods, 1986); in the present study, a wide variety of drugs were compared for their capacity to increase the latency for monkeys to remove their tails from 50° and 55°C water. Opioids as well as a variety of nonopioids (e.g., ketamine) increased the latency for tail withdrawal in a dose-related manner, however, there were marked differences among drugs in their potency, efficacy, and the susceptibility of their analgesic actions to antagonism by opioid antagonists. For example, some opioid mu agonists (alfentanil, etorphine) as well as some opioid kappa agonists (bremazocine, U-50,488) produced full analgesic effects (e.g., 20-sec latency) with both 50° and 55° C water. Other opioids that substitute as discriminative stimuli for etorphine (nalbuphine) or for ethylketocyclazocine (levallorphan) either had no effect or produced a less than maximal. analgesia. The opioid antagonist quadazocine attenuated the analgesic effects of full and partial agonists. Moreover, partial agonists attenuated the analgesic effects of full agonists. For example, the partial agonist buprenorphine produced a maximum of only 70% analgesia at a dose of 3.2 mg/kg. No analgesic effects were evident 24 hrs after large doses of buprenorphine, however, the analgesic effects of full agonists were attenuated for several weeks following administration of large doses of buprenorphine. Although nonopioids also increased the latency for monkeys to remove their tails from warm water, there were differences in the temperature- and dose-effect functions between nonopioids and opioids.

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AFFILIATION

Departments of Psychology and Pharmacology, University of Michigan, Ann Arbor, MI 48109-0626.

Relative Reinforcing Properties of Opioid Mixed Agonist-Antagonist Drugs

N. Mello, S. Lukas, M. Bree and J. Mendelson

The opioid mixed agonist-antagonist drugs are powerful analgesics with minimal capacity to induce physical dependence. The clinical usefulness of these compounds will be influenced by their abuse potential, but little is known about the reinforcing properties of the newest opioid mixed agonist-antagonists available for medical use. The relative reinforcing properties of nalbuphine (Nubain®) and butorphanol (Stadol®) were compared with pentazocine (Talwin®) in male rhesus monkeys (Macaca mulatta). A progressive ratio procedure was used to provide a quantitative index of the number of responses that a monkey will emit for a single drug injection. Monkeys worked at an operant task for food (1 gm banana pellet) and for nalbuphine (0.010, 0.032, and 0.100 mg/kg/inj); butorphanol (0.0010, 0.0032, and 0.0100 mg/kg/inj); pentazocine (0.10, 0.32 and 0.56 mg/kg/inj) and saline on a second order schedule of reinforcement [FR 4 (VR 16:S]. After baseline drug self-administration was stable, the second order schedule response requirement per injection was increased in increments of 64 until the monkey stopped responding for 8 consecutive sessions.

All three drugs at each dose maintained more responding and higher progressive ratio breakpoints than saline. Group average progressive ratio breakpoints for butorphanol showed dose-related increases of 576, 1173 and 1963 responses per injection. Progressive ratio breakpoints for the highest dose of butorphanol were higher than for any dose of nalbuphine. The low and high doses of nalbuphine maintained higher progressive ratio breakpoints (1600 and 1472 responses per injection) than the intermediate dose of nalbuphine (618 responses per injection). Group average breakpoints for pentazocine also showed dose related increases (864 and 1824 responses per injection) and these studies are still in progress. In contrast, group average breakpoints for saline-maintained responding ranged from 64 to 170 responses per injection. These preliminary data suggest that the relative reinforcing efficacy of these 3 opioid mixed agonist-antagonist drugs is quite similar. These data obtained in a primate drug self-administration are concordant with human abuse of pentazocine and predictions of abuse liability of butorphanol and nalbuphine. Progressive ratio breakpoints were consistently higher than we previously reported for buprenorphine (0.10 mg/kg/inj) or heroin (0.10 mg/kg/inj) (522 and 1067 responses per injection) (Mello et al., 1988). [DA-02519, DA-00101, DA-00064, DA-00115

AFFILIATION

Alcohol and Drug Abuse Research Center, Harvard Medical School-McLean Hospital, 115 Mill Street, Belmont, MA 02178

Modification in Protocols Used to Characterize Opioids Within the CPDD Drug Evaluation Program

J. Woods, F. Medzihradsky and C. France

We have recently described the use of the highly selective ligands [^{3}H]sufentanil, [^{3}H]D-Pen2,D-Pen-enkephalin, and [^{3}H]U69,593 to quantitate binding of opiates to the mu, delta, and kappa opioid receptors, respectively (Clark et al., 1988). The selectivity index of a compound in binding to a given type of opioid receptor was expressed as the ratio of its EC50's in displacing the radiolabeled ligands. These methods were applied to brain membranes from the monkey and rat.

In addition, others of us have described the use of a tail withdrawal procedure in rhesus monkeys to assess analgesic effects (Dykstra and Woods, 1986; Woods and Winger, 1988). The procedure utilizes as a measure of analgesia the latency of tail withdrawal from water of different temperatures. A wide variety of drugs have been studied in this procedure and the results will be shown to complement other procedures currently used within the opioid drug evaluation program.

It will be suggested that the above outlined methods employed to characterize compounds submitted to the opioid drug evaluation Program.

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AFFILIATION

Departments of Biological Chemistry, Pharmaciology, and Psychology, University of Michigan, Ann Arbor, MI 48109-0626

The Intermittent Antagonist Paradigm: Intermittent Naloxone Attenuates the Development of Physical Dependence on Methadone in Rhesus Monkeys

J. Krystal, M. Walker and G. Heninger

Rhesus monkeys that received 15 daily injections of methadone (METH) (2 ml/kg i.m.) exhibited opiate withdrawal after injection of naloxone (0.5 mg/kg i.m.) on day 16. In comparison, naloxone (0.5 mg/kg i.m.) once every two days during a similar 15-day METH treatment period in these monkeys significantly decreased the severity of opiate withdrawal symptoms exhibited after naloxone injection on the sixteenth day. Each naloxone injection during the 15 day METH treatment period elicited a mild opiate withdrawal syndrome that did not significantly differ on each of the 7 days it was given. This mild withdrawal syndrome was less severe than the syndrome observed when naloxone was administered on day 16 following 15 days of METH without intermittent naloxone. The lack of increments in the withdrawal response to the 7 naloxone injections during the 15 days of METH treatment and the attenuation of the withdrawal response to naloxone administration during the 15 day METH treatment period support the hypothesis that naloxone modifies opiate receptor mechanisms so that they revert to an agonist-naive state following antagonist exposure. These findings would suggest that agonist and antagonist drug combinations or partial agonist drugs could be useful in the clinical management of situations where physical dependence on opiates is a problem.

AFFILIATION

Abraham Ribicoff Research Facilities, Department of Psychiatry
Yale University School of Medicine, New Haven, CT

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Opioid Receptor Selectivities Induced by N-Substituent Variations

L. Toll, W. Polgar, J. Lawson and G. Loew

N-substituent variations in families of fused-ring opioids are known to produce change in efficacy, in some cases leading to the development of agonist activity. However, the effect of these N-substituent variations on relative receptor affinities has not been systematically evaluated. We report here studies of the binding properties of N-methyl, N-allyl, and N-cyclopropylmethyl (N-cpm) analogs of five opioid families, including fused-ring benzomorphan, morphine, oxymorphone, and oripavine analogs, and the flexible 4-phenyl piperidines. In the morphine, oxymorphone, and benzomorphan families, the N-methyl analogs are p-selective, with lowest affinity at the K-receptor. Replacement of the N-methyl by allyl or cpm increases affinity at μ, δ, and κ receptors, however, affinity at κ is increased disproportionally. The result is that the N-cpm analogs have equal affinity at μ and κ receptors in these three families. For the oripavine etorphine, and analogs, there is little effect of change in the N-substituent on receptor selectivity. All three analogs bind with high affinity at all receptors, confirming this class of compounds as universal ligands. The 4-phenyl piperidines also show little effect of N-substituent variation, but unlike the etorphines, have only moderate affinity at μ and κ receptors, and low affinity at δ. For most families of fused-ring opiates, an increase in κ affinity correlates with a decrease in μ efficacy, leading to analgetic antagonism. However, high κ affinity does not necessarily lead to μ agonism, as etorphine is a super high efficacy μ agonist with high κ affinity. Furthermore, N-allyl and N-cpm analogs of the oripavines have decreased μ efficacy without any increase in relative μ affinity. This suggests that efficacy and selectivity may be modulated by separate effects of the N-substituent.

AFFILIATION

Molecular Thebry Department, Life Sciences Division, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025

A Novel Peptidic Mu Opioid Antagonist with Exceptional Potency and Specificity

T. Kramer, W. Kazmierski, J. Shook, V. Hruby and T. Burks

A novel, cyclic octapeptide derivative of somatostatin, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-Nh_2 (CTAP), a high affinity, selective ligand for mu opioid receptors in rat brain was tested extensively in vitro and in vivo. In vitro bioassays included the guinea pig ileum longitudinal muscle/myenteric plexus (GPI), and the mouse (MVD) and rabbit (LVD) vasa deferentia. In vivo tests, performed in mice, included hotplate analgesia with intracerebroventricular (i.c.v.) dosing, and intestinal transit inhibition (geometric center method), with intrathecal (i.t.) dosing.

In the GPI, CTAP showed no intrinsic cpioid agonist activity at concentrations as high as 10 uM. CTAP competitively antagonized the mu selective cpioid agonist PL017 in the GPI; Schild analysis gave a pA_2 value of 7.12±0.08. CTAP selectivelv protectedm mu, but not kappa, receptors in the GPI from alkylation by the non-selective irreversible opioid antagonist beta-chlornaltrexamine. In the MVD, CTAP showed agonist activity at high (>3000 nM) concentrations which were blocked by the delta selective opioid antagonist ICI 174,864. CTAP competitively antagonized PL017 in the MVD (pA_2=7.03±o.064), but did not antagonize the delta agonist DPDPE at concentrations up to 10,000 nM. CTAP was completely devoid of intrinsic activity in the LVD. In vivo, CTAP alone did not produce analgesia, inhibition of intestinal transit, or noticeable toxicity when administered i.c.v. or i.t. in doses as high as 10 ug. CTAP antagonized the analgetic effects of PL017, the in vivo pA_2=11.18±0.4, appearing more potent than naloxone (pA_2=10.14±0.40). Similarly, CTAP antagonized the antitransit effect Of PL017, the in vivo pA_2=11.82±0.75, appearing markedly more potent than naloxone (pA_2=10.14±0.39). We conclude that CTAP is a highly selective and potent mu opioid antagonist which should prove useful in future studies of opioid neurobiology.

AFFILIATIONS

Departments of Pharmacology[1] and Chemistry[2], University of Arizona, Tucson, AZ 85724. Behavioral and Neurological Toxicology[3], NIEHS, Research Triangle Park, NC 27709. Supported by USPHS grants DA02163, DK36289, and DA04248.

Differential and Selective Modulation of Opiate Mu Agonist Effects by Delta and Kappa. Agonists *In Vivo*

J. Heyman, R. Sheldon and F. Porrecca

In spite of evidence suggesting that the opiate receptors exist as separate macromolecules with anatomically distinct localization and pharmacology, evidence has also begun to accumulate which suggests that, in some cases, opiate receptors may exist in a physically or functionally coupled states. Much of the evidence supporting receptor interaction stems from observations which showed that the analgesic effect of morphine is potentiated or antagonized by the co-administration of Leu-enkephalin or Met-enkephalin, respectively. Our previous work has demonstrated that opioid κ agonists such as U50, 488H, tifluadom and ethylketocyclazocine can antagonize the suppression of volume-induced micturition contractions of the rat bladder produced by selected opiate μ agonists such as morphine and normorphine. These κ agonists, however, do not affect the agonist properties of other μ agonists such as DAGO, PL017, meperidine and phenatocine. The data have been interpreted to suggest the possibility of μ receptor subtypes within the central nervous system which could be activated by morphine and normorphine and modulated by κ agonists, with other μ receptor types activated by compounds such as DAGO and PL017 and not associated with κ receptor modulation. In an attempt to expand this hypothesis, our present experiments have extended these observations by (1) determining whether those μ agonists modulated by U50,488H, ethylketocyclazocine and tifluadom are the same as those modulated by either a δ agonist (i.e., DPDPE) or an endogenous κ agonist, dynorphin using the bladder model; and (2) determining whether δ and κ ligands at subagonist doses can modulate the analgesia produced by this series of μ agonists. Support for the hypothesis would gained if the same μ agonists were modulated by both δ and κ agonists, and if the modulation profile were observed in two endpoints, inhibition of the micturion reflex and production of analgesia. Our data showed that DPDPE could potentiate the bladder and analgesic effects of morphine. normorphine, and etorphine but failed to affect the actions of DAGO. PL017, meperidine, methadone, phenazocine and sufentanil, in either endpoint. The DPDPE potentiation, but not the direct actions, of morphine were prevented by co-administration of ICI 174,864. Similarly, dynorphin A-(1-17) antagonized the bladder effects of morphine and normorphine, but did not affect the actions of the other μ agonists in the bladder model.

The collective results of these studies show that μ agonists are generally separated into two groups - those which are modulated by δ and κ agonists, and those which are not. These data are interpreted as supporting the view that opioid receptors may exist in both complexed forms involving μ, δ and κ sites, as well as in individual non-complexed forms.

AFFILIATION: Department of Pharmacology, University of Arizona Health Sciences Genter, Tucson, Arizona 85724

Excitatory Amino Acid Neurotransmission and the Behavioral Pharmacology of Phencyclidine-like Drugs

R. Balster, J. Willetts, P. Beardsley, B. Hayes and A. Rice

Recent evidence suggests that some of the effects of phencyclidine (FCP) and related drugs may result from their interaction with a PCP receptor associated with an excitatory amino acid receptor-regulated ion channel. Specifically, PCP has been found to selectively and noncompetitively antagonize many of the biochemical and electrophysiological effects elicited by N-methyl-D-aspartate (NMDA). Thus, it is possible that NMDA receptor-mediated excitatory amino acid neurotransmission may be an important factor in the transduction of the behavioral effects of PCP and that potentially therapeutic competitive NMDA antagonists may have PCP-like side effects. In order to address these possibilities, we have used a variety of behavioral approaches to examine the effects of drugs acting upon this receptor complex. Differences were found in the effects of competitive and noncompetitive NMDA antagonists. In both rats and rhesus monkeys, the noncompetitive antagonist MK-801 completely substituted for the PCP stimulus. Both PCP and MK-801 had reinforcing effects in rhesus monkeys as well, although some differences were obtained depending upon whether the animals' responding was normally maintained by cocaine or PCP. Studies in rats provided evidence that the competitive antagonists CPP and NPC 12626 only partially substituted for PCP, and only did this at doses that had other direct effects on behavior. The discriminative stimulus effects of CPP and NPC 12626 were no more similar to PCP than were those of barbiturates. In rats trained to discriminate NMDA from saline, CPP completely antagonized the NMDA stimulus at doses less than those which produce any PCP-like effects in PCP-trained animals. On the other hard, PCP and MK-801 were much less effective as antagonists of NMDA discrimination. Our results suggest that therapeutically useful competitive NMDA antagonists are less likely to produce FCP-like side effects than are noncompetitive NMDA antagonists acting upon the PCP receptor. They also suggest that NMDA antagonism may not be the sole mechanism for the behavioral effects of PCP relevant to its abuse. (Supported by NIDA Grant DA-01442).

AFFILIATION

Department of Pharmacology & Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Kinetics of a Receptor-Effector System Capable of Addictive Behavior: Abstinence Responses to Withdrawal of Opiate Agonist

J. Villarreal, S. Cruz and L. Salazar

Analysis of the early development of experimental opiate dependence led our group to elaborate a theory of the nature of this type of dependence (NIDA Research Monographs 67:105, 1986). The core of the theory is a quantitative model of drug-receptor-effector interaction whose mathematical behavior reproduces the diverse pharmacological characteristics of abstinence responses precipitated by antagonists under various conditions. We now report on the mathematical behavior of the model system with reference to abstinence responses produced by the withdrawal of opiate agonist.

The responses of the model were obtained by computer as numerical solutions to the set of differential equations of the system. The receptor-effector model was in the appropriate form to study agonist withdrawal as described by us at the 1987 CPDD meeting (NIDA Research Monographs, in press).

It was found that the new model, of drug-receptor-effector interaction is capable of producing. robust abstinence responses when the opiate agonist drug is removed from the medium. The intensity of such abstinence responses is proportional to the dose of agonist employed and to the magnitude of dependence present. Also, abstinence can be relieved in a graded fashion by new doses of opiate agonist. Dependence could be demonstrated to cause an important degree of tolerance towards the neurodepressant effects of agonist.

Unlike the present model of drug-receptor-effector interaction, the model of classical occupation theory does not have a capacity to generate abstinence responses to agonist withdrawal. Furthermore, when the new model is studied for conditions where dependence quantitatively approaches zero, the model's behavior tends to a mathematical limit defined by the classical occupation equation. Thus, for the pharmacology of opiate agonists, occupation. theory is a particular limiting case contained in the wider domain of the present model.

AFFILIATION

Department of Pharmacology and Toxicology. CINVESTAV-IPN, Czda. Xochimilco 77, 14370 Mexico, D.F., Mexico.

A Response Competition Model of Tolerance and Sensitization

D. Newlin

Siegel's (1975) Pavlovian conditioning model of drug tolerance has provided a central impetus for research concerning behavioral factors in adaptation to drugs of abuse. However, empirical research over the past decade has failed to support key predictions of the model. The response competition model (RCM) is intended to account for these discrepancies, as well as to make new predictions that are unique to the RCM.

The primary assumptions of the RCM are that when the conditioned response (CR) to drug cues has a different response processing mechanism from the unconditioned response (UR) or drug effect, the result will be an inhibitory interaction between CR and UR that leads to tolerance: conversely, when the CR and UR have similar or identical response mechanisms, there will be a synergistic inter-action between CR and drug effect that leads to sensitization. Unlike Siegel's model, the RCM does not assume that the CR to drug cues is necessarily opposite in direction to the drug effect, and the RCM assumes that the interaction between CR and UR is clearly nonadditive.

A major problem for Siegel's (1975) Pavlovian conditioning model is that most, if not all, investigators have failed to replicate his results showing a hyparaigesic CR to morphine cues. In addition, although alcohol conditioning studies have found consistently a small hyperthermic CR to alcohol cues, the CR has proved too small to account for situational specificity of tolerance to alcohol. In pyretic conditioning to morphine, more recent results show sensitization rather than tolerance and hypedhermic rather than hypothermic CRs to morphine cues. Finally, drug conditioning designs that allow independent assessment of UR and CR have found consistently that the relationship between responses is nor additive. The RCM accounts well for these discrepancies.

The basic assumptions of the RCM lead to several new predictions: a variety of opioid responses will combine synergistically with the response to morphine, but nonopioid responses will combine in an inhibitory interaction with morphine due to response competition. Both opioid and nonopioid responses will inhibit the response to alcohol. (Supported in part by NIAAA grant #AAO6433)

AFFILIATION: NIDA/Addiction Research Center, Baltimore, MD 21224

Familial Alcoholism in Opiate Addicts

T. R. Kosten and B. Rounsaville

Familial substance abuse was examined among 201 opioid addicts using modern methods on a total of 877 first degree relatives. We addressed specificity of drug versus alcohol transmission, and sex-related differences in transmission. Supporting specificity of alcoholism transmission, a strong association of familial alcoholism with alcoholism among the proband addicts was found, with 30% of alcoholic addicts having alcoholic first degree relatives. Using the 477 siblings, we also found some specificity of drug versus alcohol abuse, since 55% Of parents with drug abuse had children with both drug abuse and alcoholism, but none of their children had alcoholism alone. Furthermore, addicts' siblings with alcoholism alone did not have parents with drug abuse. We found that both female probands and their female siblings had higher rates of parental alcoholism than did males, suggest in greater female "loading". This increased loading in females has not been observed in pure alcoholism.

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AFFILIATION

Yale University School of Medicine, Psychiatry Department, Substance Abuse Treatment Unit, New Haven, CT 06519

Characteristics of Women Receiving Mandated Alcoholism or Polydrug Treatment in Massachusetts

B. Lex, S. Teoh, I. Lagomasino and J. Mendelson

The first 20 women admitted for treatment following civil commitment by Massachusetts courts were selected for study. All received physical examinations, psychological assessments, and laboratory studies, and answered questions about alcohol and drug use histories, reproductive histories, and social, legal, and economic circumstances.

Most patients had completed high school, and 50% were divorced. Patients also had few marketable economic skills, with 35% supported by public assistance. Only 25% had no family history of alcoholism.

Patients were diagnosed as either alcohol dependent (n = 12) or multiple drug dependent (n = 8). Alcohol dependent women were older at admission, at age of initial alcohol use, at age of onset of regular alcoholic use, and at first treatment admission.

Of the 20 women studied, 25% had high MCV. Two were anemic, and required investigation of the gastrointestinal tract. Five women had elevated SGPT (range 60-100), and 2 had marginally elevated sGOT.

Of the 20 women studied, 13 were of reproductive age and 7 were postmenopausal. Hormone level analysis of two of 12 reproductive age women showed secondary amenorrhea of 12 and 24 months' duration, but both had normal prolactin levels. The remaining 10 women had normal LH, FSH, E^2, and progesterone levels consistent with their reported menstrual cycle phase, but four patients had elevated prolactin levels (range 23.8.87.S ng/ml). Plasma LH, FSH, E2, and progesterone were within normal range for the 7 postmenopausal women, but 4 postmenopausal women had elevated plasma prolactin levels (22.3.37.5 ng/ml).

Women who undergo court-ordered alcohol and drug dependence treatment had multiple health, social, and economic problems.

AFFILIATION

Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, 115 Mill St. Belmont, MA 02178.

The Relationship Between Familial Risk for Alcoholism and Drinking Behavior in College Men

A. Alterman, J. Searles and J. Hall

Groups of high risk (alcoholic fathers), middle risk (second degree alcoholic relatives) and low risk (no first or second degree alcoholic relatives) male college students were compared with respect to drinking behavior, sociodemographic variables, personality, and mental health and drug use problems in themselves and in family members. The groups differed significantly on only one of a number of sociodemographic variables. No significant group differences were revealed in drinking behavior or alcohol-related symptoms/consequences. High risk subjects reported significantly more childhood attentional/social problems than low risk subjects. No group differences were found with respect to other childhood problem behaviors, and subject or family drug use and mental health problems. The findings are discussed in terms of the questions they raise concerning the results of high risk studies and the contribution of genetic factors to alcoholism.

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AFFILIATIONS

Research and Psychiatry Services. VA Medical Center and Department of Social Science, Drexel University, Philadelphia, PA 19104

Gender Differences in the Prediction of Psychotherapeutic Medicine Use

A. Trinkoff and J. Anthony

One of the intriguing relationships observed by Yamaguchi and Kandel was the association between illicit drug use and psychotherapeutic medicine use. Building on this research, we investigated the possibility that this relationship might be due to the presence of psychiatric conditions. As we reported elsewhere, illicit drug use was associated with an increased likelihood of psychotherapeutic medicine use, although the presence of psychiatric conditions did not account for this finding.

Research on the etiology and prevalence of psychotherapeutic medicine use has revealed many gender differences. Therefore, the authors felt that it was appropriate to examine the relationship between illicit drug use, psychiatric conditions, and psychotherapeutic medicine use using separate multivariate models for males and females.

Data for these analyses were obtained from the Wave I household interviews of the Eastern Baltimore Mental Health Survey of the NIMH Epidemiologic Catchment Area Program. For this analysis, the sample was restricted to 17 16 respondents aged 18-44 (668 males, 1048 females).

Analyses were conducted using Cox proportional hazards models with time-dependent covariates. These models produce parameter estimates (betas) which are interpreted similarly to estimates produced using multiple logistic regression. However, unlike multiple logistic regression, the Cox models were able to use information on the age of onset of illicit drug use and psychiatric conditions, and relate it to the initiation of psychotherapeutic medicine use.

Both males and females had an increased likelihood of psychotherapeutic medicine use in relation to antecedent illicit drug use, although the association was stronger among males (estimated rel. risk = 4.0 for males, 2.2 for females). The presence of psychiatric conditions did not change this finding for males or females.

AFFILIATION

Addiction Research and Treatment Services, Department of Psychiatry, University of Colorado School of Medicine, Denver, Colorado 80262

Health, Happiness and Medicine Use in an Elderly Population

W. McKim, M. Stones and A. Kozma

In this study, a number of demographic, health, and personality measures including Significant Life Events, Satisfaction with Housing and Finances, Religiosity Locus of Control and use of prescription and nonprescription drugs were collected on two separate occasions 12- 18 months apart from 380 people over 65 years of age. The subjects were selected randomly from urban, rural and institutionalized populations living in Newfoundland. All these measures were then regressed on the number of medicines used. Only variables which were significantly related to the dependent variable on both testings were considered reliable. The analyses showed that the primary predictor of medicine use is Disease Severity; a combination of the number of different diseases or disorders and the extent of their severity. This factor accounts for about 26-30% of the variance. The next most important factor is Self-Rating of Health; how the individual perceives his or her own health. This factor accounts for 6 to 10% of the variance. The only other significant predictor is Word Fluency, accounting for 1 to 3% of the variance. No other social or demographic factor such as sex or age are significant predictors once variance due to these variables was removed from the equation. Health Rating and Disease Severity, make additive contributions to predicting medicine use and do not interact. Both of these variables predicted Medicine use 12- 18 months later. Disease Severity is significantly predicted by Health Rating, but the Number of Stressful Life Events also makes a significant contribution to Disease Severity. Likewise, Health Rating is primarily influenced by Disease Severity, but Happiness, as measured by the Memorial University of Newfoundland Scale of Happiness, also makes a significant independent contribution to Health Rating. This model applies to both males and females and urban and rural populations, but the influence of Health Rating disappears in institutionalized populations, leaving Disease Severity as the only predictor of medicine use.

AFFILIATION

Department of Psychology and Gerontology Centre, Memorial University of Newfoundland, St. John's, Newfoundland, CANADA, A/B 3X9

Drug Abuse in Poland: Problems and Trends

T. Chrusciel

It is estimated that in 1986 there were in Poland 200,000 occasional users among which 30,000 to 35,000 can be qualified as regular users. Among schools of all grade students reported as using drugs, 64% was in the primary and middle level schools, 31% in the occupational schools and 4% in high schools. Index (1/10,000) was 5.9: 10.2 and 4.6 resp., indicating the greatest involvement among 14-18 year-old students of occupational schools. The main substances of abuse are opiates but poly-drug use prevails among users. Illicit production of concoctions from home grown opium poppy for oral use ("makiwara") or for injecting ("kompot") as well as of amphetamines is on-going).

The model of use is being changed rapidly. There is somewhat less use of opiates and more use of psychotropic medicaments, inhalants, hallucinogens (mushroom Psyloche lanceata) and more cannabis smoking. Less users currently report for medical treatment. Police data indicate in 1986 and 1987, 16,675 and 16,229 resp. users involved in criminal activities. Illicit production, originally limited for self-use of the producers, is growing and non-users are more frequently involved. A previously non-existing black market begins to emerge and attempts to smuggle out from the country home made products are increasing. Responses of health and educational sectors are developing but are very slow because of lack of qualified personnel. At the community level several societies were created (Soc. for Prevention of Drug Abuse, Return from u, Monar and Forge).

AFFILIATION

Dpt. of Clin. and Social Pharmacology, PMEC, Warsaw, Poland

Methadone Exposure *In Utero:* Assessing the Risk Factors

K. Kaltenbach

Although methadone maintenance Is widely recognized as appropriate treatment for pregnant narcotic drug dependent women, there is continuing debate regarding the effect of in-utero exposure to methadone on the developmental outcome of their progency. This study was designed to assess the -outcome of infants exposed to methadone in-utero through the first five years of life. All of the drug dependent mothers were participants in Family Center, a comprehensive program that provides methadone maintenance. prenatal and obstetrical care and intensive psychosocial counselling for pregnant drug dependeat women. Two hundred and sixty-eight infants: 141 infants born to Family Center women maintained on-methadone -during pregnancy and 127 comparison infants born to non drug-dependent women were enrolled in the study. All infants were healthy term newborns. Perinatal, developmental and psychosocial variables were examined. The methadone exposed infants had smaller birth weight (x=2953 grams) and smaller head circumference (x=33.29 cm) than comparison infants (x=3210 grams: x=33.94 cm) but they were not small for gestational age. One hundred sixty-eight infants (105 methadone exposed and 63 comparison infants) were evaluated at 6 months of age. No differences were found between groups on the Bayley Scale of Mental Development and a comprehensive neurological examination. Meao scores were 103 and 105 for methadone exposed infants and comparison infants, respectively. Nor were any differences found in infants assessed during the first two years of life. Mean MDI Scores at 6, 12 and 24 month; were 105, 103 and 99. respectively, for methadone exposed infants and 106, 109 and 104 respectively, for comparison infants. Cognitive ability for the children at preschool age was also similar between groups. No differences were found in McCarthy General Cognitive Index (GCI) or on any of the 5 subscales. Mean scores for the methadone exposed children were GCI = 106.51; Verbal = 53.44; Perceptual = 55.51; Quaotitative = 51.33; Memory = 49.51; and Motor = 52.29. Mean scores for the comparison children were GCI = 106.5; Verbal = 54.33; Perceptual = 53; Quantitative = 53.88; Memory = 52.27; and Motor = 50.44. These data indicates that when methadone maintenance for pregnant women is provided within a comprehensive prenatal treatment program the outcome of their progeny is favorable.

AFFILIATION

Department of Pediatrics, Thomas Jefferson University, Philadelphia, PA 19107.

Alcohol, Drugs and Sex: Risky Behaviors in the General Population. A Report From the St. Louis ECA Survey

L. Cottler and J. Helzer

In general, our knowledge of AIDS risk factors has come from treated samples, or groups selected as proxies for the general population, such as new military recruits. As part of the St. Louis NIMH Epidemiologic Catchment Area survey (EGA), a community study of the incidence and prevalence of mental disorders, data on illicit use of psychoactive drugs and a history of sexual behaviors was obtained on a sample of 3,200 subjects. We classified subjects into five mutually exclusive groups according to their patterns of substances used. Data on sexual behaviors included lifetime history of sexual behaviors such as homosexual relationships, sexual promiscuity, infidelity, and prostitution.

The proportion of men who were classified as hard drug users (cocaine, heroin, other opiates) was 10%-- twice the rate for women. Marital infidelity was associated with hard drug use. Sexually promiscuous women (10+ partners/yr) were more likely than promiscuous males (35% vs. 23%) or female prostitutes (19%) to report a history of hard drug use. Persons who had ever had homosexual relations were also at greater risk for hard drug use compared with heterosexuals and virgins. These data can be used to estimate the extent to which the untreated are at risk. For example, 80% of hard drug users 18-24 years of age have not been in drug (or alcohol) treatment. Our data also show the progression of behaviors to be drinking, smoking, risky sex and drugs, except for the youngest cohort which indulges in drugs slightly earlier than in risky sex. These data are interesting for several reasons: they are general population data; they were collected prior to AIDS education campaigns; and they can be weighted to national distributions. As expected, illicit use of drugs tends to be reported by persons who also report risky ‘sexual behaviors. These estimates may help answer the question of who in the general population is potentially "at risk" for HIV infection.

AFFILIATION

Department of Psychiatry, Washington University School of Medicine, 4940 Audubon Avenue, St. Louis, MO 63110.

Pilot Trial of Small Group AIDS Education with Intravendus Drug Abusers

J. Sorensen, D. Gibson, C. Heitzmann, A. Calvillo, R. Dumontet, E. Morales and A. Acampora

In a random assignment study, preliminary evaluation indicates that AIDS educations groups in a therapeutic community increase residents' knowledge about AIDS and change their attitudes toward AIDS.

Drug treatment programs may play a vital role as centers for prevention of AIDS among addicts, but most interventions have little evidence of efficacy. We have developed and are evaluating the impact of a small-group AIDS education program for IV drug abusers completing residential treatment. In a pre, post, follow-up design subjects (Ss) are randomly assigned to six hours of small- group education or to receive brochures only. Measures assess knowledge and attitudes about AIDS and the behaviors that put Ss at risk of HIV infection. Follow ups occur 6 and 12 months post intervention. The intervention focuses on safe sexual practices and avoiding HIV infection if Ss relapse to IV drug use.

The first 79 Ss in the first 5 cohorts (114 Ss will be involved in the complete study) attended an average of 82% of the group sessions; 82% were reached for post-interviews, and 89% of the first 63 Ss have been reached at 6 months. In pre-post comparisons workshop Ss gained more knowledge than controls ($p<.05$), belief in their self-efficacy to avoid HIV infection ($p<.005$), confidence in their ability to communicate in risky situations ($p<.05$), and displayed a trend ($p=.07$) toward greater response efficacy. Both groups reported low post-interview levels of drug use and sexual activity (Ss were still in treatment). At the 6-month followup drug use was still infrequent, and the groups did not differ in their use of condoms. The differences in knowledge endured to the 6-month followup.

AFFILIATION AND ACKNOWLEDGMENTS

UCSF School of Medicine, Dept. of Psychiatry, S. F. General Hospital, 1001 Potrero Avenue, San Francisco, CA 94110.
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Topographic Mapping of Quantitative EEG Variables in Chronic Heavy Marijuana (THC) Users

F. Struve and J. Straumanis

Previous quantitative EEG studies of acute THC exposure by others have shown placebo controlled dose dependent transient increases in posterior alpha activity. Persistent EEG change from chronic THC use has not been-reported. Our data suggests that-the topographic distribution of quantitative EEG variables may identify regional characteristic EEG features associated with chronic heavy THC abuse.

Ten psychiatric patients who used THC daily for 3 to 12 years were age and sex matched with patients never using THC. Ten normals also served as controls. All subjects had a 21 channel EEG with ocular monitoring from which 40 to 60 artifact free 2½sec. raw EEG epochs were selected for quantitative analysis. Topographic EEG mapping and between group statistical comparisons at all 21 electrode sites showed clear EEG profile differences between THC users and both non-user groups. THC use was significantly associated with (1) elevations of both absolute and relative power of alpha at all 21 electrode sites, (2) elevated interhemispheric coherence of alpha over frontal regions, (3) a less marked elevation of absolute power of other frequencies at many but not all electrode sites and (4) a decrease of relative power of all non-alpha frequencies. THC users were confined inpatients with no known access to marihuana and had not used THC for several days prior to testing. When EEG variables are expressed as Z-score departures from a normative data base, a marked "hyperfrontality" of alpha rhythm is seen. The most extreme or deviant elevations of relative and absolute power and coherence of alpha for THC users occur over prefrontal and frontal cortex. In almost all people alpha is posteriorly dominant and rarely well developed over frontal cortex. Thus in our THC users the regional distribution of alpha activity is quite different from the expected alpha topography which characterizes the population at large.

Intergroup medication or diagnostic differences are not plausible explanations for our results. Confounds in our preliminary study are discussed and methodological issues for further research introduced. The possibility that these findings may have relevance as CNS markers for chronic THC exposure is entertained.

AFFILIATION: Department of Psychiatry, Louisiana State University School of Medicine at Shreveport, PO Box 33932, Shreveport, LA 71130

Ethanol Effects on Marijuana-Induced Intoxication and Electroencephalographic Activity

S. Lucas, J. Mendelson, J. Henry, L. Amass and A. Budson

Ethanol/marijuana combinations result in enhanced electroencephalographic (EEG) and behavioral responses. Adult male volunteers provided informed consent and were prepared with scalp electrodes for EEG and physiologic recording and an i.v. catheter for blood withdrawal. A within-subject design was used such that each subject participated on three separate days spaced 1 week apart. Subjects received either placebo, low-dose (0.35 g/kg) or high-dose (0.70 g/kg) ethanol 30 min before smoking either a placebo, low-dose (1.24% Δ^9-THC) or high-dose (2.65% Δ^9-THG) marihuana cigarette. The design was blocked so the ethanol pretreatment was varied while each subject smoked the same strength cigarette each day.

Some of the behavioral effects of ethanol and marihuana combinations were similar to those we noted previously when each drug was given alone: subjects reported that they experienced transient, paroxysmal episodes of euphoria which *were* clearly differentiated from drug detection. There were some notable differences when ethanol and marihuana were administered together. First, ethanol and marihuana combinations frequently resulted in brief episodes of dysphoria. Second, the number of euphoric episodes was maximal after intermediate doses of each drug. Third, while ethanol failed to alter marihuana-induced subjective effects, concurrent administration of marihuana resulted in an increase in ethanol-induced intoxication. Finally, marihuana delayed the appearance of peak plasma ethanol levels which may have been due to slower ethanol absorption.

EEG alpha a tivity during marihuana-induced euphoria was greater after ethanol pretreatment than after placebo. These effects on alpha activity after both drugs are the largest we have observed. The results of prior studies suggest that increased EEG alpha activity is associated with a pleasant mood state. Thus, drug-induced increases in EEG alpha may be related to the reinforcing properties of drugs of abuse. While the EEG response was qualitatively similar to that observed after each drug alone, the observed increase in EEG alpha power suggests that the degree of euphoria during ethanol and marihuana combinations may differ in intensity. These results also offer promising insights to the neurophysiological correlates of drug-induced euphoria and provide a framework for studying the pharmacological and behavioral bases of polydrug abuse.

AFFILIATION

Alcohol and Drug Abuse Research Center, Harvard Medical School/McLean Hospital, Belmont, Massachusetts 02178. Supported by NIDA Grant DA03994 and Research Scientist Development Award DA00115.

Dependence Upon Anabolic Steroids

F. Tennant

The author has studied a group of six weight lifters and body builders in Southern California who have used anabolic steroids (AS) on a daily basis for three to ten years. Type of AS, dosage, side-effects, psychogenic properties and self-reported dependence has bee assessed, and AS use was confirmed by urine analysis. Preliminary results indicate that some users report euphoric effects when AS are consumed and experience withdrawal symptoms when they are discontinued.

One 23-year old weight lifter voluntarily sought medical withdrawal from "addiction to AS." On initial evaluation, this individual was 5'3" and weighed 230 lbs. He ceased AS 72 hours prior to evaluation and was currently in withdrawal and "sick." Physical examination revealed a dilated pupil, diaphoresis, and an elevated pulse rate, blood pressure, and respiratory rate. A naloxone challenge (.2mg) precipated a severe reaction of nausea, vomiting, hypertension, diaphoresis and diarrhea which lasted four hours. During the ensuing five days he was treated with clonidine and withdrawal scores dropped each day to zero on the fourth treatment day. His urine contained the following AS: 19-Norepiandrosterone; (4.5mcg/ml); 19-Noretiocholanolone (1.9mcg/ml); 19-Norepiandrosterone; and a testosterone/epitestosterone ratio of 21.6:1.

In an epidemiologic study of 215 football players who demonstrated AS in their urine, nadrolone, testosterone, methandienone, and stanozolol were the most commonly used As. Only 22 (10.2%) subjects, however, demonstrated nandrolone (19-norandrosterone) and its two metabolites, 19-norepiandrosterone and 19-noretiocholanolone. These individuals were presumed to be using high dosages of nandrolone and are likely dependent upon it.

AFFILIATION:

Community Health Projects Medical Group 336 So. Glendora Ave., West Covina, California 91790

Experimental Analysis of Triazolam and Pentobarbital-Induced Memory Impairment

J. Roache, T. Kirk and R. Griffiths

The effects of placebo, triazolam (TZ: 0.25, 0.5 and 0.75 mg) and pentobarbital (PTB: 100, 200 and 300 mg) were compared in 7 subjects utilizing a double-blind within-subject Latin Square design. Healthy paid male high school graduates, 27-38 yrs old, who weighed 68-88 kg and reported regular use of only modest amounts of tobacco, alcohol and marijuana participated 3 d/wk (M,W,F). After initial training and placebo sessions, the 7 test doses were administered orally at 1000 hr on 7 consecutive sessions. Prior to and at hourly intervals for 5 hr following drug administration, subjects completed subject ratings, psychomotor performance tasks and two versions of a short-term memory number recall task. The recall task involved the use of a numeric keypad to reproduce an 8-digit number (stimulus) displayed on a video screen. In one version, the length of the stimilus presentation time was varied (3, 6 or 9 sec) after which, subjects were immediately prompted to recall the stimulus; a total of 5 trials at each stimulus presentation time were randomly intermixed. In another version, subjects had up to 12 sec to correctly reproduce an 8-digit number continuously-displayed on the video screen (matching), following which, the screen was cleared and subjects were prompted to recall the number following an immediate (0.1 sec) or a 10 sec delay interval; a total of 5 trials of each delay condition were randomly intermixed.

Both TZ and PTB produced generally comparable dose-related effects; TZ was 270-384 times more potent than PTB on measures of sedation and psychomotor impairment but was 406-647 times more potent than PTB on measures of recall impairment. These relative potency comparisons indicate that TZ may have a greater amnestic liability than PTB. Analysis of the variable stimulus presentation time task showed that recall deficits produced by both TZ and PTB at the 3 sec presentation time were attenuated or antagonized at the longer stimulus presentation times. With the variable delay recall task, TZ impairments were only observed under the delay condition while PTB impairments were observed at both delay intervals and were not affected by increases in the delay. These data suggest that both TZ and PTB quantitatively impair acquisitional processes but that TZ may produce qualitatively different effects than PTB to enhance the rate of forgetting. Such analyses of short-term recall performance permit assessment of drug effects on the time-related establishment and maintainence of stimulus control processes related to short-term memory phenomena.

The University of Texas Medical School at Houston (JDR) and The Johns Hopkins University School of Medicine (TK and RRG).

Evaluation of the Abuse Potential of Methocarbamol in Man Compared to the Benzodiazepine Lorazepam

J. Guarino, K. Preston, W. Kirk and R. Griffiths

The subjective and behavioral effects of orally administered methocarbamol, lorazepam, and placebo were studied in a non-residential group of adult male volunteers with histories of recreational substance abuse including sedative/hynotics. In the first phase of the investigation, a dose run-up of methocarbamol (up to 12 mg) was conducted in six subjects to determine appropriate doses. In the second phase, a randomized block cross-over study using 14 subjects was conducted. The following drug conditions were tested in the cross-over phase: placebo, lorazepam 1, 2 and 4 mg, and methocarbamol 2.25, 4.5 and 9 gm. Drug conditions were tested under double-blind conditions. Psychomotor and cognitive performance measures and subject- and observer-rated behavioral responses were measured daily before dosing and for 5.5 hours after drug administration. The results showed that both lorazepam and methocarbamol produced statistically significant dose related increases in subjects' ratings of drug effect and liking, although only lorazepam increased MBG scale scores. Methocarbamol also increased ratings on measures indicating the emergence of dysphoric and other side effects at high doses. Both drugs impaired psychomotor and cognitive performance, with lorazepam generally producing greater effects than methocarbamol. The results indicate that methocarbamol, at doses well above those used therapeutically, has some potential to be abused by persons with histories of sedative/hynotic abuse; however, this potential for abuse is probably decreased by the accompanying side effects at high doses and is probably less than that of lorazepam.

AFFILLIATION

Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21224

Developmental Time Course of Acute Opioid Physical Dependence in Humans

S. Heishman and M. Stitzer

Previous studies in our laboratory have documented the occurrence of naloxone-precipitated withdrawal 6 hr, following acute morphine administration (acute physical dependence) in non-dependent humans and demonstrated that the intensity of withdrawal signs and symptoms was directly related to the size of morphine and naloxone doses. The purpose of this study was to determine the minimum length of agonist exposure necessary to observe antagonist-precipitated withdrawal by manipulating the time interval between the morphine and naloxone doses.

Participants were 5 male community volunteers reporting prior opiate use and average current opiate use of 11 times per month. Subjects participated in four experimental sessions in which they received i.m. injections of morphine (18 mg/70 kg) followed by naloxone challenge (10 mg/70 kg) at 0, 15, 45, and 90 min postmorphine. Experimental sessions involved baseline measurements prior to the morphine and naloxone injections, followed by a 60-min post-naloxone assessment period. Physiological measures were recorded continuously throughout sessions and a battery of pupil photographs and subjective reports and observer ratings of withdrawal was completed at baseline and 5, 15, 30, 45, and 60 min postnaloxone.

The onset of agonist effects, as measured by pre-naloxone baseline values, was evident by 45 min post-morphine, with little further change at 90 min. Pupillary diameter and respiration rate were decreased and subjective measures of good drug effect and drug liking were increased from pre-morphine baseline levels. Naloxone fully reversed these agonist effects. The onset of naloxone-precipitated withdrawal paralleled that of agonist effects. Naloxone produced no measurable effects at the 0 and 15 min conditions, but at 45 and 90 min post-morphine, naloxone clearly precipitated subjective report symptoms and observer rated signs of withdrawal.

This study found that the onset of naloxone-precipitated withdrawal occurred simultaneously with the onset of morphine agonist effects. Additionally, these data suggest that the minimum duration of agonist exposure necessary to observe antagonist-precipitated withdrawal is between 15 and 45 minutes. This implies that the development of opioid physical dependence begins within minutes following acute exposure to an opiate drug and does not require chronic exposure.

AFFILIATION

Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21224

Predictors of Opiate Use During the Course of a 90-Day Methadone Detoxification

M. Iguchi, M. Stitzer and I. Liebson

The behavioral circumstances related to opiate drug use were examined during a 90-day, outpatient methadone detoxification (DTX). Seventy one subjects (55 male and 16 female) were followed from the day of intake to treatment termination Data were collected by means of a weekly structured interview. Questions were asked about each occasion of opiate use in the previous week with respect to time, source, cost, social circumstance, etc. Monitored urine samples were tested 3 days/wk to verify verbal reports. A backward stepwise regression (BSR) was used to derive predictors of treatment outcome. Standard demographics were entered into the analysis along with a number. of opiate use-related measures including: duration of the current episode of opiate use. the avg. number of opiate use episodes/wk, the avg. estimated dollar value of opiates consumed/wk, the avg. number of different opiate sources/wk, and the avg. number of opiate related contacts/wk. "Opiate related contacts" refers to the number of times an individual does not use drugs while: 1) being in the same room with others who are using opiates; 2) selling opiates; 3) turning down opiate drugs when offered; and 4) seeing a regular drug source without buying. Four variables were identified by the BSR with number of opiate free urines as the dependent variable. The number of opiate free urines was chosen as the dependent measure because it combined days in treatment with opiate free behavior. The overall regression was significant ($r=.566$; $p<.00001$; $F=7.776$; $df=4,66$), with gender ($p<0.05$; $t=2.087$; $beta=.215$), race ($p<.001$; $t=-3.592$; $beta=-.372$), and the estimated dollar value of baseline opiate use/week ($p<0.03$; $t=-2.288$; $beta=-.238$). identified as independently significant Current duration of continuous opiate use was also marginaly significant ($beta=-.194$). Those who submitted more opiate free urines tended to be female, white, estimated their opiate use to be of less $ value at baseline, and indicated a longer duration of continuous opiate use prior to treatment entry. A second identical analysis was conducted with race and gender removed from the list of independent variables. Three variables emerged from the analysis with the overall regression still significant ($r=456$; $p<.0013$; $F=5.860$; $df=3,67$). An inverse relationship was noted between the number of opiate. free urines submitted during the course of treatment and: number of different opiate sources/week at baseline ($p<.05$, $t=-2.034$; $beta=-.240$); number of opiate related stimulus exposures/week at baseline ($p<.05$, $t=-2.024$; $beta=-.243$); and episodes of opiate use/week at baseline (marginally significant, $beta=-.240$). It appears that global identifiers such as race and gender may obscure important environmental factors. Knowledge of the impact of such factors on treatment outcome, independent of race and gender, may help us to better understand the role of the environment with respect to opiate use. It may also help us to better direct our limited resources and to better focus our treatment interventions.

AFFILIATION: Johns Hopkins University School of Medicine and Francis Scott Key Medical Center, BPRU, D-5-West, Baltimore MD, 21224

Opioid Detoxification Using Buprenorphine

T. R. Kosten, J. Krystal, C. Morgan, D. Charney, L. Price and H. Kleber

Thirty-nine opioid dependent patients were placed on sublingual buprenorphine at doses ranging from 2 mg to 8 mg daily for a 30 day outpatient trial. During this outpatient trial 12 patients dropped out (69% retention) with an overall mean stay of 25 (+8) days. Illicit opioid use during this outpatient trial decreased from 40% Of urines during the first week to 27% during weeks 2-4. Out of the 27 completers, 10 unsuccessfully attempted an outpatient induction onto naltrexone. The other 17 patients went on to a 4 day inpatient phase involving double blind buprenorphine discontinuation and challenges with either low dose naltrexone (1 mg oral, n=12) or high dose naloxone (0.5 mg/kg IV, n=5). Among the 12 naltrexone challenged patients, withdrawal symptoms remained at baseline with either 1 mg naltrexone or placebo. Blood pressure and MHPG changes were also indistinguishable from placebo response, but all three assessments were significantly different from responses to 1 mg naltrexone in methadone maintained patients. Five other patients who got high dose naloxone challenges had withdrawal symptoms and blood pressure responses less than in methadone patients given 1 mg naltrexone. Following these challenge paradigms, patients were offered naltrexone maintenance at 50 mg daily. Only one naltrexone (1 mg) challenged patient and three out of the five naloxone challenged (0.5 mg/kg) patients remained on naltrexone (50 mg/d) maintenance. This suggests that detoxification and naltrexone induction may be possible using an outpatient stabilization on buprenrphine followed by very rapid inductioin onto high dose naloxone and subsequent oral naltrexone at 50 mg daily.

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AFFILIATION

Yale University School of Medicine, Psychiatry Department, Substance Abuse Treatment Unit, New Haven, CT 06519

Difficulties in a Clinical Application of Methadone Dose Contingency Contracting

D. Nolimal and T. Crowley

Among methadone maintenance patients supplemental drug use may be fatal in these days of AIDS. Retention and treatment may be life-saving. We have investigated the feasibility and efficacy of methadone dose contingency contracting (MDCC) in a clinical environment. Retrospective chart review (1982-1987) of methadone patients treated in an outpatient clinic was employed.

Fourteen especially problemmatic drug-abusing patients, who did not respond to other therapeutic modalities, were given a choice of MDCC or detoxification and discharge. Methadone doses were reduced (usually 5 mg) after each "dirty" urine, and were increased 5 mg after each "clean" sample (up to the original dose).

Compared to our other methadone patients these 14 had longer periods of drug use, abused a wider variety of drugs, and had more drug-related problems. In three pre-MDCC months, 38% of urine samples collected were clean, compared to 55% during three months of MDCC. Marked improvement occurred during the first month of intervention, while later the MDCC effect gradually faded. Nine patients discontinued or reduced their drug use and remained in treatment. Five patients continued drug use, leading eventually to termination and discharge.

Of 14 patients facing discharge, 64% remained in treatment, and their supplemental drug use declined (although that effect faded in time). We discuss procedural changes which might sustain the discharge of others, it remains an open question whether MDCC for problemmatic patients may reduce drug-related mortality, including that of AIDS.

AFFILIATION

Addiction Research and Treatment Services, Department of Psychiatry, University of Colorado School of Medicine, Denver, Colorado 80262

Relationship of Client Characteristics with Non-Compliance in Methadone Maintenance Treatment

A. Tommasello, K. Nilsen and J. Ball

To determine the association of non-compliance (NC) with client characteristics the dispensing records of 6 programs in 3 cities were reviewed for a 1 week period. Each client's daily methadone dose, sex, # of take home days, and any missed scheduled on-site methadone ingestion during the week was noted. Missing an on-site methadone dose ingestion is our measure of NC. The 1928 clients are described as follows: 72.1% male, 77.2% received <3 take home days per week, 18.9% received 0-29 mg/day of methadone, 44.6% received 30-59 mg/day and 36.5% received 60 mg/day or more. The crude rate of NC for the week was 15.7 per 100 clients.

Odds ratio of NC for females compared to males is 1.14 (χ^2_1=0.88; p:NS); and 15.4 among those receiving <3 days take home compared to those receiving $\geq$ 3 days (χ^2_1= =86; p<.001). Association of dose with NC is significant (χ^2_2= =85; p<.001). NC is greatest among low-dose clients (27.7%), moderate in the mid-dose range (18.0%), and lowest in the high-dose range (6.8%). The NC rate is significantly higher in some programs than in others (χ^2_5==86; p<.001). After stratification by dose to control for the confounding effect of dose on NC, the association of program affiliation with NC was no longer significant in the low dose category (χ^2_5=8.88; p:NS) but remained significant in the middle (χ^2_5=20; p<.005) and high dose categories (χ^2_5=37.6; p<.001). Dose adjusted NC rates in programs ranged from 2.5 to 24.8 per 100 clients.

NC interrupts medication, and increases the likelihood of failure to receive other scheduled services (urine collection, Physical exam, or counseling session). Clients receiving <30 mg/day of methadone display the highest risk of NC and program affiliation does not influence NC in this dose range. In contrast, the mid- and high-dose client NC rate is program related. These findings indicate that program policy, treatment procedures, or staffing patterns may influence compliance rates and suggest that low dose methadone may lack efficacy. They also demonstrate that programs can achieve high compliance rates in mid- and high-dose clients.

AFFILIATION:
University of Maryland School of Pharmacy, Baltimore MD 21201.

Comparative Effectiveness of Reducing Cocaine Abuse Among Methadone Patients Using Phase Lowering or Dose Reduction

R. Wang and M. McCarty

In an attempt to control the incidence of cocaine abuse among methadone maintenance pt, the present study was undertaken from March 1986 to May 1987 at the Zablocki VA Medical Center. The outpatients in two groups were informed in advance of the interventions to be used either the phase or the dose drop. All received intensive counseling. Supervised urines were 2x/wk. Pts. were given two written warnings. If cocaine use continued after 2 warnings the phase reduction group will lose a phase. Dose reduction group would receive a cut of methadone dose (5 mg) if cocaine was continued. This reduction of dose was given q2wks up to 20 mg if cocaine was continued. Phase drop always precede dose drop. Both groups would earn back their phase or dose with 2 wks of consecutive cocaine free urine specimens. Sixteen male methadone patients abusing I.V. cocaine weekly were studied. There were 27 separate episodes of cocaine abuse among the 16 pts. In the phase reduction group 6 of 10 pts. (60%) responded by discontinuing cocaine use within 1-4 wks. after the warnings. The remaining 4 of the 10 phase reduction pts continued to use cocaine requiring both phase lowering plus dose reduction. Of that group one responded 5-8 wks, one responded 9-12 wks, and two continued to use cocaine for 13 weeks or more. On the other hand, in the dose reduction group only 1 of 6 pts (17%) discontinued cocaine use within 1-4 wks after the warnings. But 4 of the 6 (66%) pts in the dose reduction group discontinued cocaine use 5-8 wks after the warnings. Four of six pts (67%) in the phase reduction group remained cocaine free for 9-13 or more wks following their first episode of cocaine abuse and only 1 of the 6 pts in the dose reduction group remained cocaine free from 9-12 wks. The results of the present study showed that methadone maintenance pts responded by discontinuing cocaine use when their phase or dose was reduced. A greater response occurred with a phase drop intervention and these pts also remained cocaine free for longer periods of time.

AFFILIATION

Zablocki Veterans Administration Medical Center, Medical College of Wisconsin, Milwaukee, Wisconsin 53295.

Basic Immunology Issues in Drug Abuse

P. Kind

Interest in the effects of drugs of abuse on the immune system has increased recently because of the AIDS epidemic. If drugs of abuse compromise the immune system, use of intravenous drugs may be a predisposing factor for the development of AIDS in this high risk group or may enhance the progression of the disease. Even in the absence of AIDS, modulation of the immune system by drugs is an important topic. Clearly a compromised immune system predisposes the individual to infectious diseases and may complicate drug abuse even in the absence of AIDS. Moreover, study of immunomodulating agents, including drugs of abuse, and their mechanisms of action may lead to insights into the regulation of immune responses. The following is a brief overview of some of the cells involved in host resistance and some of the major mechanisms of their regulation. Immune modulating drugs could act at any stage in the development, action or regulation of these cells.

The immune response contributes to the resistance to infectious disease and probably resistance to tumors in a variety of ways. These include specific immune responses and the modulation of non-specific defence mechanisms. Cells that contribute directly to resistance are often called effector cells. Specific effector cells include cytotoxic T lymphocytes and B lymphocytes. Non-specific effector cells include macrophages and natural killer (NK) cells.

Macrophages are phagocytic cells that have several important functions in host resistance. These include phagocytosis and destruction of infectious agents, tumor killing, production of immunomodulating and cytotoxic cytokines, and processing and

presentation of antigen.

NK cells are found in normal, unimmunized animals. They were discovered because they can kill certain tumors. They may also be important in resistance to viral infections and perhaps in rejection of allografts. Killing requires contact between NK cells and their targets but how NK cells recognize their targets is unknown. Morphologically, NK cells are large granular lymphocytes; they are neither T cells nor B cells.

Immunologically specific effector cells have antigen specific receptors in their cell membranes. This receptor is antibody in the case of B lymphocytes. In general, antibodies recognize epitopes on the surface of native antigens. When stimulated by antigen, B cells secrete antibodies of the same specificity as the membrane receptor. Thus, B cells and their antibody products are important in the resistance to extracellular infectious agents, those that colonize and live on anatomical surfaces such as heart valves or infectious agents and their products in body fluids. Antibodies may contribute to resistance by inhibiting colonization, neutralizing toxins, neutralizing viruses and preventing their dissemination in body fluids or by enhancing phagocytosis.

Cytotoxic T cells usually are found in the subpopulation of T lymphocytes that expresses CD8. These cells are important in resistance to certain viral diseases, resistance to tumors and rejection of allografts. Contact between cytotoxic T cells and their targets is required for cytotoxicity to proceed. Cytotoxicity appears to be caused, at least in part, by release of granules into the intracellular space. The granules contain a protein called perforin that destroys the integrity of the cell membrane. NK cells kill by the same, or very similar, mechanism but recognize target cells non-specifically.

T lymphocytes recognize antigen on the surface of cells in association with glycoproteins encoded by structural genes in the major histocompatibility complex (MHC). CD8 T lymphocytes recognize antigen associated with class I MHC glycoproteins while CD4 T lymphocytes recognize antigen associated with class II MHC glycoproteins. The antigen-specific T cell receptor is a heterodimer homologous to immunoglobulin that appears to bind to both antigen and MHC glycoprotein. The adhesion between the T cell and the antigen-presenting cell or target cell

appears to be enhanced by CD8 binding to class I MHC glycoproteins or by CD4 binding to class II MHC glycoproteins as well as by association of other adhesion molecules such as CD2 and LFA-1 with receptor molecules on the target cells.

Signal transduction in T lymphocytes appears to be mediated by a transmembrane protein, CD3, which is intimately associated with the antigen-specific T cell receptor. Signal transduction includes activation of protein kinase C, an increase in intracellular Ca^{++}, and activation of phospholipase C by a G protein-dependent mechanism.

Activation of T and B lymphocytes by antigen, mitogens or agents that mimic antigen action initiates a cascade of events that results in an immune response. The molecules that regulate these events are cytokines. Antigenic stimulation causes certain cells to produce cytokines and/or to express a membrane receptor for the cytokine. Specificity of the immune response is maintained because the cells expressing a high affinity receptor for a cytokine are more likely to be stimulated by the cytokine than bystander cells expressing little or no receptor.

Many cytokines are produced by helper T lymphocytes after interaction with antigen-presenting cells and production of cytokines is the major mechanism by which helper T cells act. The system is complicated by the fact that many cytokines can be made by more than a single type of cell and can act on many different cells causing a variety of biologic effects including stimulation of the specific immune response, of hemopoiesis, of NK cells or of macrophages.

Interleukin 1, (IL 1) is produced by macrophages well as fibroblasts and epithelial cells. many diverse biological effects including stimulation of helper T cells to produce interleukin 2 (IL 2), stimulation of B cells to be responsive to other cytokines, stimulation of the central nervous system to produce fever.

Interleukins 2 through 6 are produced by helper T cells. IL 2 is a growth factor for both T cells and B cells and also stimulates NK cells. IL 3 is a colony stimulating factor for macrophages, granulocytes, mast cells and eosinophils. IL 4 is a growth factor for both B and T cells, induces expression of class II major histocompatibility glycoproteins on B cells and macrophages, and

enhances production of IgG1 and IgE by B cells. IL 5 is a B cell growth factor, a colony stimulating factor for eosinophils, and enhances IgA and IgE production by B cells. IL 6 causes differentiation of B cells.

Other important cytokines include interferons and tumor necrosis factor. Interferon gamma is produced by T cells. It has a number of important biological effects including activation of macrophages, induction of expression of class II MHC glycoproteins, stimulation of NK cell activity, interference with the action of IL 4 and IL 5 as well as anti-viral effects. In addition to killing tumors, tumor necrosis factor activates macrophages and stimulates B and T cells.

Immunomodulation by cytokines is very complex and is a field of active research. Genes for most of the cytokines mentioned above have been cloned and studies of activities of the purified proteins are in progress. The number of types of cells that can produce each cytokine and the biologic activities associated with each cytokine tends to increase as the polypeptide is understood in more detail. Clearly further definition of the effect of drugs of abuse on the production and action of cytokines will add to our understanding of the roles of each cytokine in the immune response in inflammation and in neuroimmunology. This field is and will continue to be a fruitful research area.

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Department of Microbiology, George Washington University Medical Center, Washington, D.C. 20037

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Immunological Approaches to Clinical Issues in Drug Abuse

M. J. Kreek

Diverse immunological abnormalities have been observed consistently in narcotic addicts and former narcotic addicts in treatment. Observations of immunological abnormalities in these populations began long before the current AIDS epidemic and before effective pharmacological treatment for narcotic addiction was first introduced in 1964 by Dole and colleagues. Groups working with heroin addicts both in New York and at the USPHS Hospital at Lexington had made early observations of immunological abnormalities and the Medical Examiner's Office in the city of New York had made observations of diffuse lymphadenopathy at time of post-mortem examination of most heroin addicts.[2,21,30] Therefore in any consideration of the interrelationships between parenteral drug abuse (including now both parenteral heroin and cocaine use), immunological dysfunction and severe diseases, especially AIDS, hepatitis B and hepatitis delta, along with any consideration of the possible immunological effects of drugs of abuse, drugs used to treat abuse or endogenous ligands and receptors such as the endogenous opioid system, one must consider what clinical observations were made prior to the advent of the AIDS epidemic and then also examine with rigor the possible relationships between research findings made in in vitro systems, in animal models and in humans, both normal subjects and patients with addictive disease, before making inappropriate extrapolations. It is now estimated that at least two million persons have used heroin at some time mostly by a parenteral, usually intravenous, route.[14]

It also estimated that there are currently approximately 500,000 "hard-core" heroin addicts, that is, persons who have used multiple injections of heroin daily for one year or more, with development of tolerance, physical dependence, and drug seeking behavior or addiction. It is currently estimated that 22 million persons in the United States have used cocaine at some time; of these, approximately 5,800,000 are estimated to be regular users and 10 and 20 percent of these will use cocaine by the parenteral route some or all of the time. All parenteral drug abusers are at risk both for diseases spread by contaminated injection paraphernalia and for complications related to the multiple foreign substances used to extend drugs. Thus many factors may contribute to immunological disorders in

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this population, of which only one factor is the possible effect of drugs themselves.

When considering the possibility of drug effects per se, it is essential to consider both possible direct drug effects and indirect drug effects on any system under study. With respect to the immunological effects of drugs of abuse or drugs used to treat drug abuse, the direct drug effects would imply direct effects of the drug either on the cells of the immune system or on sites of production of humoral factors of the immune system. Conversely, indirect drug effects could involve direct drug effects on other physiological systems, such as the neuroendocrine system. Direct effects on the neuroendocrine system which lead to alterations in neuroendocrine and peripheral. endocrine function might, in turn, alter immunological function in a significant way. Short-acting narcotics such as heroin and morphine may have profoundly different effects on physiological systems of humans when used on chronic basis as compared with long-acting opiates, such as methadone, which is use in the pharmacologic treatment of hard-core heroin addicts.[13] Also, and possibly related to the different physiological effects, the pharmacokinetic differences of these agents must be recognized in any consideration of any possible direct or indirect immunologicall effects of drugs of abuse or drugs used to treat drug abuse.[11,17] Heroin has very limited systemic bioavailability after oral administration in humans, necessitating parenteral use for full effectiveness. In man, the half-life of heroin is approximately one to two hours. Conversely, methadone, the principal drug used pharmacologically in effective treatment of narcotic addiction, has a much different pharmacokinetic profile. It has essentially complete systemic bioavailability after oral administration. The racemic mixture, which is the form of methadone commercially available, has approximately a 24 hour half-life in humans.[11,17]

The safety and efficacy of methadone maintenance treatment of narcotic addiction, when appropriately carried out with medical care, counseling and support staff available, has been repeatedly shown to be greater than any other previously available approach for the treatment of this disorder.[14] Currently there are over 100,000 former heroin addicts in treatment; the voluntary retention in treatment for two years or more has ranged from 55 to 80 percent in various programs over the last 24 years. The prevalence of any heroin use, after stabilization in treatment for six months or more, has been less that 10% in reported studies. However, relapse rate after cessation of methadone maintenance treatment has been shown repeatedly to be greater than 80%. Thus, irrespective of treatment modality used, less than 20 to 30 percent of hard-core former heroin addicts are able to stay narcotic free. The actions of methadone treatment have been shown to include prevention of the withdrawal syndrome, and also of "drug hunger" and blockade, through the mechanism of cross-tolerance, of any euphoric or other narcotic effect following superimposition of a short-acting narcotic. The mechanism of action of methadone, which may be especially important in considering the possible roles of drugs of abuse or drugs used to treat drug abuse on specific indicies of immune function, is that as a long-acting narcotic, it provides a steady-state perfusion of opioid receptors at their various specific sites. Thus in a

consideration of possible direct or indirect effects of short-acting narcotics of abuse, such as heroin and long-acting opioids used to treat narcotic addiction, such as methadone, these pharmacokinetic differences and the availability at receptor sites must be considered.

In studies initiated prior to the widespread introduction of pharmacological treatment of narcotic addiction with methadone, several groups described diverse immunoglobulin abnormalities in heroin addicts; biologically false positive tests for syphilis known to be dependent upon the presence of abnormal amounts or types of IgM antibodies, were also reported.[7,12,16] In early studies, liver disease of then undefined types was also found to be very common. It was assumed that the immunological abnormalities were probably related to liver disease or to the multiple foreign substances used to extend or "cut" drugs of abuse on the street. Also, in very careful postmortem studies carried out in the City of New York by Helpern and colleagues, diffuse lymphadenopathy was reported in most cases of death from narcotism.[10]

In 1964, as part of the initial studies of the possible efficacy of use of the long-acting opioid methadone in the pharmacological treatment of heroin addiction, prospective studies from time of admission of long-term heroin addicts to treatment were initiated to determine the physiological effects and medical safety of use of methadone on a long-term basis.[12,13,16] Within a short time after the initial studies of use of methadone for treatment of addiction, the efficacy of this treatment allowed early proliferation, and resultant effective treatment programs made patients available for studies of immunological function at other medical centers.[7,12,16] prospective studies of the first 214 patients ever admitted to methadone maintenance treatment beginning in 1964 and also in retrospective studies of the first 1,435 patients admitted for methadone maintenance treatment between January 1964 and April 1970, several findings were made which could be related to the observed immunological abnormalities found in these and in later studies. [12,16] Biochemical evidence of chronic liver disease was found in over 50% of patients in both the prospective and retrospective study groups. Subsequent studies shown that chronic liver disease in those patients was due to hepatitis B infection and chronic alcohol abuse or a combination of both.[13] More recently, since the mid-1970s delta hepatitis has also been a cause of chronic liver disease.[14,15,18] Serum Protein abnormalities with diffuse elevations in serum globulin levels were found in over 30%. of patients in both studies. Lymphocytosis was found in over 20% of patients both at time of admission and after three years or more of methadone maintenance in the prospective study group. B cell function, as reflected by serum immunoglobulin levels, was examined. After three years or more of moderate to high dose methadone (80 to 100 milligrams per day) treatment, 76% of the patients had elevations in levels of immunoglobulin M; IgM was very significantly elevated levels present in 45%. Also 48% of the patients had elevated levels of immunoglobin G.[12,16] In other studies, we have shown that both the numbers of patients with immunoglobulin abnormalities (IgM and IgG) and the mean level of elevation of immunoglobulin

levels decreased with time in methadone maintenance treatment.[18] Other than a biological false positive tests for syphilis, no other factors, including history of hepatitis, biochemical evidence of chronic liver disease, or any other test results, correlated with these immunoglobulin abnormalities.[16]

In another study, by Brown, Stimmel and colleagues, in which patients were studied first as heroin addicts at time of entry into methadone maintenance treatment and at one time point following entry into treatment, elevated levels of IgM were reported in 87% of addicts at entry; 76% had abnormal values after 1 to 18 months in treatment, (a much shorter time interval than the studies from our laboratory when all patients were studied after three or more years of high dose treatment).[1] In this study, 63% of heroin addicts had elevated levels of IgG and the percentage of patients with abnormalities fell to 48% during methadone maintenance treatment; also the magnitude of levels of elevation of immunoglobulins fell during treatment. As in our own population of patients, this group reported diffuse lymphadenopathy in the majority of heroin addicts seeking treatment, a finding reported earlier by Helpern and colleagues.[9,10] This group also performed some studies of T-cell function, the in vitro response of lymphocytes in culture to three different mitogens. A significantly reduced ability of lymphocytes to respond to all three mitogens was shown in heroin addicts at time of entry to methadone maintenance treatment; however after stabilization for 1 to 18 months in treatment, 70% of the patients restudied showed normalization of cellular response to these mitogens. In these studies, as is in our own, over 60% of patients had evidence of chronic liver disease, yet the abnormalities in different indicies of immune function, both tests reflecting B and T cell function did not correlate with the presence or absence of liver disease. later studies by many groups showed that hepatitis B markers, including both antigen (5 to 15%) and core and surface antibodies are present in 60 to 80% of unselected heroin addicts as well as patients in methadone maintenance treatment; alcohol abuse has been found to be a problem in from 20 to 50% of all patients in treatment.[14]

In another sequence of early studies both of street heroin addicts and also addicts entering and during methadone maintenance treatment, Cushman, Greico and colleagues extended these findings.[3,4,5] This group also found that IgM levels, and to a lesser extent IgG levels, were increased in a significant number of patients, with elevations of IgM levels in 75% of adult heroin addicts seeking treatment and in 65% of adolescents. During long-term methadone maintenance treatment and also during abstinence treatment, the numbers of patients with elevated levels of IgM and the degree of elevation of these levels decreased. As in the other two sets of studies, history of hepatitis and biochemical evidence of chronic liver function abnormalities did not correlate with the immunoglobulin abnormalities. In a later study from this research team, also performed in the mid-70's before the HIV epidemic, T cell function was examined in a group of methadone maintenance patients.[4] Abnormal percentages of T-rosette forming cells were found in 20% of methadone maintenance patients studied; percentages of B-rosette forming cells were increased above

normal in 40% of methadone maintenance patients, reduced below the normal range in 27% and were within normal range in the remaining 33% of methadone maintenance patients studied. In a fourth set of studies also begun before the AIDS epidemic, Falek, Donahoe, Madden, et. al. found that 24% of heroin addicts had severely depressed ratios of T helper to T depressor cells. In their studies, T-rosetting, reflecting absolute T cell numbers, was also reduced in some heroin addicts studied.[8] They reported, however, that none of the study subjects who had used heroin for less than ten years had depressed helper-suppressor ratios.[8]

In another set of studies, probably performed before the AIDS epidemic hit Italy, of the possible impact of both morphine and methadone on human phagocytic physiology, Tubaro and colleagues reported that heroin addicts submitted to treatment with morphine showed a severe depression of phagocytosis, and also depressed killing properties and depressed superoxide production by both polymorphonuclear leukocytes (PMN) and monocytes.[31] However, methadone-treated subjects showed a much smaller decrease of phagocytic function, an unexpected increase (rather than decrease) in killing after phagocytosis by monocytes, and normal, not reduced, levels of superoxide anion production.[28] These differences might be explained by a variety of factors including possibly indirect, as contrasted to direct, opiate effects, which we have shown to be profoundly different during chronic treatment with the long-acting opiate methadone in humans, as compared with chronic use of short-acting opiates.[13,19,20]

Heterogeneity in levels of circulating antibodies, absolute cell numbers, and later in levels of modulators of immune function, as well as variability in responsiveness of cells of the immune system to various in vitro provocative tests in heroin addicts and frequently also methadone maintained patients have been described. However, even prior to the AIDS epidemic arriving in the United States around 1978, a high proportion and sometimes all heroin addicts had abnormalities of each index of immune function studied. It is also clear that improvement in every immune function index studied occurred during short-term or long-term methadone maintenance treatment, with complete normalization of specific indicies of immune function in some or even a majority of former heroin addicts in chronic treatment with this long-acting opioid. In retrospect, the observed partial or complete normalization of specific indicies of immune function, as observed in these early studies as well as our recent studies, strongly suggest that there is no direct, adverse "drug effect" of opiates on at least the indicies of immune function discussed herein, since during methadone maintenance, the daily dose of methadone was usually moderate to high (60mg/day or greater) in these reported studies. Thus the 24 hour "opioid load", that is exposure of various cells and, tissues to opioid, was greater during methadone maintenance treatment than during heroin addiction; a larger actual amount (mg/24 hours) of exogenous opioid was usually presented to the maintenance patients than self-administered by the heroin addicts. Also the half-life of methadone (around 24 hours) is at least twelve times longer than the half-life of heroin

(approximately two hours) in humans. This argument does not rule out the possibility that an indirect opiate effect, that is, a direct opiate effect on some physiological function other than the immunological system, but on a function which in turn might affect immune function, could contribute to the abnormalities observed in heroin addicts. All of these early data strongly suggest that the immunological abnormalities observed in heroin may be multifactorial, due to chronic diseases such as chronic liver disease (alcoholic, hepatitis B, and now also hepatitis non-A, non-B, and hepatitis delta), other infectious diseases (including tuberculosis, endocarditis, and cellulitis) and as well as to the chronic self-administration of a wide variety of foreign substances and possibly also indirect effects of drugs of abuse.

In 1973, with the description of the specific opiate receptors, followed in 1975. with the first characterization of an endogenous opioid, there has been enormous amount of interest on the possible role of the endogenous opioid system in immune function. It is now known that there at least three distinct sub-types of opioid receptors mu, delta, and kappa and that there are three separate classes of endogenous opioids, enkephalins, dynorphins, and beta endorphin, which is derived from proopiomelanocortin. This last family of peptides has been of special interest since proopiomelanocortin is processed to yield equal amounts of ACTH, ß-LPH and ß-endorphin, along with other peptide hormones including **α**-MSH and ß-MSH. All of these peptides-have been implicated as possibly having some role in immune modulation; ACTH released from the anterior pituitary in man controls cortisol production by the adrenal cortex; ACTH and cortisol are the two most important hormones involved in the stress response and both have been well-established to have immunomodulatory functions. Recent studies have suggested that there may be both positive and negative feedback loops between both cellular and humoral elements of the immune system (including the cytokines) and ACTH, as well as possibly other peptides of the neuroendocrine system. To understand how short-acting narcotics such as heroin or morphine might have very different immunomodulatory functions than the long-acting opiate, methadone, in humans, it is important to. consider both the acute, subacute, and chronic neuroendocrine effects of each of these opioids in humans. In humans, as sharply contrasted to rodents, an acute dose of either a short-acting or a long-acting opiate (heroin, morphine or methadone) causes inhibition of release of ACTH and also probably beta-endorphin from the anterior pituitary; this in turn leads to altered adrenocortical release of cortisol, with lowered levels or flattened circadian variation of levels.[13,14] In addition, acutely opiates inhibit LH release, which in turn alters levels of testosterone and possibly estrogens, which may also may be involved in immunomodulation. Conversely, opiates acutely cause release of prolactin, which also may be involved in immunomodulation. During chronic use of short-acting narcotics, each of these same effects persist with no significant development of tolerance or adaptation. However in the setting of narcotic withdrawal, which may occur to a limited extent whenever a heroin addict is unable to self-administer the next dose of heroin within 4 to 8 hours after the previous dose, the opposite neuroendocrine

observations may be made, with elevations in levels of ACTH, ß-endorphin and cortisol, reflecting the stress of early or full-blown withdrawal.

Conversely, in studies both completed and in progress, we have shown that most indices of neuroendocrine function normalize in part or completely during chronic, steady dose, methadone maintenance treatment, including normalization of both levels and circadian rhythm of release of ACTH, beta-endorphin and cortisol in patients who are not abusing cocaine or any other drugs including alcohol and who have no significant progressive medical problems. Also using provocative tests, including the dexamethasone test of integrity of the negative feedback loop by cortisol of ACTH release and the metyrapone test of hypothalamic-pituitary reserve in response to induced stress, we have shown that neuroendocrine function in the long-term methadone maintained patient becomes normalized.[13,14,19,20] The effects of short-acting narcotics such as heroin and morphine and also of the long-acting opioid methadone on synthesis, release, processing and degradation of opioid peptides of the other two classes, enkephalins and dynorphins, in humans have not yet been elucidated. Over the past decade, there have been many studies of the effects of the endogenous opioids of all three classes, as well as exogenous opiates, on various indices of immune function, including cellular and humoral aspects, using both human and animal cells and model systems. However, the published data are frequently conflicting and underscore the need to combine knowledgeabout opioids with technical expertise in immunology. The conflicting data resulting from animal versus human studies underscore the need to carry out more controlled studies in various well-characterized human patients in defined subgroups.

The next major clinical issue which demanded reassessment with respect to the immunological state of intravenous drug abusers and the possible role of drugs of abuse and drugs used to treat drug abuse on immune status, was the recognition and identification of the entry of the HIV virus and AIDS disease into the parenteral drug abusing population in the United States. In retrospective studies using banked bloods, we found that HIV first entered the parenteral drug abusing population in New York City around 1978; the disease was first identified in 1981 and parenteral drug abusers were recognized as the second major risk group by 1983. In a studies carried out by Des Jarlais and by our group in 1984, approximately 60% of parenteral drug abusers in New York City were positive for the HIV antibody and around 50 to 60 percent of former parenteral drug abusers who had. entered methadone maintenance treatment after 1982 were positive for anti-HIV antibody. However we found that less than 10% of former IV heroin addicts who had entered effective methadone maintenance treatment prior to the AIDS epidemic hitting in 1978 were positive for the HIV-antibody. All studies of immune function in heroin addicts, other parenteral drug abusers and also in methadone maintenance patients carried out after 1978 and especially after the increasing prevalence of HIV infection by 1981, are essentially uninterpretable with respect to causes of any abnormalities observed, unless HIV testing has been performed, since HIV

infection itself causes a very wide spectrum of immunological abnormalities including profound alterations of both B cell as well as T cell function. Also, in addition to AIDS infection, by the early 1980s it was also well recognized that hepatitis delta infection, as well as hepatitis B infection, was contributing significantly to chronic liver disease in heroin addicts and former addicts in methadone maintenance treatment. potentially contributing to observed immune abnormalities.[14,15,18,25]

In a recent study, we have shown that in the setting of active AIDS disease, patients are apparently unable to raise antibodies to delta virus and to hepatitis B virus, or alternatively that antigenemia reappears in the setting of AIDS disease. Such a state would both potentially increase severity of liver disease in the patients and increase infectiousness to others at risk for exposure to these patients, including health care workers.[15] We have also found that in alcoholic abusing parenteral narcotic addicts, the false-positive rate for anti-HIV is greater than in other populations studied; these false positive test results are correlated with hyperglobulinemia.

We have recently focused our attention on studies of human natural killer (NK) cells, a very important aspect of immune surveillance since they are the first line of defense against both viral infections and tumor invasion. Previous studies have reported uniformly that levels of natural killer cell cytotoxicity, activity are significantly reduced in heroin addicts[22,24] In a study performed in an unselected group of former narcotic addicts in methadone maintenance treatment for varying periods of time, including patients without and with continuing polydrug and alcohol abuse, we found that 53% of subjects had normal natural killer cell cytotoxicity activity.[28] In another study of very long-term stabilized methadone maintained patients with no ongoing polydrug or alcohol abuse and no HIV infection, natural killer cell activity and also absolute B and T cell subset numbers were not significantly different from normal control subjects, whereas active heroin addicts had both significantly reduced NK activity and abnormal absolute cell numbers.[26] In related <u>in vitro</u> studies in progress, we have shown that neither the active or inactive enantiomers of the opioid antagonist, naloxone. or the opioid agonist, methadone, alter NK cytotoxicity until concentrations of drug greater than 10^{4}M, above pharmacological levels, are reached, at which point both enantiomers of both compounds reduce NK activity in parallel responses.[27] These studies suggest that action by opioids at specific opioid receptors do not modulate natural killer cell cytotoxicity activity of human cells, at least in this <u>in vitro</u> system, nor are their direct opioid effects of methadone on NK cell activity in humans <u>in vivo.</u> However, indirect opioid effects, possibly on neuroendocrine function, may contribute to the NK abnormalities observed in heroin addicts.

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Drug Abuse and Immune-Neuroendocrine Connections

D. Harbour and E. Smith

An increasing body of evidence suggests that the immune system is affected by drug abuse. These effects are probably both directly and indirectly mediated by the abused substances. The mechanisms of these actions is an active area of research, much of it described in this volume. The theme of this article is that the overlying mechanism may be based on the fact that lymphoid cells both produce and respond to nervous system components. Many of these appear involved in substance abuse, in particular, lymphocytes appear to synthesize and respond to endogenous opiates (for review see Plotnikoff et al, 1986, Harbour and Smith 1988). Glucocorticoid hormones have been known to modulate immune responses, for some time but it is now being shown that there are other means for the immune and nervous systems to communicate in a regulatory fashion. Direct innervation of lymphoid tissues and organs with modulation through neurotransmitters might be one level of interaction (Felton, et al 1985). Another level might be through interaction with the neuroendocrine systems. It is at this level that we have been investigating and feel substance abuse of opioids might have its major direct effects. The soluble mediators that could function in this interaction include neurotransmitters, neuroendocrine hormones and cytokines from the immune system that function in autocrine and paracrine fashion among both systems. These mediators react with shared receptors or binding molecules originally thought to be unique to each system. As a result of these shared signal molecules it seems apparent that a response to an environmental stimulus by one system eventually results in an alteration of the other system in a regulatory loop (Blalock and Smith 1985) to bring about a total body homeostatic response that has been referred to as a psychosomatic network (Pert et al., 1985).

This brief introduction has pointed out general pathways of interaction between the immune and neuroendocrine systems. More detailed reviews have been published elsewhere (Blalock et al., 1985; Harbour-McMenamin and Blalock 1988; Smith and Blalock 1988). It is the intention of this report to emphasize the importance of viewing the immune and neuroendocrine systems as a totally integrated circuit. In this manner one can readily see that environmental stimuli which manifest alterations in one system result in manifestations

and alterations in the other system. Thus drugs of abuse such as narcotics have been shown to have effects on both the neuroendocrine and immune systems. In this regard, our work has centered on the production and action of endogenous opioids as regulators of homeostatic function. Since the opioids also function as drugs of abuse (heroin, morphine) the emphasis of this report will be the normal involvement of opiates in immune function which should help explain the actions of exogenous opiates.

Opioids and the Neuroendocrine System

Endogenous opioids, endorphins and enkephalins are a family of related opioid peptides originally isolated from brain tissue and found to have potent analgesic and euphoric activities. The endogenous opioids are synthesized as as large biologically inactive polypeptide precursors pro-opiomelanocortin, preproenkephalin and pro-dynophin (Marx 1983; Morley 1983; Evans et al 1986). These prohormones produce numerous bioactive hormones including the neuropeptides beta (ß)-endorphin (END), delta (δ)-END, alpha (α)-END and gamma (τ)-END, met and leu-enkephalins (ENK) and the dynorphins. The processing of POMC occurs differently depending on the tissue location seemingly as a result of tissue specific proteases (Krieger 1983; Douglass et al., 1984). The differential processing provides a family of peptides capable of bringing about diverse biological effects, which include paracrine, endocrine, neurotransmitter and neuroendocrine modes of action. On a molecular basis this may be brought about by interactions with a single specific receptor from a heterogeneous family of opiate receptors mu (μ), epsilon (ε), sigma (σ) and kappa (k) present on various target cells (Chang and Cuatrecacas 1979; Lee 1983; Holaday 1984). The opioid family of peptides function directly and indirectly in a regulatory or adaptive capacity in peripheral and central homeostatic mechanisms (Rossier and Bloom 1979; Morley 1983; Kreiger 1983; Holaday 1984). This is particularly true with regards to the physiologic response to stress in which circulating levels of END and ENKs are both elevated (Bernton et al., 1985). Their pharmacologic role in analgesia is well documented particularly with regards to the potent opioid B-END which appears to mediate the majority of its effects through the μ-(morphine) and ε opiate receptors (Krieger 1983; Lee 1983; Holaday 1984). The other opioid peptides are less active. In addition the μ receptor has also been shown to mediate euphoria, respiratory depression, changes in body temperature and catalepsy (Holaday, 1984; Bernton, E.W et al., 1985). The delta (δ) receptor mediates similar actions and Leu ENK appears to bind preferentially here (Chang and Cuatrecacas 1979; Holaday 1984). The END and ENK have been shown to facilitate memory by aquisition of avoidance behavior and maintenance of that learned behavior, plus they have neuroleptic or exicitory behavioral effects and integration of emotions (Kreiger 1983; Barchas and Sullivan 1982; Kiraly and Van Ree 1984; Dougherty and Dafny 1988). Increased endogenous opioid activity has been implicated in the pathophysiology of schizophrenia. In addition to regulatory homeostatic pathways, pain modulation and behavioral effects, there is a well documented endogenous opioid component that mediates in part the pathophysiologic response to shock, in par-

ticular endotoxic shock (Holaday 1984; Bernton et al., 1985). Thus it appears that the endogenous opioid families have a multitude of effects, mediated through a family of opiate receptors present on central and peripheral tissues, including cells of the immune system. In view of the numerous locations and actions of the endogenous opiates and their receptors it is not surprising that abuse of exogenous opiates have potent and detrimental actions on total body homeostasis.

The following section is an in vivo situation in which excess production of endogenous opiates mediate some of the pathophysiologic effects of endotoxic shock and may represent processes involved with exogenous opioids.

ENDOGENOUS OPIOIDS FROM THE IMMUNE SYSTEM

A. Hypothesis for LPS induction of Leukocyte derived Endorphins

Endogenous opioids have been implicated as possible mediators of some of the pathophysiological changes induced during endotoxic shock and gram-negative sepsis (Holaday and Faden 1978; Faden and Holaday 1979). This implication was a result of the ability of the potent opiate antagonist, naloxone. to alleviate endotoxin-induced hypotension and changes in body temperature by apparently blocking the effector molecule (Faden and Holaday 1979; Mamazza et al., 1984). Additionally, naloxone treatment improved survival rates, and plasma endorphin levels have been shown to rise significantly in sheep injected with Escherichia coli endotoxin [lipopolysaccharide (LPS)] (Reynolds et al., 1980; Carr et al., 1982; Traber et al., 1983). Holaday coworkers have shown that this endogenous opioid component specifically binds to delta (6) opiate receptors which then induced the pathophysiologic respones (Holaday 1984; D'Amato et al., 1984). They have shown that these responses can be mimicked in vivo by treatment with DADLE, a δ antagonist.

Blalock and Smith reported the production of ACTH and END-like molecules from Newcastle disease virus infected and corticotropin releasing factor (CRF) treated leukocytes (Smith et al., 1982; Smith et al., 1986). In addition, Lolait and coworkers reported a similar immunoreactive (ir)-END present in mouse spleen macrophages (Lolait et al., 1984; Lolait et al., 1986). This data is important when one considers that depletion studies indicate that leukocytes may also mediate some of the aforementioned endotoxin effects (Bohs et al., 1979; Traber et al., 1983). Therefore we postulated that leukocytes may serve as an extrapituitary source of END-like molecules that are produced in response to bacterial endotoxin.

8. LPS induced mononuclear cell synthesis of ir endorphins

We chose to study the production of the ir-END by using C3H/HeJ, LPS resistant and C3HeB/FeJ, LPS sensitive inbred mice. We present our data studying this mouse model in a summarized form in Table 1. Our rationale for using these mice came from the fact that C3H/HeJ mice do not experience the pathophysiologic responses seen in endotoxic

shock (Morrison and Rifan 1979). Such resistance increases their survival time when exposed to endotoxin as compared to the endotoxin sensitive C3HeB/FeJ mice. We postulated that lack of production of leukocyte endorphin may contribute to the lack of response and increased survival of the LPS resistant mice. To test this hypothesis, we initially determined whether the spleen cells of these mice were able to synthesize ir-END *in vitro* in response to LPS. The cells were fractionated into macrophage depleted, T and B cell populations prior to *in vitro* treatment with LPS (Harbour-McMenamin *et al.*, 1986).

Table 1. Characteristics of *in vitro* LPS induced ir-END in C3HeB/FeJ versus C3H/HeJ mice.

Strain	Molecular weight	*in vitro* binding to opiate receptors	*in vivo* production and bioactivity	POMC peptides protease activity
C3HeB/FeJ (LPS sensitive)	1,800	Yes	Yes	Yes
C3H/HeJ LPS resistant	31,000	No	No	No

Immunofluorescent analysis of cultured cells demonstrated that significant staining with the monospecific antibody to T-END occurs only in LPS treated 8 lymphocyte but not T lymphocyte enriched populations. Radiolabeled material from LPS treated but not control cultures was purified by affinity chromatography on an anti-l- END antibody sepharose affinity column. Subsequent sizing of the LPS-induced radiolabeled immunoreactive (ir)-END by gel filtration showed a peak of radioactivity which comigrated with α and τ-END at approximately 1,800 daltons. Therefore, LPS but not mock treatment seemed to induce the C3HeB/FeJ mouse spleen cells to synthesize *de novo* a molecule that is antigenically and structurally related to α or τ-END. In contrast, LPS treatment of the LPS resistant, C3H/HeJ mouse spleen cells induced the production of a large molecular weight, approximately 31,000 dalton molecule (Harbour *et al.*, 1987). It is tempting to speculate that the larger molecular weight material produced by the C3H/HeJ leukocytes is POMC, the 31,000 dalton molecular weight precursor to ACTH and endorphins. Thus it appears that while LPS may induce POMC synthesis, the C3H/HeJ splenocytes lack the ability to process the precursor into a smaller ß, α or τ ir-END.

Since ligand binding to the 6 opiate receptor has been shown to elicit similar pathophysiologic responses, hypotension and hypothermia, as seen in endotoxic shock, the T and B cell supernatant culture fluids were tested for the ability to bind 6 opiate receptors (Holaday 1984; Holaday and Tortella 1984). Radioreceptor

assays were performed with the agonist DADLE on the neuroblastoma cell line NG108 which carries the δ opiate receptor. The results of these experiments showed a significant inhibition of the labeled δ agonist with the supernate of the B cell enriched population treated with LPS and no significant inhibition with the T cell-derived material. Thus, it appears that B cells are the major cell type of the C3HeB/FeJ spleen producing the LPS induced ir-END and this can bind the 6 opiate receptor, providing a mechanism whereby B cell derived ENDs could effect pathophysiologic responses of endotoxic shock. In contrast to the bioactive α or τ-END produced by the LPS sensitive mice we were unable to show specific binding to brain opiate receptors with the material derived from the C3H/HeJ LPS induced 8 lymphocytes. This finding is consistent with our data showing the production of an immunologically cross reactive molecule with the molecular weight of about 31,000 daltons. The speculation of the POMC precursor is consistent with a biologically inactive molecule.

In order to determine whether the B lymphocyte derived END was produced concomitantly with ACTH and therefore probably a cleavage product from pro-opiomelanocortin, we also tested the culture supernatant fluid for the presence of ACTH. Our results strongly suggested concomitant de novo synthesis of a bioactive ACTH (1-22 to 1-26) like molecule produced in vitro by LPS treated mononuclear cells (Harbour et al., 1987; Harbour-McMenamin et al., 1985). Thus mononuclear cell production of these pro-opiomelanocortin derived peptides in response to LPS seemed analagous to that observed in the pituitary gland.

B. In vivo production of ir-endorphin precedes pathophysiologic responses

We postulated that lack of production of leukocyte endorphin may contribute to the lack of response and increased survival of the LPS resistant mice. To test this, we injected groups of LPS sensitive and resistant mice with LPS and monitored pathophysiologic changes (i.e. body temperature) (Harbour et al., 1987). The in vivo response of the two strains of inbred mice became significantly different after one hour. The LPS injected, but not media injected, C3HeB/FeJ mice presented with marked hypothermia and respiratory alterations. These pathophysiologic responses were. preceded by positive immunofluorescence of the spleen cells stained with anti-τ-END from the C3HeB/FeJ: mice (peak immunofluorescence was 30 minutes) suggesting that the in vivo production of ir-END may in part mediate some of LPS induced pathophysiologic responses. In contrast, the LPS resistant C3H/HeJ mice did not manifest significant pathophysiologic changes nor did their splenocytes fluoresce when stained with antisera to τ-END.

C. B lymphocyte derived ir-END functions in vivo to elicit endotoxic shock-like responses.

It was necessary to demonstrate that the in vivo pathophysiologic effects observed by the administration of LPS were mediated by the

released leukocyte derived ir-endorphin. In order to determine this, affinity purified ir-endorphin produced by the in vitro stimulation of C3HeB/FeJ B cells with LPS was injected intraperitoneally into C3H/HeJ and C3HeB/FeJ mice (Harbour et al., 1987). As a control for de novo synthesis of the B cell derived ir-END, the mice were also injected with affinity purified material from the supernatant fluid of mock treated C3HeB/FeJ B cells. The injected LPS treated B cell derived END but not the mock elicits a similar hypothermic response pattern as seen when mice are injected with LPS. This hyperthermia was coupled with lethargy, an observed reduction in respiration rate, catatonia, and discharge from the eyes. The fact that the LPS resistant mice are capable of presenting with similar pathophysiologic responses as those observed in the LPS sensitive mice indicates that these mice are capable of responding to END but they probably do not synthesize the eliciting opioid components when injected with the LPS. This evidence strongly suggests that the B lymphocyte derived ir-END mediates some of the pathophysiologic responses seen in endotoxic shock.

D. Alternative processing of POMC in the immune system

An interesting finding from our results of purification of the LPS induced ir-endorphin was the possibility of alternative processing of the POMC derived peptides as represented in Table 2. NDV or CRF elicit the production

Table 2. Stimulus Dependent Production and Processing of Leukocyte Derived Endorphins and Enkephalins

Cell Source	Stimulus	Major Product	Reference
unfractionated cells	NDV, CRF	ß-END	Smith et al., 1982; Smith et al., 1986; Westley et al., 1986 Harbour et al., 1987
macrophages	constitutive	ß-END	Lolait et al., 1986; Lolait et al., 1986
mouse B cells	LPS	α or τ-END	Harbour-McMenamin et al., 1985; Harbour-McMenamin et al., 1986; Harbour et al., 1987
mouse B cells	CRF	ß-END	Harbour et al., 1987
T cells	consitutive	Pro-ENK mRNA only	Zurawski et al., 1986

of mRNA and full-length ACTH 1-39 and ß-END from cultured leukocytes. LPS elicits the production of ACTH and endorphins which

correspond to the molecular weight of ACTH 1-22 to 26 and a or τ END from B lymphocytes. We have hypothesized that the truncated ACTH and endorphins represent novel proteolytic cleavage products from ACTH 1-39 and ß-endorphin (Harbour-McMenamin et al., 1985).

This alternative processing was confirmed in the LPS mouse model in which we showed that CRF induced the LPS sensitive mouse spleen cell production of B-END and ACTH 1-39 (nature) whereas LPS induced a smaller species, a or r-End size and ACTH (1-22-26) as the major species with ACTH 1-39 as the minor species. (Harbour et al., 1987; Smith et al., manuscript submitted for publication). Taken together, these data suggest the existence of stimulus dependent processing of POMC by splenocytes. This statement can be expanded to include a stimulus dependent production of opioid peptides in the immune system since con A stimulated mouse spleen cells produced ENK mRNA (Zurawski et al., 1986).

E. LPS induces a novel protease activity

Since induction of the POMC peptides appeared to be cell and stimulus dependent, we determined whether the LPS would induce or activate an enzymatic activity that would process the POMC derived peptide ACTH (1-39). We incubated ^{125}I ACTH (1-39) with the cell sonicates from LPS or mock treated B lymphocytes of C3HeB/FeJ and C3H/HeJ mice (Harbour et al., 1987). The LPS treated C3HeB/FeJ B lymphocyte sonicates possess an enzymatic activity at pH 5 which cleaves ACTH 1-39 into a 2,900 dalton species. This same activity was not seen at pH 7 nor did the mock treated sensitive B cells possess significant, proteolytic, activity. In contrast, neither mock nor LPS treated B lymphocytes from C3H/HeJ (LPS insensitive) mice express proteolytic, activity at pH 5. Thus, this novel proteolytic enzyme does not appear to be induced or activated in the LPS resistant C3H/HeJ B cells and its absence may well be the reason for the lack of precursor processing.

These data, summarized in Table 1, provide evidence that LPS treated B lymphocytes from C3HeB/FeJ, LPS sensitive, but not C3H/HeJ, LPS resistant mice synthesize and process ACTH and ß-endorphin-like molecules into smaller peptides. Whereas, CRF elicits the production and processing of ACTH 1-39 (4,500 daltons) and ß-endorphin (3,500 daltons) from POMC: LPS causes the activation of a novel proteolytic activity which further cleaves these molecules into ACTH 1-22 to 26 (approximately 2,900 daltons) and a or τ END (approximately 1,800 daltons), respectively. The processing of the precursor POMC and its products appears to be a major pathway for production of different families of bioactive hormones and had led researchers to postulate that different enzymes may be responsible for such differential processing (Rossier and Bloom 1979; Douglass et al., 1984; Holaday 1984). This type of regulation, at the level of post translational cleavage, is seen with many hormones and particularly in several families, of endogenous opioids including ENK and END (Herbert 1981). Numerous membrane bound enzymes have been reported to be responsible for many of these activities; however, these enzymes often function at neutral pH and some investigators believe

prohormone cleaving enzymes must be active at the pH of secretory vesicles which is around 5 to 6 (Benuck et al., 1987; Loh et al., 1985). Interestingly, the enzyme activity we have demonstrated-appears to fulfill such a criteria since it has a pH optimum of approximately 5.

IMPLICATIONS

Other chapters in this volume have detailed the many actions of opiates on immune responses. The results reviewed above suggest that the reason opiates affect the immune system is that endogenous opiates may be a regular component of the immune process. Lymphoid cells possess classical and non classical opiate receptors (Hazum et al., 1979; Lopker et al., 1980; Schweigerer et al., 1982; Wybran and DuPont 1982; Mehrishi Mills 1983; Schweigerer et al., 1985; Falke and Fisher 1986; Carr et al., 1987) as well as synthesize END and ENK molecules (see Table 2).

The species of opiate, whether exogenous or a processed endogenous opiate are important determinants of activity. For example, ß-END is an enhancer of in vitro antibody production, CTL, NK function and mitogenesis, while a-END is a potent suppressor of in vitro antibody response (for review see Harbour and Smith 1988). Similarly, ACTH (1-39) suppresses antibody response and macrophage function but ACTH (1-24) has no effect (Johnson et al., 1982). Therefore, one can readily see that the production of different opioid peptides by various stimuli or with different kinetics may result in a variety of immune responses. In addition, since ß, α and τ END have different effects on body temperature as well as differences in their mobility across the blood-brain barrier, the overall physiologic response of the body could be significantly altered by exposure to different sized opioids peptides.

Thus it seems clear that endogenous opioids interact with the immune and neuroendocrine systems during normal responses of both of these systems. However, as evidenced by our own work, the excess production of these endogenous opioids may function in a detrimental fashion. Considering the fact that the neuroendocrine and immune systems both produce and respond to opioids, narcotic drugs of abuse could have devastating effects on total body systemic functions and interactions. It may be that endogenous opioids amplify the effects of exogenous opioids. This possibility becomes even more intriguing when one considers recent evidence provided by Dougherty and co-workers showing that immunomodulatory agents such as interferon alpha (IFN-α), cyclosporin A and cyclophosphamide attenuate the severity of opiate withdrawal by lessening the physiologic side effects (Dougherty et al., 1987; Pellis et al., 1987). In addition, Dafny and coworkers have shown compelling evidence that the immune system participates in opiate addiction. This was evidenced by reductions in severity of opiate withdrawal signs after inactivation of the immune system by irradiation (Dafny and Pellis 1985). The adoptive transfer of these cells restored the severity of opiate withdrawal. In light of our findings with the inbred LPS sensitive and resistant mouse model concerning the production and processing

of the lymphocyte cell derived endogenous endorphin, it may be very interesting to study this model with regards to opiate addiction and withdrawal patterns.

These studies provide in vivo evidence that the immune system and the neuroendocrine (CNS) communicate in an integrated homeostatic function on behavioral mechanisms. In elucidating pathways of interactions, one can imply the significance that drugs of abuse, such as narcotics. will have on modulating or altering CNS, endocrine and immune system activity.

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AUTHORS

Deborah V. Harbour, Ph.D.
Eric M. Smith, Ph.D.
Department of Psychiatry and Behavioral Sciences
University of Texas Medical Branch
Galveston, Texas 77550

Immunologic Effects of Drugs of Abuse

R. Weber

The major function of the immune system is the identification of, response to, and destruction of naturally occurring and synthetic subtances in the biosphere, which gain access to the body and are subsequently identified as foreign or "not self". This capability is founded in- an extraordinary array of molecular and cellular components which comprise the immune system, and act in a concerted fashion to stimulate the production of effector molecules (antibodies), and effector cells (cytotoxic T-cells, natural killer cells, T-helper cells, T-suppressor cells, etc.), which exhibit exquisite specificity and high affinity. These effector cells function in biochemically distinct ways to maintain immunocompetence in the intact organism. The immune system is a complex network of diffuse cells which are capable of internal self-regulation. There is an increasing awareness that the immune system is capable of certain complex regulatory interactions with other organs, including the central nervous system, and the endocrine, adrenal, and gonadal systems (Blalock, 1985; Weber and Pert, 1984). These regulatory processes are founded on both direct and indirect interactions among these systems, either through neuroendocrine pathways or via the sympathetic nervous system. A pervasive theme in neurobiology is that psychotropic drugs act to mimic naturally occurring endogenous ligands, such as the example of opiates derived from plants mimicing the members of the endogenous tripartite opiate gene family. Previous observations that neuroendocrine systems share receptors and regulatory molecules with the immune system provide direct mechanisms to account for immunological effects of psychotropic drugs and their endogenous ligands. Indeed, activation of certain receptors in the central nervous system can lead to alterations in immune function, providing indirect immunomodulatory pathways to account for the immunological effects of psychoptropic drugs of abuse.

Rather than catalog a voluminous literature regarding the effects of drugs on the immune system, the author refers the reader to several reviews and texts (Descotes, 1986; Mellors, 1976; and Sheagren and

Tuazon, 1977). The following will consist of a brief discussion of some immunological effects of drugs of abuse, as well as an overview of some of the issues and problems one must consider when studying this subject. Finally. examples of work will be provided, particularly on the effects of opiates on the immune system, which may offer promising approaches to understanding more completely the precise nature of central actions of drugs of abuse and their subsequent effects on the immune system.

DRUGS WHICH ARE COMMONLY ABUSED AND HAVE EFFECTS ON THE IMMUNE SYSTEM.

Virtually all drugs which are abused have been analyzed for their ability to exert effects on immune function. A partial list has been compiled (Descotes, 1986; Mellors, 1976; and Sheagren amd Tuazpm. 1977) as shown in Table I. Each of these drugs can be demonstrated to produce suppressive effects on the immune system, and occasionally the same category of drug can be shown to be immunoenhancing, under the appropriate conditions. Though these differential effects seem to present a dichotomy, the following discussion will attempt to reconcile some of these observations, by considering several parameters that are important when evaluating the immunological effects of drugs of abuse.

TABLE I

	EFFECT	
	Enhancement	Suppression
OPIATES	+	+
COCAINE	+	+
PHENCYCLIDINE		+
MARIJUANA		+
BENZODIAZEPINES	+	+
BARBITURATES	-	+
ALCOHOL	-	+
NICOTINE	+	+
CAFFEINE	+	+

PARAMETERS ON WHICH IMMUNOLOGICAL EFFECTS OF DRUGS OF ABUSE MAY BE DEPENDENT

Much of the reported data describing effects of drugs on immune parameters is work conducted in vitro, and centers primarily on studies involving antibody production, mitogen activation, and natural killer cell activity. When discussing immunological effects of drugs of abuse, it is important to make the distinction from effects

on immunity. That is, an effect of a drug on a parameter of immunoresponsiveness measured in vitro, does not allow one to make statements regarding the effects of that drug on the overall immune function of the health of the individual.

There exists a lack of consensus on whether certain drugs enhance, suppress. or have no. effect on immune function. The variable effects of drugs on the immune system may be related to which immune effector arm is being measured. For example, depending upon which parameter of immune responsiveness one chooses to measure may determine whether enhancement, suppression or no effect is observed. Certain other factors should be considered, such as physiological versus pharmacological doses, the ability to block the observed effect with classical antagonists, the stereospecificity of the effect, as well as establishing a structure-activity relationship. The effects of benzodiazepines on monocyte chemotaxis satisfy several of these requirements (Ruff, *et al.,* 1985).

Reliable in vitro results may then prompt the investigator to studies in animals and, where appropriate, the drug may be evaluated under controlled clinical protocols or through the analysis of epidemiological data. One can then make rational statements regarding the effects of a drug on the immune system and subsequently, the potential effect of the drug on human health. The effects of opiates on the immune system are illuminating in this regard since they can be shown to have in vitro effects (Weber and Pert, 1984; Johnson, *et al.,* 1982). alter immune function in animals (Shavit *et al.,* 1986; Weber and Pert, 1988; Bryant, et al., 1987; Weber, *et al.,* 1987). and clearly have adverse effects on the immunological status and health of heroin addicts (Brown, *et al.,* 1974; Louria, *et al.,* 1967; Kreek, 1973).

The brain and the immune systems share receptors and regulatory molecules. Receptors for opiates, phencyclidine, benzodiazepines. and dopamine have been described on cells of the immune system. Opiate receptors are present on human peripheral blood leukocytes (Madden, *et al.,* 1987), and delta type opiate receptors are expressed on mouse leukocytes (Carr, *et al.,* 1988). Furthermore, a variety of opiate ligands, both endogenous and synthetic, have been shown to have effects on immune function. Given the complexity of opiate receptor subtypes, in addition to the large variety of opiate ligands employed, it is not surprising that results in this area have often proved difficult to reconcile. To further complicate matters, certain *in vivo* effects of opiates on immune function are mediated indirectly through Interactions with opiate receptors in the brain (see below). Similar problems may be encountered in the study of other drugs of abuse.

Finally, the subject one chooses for study may determine the outcome of the effects of drugs of abuse on immune function. For example, opposite effects of opiate ligands on immune function have been observed among species, as well as varied responses between strains of the same species. Neither should the investigator ignore

the state of the individual subject, with regard to stress or other physiological characteristics. Though on the surface these apparent complexities seem somewhat discouraging, the recognition of the key variables involved in the mechanisms by which drugs of abuse modulate immunity may provide us with the means to understand and manipulate the immune system in clinically important ways.

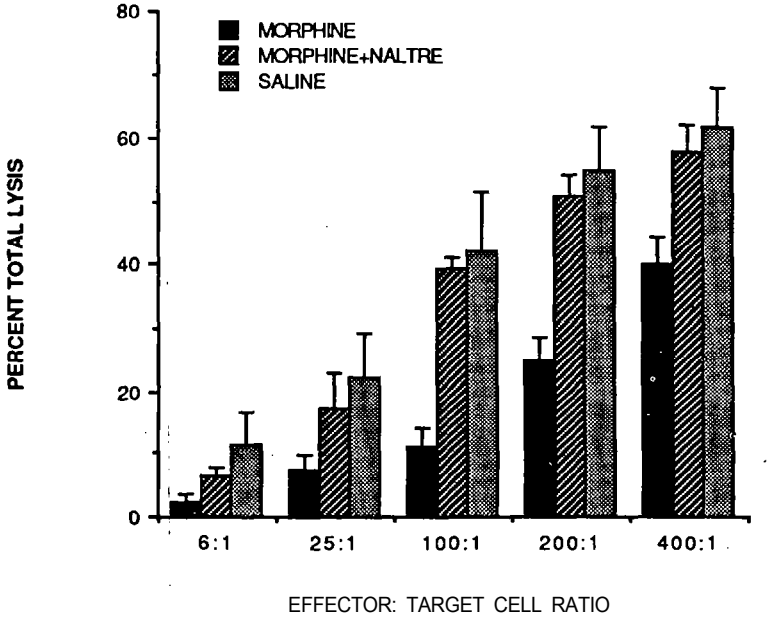
OPIATE INDUCED IMMUNOSUPPRESSION AS A MODEL FOR THE THE STUDY OF IMMUNOLOGIC EFFECTS OF DRUGS OF ABUSE

Opiates are known to exert profound effects on immune function (Weber and Pert, 1984). Previous work has shown that opiate agonists in vitro alter antibody production (Johnson, *et al.,* 1985). produce changes in the ability of leukocytes to respond to mitogenic stimuli (Gilman, *et al.,* 1982; McCain, et al., 1982). increase cytotoxic activity of natural killer (NK) cells (Mandler, *et al.,* 1986). and cytotoxic T-cells (Carr and Klimpel, 1986), and alter monocyte chemotaxis (Van Epps and Saland, 1984; Ruff *et al.*. 1985). The presence of opiate receptors on leukocytes (Weber, 1987, Madden, *et al.,* 1987; Carr, et *al.,* 1988). coupled with the identification of endorphin-like substances being produced by these cells (Smith and Blalock, 1981; Westphal, *et al.,* 1986), 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 neuroendocrinimmune network.

Opiates have been shown to produce effects on immune function *in vivo* (Weber and Pert, 1984; Yahya and Watson, 1987). Clinical observations that opiate addicts have increased susceptibility to infections (Hussey and Katz, 1950; Louria, *et al.,* 1967), were subsequently shown to be related to deficits in immune function (Brown *et al.,* 1974). More recent work has demonstrated that opiate agonists alter the response of leukocytes to mitogenic stimuli (Bryant, *et al.,* 1987), and decrease cytotoxic activity of natural killer cells (Shavit, et al., 1986). and suppress antibody production (Weber, *et al.,* 1987). These immunosuppressive effects of opiates result in decreased survival in tumor-bearing animals (Lewis, *et al.,* 1984), and increased susceptibility to bacterial and fungal infections (Tubaro *et al.,* 1983), as well as murine retroviral infections (Watson *et al.,* 1988).

We, as well as others (Shavit, *et al.,* 1986), have previously shown that opiates interacting with opiate receptors in the brain are implicated in suppression of NK activity. Morphine injected intracerebro-ventricularly suppresses NK activity at a dose 1/500 of the dose required to produce the same effect when given peripherally. Furthermore, N-methyl morphine, which does not cross the blood-brain barrier, has no effect when given peripherally. Although this data demonstrates that morphine acts in the brain, a precise neuroanatomical site regulating immune function was not known. In order to address this problem the following approach was taken. Male Fisher 344N rats were anesthetized and implanted stereotactically with cannulae guides

aimed at the several opiate receptor containing brain structures. Injections (6.6 nmoles in 1 ul saline) were made into the various structures, and three hours following administration of morphine the rats were sacrificed by decapitation, brains removed for subsequent histological examination, and spleens processed for measurment of NK cell activity. We demonstrated that opiates act in the periaqueductal gray matter of the mesencephalon (PAG), but not several other neuroanatomical sites, to produce suppression of natural killer cell activity (Weber and Pert, 1988). The observed suppression is mediated through opiate receptors in the PAG since it can be blocked by naltrexone. In addition, saline injections had no effect, indicating that the suppression of NK cell activity following injections of morphine into the PAG is specific (Figure 1).



The central or periaqueductal gray consists of neurons surrounding the cerebral aqueduct. These neurons are influenced by extensive afferent projections, originating in motor, sensory, and limbic structures. In addition, certain neuroanatomical sites, such as the medial hypothalamus, and the reticular formation, which project to the PAG, receive reciprocal inputs from this structure. The large number of afferent and efferent inputs which this structure

integrates probably determines the wide variety of functions the PAG subserves, including analgesia, reproductive behavior, vocalization, aggressive behavior, as well as regulation of immune function that we have demonstrated. Autoradiographic data indicates that opiate receptors surround the central aqueduct in rat, cat, and human brain. Enkephalins have also been detected in this brain site. This evidence opens the possibility that endogenous opiate peptides and opiate receptors in the PAG are important regulators of immune function under normal physiological or pathological conditions.

Furthermore, Seeger, *et al.,* 1986, measured local changes in opiate receptor occupation in intact rat brain, and assessed the neuroanatomical effects of certain behavioral manipulations on endogenous opiate release. This technique is based on the decrease in ^{3}H diprenorphine binding in areas where endogenous opiates are released. Following exposure to cold swim stress or prolonged intermittent foot shock, both of which have been shown to increase nociceptive threshholds in a naloxone-reversible manner, a decrease in ^{3}H diprenorphine binding in the PAG was observed. This indicates that endogenous opiates are released in this brain structure during stress.

Consider the previous observations that: a) electrical stimulation of the midbrain (a brain region containing the PAG), results in an increase in pulmonary metastases to Walker 256 sarcoma in rats, b) endogenous opiates are released in the PAG in rats during stress, c) sress causes morbidity, an increase in tumor growth and metastases. as well as a decrease in survival in tumor-bearing animals. Our findings (Weber and Pert, 1988), together with the observations cited above, suggest that opiate release and subsequent action in the PAG are components of an important neural pathway involved in stress-induced immunosuppression and illness and neoplasia associated with it. This allows us to consider novel CNS mechanisms through which the immune response may be regulated and raises the possibility that additional roles for the brain will be discovered regarding the control of immune function. It seems appropriate for this meeting and the special topic of this symposium, that the study of drugs of abuse, their receptors, and subsequent actions in the immune and central nervous systems, has led us to this hypothesis.

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Affiliation

Section on Drug Design and Synthesis,
Laboratory of Neuroscience,
National Institute of Diabetes and Digestive and Kidney Diseases,
National Institutes of Health
Bethesda, MD 20892

Opiates as Immunocompromising Drugs: The Evidence and Possible Mechanisms

R. Donahoe

Hyperplastic lymph nodes (addicts' nodes) and high susceptibility to repeated infection have been recognized as characteristic symptom of chronic street heroin addiction for many years (1,2) and are now acknowledged as hallmarks of ARC or AIDS. Historically, these facts illustrate that, long before AIDS, there was evidence of the immunocompromising potential of heroin addiction. The present report reviews data supporting the notion that heroin addiction can induce immunodeficiency and that the drug itself is a principle etiologic factor in such effect.

Immunomodulation by opiates was first noted in 1907 when Archard (3) showed that morphine depresses human phagocytic function in vivo and in vitro. Interest in this topic escalated in the 1960's when blood supplies were threatened by contamination with viral hepatitis transmitted by drug addicts (2,4). In 1972, Brown et al. (5) reported that heroin addicts exhibited depressed T-cell mitogenic responsiveness and enhanced immunoglobulin production. While establishing the immunocompromising potential of opiate addiction, this finding did not determine whether such effects were due to opiates themselves or the then favored hypothesis of immune paralysis

In the decade since the report of Brown et al (5), considerable evidence was accumulated to support the direct role of opiates in the immunodeficiencies of addiction. Falek et al. (6), in 1976, showed that opiate addicts frequently experienced elevated incidences of chromosomal damage detectable in their T lymphocytes. Since the immune paralysis concept did not provide a satisfactory explanation for this phenomenon and contemporaneous findings indicated that alkaloid opiates (7,8,9) and endogenous (10) opioids exert their effects through interaction with cell surface receptors, the Falek group explored the possibility that functional attributes of T cells were altered in opiate addicts in a receptor specific way. This approach resulted in the finding that T cell E-rosette formation was depressed in heroin addicts and that this depression was naloxone reversible (11). As this work was ongoing, Wybran et

al. (12) reported that E-rosetting was depressed in vitro by morphine but not by behaviorally inactive opiate enantiomers, and that such depression was also reversible by naloxone. They showed too that enkephalins enhance E-rosette formation. Contemporaneous studies of Singh (13) also showed that E-rosette formation was depressed by methadone and Lopker et al. (14) reported that human peripheral blood phagocytes possessed pharmacologically detectable opiate receptors wich was soon followed by others demonstrating opiate receptors on lymphocytes (15) and lymphoblastoid cell lines (16). Collectively, these data strongly implied that T cells have physiologically relevant opiate receptors that are likely to be responsible, at least in part, for modulating T cell functions.

Since these studies, extensive documentation of the immunomodulatory potential of morphine and also endogenous opioids has occured. As reviewed by several authors (17,18,19), all aspects of immunoresponsiveness seem influenced by opiates or opioids: cell mediated aspects [chemotaxis (20), phagocytosis (21,22) NK activity (23,24), blastcgenesis (25), T-cell functions (26,27) as well as those that are humoral [interferon production (28), antibody levels (29)]. Most importantly, as regards the the potential of opiates to actually compromise host immune defenses, several reports have documented such effects by showing that opiates increase the susceptibility of experimental animals to infection (30,31). Thus, the evidence for immunomodulatory, and even immunocompromising potential of opiates is compelling. Still, understanding of the effects of opiates on the immune system is incomplete. As discussed further below, controversies concerning data collected in this area (18,19) need resolved and important unanswered questions addressed.

DO OPIATES DIRECTLY COMPROMISE THE IMMUNE STATUS OF HUMANS?

The only way to unequivocally answer this question is to conduct controlled prospective studies using addicted human subjects. Of course, ethical considerations obviate such an approach except in studies of acute effects of opiates. Yet, studies of acute effects are of limited values since clinical experience with opiates and epidemiological evidence indicate that chronic exposure is a prerequisite to induce an immunocompromised state. Thus, determination of the immunocompromising potential of opiates for humans seems impractical except by circumstantial means. Of course, the circumstantial evidence derived from retrospective analyses of human addiction and animal model studies, as discussed previously, strongly supports an immunocompromising role for opiates.

Our initial retrospective studies on the immunological effects of addiction resulted in the demonstration that addicts have depressed levels of E-rosette formation (11). These studies were conducted with addicts who used opiates as their drug of choice. Also, they were conducted before 1980, which serendipitously avoided questions of the involvement of AIDS. Since depressed E-rosette formation is associated with compromised immune function (32) and was reversible in this study with naloxone (11), and reproducible in vitro (12,33), We concluded that the addicts studied were immunodepressed as the probable direct result of the opiates themselves. This conclusion

was bolstered by studies on effects of human opiate addiction on expression of leukocytic antigenic markers detectable by cytofluorometric analyses. We (34,35) and Layon et al. (36) found that heroin addicts frequently evidence depression of the ratio between their T-helper and T-suppressor lymphocytes(Th/Ts) which is a commonly accepted sign of imunodepression (32). We also found that addicts not expressing depression of Th/Ts, as a group, show increasingly elevated levels of Th expression correlating with their duration of addiction (35,36). These findings were corroberated by Des Jarlais et al. (37) and our own recent data, shown in Table 1, which illustrate that even heroin addicts free of serological evidence for infection with HIV1 have depressed Th/Ts. Table 1 also presents findings from a recently initiated study of the effects of morphine on Th/Ts in rhesus monkeys (Macaca mulatta). Comparisons of paired samplings of Th/Ts status for 10 separate monkeys are presented. They were sampled 1-month apart, with the second sampling coming 2 weeks after initiation of morphine injections (4x/day, every 6 hrs, 3mg/kg). Depression of Th/Ts is evident in 6/10 monkeys but the differences in Th/Ts values before and after morphine exposure were only significant at $p \leq 0.15$ (paired t-test). Still, this difference separates this group from the paired placebo-controls and is very similar to that seen with analysis of human heroin addicts. Should such morphine influemce over Th/Ts continue throughout the longitudinal assessments of Th/Ts as are planned for this study, the immunodepressing effects of opiates in this monkey model will be confirmed. Such confirmed phylogenetic conservancy of effect will provide strong evidence of the immunocompromising potential of opiates for humans.

TABLE 1. Comparative effects of opiate addiction on Th/Ts ratios in humans and monkeys.

Th/Ts Ratios					
Humans		Monkeys			
		Placebo controls		Morphine exposed	
Control	Addict	Before	After	Before	After
1.99	0.36	0.80	0.60	0.90	0.50
1.92	1.60	0.70	1.20	0.60	0.50
1.40	0.77	3.00	2.80	1.70	1.90
1.08	0.87	2.50	2.20	0.70	1.00
1.84	1.66	0.70	0.80	1.40	0.60
1.90	1.58	1.80	1.50	1.80	1.30
1.41	0.98	0.90	1.10	2.00	0.90
-	-	1.40	1.50	1.30	1.30
-	-	0.90	0.90	0.90	0.80
-	-	1.50	1.50	1.90	2.00
Mean 1.69	1.11	1.35	1.32	1.39	1.17
S.D. ±.37	±.51	±0.72	±0.61	±0.63	±0.64
(t-test)	$p < 0.04$		$p < 0.75$		$p < 0.15$

Knowledge of compromised immune function in addicts is important from several clinical perspectives. From the public health perspective, concerns are mainly focused on problem with AIDS and hepatitis. Methadone therapy is also of concern since this drug too has been shown to have immunomodulatory effects (13,38). In this regard, some of our work (11,39) and that of Kerman (39) indicate

that the immunological effects of methadone are probably dose dependent. At doses below 75 mg/kg, reversal of the immunodepressive effects of heroin may occur which argues for the efficacy of this treatment modality. Owing to the fact that addicts are frequently immunodepressed, it seems clear that more consideration should be given to treating this depression. Such an approach may eve be valuable in treating addiction itself since neurobehavioral effects of such therapy may be beneficial in reducing withdrawal symptoms (40,41).

IS ANY PARTICULAR OF IMMUNE PERTURBATION ATTRIBUTABLE TO OPIATES MORE CRITICAL THAN ANOTHER TO LOST IMMUNOCOMPETANCE?

As discussed above, opiates and opioids seem to have receptors on many, if not all, cells of the immune system. Since immunological effects related to practically all types of immunoresponsiveness have been recorded with opiates, the answer to this question is likely as complex as the immune system itself. Innate defense capacities of the affected host are doubtlessly involved and cell specificity of opiate effects will need to be determined since pleiotropic effects of opiates on different cells of the immune system may vary on pharmacological grounds, on the stage of the growth cycle, and on the functional activation of the cell. Furthermore, responses of immune cells may be actualized in different ways depending on the nature of the associative pathology involved. The situation is made even more complex because considerable immunoregulatory interplay occurs between different aspects of immune function and between the immune and other systems, particularly the neuroendocrine system. Consequently, attribution of the immunocompromising effects of opiates to any single branch of the immune system is liable to be difficult, at best. Animal-model studies may provide only partial answers because they impose a burden of proof on investigators to show that the selected model is actully relevant to the human circumstance.

CAN THE SOMETIMES DICHOTOMOUS EFFECTS OF OPIATES ON CERTAIN IMMUNE PARAMETERS BE EXPLAINED?

Generally, the immunological effects of opiates are depressive. However, for E-rosette formation, morphine has been reported as both enhancing and depressive (18,19). Furthermore, enkephalins appear to enhance E-rosette formation (12) and host immunoresponsiveness (42) while endorphins are depressive (24). Similarly, Th/Ts ratios are both increased and depressed for different heroin addicts (35,36). Also, NK activity is enhanced (43,44) and depressed (45) by opiates and endogenous opioids.

As regards E-rosette formation, the fact that some investigators find depression and others enhancement in the presence of morphine in vitro probabaly best explained by differences in the assay protocols used since E-rosette assays are quite sensitive to experimental variation. Our studies of the effects of morphine on the kinetics of E-rosette formation in vitro (33,36) showed that such E-rosette formation was temperature- and time-dependent. Variable, cyclical periods of morphine-induced enhancement and

depression were demonstrated with one test sample since morphine altered cyclical patterns of E-receptor fluctuations by interfering with up-regulation of receptors from a dormant pool within the plasma membrane. We believe such effects could contribute to spurious results on an intralaboratory basis. However, this source of variability does not explain why E-rosette formation in the presence of morphine versus enkephalins differs since these differences were seen in the same study (12). Such observations appear to reflect physiological differences related to stimulation of different opiate receptor subtypes.

In regard to, questions raised by findings that Th/Ts ratios differ among addicts, there are too many potential contributing factors inherent to the addiction milieu to resolve such differences. The probability is increasing, however, that such effects are related to opiate exposure per se since in vitro studies (35,46,47) have delineated effects of opiates on the differential expression of Th and Ts cells since and since Th/Ts expression in monkeys seems also to be depressed by morphine (Table 1).

Finally, morphine has been reported to have no effect, slight enhancing effects or depressive effects on NK activity (18,19). Endorphins, in particular, have been shown in vitro to largely be enhancers of NK activity along with several of their derivatives. Since various in vivo and in vitro systems and varying experimental coditions were employed in the studies involved, the reasons for the differences reported remain obscure. However, the findings of Shavit et al. (45), that morphine effects on NK activity can be centrally mediated are quite important since opiates and opioids clearly also have direct effects on NK and other cells of the immune system.

In conclusion, opiates and/or opioids can have contrasting effects on immune function which appear attributable to diversity in the biochemistry of these reagents and in the response systems they affect. Therefore, findings of contrasting effects should not detract from the thesis that opiates are immunocompromising substances.

WHAT ARE THE CELLULAR MECHANISMS INVOLVED IN THE IMMUNOLOGICAL EFFECTS OF OPIATES AND ARE THEY MEDIATED BY OPIATE RECEPTORS?

The problems inherit to this question are the same as those facing classical studies of opiate effects on cell physiology with the additional burden (opportunity) of defining effects that are discriminated exclusively on an immunological basis. In this regard, we have been studying in vitro effects of opiates on expression of CD2, CD4 and CD8 antigenic markers of T-Cells. [Note: CD2, CD4 & CD8 are cluster-of-differentiation designations for the following respective molecules: E-receptors; T-Helper markers; T-suppressor cell markers]

As discussed previously, a kinetic analyses of the effects of opiates on E-rosette formation was useful in delineating their role in modulation of E-rosette formation (33,36). Since CD4 and CD8

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marker expression cannot be measured by E-rosetting techniques, the methods used in the kinetic E-rosette assay were adapted to a cytofluorometric approach so opiate effects on CD4 and CD8, as well as CD2 expression could be identified. This adaptation required that the propensity of the E-receptor to be naturally crosslinked by ligand (E) be mimicked experimentally since CD2-cross linking is required to modulate CD2 (48,49). As detailed elsewhere (36,46,47), this was accomplished by exposing CD2 to anti-CD2 monoclonal antibodies and then crosslinking the resulting antigen-antibody complexes with goat-anti-mouse antibodies. This kinetic antibody-directed marker modulation assay yields data reflecting kinetics of modulation of antigenic markers in terms of percent cells-affected and the fluorescence intensity of the markers that combine with fluorescently conjugated monoclonal antibodies used in staining. The latter feature is most important since it allows measurements of changes in debsity of the markers being studied.

This assay has been used to show that morphine alters expression of CD2, CD4 and CD8 markers for both human (36,46,47) and monkey (46) lymphocytes. The morphine-effects seen with this assay are essentially identical to those found with the kinetic E-rosetting assay mentioned previously (33,36) in that characteristic cyclical fluctuations in kinetics of surface marker density are detected. Because of this, we have assumed the mechanisms involved are also similar. Thus, we proposed (36) that receptor modulation by morphine results from its ability to interfere with regulation of antigenic markers (receptors) lying dormant within the plasma membrane which, intervenes in cyclical processes of receptor modulation that interconnect up- and down-regulation of receptors and receptor-ligand complexes and cause alterations in the kinetic patterns of the receptor/marker fluctuations in our assays. As detailed below, interference in receptor up-regulation appears to transpire by the same mechanisms in assays. However, there is a quantitative difference in the way receptors are downregulated in each assay which depends on the nature of the ligands involved. For the E-rosetting assay, (36) down-regulation depends principally on capping and shedding of E with subsequent endocytosis of any remaining receptor-ligand complexes. Capping is required because the large size of the E-ligand prevents it from being endocytized as a whole by T cells. On the other hand, for the antibody-directed assay, receptor downregulation appears to be actualized mainly through direct endocytosis of antigenic marker antibody complexes.

The anitbody-directed assay has improved understanding of the nature of the processes involved in morphine-induced interference with up-regulation of T cell receptors/markers (47). Recent studies have allowed us to conclude that a major force in inhibiting up-regulation of receptors involves transmodulation which is a process whereby adjacent receptors are regulated inter-receptor interactions transpiring directly at the plasma membrane (49a). We have concluded that this process is responsible for the findings of our studies since it is favored by cold (0°C) thermal conditions as used in this study (47) and the fact that the effects observed occured in the presence of sodium azide, conditions which exclude capping, endocytosis and other

metabolically driven processes from consideration. A separate study, showing that CD2 and CD4 markers down-regulated each other through transmodulatory processes in the absence of opiates, also supports this conclusion (50).

Thus, kinetics of receptor/marker expression in both the E-rosette assay and the antibody-directed marker-modulation assay (33,35,37) fluctuate due to inter-reciprocating processes of up- and down-regulation of receptor expression with dawn-regulation controlled principally by capping and endocytosis, and up-regulation by inter-receptor transmodulation. Accordingly, opiates transmodulate with various T cell antigenic markers to intervene with their expression from dormant pools within the plasma membrane and the markers themselves transmodulate in various ways to influence these opiate effects and their own self-expression. A schematic illustration of such effects, as measured with the antibody-directed assay, appears Figure 1.

An ability of morphine to influence T-cell antigenic markers via transmodulatory processes is important to understand the pharmacological basis for opiate effects on T-cells. We have recently demonstrated low-affinity (5OnM K_D), naloxone-specific binding sites on purified, non-dividing, human T-cells (51). In reference to a review on the subject of opiate-binding by Sibinga and Goldstein (19), our study to be the closest one yet to demonstrating opiate-binding sites in a pharmacologically rigorous way. Still, stereospecificity of these receptors has yet to be completely characterized and high-affinity sites identified. Notably, the Will coefficients in our study (51) were indicative of cooperativity between naloxone-binding sites. Owing to findings with our kinetic E-rosetting and antibody-directed assays as discussed above, it is very likely that such cooperativity is, in same measure, due to inter-receptor transmodulation. Indeed, since our findings suggest an intimate linkage between transmodulation and endocytic processes in regulating receptor expression, it is understandable that there has been considerable difficulty in demonstrating T cell opiate receptors by classical pharmacological means. In this regard, Laduron (52) has thoughtfully pointed out that classical precepts of receptor pharmacology may not be attainable with systems where ligand uptake plays a major role, as we have shown to be the case with T cells. We believe receptor transmodulation should also be added to the list of variables that might seriously alter classical receptor-binding studies.

Interestingly, receptor transmodulation may also account for the reason why naloxone does not reverse the effects of opiates in our antibody directed assay while having a modulatory effect itself (17,18). The most parsimonius explanation for such effects is simply that naloxone, like morphine, can mediate receptor expressions via transmodulation. Presumably, such effects are separable from opiate-directed effects that are reversible with naloxone. Perhaps these reversible effects occur with opiate-stimulated processes that temporally follow transmodulation and have metabolic requirements for their actualization. These notions deserve further study since they imply naloxone reversibility of opiate effects need not be

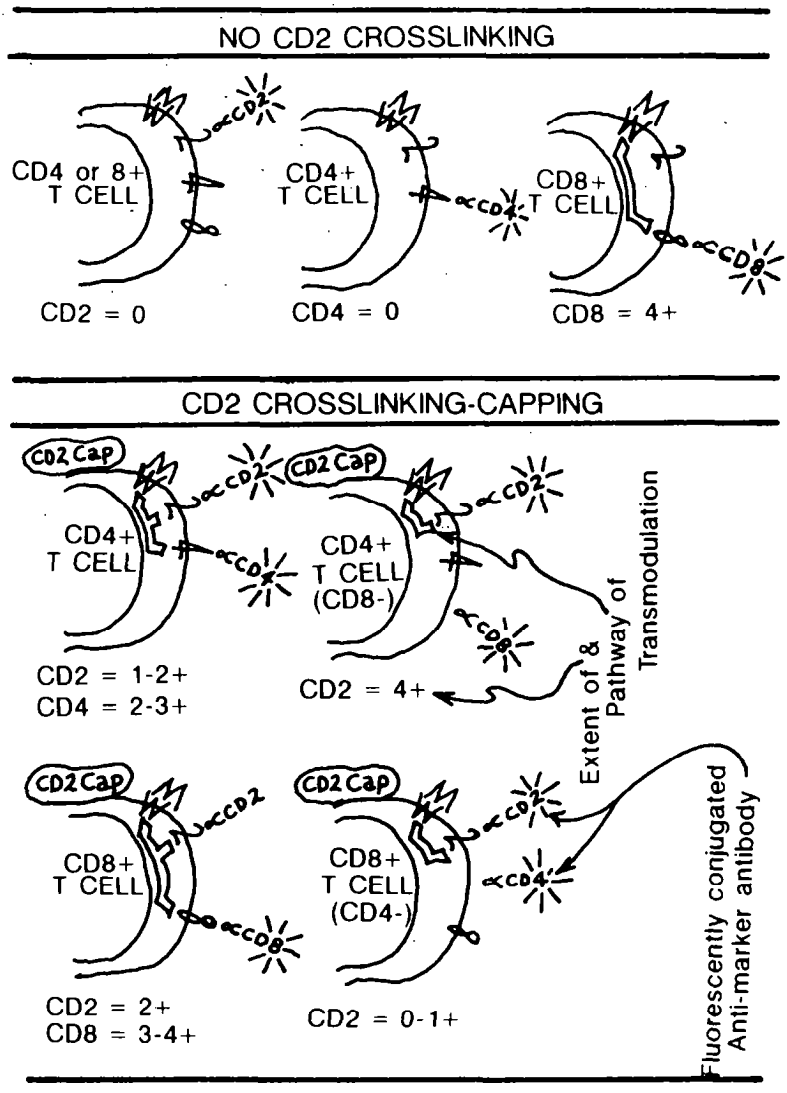

FIGURE 1. Extent of transmodulatory effects of morphine (M) upon various T-cell antigenic markers determined under variable CD2-crosslinking and staining conditions. CD4-negative (CD4-) and CD8-negative (CD8-) T-cells are assumed to be CD8-positive and CD4-positive cells, respectively. Extent of modulation by morphine is indicated at the bottom of each figure and represents a subjective estimate from cytofluorometric data collected through application of the antibody-directed marker-modulation assay.

a hallmark by which opiate receptors are classified; also, that naloxone itself can actuate Physiologically relevant processes through their interaction with opiate receptors, as suggested by numerous studies, including our own

These considerations are particularly relevant to T cell physiology because these cells have extremely pluripotent receptor networks available for transmodulation. Since CD2 antigenic markers appear to be a focus of receptor transmodulation by opiates in conjunction with other T cell markers (CD4, CD8 and CD3, in particular) and since the supply of CD2 markers is practically inexhaustible during acute phase responses due to their large dormant reserve within the T cell plasma membrane (33,48,49), it is probable that CD2 markers serve as master molecules for inter-receptor regulation of T-cell responsiveness Such a role has been proposed for other profuse dormant receptor types like those receptive to a trial natriuretic factor in the kidney (53). In this regard, it is interesting that we have noted that rhesus monkeys seem to have a smaller, less flexible CD2 receptor reserve than humans (46). This may reflect phyloginetic differences between these species that are relevent to their comparative adaptability to environmental stimuli.

CONCLUSIONS

We believe that host immune defenses my well be sensitive to the mechanisms discussed here concerning effects of opiates on T cell function. In particular, the work of Puppo et al. (54) support this notion. They found that transmodulation-like processes down-regulated CD3 and CD4 in response to endorphin for up to 24 hours. Linked with our data showing that CD2, CD4 and CD8 markers are modulated similarly in vivo (35, 11, Table 1), such findings suggest that transmodulatory effects evident after in vitro exposure of T cells to opiates represent a potential for long-range influence of these effects over T cell function.

Interestingly, the receptor-modulating effects discussed herein for opiates are also relevant to the immunocompromising potential of other drugs of abuse like cocaine and alcohol (57,56), and, presumably, to all manner of receptor-driven behaviorally modifying stimuli. Indeed, the fact that T cells produce endogenous opioids (57,58), indicates that autocrine processes are also involved in opiate-receptor mediated regulation of immune function. Presumably, these processes are driven, in part, by the mechanisms discussed in this report.

Clearly the specifics of the immunological relevance of receptor modulation by transmodulation and endocytosis need further elaboration. Nonetheless, it is heuristically satisfying to conclude that changes induced in cell-receptor expression by such mechanisms are bound to alter immunocompetence. In consideration of the obvious problems drug addicts have with opportunistic diseases including AIDS, this is an important consideration. The actual role of the opiate receptor in the phenomena reported herein also needs further elaboration. However, the present information clearly indicate that the immunological effects of opiates are mediated

directly by opiate receptors, both within the immune system itself and the interconnected neuroendocrine system.

Undoubtedly, the receptor-modulating mechanisms discussed herein are coupled with many other physiologic processes that control receptor expression as are now under investigation in laboratories throughout the world such as signal transduction pathways linking cyclic nucleotides, phospholipid breakdown products, guanine-binding regulatory proteins, kinase systems, ion channels and cytoskeletal elements. It is a goal of our future studies to better understand the link between the biological phenomena we have observed, in regard to opiate effects on T lymphocytes, and such processes.

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AUTHOR

Robert M. Donahoe, Ph.D.
Department of Psychiatry
Laboratory of Psychoimmunology
Emory University School of Medicine
Georgia Mental Health Institute
1256 Briarcliff Road, N.E.
Atlanta, GA 30306

IN MEMORIAM: LEO BERGER

1918-1987

The Special Chemistry Session of the 50th Annual Scientific Meeting of the Committee on the Problems of Drug Dependence is respectfully dedicated to Leo Berger.

Leo Berger was born in Manhattan, New York, in 1918. He attended the DeWitt Clinton High School, received a Bachelor's degree from Long Island University, and a Master's degree from New York University.

In 1941 he joined Hoffman-La Roche Inc., as a Staff Chemist, reporting to Dr. John Lee, who started the Organic Chemistry Division of Roche in Nutley, New Jersey. Early in his career Leo worked on the commercial synthesis of riboflavin, and can be credited for the first practical method for purification and crystallization of ribose. In addition, he was involved in synthetic studies of sulfonamide diuretics and salicylamide anti-inflammatory agents. It was during this time that Leo began his long association with analgesic research. His synthetic work in this area led to the development of alphaprodine (Nisentil), a

short acting analgesic of the pethidine class. Nisentil proved a boon to obstetric analgesia. Its short term of action allowed analgesic relief for the mother without the need for subsequent administration of narcotic antagonists to the newborn. His work also contributed to the successful levorphanol (Dromoran) projects and to the development of spasmolytic agents of the phenylalkylamine class.

Leo Berger devoted the late 1940's to-the isolation and purification of heparin, providing the USP standard in 1947. His other natural product research resulted in the isolation and determination of the structure of the laxative principal of casanthranol in 1949. Throughout the 1980's he continued to work on heparin and heparin substitutes, leading to the development of Treburon. He also worked extensively on vitamin K (Synkavite) and analgesics.

In the 1960's Berger contributed to the Roche benzodiazepine program, as well as developing analgesic compounds with spiroisoindoline and spiroisoquinoline ring systems. In the late 1960's. he began his extensive study of carbazole chemistry, working with Dr. Willy Leimgruber. This research culminated in the development of carprofene (Rimadyl), a non-steroidal anti-inflammatory drug with good analgesic properties. His interest in indole and carbazole chemistry led him to explore the tetrahydroindolone antipsychotic class, and resulting in the discovery, with Dr. Gary Olson, of piquindone. an antipsychotic agent.

Throughout his career, Berger developed a broad understanding of the pharmacology and clinical issues that impact upon the practice of medicinal chemistry, and was a strong supporter of the chemist's role in the development of drug candidates. He became Chairman of the Analgesic Anti-inflammatory Project Group at Roche, and was promoted through several levels to Assistant Director of Medicinal Chemistry.

Berger retired in February, 1985, after 44 years at Roche. He continued to serve Roche as a consultant in Medicinal Chemistry. His anti-inflammatory drug, Rimadyl, was approved by the EDA just a week before his death, and will be marketed by Roche in the United States late this year.

Leo Berger showed consistent interest in the Committee on the Problems of Drug Dependence, and regularly attended its annual scientific meeting.

AUTHORS:

Gary Olson and Andrew Thurkauf

Ligands for Imaging Opioid Receptors in Conscious Humans by Positron Emission Tomography (PET)

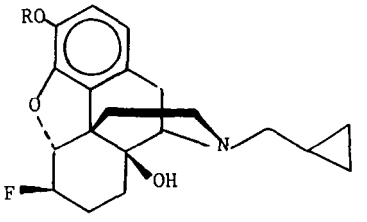
A. Newman, M. Channing, R. Finn, B. Dunn, N. Simpson, R. Carson, N. Ostrowski, R. Cohen, T. Burke, S. Larson and K. Rice

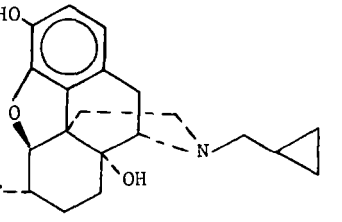
Positron Emission Tomography (PET) is a unique noninvasive technique applicable to the study of biochemical function in the conscious human brain and body. The technique has been exploited widely in metabolic studies involving utilization of glucose and in drug receptor studies of the central nervous system (Phelps and Mazziotta. 1985; Frost, 1986). In the latter, a drug with known receptor specificity is labeled with a positron emitting atom, administered intravenously (i.v.) to the subject and the localization of the drug in receptor rich regions can be visualized. Differences between abnormal and normal control subjects can then provide insight into the biochemical basis of disease states and potentially be used to monitor the effects of drug therapy. Ideally, the radioligand would have a receptor affinity in the one nanomolar range, penetrate the blood-brain barrier readily, be free from interfering metabolites and have no detectable pharmacological

studies of drug receptors are F ($t_{½}$ 110 min) and ^{11}C ($t_{½}$ 20 min) . Although this technique is relatively new, it has excellent future potential not only as a research tool, but in the diagnosis of mental dysfunction as related to receptor and (or) endogenous ligand abnormality. In this regard, the capability to quantitate opioid receptor subtype density and determine subtype affinity for the imaging ligands is highly desirable. Such studies require the design, synthesis and identification of suitable ligands, and subsequent development of efficient routes for their synthesis.

The opioid receptor-endorphin system is a major network for neurotransmission in the mammalian central nervous system (CNS) and is currently believed to be involved in the human perception of pain, pleasure, mood and other aspects of CNS function. The structure and function of this system is currently under investigation in many laboratories, and progress in this area has recently been summarized (Pasternak, 1988). One line of investigation currently under study in our laboratory and that of others (Luthra et al., 1987) is the development of agents suitable for the *in vivo* visualization of opioid receptors in

the conscious human brain. We prepared (-)-17-cyclopropylmethyl-3,14-dihydroxy-4,5-epoxy-6ß-fluoromorphinan (1, cyclofoxy) as a potential ligand for study of opioid receptors in humans via PET (Burke et al., 1985). This compound demonstrated high affinity for opioid receptors (Pert et al., 1984), and was a more potent narcotic antagonist in animals (Aceto et al.. 1986) than naloxone and naltrexone. The drug was subsequently tritiated (Ostrowski et al., 1986, 1987) and also prepared as the ^{18}F-3-0-acetyl derivative 2 (Channing et al., 1986). Initial PET studies with this drug in baboons revealed this





1: R = H (Cyclofoxy)

2: R = Ac (3-O-Acetylcyclofoxy)

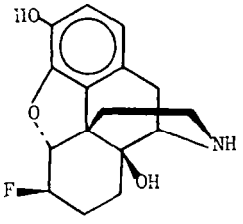
3: (+)-Cyclofoxy

radiopharmaceutical readily penetrated the blood brain barrier and localized in opioid receptor rich regions of the brain including the thalamus and caudate nucleus (Pert et al., 1985). This presumably occurs after metabolic cleavage of the acetyl function in a manner analogous to the rapid conversion (Inturrisi et al., 1984) of heroin to 6-acetylmorphine in humans. The accumulation of ^{18}F-cyclofoxy could be rapidly and completely displaced by treatment with a low, pharmacologically relevant dose of the narcotic antagonist (-)-naloxone but not with an equal dose of the inactive (+)-stereoisomer, thus unequivocally confirming visualization of opioid receptors. Autoradiographic studies using ^{3}H-cyclofoxy revealed the phenolic compound also readily penetrated the blood brain barrier, and in a dose related manner localized in opioid receptor containing regions of the rat brain in a pattern essentially identical to ^{3}H-naloxone (Ostrowski et al., 1986, 1987).

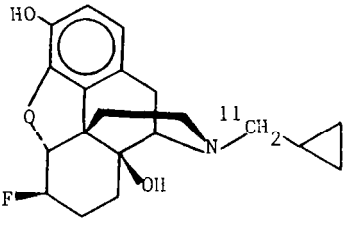
The initial synthesis of cyclofoxy was from (-)-naltrexone by reduction of the carbonyl group to afford a mixture of the α and ß-naltrexols, phenolic acetylation and chromatographic purification of the 6α epimer which was converted in three steps to cyclofoxy (Burke et al., 1985). The successful application of ^{18}F-cyclofoxy as a PET imaging ligand of opioid receptors in the primate brain suggested synthetic studies to determine the most efficient route to this compound, and in particular to the (+)-enantiomer (3) from intermediates available by the NIH Opiate Total Synthesis (Rice, 1980, 1981, 1985). These studies would also facilitate preparation of multigram quantities of

cyclofoxy for behavioral studies. Since it has been demonstrated that (+)-naloxone exhibits 10 fold less affinity for the classical opioid receptors than the (-)-enantiomer (Iijima et al, 1978), it seemed likely that ^{18}F-(+)-cyclofoxy could serve as the ultimate control for nonspecific binding in PET and hence could be used for quantitation of occupied opioid receptors by ^{18}F-cyclofoxy. Such quantitation could be essential in detecting small but highly significant differences in receptor density or endogenous ligand concentration between clinically normal and abnormal human subjects.

A final goal of this work was to prepare (-)-norcyclofoxy (4) which could serve as a direct precursor of unlabeled cyclofoxy. and after introduction of the ^{11}C cyclopropylmethyl function would afford ^{11}C-cyclofoxy (5). The availability of ^{11}C-cyclofoxy ($t_{½}$ 20 min) would permit repetitive studies with the same subject in a single day, and also would allow study of the metabolism of the cyclopropylmethyl function.



4: (-)-Norcyclofoxy



5: ^{11}C-Cyclofoxy

PET STUDIES IN CONSCIOUS HUMANS AND FUTURE DIRECTIONS

Administration (i.v.) of 4 mCi of ^{18}F-cyclofoxy (specific activity 5 Ci/μmol) in normal male subjects resulted in rapid entry of the radioligand into the CNS followed by localization in brain regions of high opioid receptor density with concomitant clearance from brain regions with lowest receptor density. Brain regions with highest accumulation were the amygdala, thalamus and caudate nucleus (Cohen et al., 1988). We have now synthesized (-)-norcyclofoxy and its conversion to ^{11}C-cyclopropylmethyl cyclofoxy is under study. Our synthetic studies have also provided unnatural ^{18}F-(+)-cyclofoxy and (+)-cyclofoxy; the latter has been tritiated (specific activity 24.5 Ci/mmol) essentially as described for the (-)-isomer (Ostrowski et al., 1987). Preliminary *in vivo* autoradiographic studies with ^{3}H-(+)-cyclofoxy in the rat brain have shown that the drug readily penetrated the the blood brain barrier, but unlike its natural stereoisomer ^{3}H-cyclofoxy dispersed throughout all regions of the brain and did not localize.

Similar highly promising results have been obtained in preliminary studies with ^{18}F-(+)-cyclofoxy in the baboon. These observations strongly suggest that ^{18}F-(+)-cyclofoxy will play a major role in ongoing studies directed at quantitation of opioid receptors in conscious humans, thereby permitting better insight into the clinical consequences of opioid receptor abnormality.

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AUTHORS

Amy H. Newman, Ph.D.
Department of Applied Biochemistry, Walter Reed Army Institute of Research and Department of Chemistry, The American University, Washington, DC.

Steven M. Larson, M.D., Michael A. Channing, Ph.D., Ronald D. Finn, Ph.D., Richard E. Carson, Ph.D., Bonnie Dunn, Ph.D., Norman Simpson, B.S. Department of Nuclear Medicine, Clinical Center, National Institutes of Health, Bethesda, MD.

Robert M. Cohen, M.D., Ph. D. and Nancy L. Ostrowski. Ph.D. Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, MD.

T. R. Burke, Jr., Ph.D. and Kenner C. Rice, Ph.D. Laboratory of Neuroscience, National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, MD.

Phencyclidine (PCP) and Phencyclidine-like Compounds: A Molecular Graphics-Molecular Mechanics Study

F. Carroll, G. Brine and S. Mascarella

Since PCP (1) was once used clinically, its pharmacological properties are relatively well documented (Johnstone et al. 1959: Luby et al., 1959). However, its mechanism of action at the molecular level is still not known. Attempts to explain the mechanism of action of PCP have involved monoamine neurotransmitters (Doherty et al., 1980: Garey et al., 1976: Hitzeman et al. 1973: Taube et al. 1975). cholinergic receptors (Aronstam et al. 1980; Kloog et al. 1977; Maayani et al. 1974). various ion channels (Albuquerque et al. 1980: Aronstam 1982: Blaustein and Ickowicz 1983; Kloog et al. 1980: Quirion and Pert 1982), as well as other neurotransmitters. The fact that the stimulus properties of PCP are not mimicked by drugs that are known to interact with the above neurotransmitter systems suggested that PCP acted at a specific site (Browne et al. 1983; Shannon 1981). Such a site was identified in the CNS (Vincent et al. 1979: Zukin and Zukin 1979; Quirion et al. 1981). and the affinities of PCP and related compounds in inhibiting specific ^{3}H-PCP binding to this site could be correlated with their potencies in the mouse rotarod test and in mimicking the discriminative stimulus properties of PCP (Zukin and Zukin 1979; Brady et al. 1982: Holtzman 1981; Shannon 1981; Kozlowski et al. 1986).

Three other classes of compounds which share many pharmacologic properties with PCP also bind to the PCP receptor site. These are dibenzo[a,d]cycloheptenimines represented by MK801. dioxolanes such as dexoxadrol and etoxadrol and benzomorphans such as N-allyl-N-normetazocine and cyclazocine. The first two classes bind selectively to the PCP receptor site, whereas the benzomorphans bind both to the PCP and sigma binding sites with the latter being the preferred site. The phencyclidine (PCP) and sigma-type receptors were originally thought to represent a single entity (Martin et al. 1976: Vaupel 1983). More recently the separate existence of PCP and sigma receptors has been recognized (Hendelsohn et al. 1984: Su 1982: Tam 1985). In addition. Lodge and co-workers (Lodge and Aims 1982: Lodge et al. 1987) have shown that all compounds which inhibit the binding of ^{3}H-PCP also inhibit the excitation of neurons induced by the excitatory amino acid, N-methyl-D-asparate (NMDA).

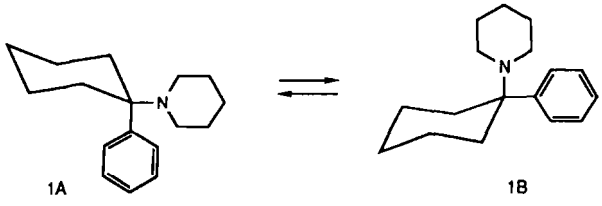
Structurally, PCP is a semirigid molecule consisting of a cyclohexane ring geminally substituted with phenyl and piperidinyl rings. The

intriguingly simple structure of PCP combined with its unique pharmacology engendered several structure-activity studies in the late 1970's and early 1980's (Geneste et al. 1979; Kalir et al. 1978; Shannon 1981).

Cone et al. (1984) reported a structure-activity relationship (SAR) study of PCP analogs in rats. The data from this study, together with the earlier SAR studies showed that the minimum structural requirements for PCP-like activity were an electron-dense small aromatic ring located in close proximity to a basic nitrogen atom. In addition. the geometrical relationship between the nitrogen and phenyl ring. which is fixed by the relatively rigid cyclohexane ring in PCP. was also found to be critical.

Based on the SAR data. Cone et al. (1984) developed a receptor model for PCP, dexoxadrol and N-allyl-N-normetazocine which consisted of an anionic site with multiple subsites. In this model PCP and its analogs were superimposed by overlaying the piperidinyl and aromatic rings of each compound. However, the difference in PCP receptor binding affinity for etoxadrol and epietoxadrol could not be explained by the Cone model (Thurkauf et al. 1988).

It is now well documented that PCP exists as a mixture of conformations 1A and 1B in solution (Manoharan et al. 1983: Kamenka and Geneste 1983). The ratio of A/B varies from 99:1 in CD_3OD/CD_2Cl_2 at -80°C to 1:11 in acetone-d_6/acetonitrile-d_3 at 25°C. Due to favorable solvation of the protonated nitrogen. PCP hydrochloride exists almost entirely with the phenyl group in an axial position which is also the conformer found in the solid state (Carroll et al. 1987; Argos et al. 1970).



1A 1B

Eaton et al. (1983) used MM2 molecular mechanics calculations to estimate that the energy difference between conformers 1A and 1B was small (0.2 kcal/mol). In addition. Eaton et al. (1983) found that the axial phenyl conformer 1A. as also observed in the X-ray crystallographic structure of the salt, was the global energy minimum conformation. We (Carroll et al. 1987) obtained similar results using the MAXIMIN2 and SEARCH options of the SYBYL software package (Tripos Associates, St. Louis. Missouri 63117).

Kamenka and Geneste (1981) suggested that PCP "is likely to take on two profiles" in its biological properties corresponding to the conformers 1A and 1B. These same authors (1983) reported that there was a correlation between the PCP analogs that favored conformation 1A and their receptor binding affinity. Since biochemical studies had indicated the presence of an acid function in the receptor site. Kamenka

and Geneste (1983) concluded that. as part of the recognition process at the PCP receptor. the freebase of PCP in conformation 1A would be protonated and stabilized in this lowest energy conformation. Taking into consideration all of the previously reported SAR data, we developed a pharmacophore hypothesis for the PCP receptor (Carroll et al. 1987). This hypothesis involved an aromatic receptor site pocket extending -3.5A above and below the plane of the aromatic ring and a hydrogen bonding site ~2.8A along the vector of the nitrogen lone pair. These three points define a triangle as shown in Figure 1 (the side of the triangle passing through the aromatic ring is 7.OA. and the remaining sides are 6.7 and 7.7A in length). In addition. as a working hypothesis, we suggested that a 1.OA diameter volume close to the hydrogen-bonding receptor site is essential for the receptor and cannot be occupied by the ligand. This region is represented by the "chicken-wire" volume along the nitrogen vector in Figure 1.

Manallack and Beart (1987) applied molecular modeling techniques to (+)-SKF 10,047. MK801. LY154045 and meta-hydroxy-PCP to define a model for the PCP binding site. The geometry of this model is somewhat similar to our model (Carroll et al. 1988) and the MM2 derived structure for 1A of Eaton et al. (1983). The deviations between the models probably arise from differences in the derivation or construction of the molecular models and possibly to differences in the force fields used for the molecular mechanics energy minimization. An additional difference is that the PCP model of Manallack and Beart (1987) does not specify a volume constraint similar to the PCP pharmacophore hypothesis of Carroll et al. (1988).

The SYBYL software package interfaced with an Evans and Sutherland PS330 graphic system was used for the display. manipulation and superposition of molecules employed in the development of our PCP pharmacophore (Carroll et al. 1987) as well as during the new studies described in this manuscript. The host computer was a microVAX workstation. The structures were constructed using the standard fragments, bond distances and angles supplied by SYBYL. The geometry was optimized with the MAXIMIN module of SYBYL, and when necessary, systematic conformational searches using the SEARCH module of SYBYL were used to locate sterically allowed conformers and to identify local and global conformational energy minima.

Figure 2 is a plot of the low energy conformations of MK801. (+)-N-allyl-N-normetazocine. dexoxadrol. etoxadrol and ketamine overlayed with PCP and fitted using a least-square fit to the PCP pharmacophore triangle. The combined van der Waal volume of each of the compounds was generated to give the chicken-wire volume display shown in Figure 2. The hydrogen bonding volume along the nitrogen vector required by the receptor is represented by the dotted sphere shown in Figure 2. Careful inspection of these two volumes demonstrates that none of the PCP-active compounds possesses volume in the proposed critical receptor ragion.

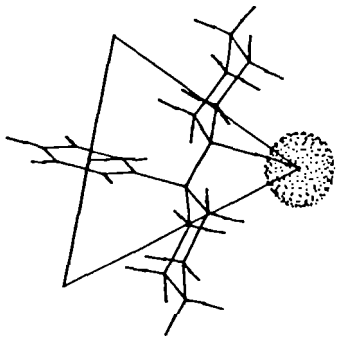


Figure 1. The PCP Pharmacophore Triangle

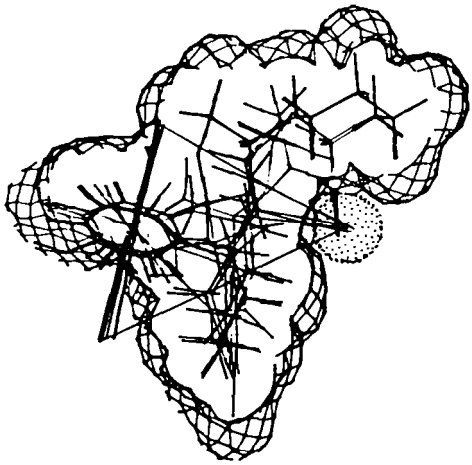


Figure 2. Combined volume of the PCP-active substances: PCP, MK-801, N-allyl-N-methylnormetazocine, ketamine, etoxadrol and dexoxadrol ("chicken-wire" volume) compared to the proposed receptor-essential volume (dot sphere)

SUMMARY

The low energy conformation of MK801. (+)-N-allyl-N-normetazocine. dexoxadrol, etoxadrol and ketamine was found to possess the unique pharmacophore geometry of a PCP pharmacophore hypothesis recently reported by Carroll et al. (1988). Moreover, the union of the volumes of these compounds in their proposed receptor bound conformation locked together such that equivalent groups were superimposed showed that none of the compounds extended into the binding site volume above and below the aromatic ring and along the nitrogen vector.

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AUTHORS

F. Ivy Carroll, Ph.D., George A. Brine, Ph.D., and S. Wayne Mascarella. Ph.D., Chemistry and Life Sciences, Research Triangle Institute, Post Office Box 12193, Research Triangle Park, NC 27709.

Structural Studies Leading to the Discovery of a Cannabinoid Binding Site

M. Johnson, W. Devane, A. Howlett, L. Melvin and G. Milne

INTRODUCTION

The medicinal chemists search far the ideal analgetic has occupied thousands of scientists over the past century. Much of this research has focused on synthetic and endogenous opiates (Johnson and Milne 1981). We sought a structurally and mechanistically distinct approach to the problem when we entered the field in the 1970's. One of the areas we followed closely at the time involved the cannabinoids. Various preparations of *Canabis sativia* have been used for a variety of social and medicinal purposes including the relief of pain (Lemberger 1980; Segal 1986). The availability of the pure psychoactive component, Δ^9-THC (1), allowed for more definitative analgetic studies (Mechoulam 1986). It wasn't until 1974, however, that evidence of structurally dissociable analgesia was presented by Wilson and May. They discovered that HHC (2), a synthetic intermediate, possessed analgetic activity nearly equal to morphine in the hot plate test (Wilson and May 1975). In this paper we briefly review our progress in the field over the past decade.

MEDICINAL CHEMISTRY

Based on the clue from Wilson and May that analgetic activity is a dissociable feature of the cannabinoid molecule and relying on structural insights from the prostaglandin overlap hypothesis (Milne and Johnson 1981) we examined modifications of the side chain, the phenolic moiety, and, most significantly, structures that lack the benzopyran ring present in THC and HHC (Johnson and Melvin 1966). In our initial studies, we found that a new grouping, the 1-methyl-4-phenylbutyloxy C-3 side chain (3), elaborates a unique lipophilic region (Figure 1). This more hydrophobic compound possessed 10-50 times the analgetic activity of HHC (Johnson et al 1981). Introduction of a weakly basic nitrogen at C-5 and deletion of the axial methyl group in the B ring, two structural changes forbidden by traditional cannabinoid SAR, resulted in a unique family of benzoquinolines with potent analgetic activity. The prototype of this series, levonantradol (4a). exhibits potent and enantiospecific analgetic and antiemetic activity (Johnson and Milne 1980). Substitution on the nitrogen to produce compounds 5 and 6 further increase analgetic activity over 4a by an average of five-fold.

Synthesis of the first AC-bicyclic cannabinoid followed from our observation that the pyran ring of HHC was not a requirement of this structural class for expression of biological activity (Johnson et al., 1982). Together, this observation and speculation about the necessity of the lipophilic side chain, phenol and alcohol for

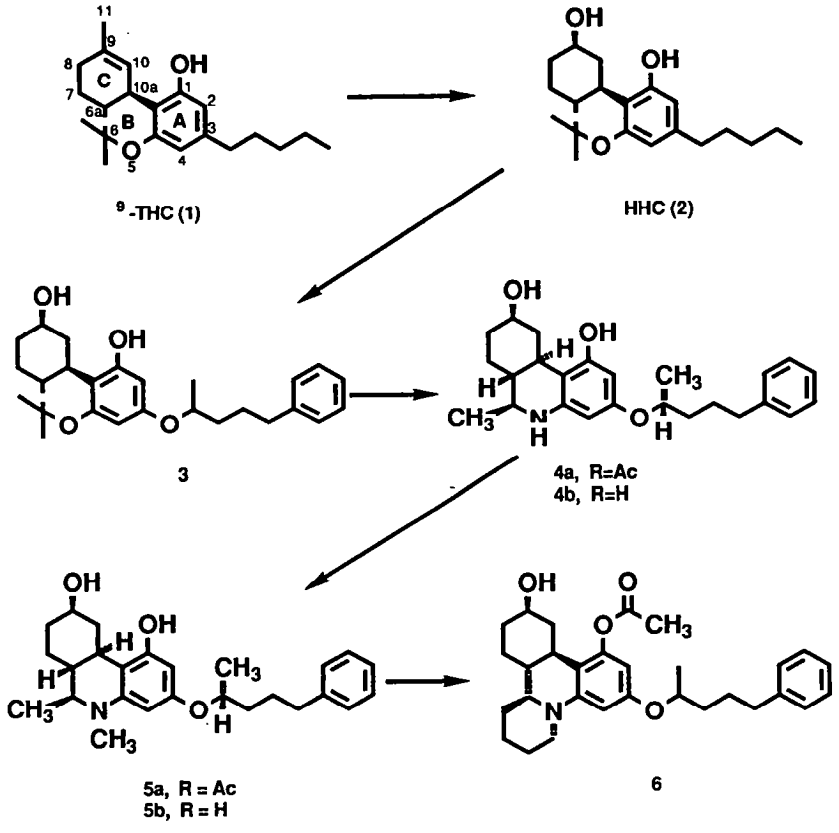

Figure 1. Evolution of ABCE-tetracyclic cannabinoids

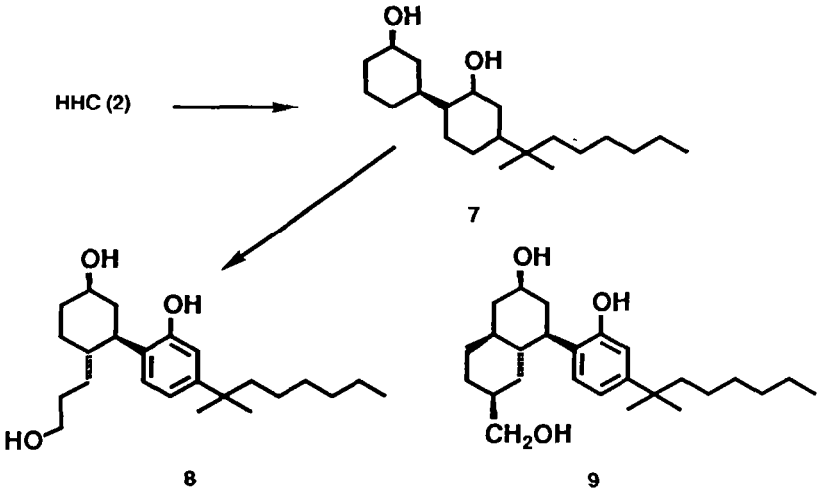

Figure 2. Evolution of AC-bicyclic and ACD-tricyclic cannabinoids

Compound	PBQ writhing	K_{inh}
	mg/kg	nM
Δ^9-THC (1)	5.9 (1.32-11.3)	430
HHC (2)	0.63 (0.26-0.97)	
(±)-3	0.07 (0.03-0.11)	70
Levonantradol [(-)-**4a**]	0.07 (0.05-0.10)	100
DALN [(-)-**4 b**]	0.062 (0.006-0.014)	7
(-)-**5 a**	0.012 (0.01-0.014)	126
(-)-**5 b**		9
(±)-**6**[a]	0.02 (0.00-0.05)	10
(±)-**7**	1.0 (0.35-1.62)	
(-)-**7**	0.55 (0.00-1.05)	79
(±)-**8**	0.29 (0.27-0.32)	
(-)-**8**	0.059 (0.017-0.113)	2.5
(±)-**9**	0.054 (0.04-0.07)	
(-)-**9**	0.018 (0.011-0.024)	5

[a] Equal mixture of two racemic diastereomers.

Table 1. Analgetic and Adenylate Cyclase Activity of Prototype Cannabinoids.

biological activity was confirmed with the synthesis of **7** (Figure 2). This compound was shown to possess a biological profile and potency similar to HHC (Melvin et al 1984). Such activity and potency was highly dependent on-the side. chain but was not as influenced by substitution in-the cyclohexanol ring. Further structural elaboration and development of SAR around **7** led to a more potent bicyclic derivative **8** (Melvin et al 1983A). The degree of importance of the three hydroxyl groups in this structure was explored and only the phenolic hydroxyl was found to be an absolute necessity. The two alcohol groups were seen to be modifiers to activity and potency. Structural optimization of this derivative was achieved by incorporation of the hydroxypropyl chain as a new fused ring and thus the synthesis of the first ACD-tricyclic cannabinoid, 9 (Melvin et al 19838). This rigid molecule again exhibits an increase in potency and significantly shows total enantiospecificity in favor of the levorotatory enantiomer. The combination of potency, stereospecificity, enantiospecificity, and well-defined SAR lead us to intensify our search for a cannabinoid receptor site.

BIOCHEMISTRY

Recent studies have demonstrated that the N18TG2 neuroblastoma cell in culture provides a suitable model system for the study of cannabinoid drugs at the cellular level (Howlett 1984). Cyclic AMP accumulation is attenuated by centrally active cannabinoid drugs in this cell line (Howlett 1984). This effect can be attributed to the rapid and reversible inhibition of adenylate cyclase (EC4.6.1.1) via a G-protein component of the enzyme (Howlett and Fleming 1984. Howlett 1985, Howlett et al 1986). Centrally active cannabinoid compounds fail to interact with the opioid, muscarinic cholinergic. or adrenergic receptors known to inhibit adenylate cyclase in membrane fractions from these cells (Howlett and Fleming 1984. Devane et al 1986). Thus, it may be hypothesized that a unique receptor exists to mediate the effects of centrally active cannabinoid compounds in the adenylate cyclase system. We sought to fully characterize the prototypes in Figures 1 and 2 to determine if a correlation exists between analgetic activity and attenuation of adenylate cyclase activity. As was the case for analgetic activity, a decrease in K_{inh} for adenylate cyclase activity occurred in going from HHC to the more potent side chain **3.** Further potent inhibition occurs with the nantradol series, compounds **4b, 5b,** and **6.** A similar trend is apparent in the series **7-9** wherein increasing analgetic potency is accompanied by increasingly potent inhibition of adenylate cyclase. Enantiospecificity is evident for the inhibition of adenylate cyclase by these highly potent compounds. Thus, it can be tentatively concluded that the receptor mediating the inhibition of adenylate cyclase *in vitro* is similar or identical to the receptor mediating analgesia *in vivo*. Based on these studies, *a* more detailed receptor model has recently been proposed (Howlett et al 1988).

BINDING STUDIES

Having determined the potent, efficacious, and highly stereospecific actions of **8** in both analgetic and biochemical models, we sought to determine and characterize a receptor site in brain using ^{3}H-**8** (Devane et al 1987). Binding equilibrium was reached within 40 min at 30°C. No further increase in nonspecific or specific binding of ^{3}H-**8** was observed in experiments carried out to 120 min. With the synaptosomal preparation, binding was linear through 150 ug protein/ml. The binding site was heat sensitive: incubating the membranes for 60 min at 52ºC reduced the specific binding to ^{3}H-**8** by 96%. Specific binding was defined as that displaced by 1uM Δ^9-THC or 100nM (-)-**8.** The pharmacology of the ^{3}H-**8** binding

site was compared to that described above for adenylate cyclase inhibition and for analgetic activity *in vivo* The rank order of potencies for DALN, **8,** and **7** to compete with the binding of ^{3}H-CP-55940 paralleled the adenylate cyclase studies. Cannabigerol and cannabidiol at concentrations as high as 1 uM failed to appreciably compete for ^{3}H-**8** binding. These compounds are not analgetically active and do not inhibit adenylate cyclase in the neuroblastoma model (Howlett 1987). In addition, stereospecificity was demonstrated for binding to this site. (-)-**8** was about 20 times more potent than the (+) isomer. Thus, the pharmacology of agonist binding to the site labeled by ^{3}H-**8** parallels biological activity both *in vitro* and *in vivo.* Furthermore, the requirement for stereospecific binding of the ligand has been met.

CONCLUSION AND FUTURE DIRECTIONS

The characteristics of this binding site are entirely consistent with characteristics observed for neurotransmitter receptor systems. The implications of this demonstration are multifold. First, these findings provide compelling evidence that a true receptor mediates at least certain actions of cannabinoid drugs. This receptor may act at the cellular level via the cyclic AMP second messenger system. However, these data do not exclude the possible interactions of the cannabinoid receptor with other second messenger systems, including those regulating Ca^{2+} and phosphatidylinositol metabolism. Second, neurons in the central and peripheral nervous systems that express receptors for cannabinoid drugs may be studied with the radioligand binding assay based on ^{3}H-**8.** Thus, pathways in the brain, in the spinal cord, and pathways innervating peripheral organs can be quantitatively assessed for cannabinoid receptor binding. Investigation of the relationship between receptor binding activity and analgesia or other behavioral effects of cannabinoid drugs will now be possible. Third, this cannabinoid receptor assay will greatly facilitate development of a functional cannabinoid antagonist. The mechanism(s) of action of cannabinoid based drugs will be more readily elucidated when such antagonists are available. Fourth, the determination of a cannabinoid receptor present on neurons implies the presence of an endogenous neuromodulator(s) to which these receptors normally respond. Using the radioligand binding assay based on ^{3}H-**8,** it will be possible to isolate the putative endogenous substance(s) that interacts with these receptors *in vivo.* These and other future studies require a truly multidisciplinary approach to studying the mechanism of cannabinoid action. Towards this end, the International Cannabinoid Study Group (ICSG) was formed on March 8, 1988 during a meeting of interested investigators at the Medical College of Virginia (VCU).

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AUTHORS

M. Ross Johnson, Ph.D.
Glaxo Research Laboratories
Research Triangle Park, NC 27709

William A. Devane
Allyn C. Howlett, Ph.D.
St. Louis University School of Medicine
St. Louis, MO 63104

Lawrence S. Melvin, Ph.D.
Pfizer Central Research
Groton, CT 06340

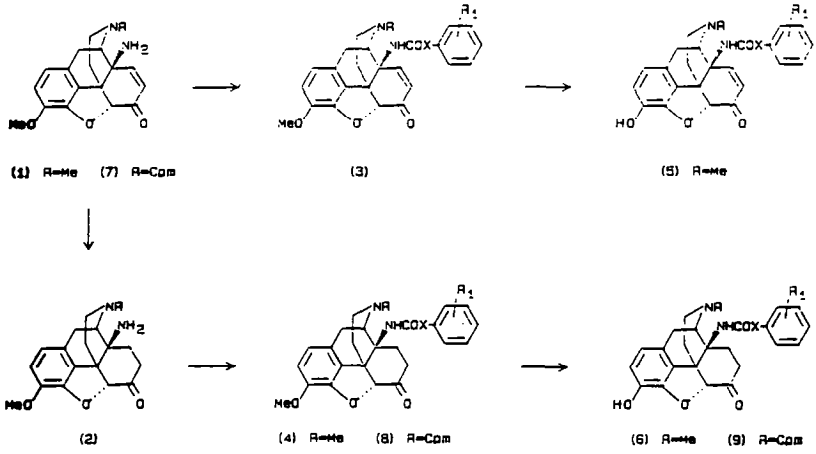
New 14-Aminomorphinones and Codeinones

J. Lewis, C. Smith, P. McCarthy, D. Walter, R. Kobylecki, M. Myers, A. Haynes, C. Lewis and K. Waltham

The effect of acylation of the C_{14} hydroxyl group of 14-hydroxy-codeinone was shown by Buckett (1965) to result in enhanced analgesic potency particularly when the acylating group was cinnamoyl. We have investigated analogous series of derivatives of 14-aminocodeinone and 14-aminomorphinone to establish detailed structure-activity relationships particularly with respect to opiate antagonist activity which was not found in the more limited 14-hydroxy series.

SYNTHESIS

14ß-Aminocodeinone (1) can be prepared in substantial quantity by the efficient synthesis of Kirby and McLean (1985). Acylation of this base or its 7,8-dihydroderivative (2, prepared from (1) with H_2/10% Pd on carbon), by the appropriate cinnamoyl or dihydrocinnamoyl chloride proceeds smoothly in dichloromethane. Demethylation of the codeinones (3 and 4) to the corresponding morphinones (5 and 6) is achieved with boron tribromide in dichloromethane at -20 .



14ß-Amino-N-cyclopropylmethylnorcodeinone (7) prepared by the Kirby and McLean method applied to N-cyclopropylmethylnorthebaine, was converted to 14ß-cinnamoylamino-N-cyclopropylmethyl-nor-7,8-dihydrocodeinones (8, X = CH=CH) and hence to the corresponding morphinones (9, X - CH=CH).

METHODS

Mouse Vas Deferens (MVD): VD of albino mice (>30g) of the strain MFI OLAC were suspended in an organ bath containing Krebs solution. Stimulation was via platinum electrodes placed above and below the tissue at 40V, 3 msec and 0.1Hz using a BBC microcomputer-controlled stimulator. A 30-45 minute period of equilibration under continuous stimulation with washing out every ten minutes was allowed before constructing dose-response curves. Cumulative dose-response curves were constructed to the test agonist by successively increasing the agonist concentration three-fold after the maximum inhibiting effect of the previous concentration was established. The bath solution was then changed for fresh Krebs containing the antagonist and at least 30 minutes contact time was allowed before construction of another dose-response curve. The agonists in this series took several hours to wash from the tissue so that the effects of antagonists were determined by use of separate tissues for each concentration of antagonist investigated.

The rat tail pressure (RTP) test used the method of Green and Young (1951) and the rat tail flick test (RTF) used the method of Janssen et al (1963) with hot water as the nociceptive stimulus. In the metabolism studies male Sprague-Dawley rats were dosed orally (3mg/kg) or subcutaneously (0.3mg/kg) with ^{3}H-RX819006. Similarly two male cynomologous monkeys were dosed with 2mg/kg of the tritiated dihydrocodeinone p.o. and s.c. respectively. All animals were fitted with an exteriorised cannula in the bile duct. Bile was collected throughout the experiment and urine at the end. Bile and urine samples were freeze dried, extracted with methanol and the extracts chromatographed on silica plates in two solvent systems along with cold markers. The residue was oxidised (Packard Oxidiser) and counted. Visualisation of metabolites was made using a radio-TLC analyser (RITA, Raytest Instruments UK).

The methods employed for the Medical College of Virginia (MCV) and University of Michigan, Ann Arbor (UM) studies are as published (Aceto et. al., (1986); Woods et. al, (1986)).

Activities of 14-Cinnamoylaminomorphinones
(Structure 5, X=CH=CH)

TABLE 1

R_1	MVD[1]	RTP[2]	RTFA[3]	R_1	MVD	RTP	RTFA
H	353	0.003	>10	p-Me	Part.Ag.	1.45	0.69
o-Me	589	0.2 (flat)	>10	p-Cl	Part.Ag.	0.1	18
o-Cl	415	0.008	>10	p-F	425	0.012	>10
m-Me	177	0.014	>10	p-OH	12	>10	>10

[1]Agonist potency (normorphine=1) in mouse vas deferens.
[2]ED_{50} (mg/kg s.c.) in rat tail pressure test.
[3]AD_{50} (mg/kg s.c.) in rat tail flick test (vs morphine).

TABLE 2

Activities of 14-Cinnamoylaminomorphinone derivatives

Cpd.No.	Structure	R_1	X	TF[1]	TFA[2]	PPQ[3]	SDS[4]
RX789041	5	p-Cl	.CH = CH.	0.5	>30	NT	6
RX779112	5	p-Cl	$.CH_2.CH_2.$	0.3	>30	0.2	2
RX789003	5	p-Me	.CH = CH.	>30	1.3	1.0	1A
RX859019	6	p-Me	.CH = CH.	>30	0.6	10(63%)	1A
RX869030	6	p-Me	$.CH_2.CH_2.$	0.5	>30	0.09	3-5

[1]AD50 (mg/kg s.c.) in rat tail flick test
[2]AD50 (mg/kg s.c.) in rat tail flick test (vs morphine)
[3]AD50 (mg/kg s.c.) in mouse phenylquinone writhing test
[4]Potency (morphine=1) in single dose suppression test in withdrawn morphine-dependent monkeys.

RESULTS

The indication that certain substituents in the para-position of the cinnamoylamino substituent had a substantial effect on intrinsic activity came from our screening results in the 14ß aminomorphinone series (Table 1). Substituents in the ortho and meta-positions had little effect on intrinsic activity or potency These compounds like the unsubstituted 14-cinnamoyl-amino-derivative were agonists in the (MVD) several hundred times more potent than normorphine, though somewhat less potent in vivo in the RTP test. The p-methyl and p-chloro-substituted compounds were partial agonists in the MVD. In the RTP test they displayed agonist activity though their potencies were much lower than that of the unsubstituted parent. In addition both showed morphine antagonist activity in the RTF assay. The antagonist activity of the p-chloro derivative was very weak and only detectable when the morphine was administered 4h after the test compound to allow for the very slow onset of action. Other para-substituents i.e. fluoro and hydroxyl - did not show this effect on intrinsic activity. The p-hydroxyl-derivative had very much lower activity particularly in vivo.

The two interesting p-substituted morphinone derivatives and closely related analogues have been tested at MCV and UM. MCV data are shown in Table 2. The higher intrinsic activity activity of the p-chloro-derivative (RX789041) over the p-methyl-derivative (RX789003) suggested by our data was clearly confirmed. RX789041 was active in the tail flick assay and suppressed abstinence in withdrawn, morphine-dependent rhesus monkeys (SDS test). RX789003 was inactive as an agonist but showed morphine antagonist activity in the RTF assay; it was inactive in the SDS test. The effect of reducing the codeinone to the 7,8-dihydrocodeinone is shown in the comparison of RX789003 with RX859019. Both compounds are partial agonists i.e. active in the phenylquinone writhing test (PPQ) and in the RTF assay active as morphine antagonists but not as agonists. The relative agonist to antagonist effects suggest that the 7,8-dihydro-derivative has the lower intrinsic activity. This was confirmed in the MVD (UM data, not shown) in which RX789003 was an agonist (64% peak depression) whereas RX 859019 was an antagonist.

The effect of hydrogenation of the cinnamoyl double bond is illustrated by comparison of RX789041 with RX779112 and RX859019 with RX869030. The data for the first pair show that they have similar profiles whereas RX859019 and RX869030 are markedly different. The former is a weak partial agonist whereas the latter is a morphine-like agonist active in RTF and able to substitute completely for morphine in the SDS test. This difference was confirmed in the MVD (UM data, not shown) in which RX859019 was an antagonist (predominantly μ) and RX869030 a μ-agonist comparable in efficacy to morphine.

A limited series of 14ß-cinnamoylamino-N-cyclopropylmethylnor-7,8-dihydrocodeinones (8, X = CH=CH) have been studied in detail. The in vivo data from MCV (Table 3) show the p-chloro-, p-bromo- and p-methyl-derivatives all to be partial p-agonists of substantial intrinsic activity since they substitute almost completely for morphine in the SDS test. They showed only weak morphine antagonist activity in the RTF test. In non- withdrawn morphine-dependent rhesus monkeys the p-chloro (RX819006) and p-methyl (RX839016) derivatives showed very long lasting withdrawal effects which were very slow in onset. The p-bromo-derivative (RX849015) had only a weak antagonist action in the same test.

In the MVD there was some discrepancy between our own data and that from UM. We found the RX819006 to be a partial S-agonist and the other two to be antagonists whereas at UM the RX819006 was found to have both μ - and δ -agonist activity and RX849015 and RX839016 to be partial p-agonists. The differences reflect the low sensitivity to p-agonists of our preparations.

Activities of 14-Cinnamoylamino-N-cyclopropylemthylnor-7,8-dihydromorphinones

(Structure 8, X = CH=CH

TABLE 3

Cpd.No.	R_1	TF	TFA	PFQ	SDS
RX819006	p-Cl	>30	6.0	0.2	3
RX849015	p-Br	>30	2.3	0.11	10
RX839016	p-Me	>30	5.7	0.1	1

Legend as for Table 2

TABLE 4

Cpd.No.	R_1	Tissue	μ^1	ke (nM) k^2	δ^3
RX819006	p-Cl	GPI	0.12	3.1	-
		RVD	0.12	-	6.4
RX849015	p-Br	MVD	0.32	27.3	8.6
RX839016	p-Me	MVD	0.28	66.3	19.3

[1]vs normorphine [2]vs EKC [3]vs DADL

Table 4 shows the relative affinities at μ- , k- and δ-receptors for the three dihydrocodeinones. Ke values were determined for RX849015 and RX839016 in the MVD; for RX819006 which had agonist activity in the MVD the guinea pig ileum and rat vas deferens (Smith and Carter 1986) were used. All three derivatives were selective for the μ- receptor with RX839016 having greatest specificity - 237:1 for μ/K and 69:1 for μ/δ.

The dihydrocodeinones were evaluated in rhesus monkeys at UM in self administration and drug discrimination studies. RX849015 maintained self administration rates in the codeine range, Rx839016 greater than saline but below codeine and RX819006, surprisingly, failed to induce self administration above saline rates. This may reflect that the dosage levels used for the p-chloro derivative were below the threshold for induction of self administration. All three derivatives generalized to codeine in the drug discriminations assay and were about 10 fold more potent than morphine.

The morphinones (9, X = CH=CH) related to the above codeinones are potent opiate antagonists with exceptionally long durations of action. In our own studies the AD_{50} in the RTF test (0.12 mg/kg) test when the antagonist was administered 24 hours before the morphine challenge was the same as when the period of pre-treatment was 45 minutes. With 6 hours pre-treatment the AD_{50} was lower (0.054 mg/kg).

Activities of 14ß -Cinnamoylamino-N-cyclopropylemthylnor-7,8-dihydromorphinonesText

(Structure 9, X = CH=CH

TABLE 5

Cpd.No.	R_1	TFA[1]	PPQ[1]	SDS[2]
RX809055	p-Cl	0.12	<30 (69%)	0.025
RX849031	p-Br	0.8	7.1	0.085
RX849030	p-Me	0.2	>30 (0%)	0.05

[1]Legend for Table 2
[2]Minimum dose exacerbating withdrawal

TABLE 6

Cpd.No.	R_1	Ke (nM)[1] μ	k	δ
RX809055	p-Cl	0.0025	0.077	0.054
RX849031	p-Br	0.003	0.25	0.21
RX849030	p-Me	0.0012	2.22	2.63
β-FNA		0.18	>6	8.55

[1]Legend as for Table 4. Tissue was MVD

The MCV in vivo data (Table 5) confirm the potent antagonist activity. In the anti-writhing test weak agonist activity was shown by the halo-substituted derivatives (RX809055, RX849031) but none by the p-methyl analogue (RX849030). Thus the rank order of intrinsic activity in the dihydrocodeinone series i.e. Br > Cl > Me was confirmed in the morphinones. In the single dose suppression test the antagonists produced severely exacerbated withdrawal effects which could not be suppressed by administration of morphine. In a separate study, a non-addicted monkey pretreated with a single dose (0.35mg/kg) of RX849031 showed no response to acutely administered morphine for two weeks indicative of an irreversible antagonist effect.

In the MVD the affinities (Ke) of the three morphinones at the μ, k- and δ- receptor subtypes have been determined (Table 6). As in the equivalent dihydrocodeines, the antagonists were specific for the μ-receptor with the p-methyl analogue again showing the greatest specificity (ca 2000: 1 for both μ/k and μ/δ.)

Preliminary metabolism studies have been conducted on the dihydrocodeinone RX819006 as the tritiated drug in the rat and cynomologous monkey by the s.c. and p.o. routes. In the former about 65% of the dose was recovered in the bile and around 3% in the urine in the first 24 h after dosing by either route. About 50% of the radioactivity in the bile was a conjugate of the morphinone RX809055. In the monkey only 18% of the s.c. dose and 9% of the oral dose was recovered in The 8 h experimental period.

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Virtually all of this radioactivity was in the bile and occurred as one product, a conjugate of the morphinone RX 809055.

DISCUSSION

We have discovered that the profiles of 14-ß-cinnamoylamino-codeinones and morphinones are substantially changed by the introduction of p-halo or methyl substituents. In particular the intrinsic activity is reduced so that in the N-methyl series the p-substituted morphinones become partial agonists instead of full agonists whilst in the N-cyclopropylmethyl series highly potent antagonists of exceptional duration of action are produced. The methyl group has the greatest effect on intrinsic activity and of the two halogen substituents, chloro has a greater effect than bromo.

Other features of the structure - activity relationships are:

(i) 7,8-Dihydrocodeinones and morphinones have marginally lower intrinsic activity than the unhydrogenated analogues.

(ii) Hydrogenation of the cinnamoyl double bond can substantially increase intrinsic activity.

(iii) The N-Cpm dihydrocodeinones in vivo are μ partial agonists which show delayed long-lasting antagonist effects. This profile may be attributable to metabolism to the corresponding dihydromorphinones.

These dihydrocodeinones. may offer the opportunity to treat opiate dependence by providing limited reinforcing effects followed by a longer period of opiate blockade.

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AUTHORS:

J. W. Lewis, C. F. C. Smith, P. S. McCarthy, D. S. Walter, R. J. Kobylecki, M. Myers, A. S. Haynes, C. J. Lewis and K. Waltham

Reckitt & Colman Pharmaceutical Division
Dansom Lane
Kingston upon Hull HU8 7DS
United Kingdom.

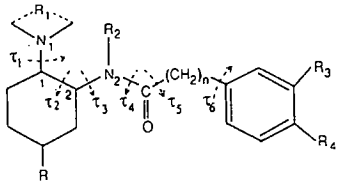
Structure Activity Studies of Two Classes of Beta-Amino-Amides: The Search for Kappa-Selective Opioids

G. Loew, J. Lawson, L. Toll, G. Frenking, I. Berzetei-Gurske and W. Polgar

The discovery of the first κ-selective analogs, U50,488 and U69,593 by investigators at Upjohn laboratories (VonVoightlander et al., 1983) has given the study of κ-mediated events great impetus. Such selective drugs could be helpful in studies to determine the unique biochemical or **in vivo** consequences of binding to κ-receptors. However, very little work has been reported designed to determine molecular properties which modulate affinity at the κ-receptors for the Upjohn (U) compounds.

We report here the systematic determination of the affinities at μ- and **κ-** receptors of a more extensive series of U-compounds (table 1) then heretofore published, with five new analogs kindly given to us by Dr. R. Lahti of Upjohn. As seen in table

TABLE 1. μ/κ Receptor affinities (K_D nM) of 1,2-cyclo-ß-amino-amides (U-Compounds) in guinea pig brain



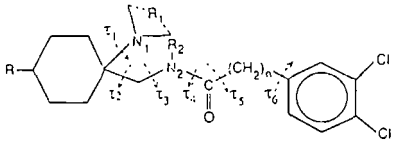
	n = 0				n = 1			
U	47109	47700	48520	50211	51754	50,488H	62066	69,593
R_1	diMe	diMe	diMe	diMe	diMe			
R	H	H	H	H	H	H		
R_2	H	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3
R_3	Cl	Cl	H	H	Cl	Cl	Cl	H
R_4	Cl	Cl	Cl	OH	Cl	Cl	Cl	H
μ	59	5.3	200	>10,000	220	430	210[a]	1,700
κ	910	910	2,900	>10,000	71	2.2	2.5	7.2
ratio μ/κ	0.06	0.006	0.07		3.1	195	84	236

[a]Literature values (Lahti et al., 1985)

1, the structural commonality of these U-compounds include a trans-1, 2-diaminocyclohexane moiety with a benzoyl or arylacetyl functionality on the second amine. Thus we refer to this family as 1, 2-cyclo-ß-amino-amides (1, 2 cyclo-ß-AAs).

We also report here progress in the development of a new class of opioids related to the U-compounds that show promise as κ-Selective analogs, the achiral 1, 1 cyclo-ß-aminoamides or S-compounds shown in table 2. These S-compounds have the same gross structural moieties as the U-compounds, i.e., a cyclohexane ring with amine and amide nitrogens separated by two carbon atoms, and an aryl acetamido group. However, there is one notable difference in that the amine and amide groups are linked by a rotatable torsion angle, τ_2, in the S-compounds, while this angle is part of the cyclohexane ring in the U compounds. Moreover, the S-analogs are achiral and much more readily synthesized than the U compounds which have two or three chiral centers. The synthesis, receptor binding, and in vitro activities of a number of these analogs is reported here.

TABLE 2. μ/κ Receptor affinities (K_D nM) of new class of achiral 1,1-cyclo-ß-amino-amides (S-compounds) in guinea pig brain



	n = 0	n = 1				
	S1	S2	S3	S4	S5[b]	S6[a,b]
R_1	diMe	diMe	diMe	N⟨ ⟩	diMe	diMe
R	H	H	H	H	>C(=O)-O-	>C(=O)-O-
R_2	H	H	CH_3	H	CH_3	CH_3
μ	10	100	620	4,500	90	1,000
κ	150	67	53	56	12	120
ratio μ/κ	0.07	1.5	11.8	80	7.5	8.3

[a]des 3,4 diCl analog

[b]These are IC_{50} from inhibition of [^{3}H]U69,583

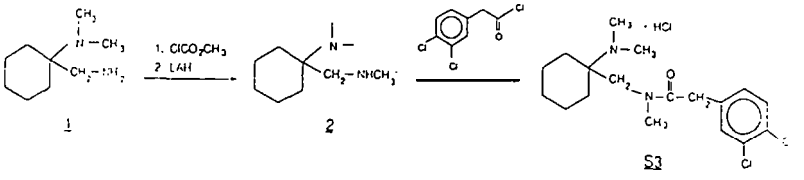
In a complementary theoretical effort, we have also calculated and compared the energy conformational profiles of the U- and S-compounds to help determine the molecular modulators of μ/κ selectivity in both families.

METHODS

Synthesis

The known diamine 1 is acylated with methyl chloroformate in ether and the resulting urethane reduced with LAH without isolation to give the N-methyl diamine 2 in near quantitative

yield. Exposure of 2 to 1.05 equivalents of 3,4-dichlorophenylacetyl chloride in dichloromethane results in the immediate formation of the amide S3. Dilution of the reaction solution with ethyl ether precipitates the product hydrochloride salt as an off-white powder, which is recrystallized from dichloromethane/cyclohexane to give an analytically correct crystalline product.



Scheme 1

The structures of each of the compounds in table 2 have been confirmed by their mass spectrum and ^{1}H-NMR (400 MHz) and are analytically correct (CHN).

Receptor Binding

All receptor binding studies were performed using basic procedures similar to those described by Pasternak (Pasternak et al., 1975) in guinea pig brain using three labeled ligands found to be most selective at μ(DAGO); δ (DSLET), and κ (U69,593). All compounds had low affinities at the g-receptor and only K_Ds at the μ- and κ-receptors are reported as determined by computer analysis using the nonlinear least-squares regression procedure in the program LIGAND (Munson and Rodbard 1980). A number of 1,2-ß-AAs were kindly supplied to us by Dr. R. Lahti of Upjohn. Their relative receptor affinities at μ- and κ-receptors as well as those for our new S-analogs are reported here for the First time.

In Vitro Activity

The longitudinal muscle strip of the guinea pig from male guinea pigs (350-450 g) was prepared as described elsewhere (Paton and Vizi 1969). The experiments were performed in Krebs solution bubbled with 5% CO_2 in oxygen, at 37°C. The composition of Krebs solution used was as follows (in mM) NaCl 288, $NaHCO_3$, 25, KCl 4.7, KH_2PO_4, 1.2, $CaCl_2$ 205, $MgSO_4$, 1.2 and glucose 11.5. Field electrical stimulation was applied; the supramaximal (1.5 times the maximal voltage) rectangular stimuli of 1 msec duration were delivered at a rate of 0.1 Hz. Contractions were recorded isometrically by means of a strain gauge coupled to a Grass amplifier-recorder system. The agonist potencies of compounds were determined from concentration-response curves and characterized by the IC_{50} values. The IC_{50} is defined as the concentration of the agonist which produces 50% inhibition of the electrically induced contraction.

THEORETICAL

For studies of the conformational properties of the two classes of ß-AAs, an empirical energy program called MOLMEC described in detail elsewhere (Oie et al., 1981) was used, with net atomic charges in the electrostatic term obtained from a MNDO method (Dewar and Thiel 1977).

Extensive energy-conformational studies were made of four analogs of the U-compounds, shown in table 1 (U51,754; U50,488H; U47,700; and U47,109), and three of the new compounds, shown in table 2 (S1, S2, and S3). The Upjohn U-compounds have five rotatable torsion angles, and four relative equatorial/axial positions of the 1-amino and 2-amido substituents on the cyclohexane ring. The new S-compounds have an additional rotatable bond, τ_2, which is fixed in the U-compounds, but have only two possible relative positions of the 1-amino, 1-methyl-amido substituents: ax/eq and eq/ax.

Similar procedures were used to explore conformational space for both the 1, 2-ß-AA and 1, 1-ß-AA analogs. As a first step, the aryl fragments were approximated by a methyl group, and the remainder of the molecule was examined, with all possible combinations of amino and amido substituents as well as both chair and boat forms of the cyclohexane ring and 8 sets of torsion angles for the U-compounds and 11 for the S-compounds. In the next step, the aromatic portions were added to the 9 lowest energy structures of the U fragments (ΔE < 9 kcal/mol) and the 11 lowest energy conformers of the S-fragments (ΔE < 3 kcal/mol). The 27 conformers of U51, 754 compound and the 33 conformers of S3 compound were then reoptimized.

The effect of substituent changes on the amino and amide nitrogen on the conformation was also explored, comparing the dimethyl amine (U51,574) to a pyrrolidine (U50,488H) in the U-series and the effect of adding a methyl group to the amide nitrogen in the U- and S-series.

RESULTS AND DISCUSSION

U Compounds (1, 2-cycloAAs)

The results of receptor binding studies shown in table 1 for eight 1, 2-ß-AAs (U-compounds) show that there is a very subtle electronic and conformational modulation of μ/κ affinities and selectivities in this family of opiates. None of the four benzamides (n = 0, table 1) compounds bind with high affinity at κ. Moreover within this series, changing the substituents on the phenyl ring greatly modulate μ-receptor affinities. The 3,4-dichloro-analog (47,700) has highest affinity at μ and is μ-Selective (μ/κ - 200). The simple insertion of a methylene bridge (n = 1) in this analog to make U51, 754, greatly diminishes affinity at μ and increases affinity at κ, making it mildly κ-selective (κ/μ = 3). As indicated in table 3, energy conformational studies of these analogs, U47,700 and U51,754,

TABLE 3. Calculated torsion angle value for low energy conformers of U and S compounds

	n = 1				n = 0[b]		
	U51,754	U50,488	S3		U47,700[c]	S1	
τ_1	- 43	- 46	- 42	179	- 43	- 41	174
τ_2[a]	45	46	46	61	46	45	72
τ_3	51	50	- 93	84	50	-105	89
τ_4	-160	-165	160	-179	-173	155	179
τ_5	66	66	67	172	84	96	96
τ_6	61	62	60	- 87			
amine/amide	eq/eq	eq/eq	eq/ax	ax/eq	eq/eq	eq/ax	ax/eq

[a]This angle is a fixed part of cyclohexane ring in U compounds (Table 1) and a flexible torsion angle in P compounds (Table 2).
[b]n=0 compounds do not have a methylene bridge between amine and phenyl ring and hence have one less torsion angle (Tables 1 and 2).
[c]This same low-energy confomer was obtained for U47,109 with a H instead of a methyl group on the amide nitrogen.

result in similar low-energy conformers, shown in figure 1, with overlapping trans-1-amino-2-amido-cyclohexane regions. As seen in this figure, the only differences between the μ- and κ-selective analogs are the relative positions of the aromatic rings caused mainly by the presence (n = 1) or absence (n = 0) of the methylene bridge. Apparently, this difference in spatial relationship of the aminoamide and aryl moieties is important in determining relative μ/κ affinities.

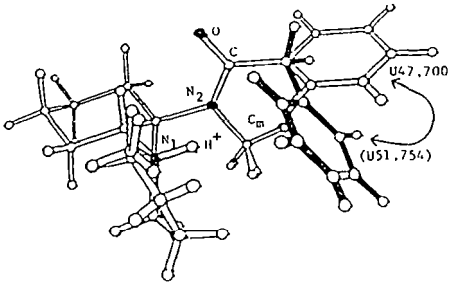


FIGURE 1. Overlap of low energy conformers of κ-selective (U51,754) and μ-selective (U47,700) 1,2ß-AA. (These analogs are shown with complete 1 amino, 2 amido-cyclohexane overlap.)

Among the (n = 1) analogs (table 1), affinity at both receptors is sensitive to changes in substituents on the cyclohexane ring, and on the amino, amido, and aryl moieties. It appears that all four groups make a crucial contact with binding sites in the μ- and κ-receptors. An example of the sensitivity to amino-N-substituent is that replacement of the dimethylamine of U51, 754 by a pyrrolidine, leading to U50, 488, slightly lowers the μ affinity but greatly enhances κ affinity resulting in the first highly selective κ-agonist reported (κ_1/μ - 200). The

sensitivity of relative μ/κ affinities to aryl substituents is shown by the observation that if the 3,4-dichloro-substituents are removed from U62,066, a drastic reduction in μ- but not K-affinity occurs, leading to the most K-Selective U-compound yet reported (U69, 593, κ/μ ~ 600). The effects of these substituent changes, one increasing κ affinity and the other preferentially reducing μ affinity, are most likely caused by electronic rather than conformational modulation. Extensive energy conformation studies of the N-dimethyl compound (U51,754) and the pyrrolidine derivative (U50,488) reveal that both have the same low energy conformation (table 3). Results of these studies indicated that the chair form of the cyclohexane ring is significantly more stable than the boat form ($\Delta E \geq 6$ kcal/mol) for all conformers studied. In addition, of the four isomeric arrangements of the 1-amine/2-amide substituents of the cyclohexane ring, the eq/eq configuration was the lowest energy ($\Delta E \leq 7.5$ kcal/mol). The similar conformations of U51, 754 and U50, 488 provide evidence that, indeed, selectivity occurs through local electronic effects of the N-substituent itself. Such N-substituent modulation of μ/κ selectivity was also observed for the rigid fused-ring opiates.

In summary, the basic 1,2-ß-AA moiety is not intrinsically a κ-selective pharmacophore. Rather, it is a framework within which variations at a) the amino group, b) the cyclohexane ring, c) the amido group, and d) the arylacid can produce a range of results From p-selective to κ-selective compounds.

S-Compounds (1,1-cyclo-ß-AAs)

Table 2 shows the results of receptor binding studies of six analogs in the new series of achiral 1,1-ß-AAs (S-analogs). These results indicate a similarity between the U- and S-compounds. Like the U compounds, insertion of a methylene bridge between the aminoamide and aromatic moieties (S1 versus S2) enhances κ Selectivity. Another similarity between the series is that μ/κ selectivity is modulated by changing the amine N-substituent. In particular, similar to the U-series, κ-selectivity is enhanced when a dimethyl group is replaced by a ring compound, in this case a piperidine ring (S2 versus S4) leading to the most selective κ-analog obtained thus far in our new Series (κ/μ ~ 100). A third similarity encountered is the ability of a 3,4-dichloro substituents on the phenyl ring to modulate affinity at both μ- and κ- receptors (S5 versus S6) leading to analog S5 with the highest K-affinity thus far obtained.

In vitro studies in guinea pig ileum, shown in shown in table 4, yield IC_{50} values of 1.9 nM for both U50,488 and U69,593, while that for S3 and S4 our most κ-selective compound were 65 and 465 nM respectively. Although there are both μ and κ receptors in guinea pig ileum, the κ-selectivity of both U compounds and S4 is great enough to insure that the activity measured is initiated primarily by binding to the κ-receptor. If we define relative efficacy as the binding affinity (K_D) at κ determined

TABLE 4. In vitro activity in guinea pig ileum assay of U and S compounds

	IC_{50} (nM)	K_D (<, nM)	K_D/IC_{50}
U50,488	1.9 ± 0.5	2.2	~1
U69,593	1.9 ± 0.1	2.7	~1
S3	65 ± 4.9	·53	~1
S4	.465 ± 132	56	~0.1

in guinea pig brain, divided by the **in vitro** potency (IC_{50}) in guinea pig ileum, the results in table 4 indicate that S4 has a factor of 10 lower efficacy than both U compounds and S3.

The results of energy-conformational studies of the S analog, S3, summarized in table 3, provides a possible explanation for the similarities between U- and S-compounds. These results indicate that there are many more low-energy conformers for S3 than For the corresponding U-analog (U51,754). In particular, while the eq/eq amino/amido conformation in the U-compounds was found to be the definitive low energy Form (Δ|E $\geq$ 6 kcal/mol), the eq/ax and ax/eq isomers of the 1,1-ß-AA compounds have equal energies. The torsion angles For the lowest energy Forms of each of these isomers for S3 indicate that the conformation of the amino-equatorial, but not the equi-energy amino-axial form, is very similar to that of the U-compounds. In particular, the value for the rotatable angle τ_2 in S3 matches that of the fixed angle τ_2 in the U-compounds. This similarity is shown in figure 2 where the lowest energy conformer of U50,488 is superimposed

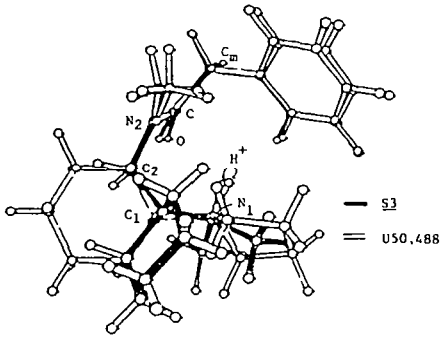


FIGURE 2. Overlap of low energy U(eq/eq) and S(eq/ax) conformers. (RMS = 0.37, $N_1C_1C_2N_2C$); $R_{H^+H^+}$ = 0.14)

on the lowest energy amino-equatorial form of S3. In this figure, the N1(amine)-C_1-C_2-N_2(amide)-C(=0)-CH2 atoms of each compound are overlapped with a root mean square of 0.37 A. The two amine protons are 0.14 A apart and point in the same direction. While there are significant differences in the position of their cyclohexane rings, the similarity in overlap

of these two families of compounds, when the S-analog is in the amino-equatorial conformer, indicates that they may bind in a similar fashion to the κ-receptor. Thus S-type analogs which have high c-affinity and selectivity should be possible to obtain.

Insights gained from the studies thus far are guiding further choices for synthesis of this new class of 1,1-ß-AA. For example, we plan to synthesize analogs of the S-compounds that will preferentially enhance the stability of the amino-equatorial comformer which most resemble the U-compound. Addition of bulky alkyl group to the 4-position of the cyclohexane ring, trans to the equatorial amino moiety, will stabilize this conformer and is expected to enhance κ-affinity and selectivity.

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AUTHORS

Dr. John Lawson, Dr. Lawrence Toll, Dr. Gernot Frenking, Dr. Ilona Berzetei-Gurske, Ms. Willma Polgar, and Dr. Gilda Loew, Molecular Theory Department, Biomedical Research Laboratory, SRI International, Menlo Park, California 94025 U.S.A.

Patient-Treatment Matching in the Management of Alcoholism

H. Annis

INTRODUCTION

The limited efficacy of the conventional approach to alcoholism treatment is now widely acknowledged in the research literature. Studies comparing the effectiveness of lengthy inpatient programs with lower cost alternatives have challenged traditional beliefs concerning the required length of inpatient treatment and the role of inpatient versus day treatment and outpatient options. Well-controlled randomized trials comparing standard multimodal alcoholism treatment programs ranging in length from several days to a few months have reported no advantage for prolonged hospitalization Similarly it has been demonstrated that both day treatment (partial hospitalization) and outpatient programs produce comparable results to inpatient programs for unselected alcoholic patients seeking treatment. (For reviews of this literature, see Annis 1986; Miller and Hester 1986a.) Futhermore, there is evidence that minimal intervention strategies (e.g., brief "advice" counselling) can be as effective under some circumstances as more intensive traditional programming (Edwards et al., 1977).

Two explanations have been offered for the apparent limited efficacy of treatment. First, it has been proposed that there is a need for the development of more-effective treatments than those methods employed in traditional multimodal alcoholism treatment programs - i.e., detoxification, AA meetings alcohol education, disulfiram, and group confrontation therapy. Miller and Hester (1986b) have argued that certain treatment methods such as aversion therapy, behavioral self-control training, social skills training, stress management, marital and family therapy, and community reinforcement approach have demonstrated specific effectiveness in the treatment of alcoholics. However, other investigators have concluded that differences in effect size between treatments are small and make only a modest contribution to outcome results. In an analysis of 384 studies of psychologically-oriented alcoholism treatment, Emrick (1975) found that differences between treatment methods had little effect on long-term outcome. Work by Cronkite and Moos (1978) and by Costello(1980) has provided evidence that treatment variables account uniquely for only 6 to 7% of the outcome variance. Nevertheless, the possibility remains that more effective treatments for alcoholics will yet be discovered.

A second explanation for the apparent limited efficacy of treatment suggests that what is needed is not so much better methods of treatment, but more judicious matching of patients to available treatment options. There is a growing consensus in the field that it is unlikely that a single treatment will be found that will be effective for all alcoholics. It is now widely acknowledged that there is wide heterogeneity among alcoholics, and that a patient with one set of characteristics may respond favorably to one type of treatment, whereas, a patient with another set of characteristics may respond more favorably to another treatment approach. The attempt to match patients to treatments to improve outcome results is referred to as patient-treatment matching or the matching hypothesis. There is general agreement in the field that differential assignment of patients to treatments has potential for improving outcome results.

STRATEGIES FOR IDENTIFYING MATCHING VARIABLES

Five approaches have been suggested as potentially fruitful avenues for the identification of patient-treatment matches (cf., Finney and Moos 1986) : 1) therapist or clinical judgment; 2) patient judgment (the "cafeteria" approach); 3) exploratory data analysis; 4) data reduction techniques; and 5) theory-driven selection of patient and treatment variables. There has been little systematic study in the alcoholism field of the operation of clinical judgment by therapists in individualizing or matching treatment approaches to different patients. Few treatment settings have offered the range of treatment alternatives that would permit such study. However, this remains a potentially fruitful avenue, particularly for the generation of hypotheses about appropriate patient-treatment matches. A second approach, namely studying the operation of patient judgment, has been called the cafeteria plan. In this approach, patients are offered several alternative treatments and permitted to choose among them (Ewing 1977). A limitation of this strategy for the identification of effective patient-treatment matches is the confound introduced by the attractiveness of the treatment; treatment attractiveness ma not necessarily relate to the effectiveness of the treatment for the client (c.f., Finney and Moos 1986; McLellan 1988). Consequently, what may be studied is patient-treatment acceptability rather than patient-treatment effectiveness.

The next two approaches, exploratory data analysis and data reduction techniques are similar except that data reduction techniques use factor or cluster analysis to reduce the number of patient and treatment variables to a few general dimensions before exploring their relationship to outcome. These techniques have not been extensive employed to date in identifying patient-treatment matches, but they could serve both to generate matching hypotheses and to provide some initial confirmatory evidence for further controlled testing.

Finally, it has been suggested by several investigators (Annis 1987; Longabough 1986; McLellan 1988), that theory-driven selection of patient and treatment variables is the approach that is most likely to advance knowledge of patient-treatment matching. The reasons why theory can be expected to be so critical in guiding empirical testing of matching effects are examined below.

COMPLEXITY OF THE MATCHING TASK

The search for patient-treatment matching effects requires the reliable assessment of patient variables, on the one hand, and treatment variables on the other. The conceptualization and assessment of salient characteristics of patients is at a more advanced stage of development than the measurement of treatment variables (c.f., Glaser 1980), although considerable progress has been made by Moos and his colleagues in evaluating certain aspects of treatment environments (Moos 1974). Table 1 presents a list of patient and treatment variables that have received some interest in the alcoholism treatment literature in relation to patient-treatment matching. Among the wide array of variables on which patients may be differentiated, a number of general background and alcohol specific variables have been proposed as typing dimensions. Work has been reported typing clients in terms of : 1 general sociodemographic variables such as marital status (Azrin et al., 1982) and social stability (Mayer and Myerson 1971); 2) the extent of environmental resources such as social supports at intake to treatment (Smart 1978) and in the post-treatment environment (Moos and Finney 1983); 3) the degree of neuropsychological deficit (Wilkinson and Sanchez-Craig 1981); and 4) a variety of personality factors including depression (Merry et al., 1976) anti-social personality disorder (Schuckit 1985), and psychiatric severity (McLellan et al, 1983). In addition, patients have been typed on a range of alcohol specific dimensions including: 5) consumption variables such as years of excessive drinking and quantity/frequency measures of alcohol intake (Smart 1978); 6) measures of alcohol dependence (Sanchez-Craig et al., 1984; Skinner and Horn 1984); 7) alcohol expectancies such as drinking-related self-efficacy (Annis and Davis in press a) and treatment outcome beliefs (Solomon and Annis in press); and finally, 8) patients have been in terms of the situational antecedents to their drinking (Annis and Davis in press b).

A similar wide array of variables exists on the treatment side of the equation as potentially important dimensions for the matching of relevant patient attributes. Among the treatment dimensions that have been proposed as the basis for differential patient assignment are: 1) treatment setting variables such as inpatient, day treatment, outpatient and halfway house options (Kissin et al., 1970; Pattison 1979); 2) intensity/duration variables such as length of hospitalization (Walker et al., 1983), brief advice versus longer-term counselling (Orford et al., 1976), and the use of self-help manuals (Heather 1986; Sanchez-Craig 1988); 3) treatment modality variables such as the use of disulfiram (Obitz 1978), Alcoholics Anonymous (Ogborne and Glaser 1981), relaxation training (Rosenberg 1979), and patient versus environmentally focused interventions (Longabough and Beattie 1985); 4) therapist-offered conditions including such variables as therapist supportiveness (Luborsky et al., 1985). non-directive versus directive counseling style (McLachlan 1974), and peer versus professional counselling (Lyons et al., 1982); 5) the goal of treatment whether abstinence or moderation (Sanchez-Craig et al., 1984; Sobell and Sobell 1987); and 6) a wide array of treatment context variables such as whether treatment is offered in an individual or group context, recruitment procedures, and the sequencing of treatment components (McLellan 1988; Pattison 1979).

TABLE 1. Patient-treatment matching

Patient Variables

1) General

SOCIODEMOGRAPHIC
(e.g., age, sex, marital status, social stability, family history of alcoholism)

ENVIRONMENTAL RESOURCES
(e.g., finances, social supports)

NEUROPSYCHOLOGICAL STATUS
(e.g., type and degree of neuropsychological deficit)

PERSONALITY
(e.g., self-esteem. locus of control, MMPI profile, psychiatric diagnosis, psychiatric severity)

2) Alcohol Specific

CONSUMPTION
(e.g., years excessive drinking. quantity, frequency)

DEPENDENCE
(e.g., degree of alcohol dependence symptomology, presence of physical withdrawal)

EXPECTANCIES/OUTCOME BELIEFS
(e.g., self-efficacy, belief in disease concept of alcoholism)

SITUATIONAL ANTECEDENTS
(e.g., types of high risk situations for alcohol involvement)

Treatment Variables

SETTING
(e.g., inpatient, outpatient, day treatment)

INTENSITY/DURATION
(e.g., brief advice, long-term therapy)

METHOD
(e.g., disulfiram, relaxation therapy)

THERAPIST
(e.g., directive, nondirective. professional, peer)

GOAL
(e.g., abstinence, moderation)

CONTEXT
(e.g., group. individual, treatment system)

The above patient and treatment variables that have been proposed as the basis for matches in the alcoholism literature represent only a subset of the vast number of potential dimensions for defining patient-treatment matches. However, even in terms of this limited number dimensions it can be seen that the number of possible matching combinations is overwhelming. The situation is further complicated by two additional factors: 1) more than a single patient characteristic may interact, in various ways, with more than a single treatment dimension; and 2) it may be necessary to consider matching factors at more than one stage of the treatment process.

In terms of the first complicating factor above, Finney and Moos (1986) have noted that a variety of complex manifestations of matching effects are possible involving non-linear effects, higher-order effects (i.e., where an interaction itself is moderated by another patient or treatment variable) and multi-level effects (i.e.. where the interaction effect is dependent, in a group or milieu context, on the patient's standing on some variable(s) within the group). An original finding of a simple first order interaction effect (i.e., one patient characteristic interacting with one treatment dimension) may result in the specification of high-order effects when tested on more diverse patient samples.

With regard to the second factor that complicates the specification of patient-treatment matches, McLellan (1988) has pointed out that matching can occur at more than one stage of treatment. Therefore, in the specification of salient patient-treatment matches, multistage matching Should be considered at the following stages of the treatment process: (a) prior to treatment initiation (patient self-selection); (b) at treatment intake; (c) during treatment; and (d) in relation to the post-treatment environment. The patient and intervention characteristics that have importance in matching may differ at each stage of the treatment process.

In summary, given the staggering array of potential patient and treatment variables to be considered, and the complexity of the possible relationships among these variables, studies that are strongly theoretically-driven are most likely to advance knowledge on patient-treatment matching.

MATCHING DESIGN REQUIREMENTS

In order to provide convincing confirmatory evidence for the presence of a matching effect, a study must minimally involve the following six research design features: 1) a reliable patient-predictor variable on which at least two

differentiated treatment conditions; 3) assignment (preferably random) of each patient type to each treatment condition; 4) sufficient sample size across study conditions to detect a moderate effect; 5) an adequate post-treatment follow-up period; and 6) objective, reliable measure(s) of therapeutic impact. (For further discussion of these design requirements, see Annis 1987.)

EMPIRICAL EVIDENCE OF SUCCESSFUL MATCHING

Based on the above design considerations, a recent review of the alcoholism treatment literature by the present author was able to locate 15 studies that provide evidence for successful patient-treatment matching-effects (see

TABLE 2. Alcohol treatment outcome studies providing evidence for successful patient-treatment matching

Matching Variables		
Patient Characteristic	Treatment Characteristic	Study
General		
SOCIODEMOGRAPHIC (marital status)	MODALITY (disulfiram)	Azrin et al. (1982)
SOCIODEMOGRAPHIC (social stability)	MODALITY (disulfiram/tranquilizers)	Mayer et al. (1971)
SOCIODEMOGRAPHIC (social/psy. stability)	SETTING/MODALITY (in/outpatient; drugs)	Kissin et al. (1970)
PERSONALITY (psychiatric diagnosis)	MODALITY (disulfiram/hypnotherapy/milieu)	Wallerstein et al. (1957)
PERSONALITY (schizophrenia)	MODALITY (LSD)	Tomsovic et al. (1970)
PERSONALITY (psychiatric severity)	SETTING (in/outpatient)	McLellan et al. (1983)
PERSONALITY (conceptual level)	THERAPIST (directive/nondirective)	McLachlan (1972)
PERSONALITY (depression)	MODALITY (lithium)	Merry et al. (1976)
PERSONALITY (self-image)	MODALITY (confrontive group)	Annis et al. (1983)
PERSONALITY (locus of control)	MODALITY/INTENSITY (structured, intense)	Hartman et al. (1988)
Alcohol Specific		
DEPENDENCE (gamma alcoholism)	INTENSITY (advice)	Orford et al. (1976)
DEPENDENCE (problem drinkers)	THERAPIST (professional/peer)	Sokolow et al. (1980)
DEPENDENCE/SOCIODEMO (degree dependence)	GOAL (abstinence/moderation)	Polich et al. (1980)
SITUATIONAL ANTECEDENTS (anxiety)	MODALITY (relaxation training)	Rosenberg (1979)
SITUATIONAL ANTECEDENTS (risk situations)	MODALITY (relapse prevention)	Annis et al. (in press b)

Annis 1987). The matching variables involved in these studies are presented in table 2.

In terms of patient variables it can be seen from table 2 that matching effects were based on a single personality dimension in 7 of the studies, a sociodemographic factor in 3 of the studies, degree of alcohol dependency in 2 studies, alcohol dependency and sociodemographic factors in 1 study, and the situational antecedents of a patient's drinking in 2 studies. The great majority of the studies (i.e., 10 of the 15) involved matching these patient characteristics to a particular type of treatment modality. Studies that have shown the largest effect sizes associated with matching tend to be those in which the choice of client and treatment matching dimensions were theoretically-driven (see Annis 1987).

CONCLUSION

Empirical evidence for matching effects is in its infancy. Nevertheless, the differential assignment of patients to treatment holds promise for improving alcoholism treatment outcome results. Studies in which the choice of client and treatment variables has been theoretically-driven suggest that matching effects can make a substantial contribution to explaining treatment outcome variance.

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AFFILIATION: Psychology, Clinical Institute, Addiction Research Foundation, 33 Russell St., Toronto, Ontario M5S 2S1

Psychotherapy for Substance Abuse

G. Woody, L. Luborsky, A. McLellan and C. O'Brien

INTRODUCTION

The recent work done to evaluate the efficacy of psychotherapy for substance abuse originated from criticisms of methadone maintenance. The original and very Positive outcome results obtained by Dole and Nyswander (1968) led to the rapid development of methadone programs and within a few years, the number of opiate addicts treated with this modality grew from a few hundred to almost 80,000. Patients who entered this expanded treatment effort were often cross-addicted or abusing other substances along with opiates, and also had a high proportion of psychiatric and behavioral problems. The staffs of the newly-formed treatment programs were often inexperienced in the management of this type patient. Perhaps as a result, the outcomes achieved by many patients were less positive than those originally reported, and the value of methadone treatment was questioned. Some said that outcome could be improved if the programs were staffed by more highly trained personnel, especially those experienced in treating psychiatric disorders.

In response to these concerns, NIDA organized a series of meetings that were designed to develop testable hypotheses that could provide more precise information about these issues. Two ideas were generated and these resulted in three contracts aimed to determine the kinds and frequencies of additional psychiatric problems among opiate addicts, and to see if professional psychotherapy added anything to paraprofessional drug counseling services in methadone maintained opiate addicts.

The results of the diagnostic studies were consistent. All showed that **about 85%** of the populations studied had experienced a psychiatric disorder in addition to substance abuse either currently or in the past. The two psychotherapy studies that were done showed different results. One showed no gains from professional psychotherapy beyond those obtained by standard drug counseling services (Rounsaville et. al., 1983). The second (Woody, et. al., 1983) showed that:

- professional psychotherapy added to traditional drug counseling produced significantly better outcomes at both 7 and 12-month follow-up than drug counseling alone.
- both psychotherapies studied (supportive-expressive and cognitive-behavioral) produced relatively similar degrees of benefit.
- the differential effects of psychotherapy over drug counseling alone were seen in those patients with moderate and high levels of psychiatric symptoms; low severity patients did equally well with counseling alone.

- there were marked differences in outcome according to therapist and these related to the ability to form a "helping" relationship as well as to the therapeutic techniques of the therapists
- sociopathy (antisocial personality disorder) alone was a negative predictor of outcome, but sociopathy plus depression carried a better prognosis.

These findings were interesting and, when combined with the results of other work, make us reasonably comfortable in saying that professional psychotherapy can provide meaningful benefits when delivered in the context of our program which is University-affiliated and research-oriented.

CURRENT WORK

The next step was to see if therapy could have comparable effects in other settings, and this is the focus of our current work in four community-based methadone programs. We attempted to replicate the administrative conditions of our earlier study by having:
- the clinical director of each program monitor and supervise the psychotherapy study as an integral part of the ongoing treatment services.
- offices for the therapists in the treatment facility, along with efforts to integrate them comfortably into the treatment program.
- the program director pay attention to coordination and cooperation between therapists and counselors, especially at the beginning of the project. The P.I. attends weekly meetings to assist in this process.
- attention given to patient compliance. Patients are reminded if they miss appointments, and attempts are made to reschedule them. A counselor was identified in each program to serve as the contact person to work with program staff, the P.I. and the project's technicians to monitor compliance.
- patients start in therapy shortly after they enter the program since that appears to increase the chances for successful engagement. The contact person and counselors help identify and recruit subjects.
- therapists who seem truly interested in the treatment of drug addicts and who feel comfortable with them.
- ongoing supervision of therapists by Dr. Luborsky.

The design of the original project was modified such that patients were assigned to receive either counseling plus psychotherapy (SE group), or to have their original counselor plus an additional counselor (DC group). This change was made to better control for relationship and time variables. In addition, only patients with moderate to high levels of psychiatric symptoms were selected for recruitment. This was done because our earlier work had shown that those with low symptom levels made considerable gains, and that the psychotherapy did not add anything to their usual clinical course.

RESULTS

Interest and Participation

As of March 20, 1987 two hundred-ten patients were asked to participate in the Psychotherapy study from all the programs combined. The numbers from each program who have followed through to date is displayed below.

Program	Patients Contacted	Patients Consented	Completed 3 Sessions	Completed 24 Weeks*	Ave. # Sessions
A	75	45	30	24	15
B	60	12	7	6	16
C	40	18	10	7	15
D	25	14	11	7	16

*Note several patients are currently active in the 24-week study

As can be seen there was generally good compliance with the study protocol although there were some differences seen among programs (Program D has had a higher retention rate than all other programs, while Program B had the worst). It is important to note that patients were not paid for attending the therapy or counseling sessions, nor were they coerced into attendance if they missed sessions. Patients who missed sessions were simply contacted by the therapist or counselor and asked if there were schedule difficulties or other problems. No programmatic sanctions were used to increase attendance.

Outcome

As seen, in the following table, there is an indication of general improvement in both the SE and DC groups, particularly in the areas of psychiatric symptoms and drug use. The combined data were analyzed between groups and over time using a repeated measures, multivariate analysis of variance (MANOVA). Results of this initial analysis indicated a significant effect of time (pre to post treatment, $p<.01$) but a non-significant effect of group ($p<.11$). Given the significant time effect, we were at liberty to make further comparisons of pre-treatment to seven-month change in each group using the paired t-statistic. Since these data are still preliminary and the sample sizes are small we have chosen to present trends ($p<.10$) as well as significant differences ($p<.05$ or less) in these analyses. As can be seen, there were somewhat more improvements in the SE group than in the DC patients as judged by the number of significant differences and/or trends seen. The SE patients showed their major improvements in the areas of employment, psychiatric symptoms and medical status. Thus, while there is some indication of better performance in the therapy group at this time, it is clear that substantial additional data will have to be collected in order to make a final determination of the overall effects of the two conditions.

CHANGE FROM START TO SEVEN MONTH FOLLOW-UP

	SE N=17			DC N=11		
	INTAKE		7-MONTH	INTAKE		7-MONTH
MEDICAL FACTOR	40	*	22	38		48
Days Medical Problems	10		7	9	*	16
EMPLOYMENT FACTOR	62		60	75		63
Days Working		*	10	5		8
Money Earned	$298	*	$560	$265		$377
Welfare Income	$175		$194	$102		$175
DRUG USE FACTOR	35		30	33	*	23
Days Opiates	9	+	5	8		4
Days Cocaine	4		3	9		5
Days Depressants	41	+	7	3		2
ALCOHOL USE FACTOR	13		8	10		7
Days Drinking	8		5	6		3
Days Intoxicated	4		2	4		2
LEGAL FACTOR	8		11	16		8
Crime Days	1		1	4		1
illegal Income	$95		$83	$93		$33
PSYCHIATRIC FACTOR	45	**	28	45	*	25
Days Psychiatric Prob.	15	**	7	18	*	8
Beck Dep Inv	19	**	9	24		20

+ p<.08 (trend) * p < .05 l * p < .01

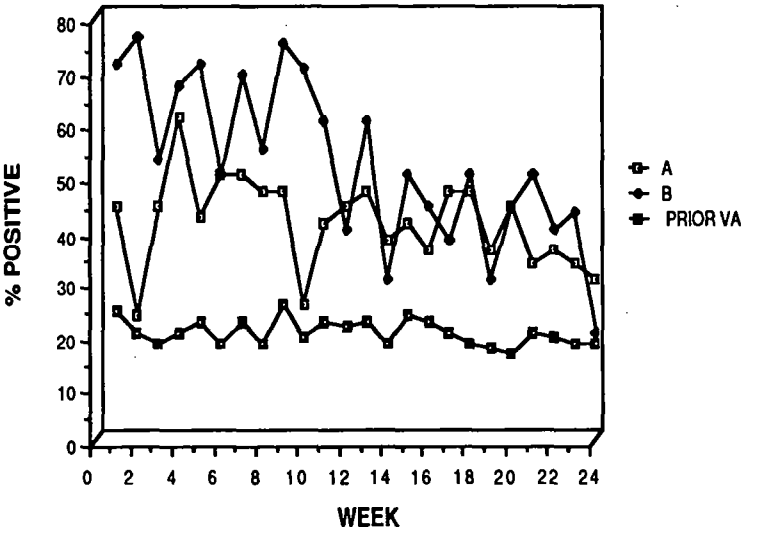
Although the initial data collected thus far on the current project are promising, it is not yet clear that psychotherapy will definitely show significantly better outcomes than drug counseling in these community-based programs. What is clear from these initial analyses is that the data are substantially more *variable than from our prior studies in the VA clinic.* This is interesting in light of the **lack of variability** among the patient samples seen at these clinics. The demographic and background stat-us variables of the patient samples are generally quite similar, with the only significant differences seen in the variables of race, days of medical problems, days of legal problems and days of alcohol use and these differences were not all associated with a single program.

Differences Between Programs

Despite the relatively small differences seen among the patient samples in the current study, we have seen dramatic differences in such fundamental outcome measures as proportion of opiate-positive urines, number of visits to the program, average methadone dose, etc. An example of the size of these differences is shown below in a figure which illustrates the proportion of opiate-positive urines for study patients over the course of the six month treatment period, in two of the programs in the

current study as well as in the original VA psychotherapy study. As can be seen these differences are quite large and in consideration of the lack of differences seen among the patient samples, suggest that there are basic programmatic differences that account for the observed performance differences.

COMPARISON OF CLINIC REPORTS FOR POSITIVE OPIATE URINES



Again, the sample sizes are not yet representative and we are aware that these initial trends could change but we feel that these preliminary indications in the outcome *data may* reflect *substantive differences in the way the programs operate.* Another potentially important variable, suggested by our initial VA study (Luborsky et al., 1985). is *the ability of the therapists.* Each of these two possibilities has important implications for the present study and for the way methadone treatment is provided.

DISCUSSION

The preliminary nature of these data makes firm conclusions about the efficacy of psychotherapy in these programs difficult to make at this time. It appears that we are obtaining a therapy effect, but that it is weaker than that seen in earlier studies done in our own program. We are finding that engagement of patients is more difficult, and we think that this is because we do not have as much control over the environment, or over the recruiting efforts as is the case when we do studies in our own clinic. The differences in outcome between programs is unexpected, but very interesting and important. It is consistent with the work recently reported by Dr. John Ball (1988) in his methadone treatment study. There is probably an interaction between psychotherapy, psychotherapist and program that influences outcome, and we are currently working to obtain data to provide information about this area.

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AUTHORS

George E. Woody, M.D.
Lester Luborsky, PhD
A. Thomas McLellan, PhD
Charles P. O'Brien, M.D., PhD

Psychiatry Department (116)
Philadelphia V.A. Medical Center/University of Pennsylvania
University and Woodland Avenues
Philadelphia, PA 19104

Reprint requests to Dr. Woody

Medical Maintenance: A New Model for Continuing Treatment of Socially Rehabilitated Methadone Maintenance Patients

D. Novick, H. Joseph, B. Richman, E. Salsitz,
E. Pascarelli, D. Des Jarlais, V. Dole and M. Nyswander

Methadone maintenance is a successful treatment of heroin addiction (Dole and Nyswander, 1965, 1976; Cooper et al., 1983). but it can be provided only by licensed clinics that are subject to Federal, State, and local regulations. These regulations, which encompass dosages, frequency of patient visits, counseling, supportive services, and many other details, were developed to deal with the problem behavior of unemployed and socially marginal patients who still participate in activities of the drug subculture. In contrast, socially rehabilitated patients who no longer need supportive services may be less well served by methadone clinics governed by such regulations. These patients have stable lifestyles, are usually employed, do not abuse drugs or alcohol, and do not have social ties with illicit narcotic abusers.

In this study, pharmacologic treatment of heroin addiction is provided to stable, long-term methadone maintenance patients in a medical setting similar to that of treatment of other chronic diseases (Des Jarlais et al., 1985; Novick et al., 1988a). In his or her office, the physician treats concomitant medical problems as well as the addictive disease. Decisions regarding treatment *are* based on the individual needs of the patient and on currently accepted medical practice rather than on explicit regulations (Dole 1965; Newman 1987). We describe herein this program, known as "medical maintenance", and the first 40 former heroin addicts who entered this treatment modality between June 1983 and January 1987. The 37 additional patients who have commenced medical maintenance since then are discussed briefly.

PATIENTS AND METHODS

Twenty-five former heroin addicts were originally admitted to medical maintenance at The Rockefeller University in June 1983. They remained there under the care of Dr. Marie E. Nyswander until 1985, when they were transferred to the care of physicians on the staff of Beth Israel Medical Center or St. Luke's-Roosevelt Hospital Center. This transfer was necessitated by

the retirement of Dr. Nyswander as well as the desire of the initial investigators to increase the number of participating physicians and test the treatment in other settings. These primary care physicians also have had experience in drug abuse treatment. During 1986 and January 1987. an additional 15 former heroin addicts were admitted to medical maintenance from the "aftercare" component of Beth Israel Medical Center's methadone program. In aftercare, rehabilitated patients remain in their methadone clinics and receive urine monitoring and physical examinations; every two weeks, liquid medication is dispensed and one dose is taken under observation (Peyser NP, unpublished data). Aftercare patients receive no ongoing counseling. From April 1987 through May 31, 1988, an additional 37 patients were accepted into medical maintenance from other methadone maintenance programs in the New York Metropolitan area as well as from the Beth Israel aftercare component.

Patients are accepted for transfer into medical maintenance after a personal interview with a medical maintenance physician, review of clinic records, and communication with staff of the referring methadone program. Table 1 lists the criteria for admission to medical maintenance. In addition to these formal

TABLE 1. Criteria for Admission to Medical Maintenance*

1. Five years in conventional methadone maintenance treatment.
2. Stable employment or other productive use of time over the past three years with legitimate sources of income.
3. No criminal involvement for the previous three years.
4. No drug or alcohol abuse for the previous three years.
5. A record of reliability in conventional methadone maintenance treatment with respect to attendance,+ lack of requests for replacement of medications, submissions of urine for testing, and adherence to clinic rules.
6. Indications that further long-term maintenance will be needed.
7. The patient should be emotionally stable.
8. Lack of social ties to illicit narcotic users who might encourage diversion of medication.
9. Recommendation from a clinician with thorough knowledge of the patient's treatment history.
10. All patients must be volunteers and willing to participate in research evaluations.

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* Determined by review of case records, communication with the referring methadone program, and a personal interview.

\+ The patient must have demonstrated reliable reporting on a weekly schedule to a methadone clinic for at least one year.

¶ Such indications include previous unsuccessful attempts at detoxification or a shared belief between the patient and physician that continued maintenance is necessary.

criteria, the patient must be deemed appropriate for medical maintenance in the clinical judgement of the accepting physician. It is likely that the admission criteria will be modified as clinical experience accumulates.

At Beth Israel Medical Center, the medical maintenance patients are seen in offices within a hospital building. At St. Luke's-Roosevelt Hospital Center, they are seen in the Family Care Group Practice, a comprehensive primary care facility at the St. Luke's site of St. Luke's-Roosevelt Hospital Center. At both facilities, the atmosphere is that of a physician's office rather than a clinic, and visits of medical maintenance patients are scheduled by appointment during regular hours along with visits of other medical patients. At each visit, the patient is interviewed, submits a urine sample for methadone as well as illicit substances, takes one dose of medication in the presence of a physician or staff member to ensure tolerance to the prescribed dose, and completes a brief questionnaire. The methadone prescribed for home use (up to 100 mg daily for 28 days) is then given to the patient as a legally prescribed medicine. Return visits are usually every 28 days but are determined clinically, reflecting the physician's judgement. At each visit, the patient pays a fee which includes costs of the

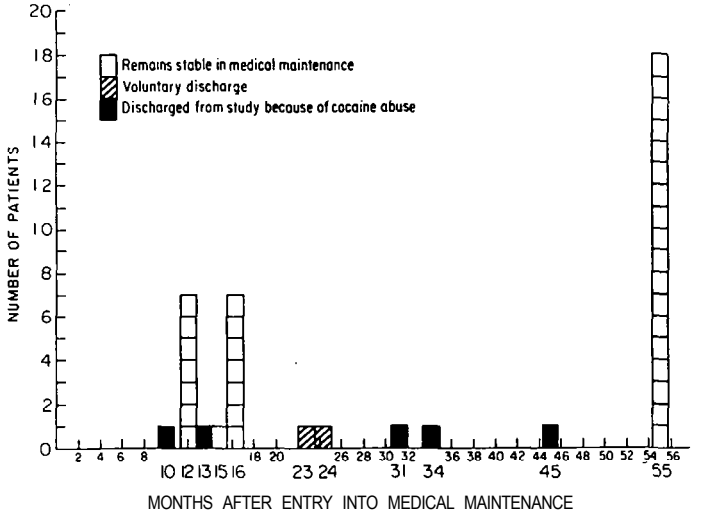

FIGURE 1. Treatment status and duration of treatment of the first 40 patients admitted to medical maintenance. Each box represents one patient. Data are from June 1983 to January 1988.

drug screening and the medication. To protect confidentiality, urines for drug screening are coded, and records are kept in locked offices. Methadone is dispensed from hospital pharmacies in amounts prescribed by the physician and stored in special narcotics cabinets on the premises of each facility until the patient visit. Cross-scored 40 mg methadone hydrochloride diskets or tablets containing 5 mg methadone are used. All patients gave written informed consent.

RESULTS

Figure 1 shows the status as of January 1988 of the first 40 patients admitted to medical maintenance. In follow-up ranging from 12 to 55 months, 33 (82.5%) of 40 had remained in medical maintenance, yielding an annual retention rate of 94%. Five (12.5%) patients had been discharged because of cocaine abuse and returned to a conventional methadone maintenance program.

TABLE 2. Characteristics of Medical Maintenance Patients

	Currently in Treatment (n=33)	Discharged* (n=7)
Sex		
Male	27 (82%)	6 (86%)
Female	6 (18%)	1 (14%)
Ethnicity		
Black	2 (6%)	1 (14%)
Hispanic	5 (15%)	
White	26 (79%)	6 (86%)
Married	25 (76%)	6 (86%)
Employed	31 (94%)+	7 (100%)
Annual Income ($)	29700±13001	36300+27600
Years education	13.5±2.6	13.0±3.3
Age first arrest (yr)¶	20.0±4.2	16.3±2.8
No. of arrests (lifetime)	6.7±5.4	9.3±7.1
Age first used heroin (yr)	18.6±5.6	17.1±2.0
Years heroin addiction	9.5±4.9	9.4±3.2
Age at entry methadone maintenance	30.1±7.3	29.7±1.0
Years in MMT	16.5±3.0	16.3±3.0

* Five discharged patients had abused cocaine, and two were discharged voluntarily. Six of the seven discharged patients returned to a conventional methadone clinic.

\+ Of the two patients not employed, one is a homemaker and one is semi-retired.

¶ Differences are, significant ($p<0.05$) by standard t test. Data expressed as mean±SEM. Arrests were mostly for property crimes and/or narcotics possession.

Two (5%) patients were voluntarily discharged from the study; one was detoxified while. the other preferred the familiar clinic setting. Of the 33 patients who remained in-the medical program, three had transient episodes of-substance-abuse; with cocaine, diazepam, and heroin, respectively. These problems were resolved with counseling and increased frequency of office visits with urine monitoring. Only four instances of lost medication occurred in the 1381 patient-months of this study. No overdoses of methadone were reported by patients or their families. Table 2 shows demographic features and addiction history of the first 40 patients.

Of the 37 patients who have been admitted to medical maintenance between April 1987 and May 1988, 36 have remained stable during 1-14 months of treatment, with no documented substance abuse. One patient was transferred back to his previous program after having repeatedly exceeded his daily methadone dose. Thus, of the 77 patients admitted to medical maintenance since its inception, 69 (90%) have remained in treatment as of this writing (June 1988).

DISCUSSION

In this study, the patients received methadone in physicians' offices rather than in formal clinics. These carefully selected patients had previously shown an excellent response to more than five years of conventional methadone maintenance treatment, manifested by stable employment, lack of criminal involvement, absence of abuse of alcohol or drugs, and reliable clinic attendance. The 94% annual retention rate in medical maintenance compares favorably with the 78% annual retention rate observed in the Beth Israel Methadone program over four years for the cohort of 607 patients admitted from-January-June 1983 (Jacknow C, personnal communication). Furthermore, six of the seven patients who left medical maintenance returned to a standard methadone clinic. This experience demonstrates, that medical maintenance is a workable arrangement for qualified patients cared for by physicians with knowledge of drug abuse treatment and deserves further trial.

The patients in medical maintenance have-perceived a number-of benefits. Most notable is that of being treated in a more professional atmosphere. They have reported improved self-esteem from being regarded as medical patients rather then drug abusers, from being rewarded with a degree of trust after many years of excellent performance in treatment, and from no longer being required to receive unnecessary supportive services (Des Jarlais et al., 1985; Novick et al., 1988a). Patients have also stated that the 28-day reporting schedule markedly reduced, problems in work attendance and maintaining confidentiality. The 28-day schedule with medication in tablets rather than as a liquid which required constant refrigeration- also allowed patients to take extended business trips or vacations. Finally,

the physician-patient relationship is improved, since treatment decisions are based on clinical indications rather than impersonal regulations (Dole 1965).

Medical maintenance is beneficial to society in a number of ways. Treatment costs are covered by patients' fees rather than public funding. Also, when socially rehabilitated methadone maintenance patients are transferred to medical maintenance, an opening in a conventional methadone program becomes available, thus allowing the admission of an active narcotic addict from a waiting list. Many methadone programs are fully subscribed, and eligable patients must remain on the streets for weeks or months awaiting admission. The need to treat all narcotic addicts who voluntarily seek treatment is particularly great at this time because of the epidemic of acquired immune deficiency syndrome (AIDS)(Boffey 1988; Novick et al., 1986a; Senay 1988; Weinberg and Murray 1987). More than 25% of AIDS patients in the United States are heterosexual or homosexual parenteral drug abusers, and more than 50% of parenteral drug abusers in the New York/New Jersey metropolitan area are seropositive (Novick et al., 1986b, 1988b; Des Jarlais and Friedman 1987) for antibody to human immunodeficiency virus, the agent of AIDS(anti-HIV). Preliminary data indicate that methadone maintenance treatment is associated with reduced prevalences of anti-HIV (Novick et al., 1986b; Tidone et al., 1987).

We emphasize that even though the patients in this study have achieved a high degree of social rehabilitation, continued maintenance on methadone is medically indicated. Methadone has a high degree of safety in long-term treatment (Kreek 1973, 1987), and other studies have shown that a majority of patients who do well in methadone maintenance treatment relapse to heroin abuse following detoxification (Cooper et al., 1983; Dole and Joseph 1978; McGlothlin and Anglin 1981). Patients with ongoing social, behavioral, or drug-related problems in treatment have almost no chance of remaining drug-free after detoxification. Detoxification from medical maintenance is therefore not encouraged but is considered on an individual basis after an informed discussion of the risks involved.

Medical maintenance is not always successful. Abuse of illicit substances, primarily cocaine, was encountered in eight (20%) of the first 40 patients in medical maintenance. In five (12.5%), the drug abuse persisted despite short-term counseling and thus necessitated transfer from this research program to a conventional maintenance program with closer supervision. In the other three, the drug abuse occured in five or fewer episodes and responded quickly to counseling along with increased visits and urine monitoring. The decision to retain these three patients was based on the judgement of the primary care physician.

Since more than three-fourths of our patients remained in

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medical maintenance in good standing, we believe that continued expansion of this study is warranted. Our future outcome data may be even better as experience in patient selection accumulates. Recruitment of additional primary care physicians for medical maintenance with limited patient loads for each physician will be particularly important in order to avoid the depersonalization that can occur in large-scale programs (Dole. 1971). The present study demonstrates that primary care physicians with knowledge of drug abuse treatment can treat rehabilitated former heroin addicts on methadone maintenance, and that a majority of such patients can function well in general medical practice.

FOOTNOTE

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AUTHORS

*# David M. Novick, M.D.
+ Herman Joseph, B.S.
* Beverly L. Richman. M.D.
* Edwin A. Salsitz, M.D.
¶ Emil F. Pascarelli, M.D.
§ Don C. Des Jarlais, Ph.D.
# Vincent P. Dole, M.D.
Marie E. Nyswander, M.D., Deceased

* Beth Israel Medical Center
First Avenue at 16th Street
New York, N.Y. 10003

+ New York State Division of
Substance Abuse Services
55 West 125th Street
New York, N.Y. 10027

St. Lukes-Roosevelt Hospital Ctr
425 West 59th Street
New York, N.Y. 10019

§ Narcotic and Drug Research, Inc
11 Beach Street
New York, N.Y. 10013

The Rockefeller University
1230 York Avenue
New York, N.Y. 10021

Management of Maternal and Neonatal Substance Abuse Problems

L. Finnegan

The magnitude of drug use during pregnancy is often underestimated as are the effects of maternal drug use on the fetus and the neonate. This is frequently related in part to a common tendency to minimize the existence of problems that one finds socially unattractive. Unfortunately, the prevalence and sequelae of both licit and illicit psychotropic drug use during pregnancy indicate that we have a significant problem in this country which must be recognized and addressed by health care delivery systems providing optimal medical care. Therefore, this overview, concerning the management of maternal and neonatal substance abuse problems, will describe the complications of pregnancy so common in these women. Secondly, it will discuss the recognition and treatment of neonatal abstinence and thirdly, the potential of child abuse and neglect of the offspring.

The major problem to address in this population is the fact that pregnant drug dependent women are most disadvantaged with regard to procreation. They present a most adverse intrauterine milieu for their fetuses. They do not partake of any of the traditional requirements during pregnancy, such as: good nutrition, good rest, and on-going prenatal care.

Moreover, today's pregnant drug dependent woman does not use just one drug during pregnancy. Therefore, the fetuses are exposed to narcotics, stimulants, tranquilizers and hallucinogens. It is not unusual to find a pregnant woman who is using four to five drugs throughout pregnancy.

Because of the above, medical complications abound in pregnant drug dependent women and include anemia, bacteremia, cardiac disease (especially endocarditis), cellulitis, hepatitis (acute and chronic), phlebitis, pneumonia, septicemia, urinary tract infections (including cystitis, urethritis, pyelonephritis), as well as sexually transmitted diseases including condyloma acuminatum, gonorrhea, herpes, syphilis, and acquired immune deficiency syndrome. Another infectious disease that has become more common in drug dependent individuals due to the prevalence of the human immunodeficiency virus is tuberculosis (Finnegan 1985) (Finnegan and Wapner 1987).

In addition to medical complications, obstetrical complications are also more common in these women. There is an increase in fetal wastage not only because of early fetal lose due to abortion, but also later lose due to intrauterine fetal death. There is an increase of infection of the fetal membranes including amnionitis and chorioaminionitis. The fetus grows poorly in the disadvantaged intrauterine milieu and therefore suffers from intrauterine growth retardation. The onset of premature labor is much more common in women who utilize psychoactive drugs indiscriminately. Premature rupture of membranes as well as postpartum hemorrhage are also more common in this population. Abruption of the placenta is a hazardous complication resulting in maternal hemorrhage and shock and the possibility of both maternal and fetal death. This frequently occurs with narcotic abuse due to the potential for withdrawal symptomatology as well as cocaine abuse in which the maternal blood pressure is increased (Finnegan 1985) (Finnegan and Wapner 1987).

Clinical studies (Ryan et al., 1987) (Chasnoff et al., 1987) have shown that cocaine use in pregnancy adversely affects-maternal and infant outcome with an increase in vasoconstriction maternal heart rate and blood pressure. There is the potential of intrauterine hypoxia, precipitous labor, preterm labor and abruption of the placenta. Infants born to cocaine drug dependent women are smaller in height, weight, and head circumference.

Medical complications in infants born to drug dependent mothers will generally be influenced by: 1) inadequacy of prenatal care; 2) the presence of obstetrical or medical complications in the mother; and 3) multiple drug use. The general medical complications seen in infants born to drug dependent women encompass those problems that are seen in the low birth weight infant. They include: asphyxia neonatorum, intracranial hemorrhage, pneumonia, septicemia, hypoglycemia, hypocalcemia, hyperbilrubinemia, and respiratory distress syndrome. In the full term infant, pneumonia and meconium aspiration syndrome are commonly seen. With the increase of human immuniodeficiency virus possitivity in this population of mothers, there is an increased chance of the infants having acquired immune deficiency syndrome (Finnegan 1985).

In a recent study (Silver et al., 1987), it was noted that with intensive prenatal care, psychosocial counselling and methadone maintenance in narcotic addicted pregnant women, there was no significant difference from a comparison population in intrauterine death, neonatal deaths or small gestational age infants. There was a significant difference in mean infant birth weights.

Once, the umbilical cord is cut, if their mother's have been addicted to narcotics, the infants have a 60% chance of developing neonatal abstinence. Signs of neonatal abstinence include: hyperirritability, increased deep tendon reflexes, exaggerated Moro reflex, increased muscle tone, tremors (undisturbed and disturbed), and high-pitched cry. Infants will have gastrointestinal dysfunction as well including regurgitation and loose stools. Although there is an increased rooting reflex, the infants will have an uncoordinated and ineffectual sucking and swallowing reflex. Less troublesome symptoms include: yawning, sneezing, mottling, fever, and increased respiratory rate (Finnegan 1986).

Infants born to drug using mothers must have careful assessment for at least 4 days utilizing a scoring system which monitors the various symptoms and their magnitude. Treatment must be initiated promptly when indicated by a high score and the dose escalated as needed. Careful detoxification is then initiated when the infant has achieved an asymptomatic clinical picture. Paregoric and other narcotic substitutes have been efficacious in narcotic dependent infants. Phenobarbitol is useful when infants are exposed in-utero to sedatives and / or hallucinogens. Clinicians must follow rigid prescribed methods in order to eliminate any untoward complications of neonatal abstinence (Finnegan, 1986).

Kron (1976) investigated the sucking reflexes of infants undergoing abstinence. Neonatal nurses who have been caring for such infants have clinically documented the poor sucking patterns seen in passively drug dependent infants. In this investigation, the infants were studied with a precise instrument that was capable of recording the sucking rate, pressure, and organization of sucking as well as the amount of nutrients consumed. Infants born to narcotic dependent mothers were compared with those who had received sedative agents due to maternal pre-eclampsia. A third group was a comparison group of children who had no drug exposure in-utero. The infants exposed to chronic narcotic use in pregnancy had an extremely low sucking reflex in contrast to the other two groups.

In order to further study the effects of abstinence and maternal drug use in pregnancy upon ventricular configuration and cerebral growth in infants, cranial ultrasound examinations were performed during the first three days of life and at the age of one month in infants with neonatal abstinence syndrome (Pasto et al., 1985). The results were compared to control infants who were not exposed. to narcotic drugs in-utero. The ultrasound images were examined for ventricular configuration, intracranial hemidiameters, area of the thalmi and width of the temporal lobes. At 24 to 72 hours and at one month of age, significantly more drug exposed than control infants had slit-like ventricular configuration. intracranial hemidiameter was significantly smaller in the drug exposed than in the control infants. All cerebral measurements except the right temporal lobe demonstrated significant growth over the first month of life in both groups of infants. By means of ancillary examinations including computerized tomography and transfontanel pressure measurements, the pathogenesis of the slit-like ventricles was found not to be related to edema or to increased intracranial pressure. Although measurements were less than the comparison population from birth to one month, the ventricles and the brain had accelerated growth from the first month of life to the sixth month of life. At the six month examination,. there were an insignificant number of infants who continued to have smaller ventricular configuration as well as a somewhat decreased cerebral growth. Moreover, evaluation of the development of the infants at that age was normal.

Of great concern is the fact that infants born to drug dependent mothers are at high risk for child abuse and neglect (Regan et al., 1987). Drug and / or alcohol abuse is known to be one of the critical factors involved

in the abuse, neglect and even the abandonment of children. In addition to the chaotic life-style and physical discomforts of addiction which inevitably place the drug dependent women at high risk for parenting problems, they must also cope with numerous financial, social, and psychological difficulties. These include: single parenthood, poor housing, inadequate income, lack of education, and emotional problems. A history of having been physically and / or sexually abused during childhood is not uncommon among these women. The following characteristics have been commonly reported in families where child abuse occurs. Many of these characteristics are seen in the lives of drug dependent women:

1) When young themselves, one or both parents have been subjected to violence;
2) One or both parents have had an unhappy, disrupted and insecure childhood;
3) One or both parents are addicted to drugs, alcohol, or are psychotic;
4) There is a record of violence between the parents;
5) Another child in the family has already been abused, or has suffered an unexplained death;
6) With the pregnancy unwanted, the baby was rejected at birth or soon thereafter;
7) Failure in early bonding;
8) Both parents are under 20 years of age, immature for their years, and socially isolated;
9) The family lives in poor housing and on a low income; and
10) The family is suffering from multiple deprivations (Frode 1981). Therefore we can expect that instead of having attachment between the drug dependent mother and her child we most frequently have detachment.

If one is to have the capability of managing the drug dependent woman and her child, it is essential that a comprehensive treatment program be provided to include intensive prenatal management for these high risk pregnant ‘women, psychosocial counselling, prenatal / parenting education classes, psychiatric therapy when necessary, and methadone maintenance. With these treatment resources, it has been shown repeatedly that one can decrease the complications associated with pregnancy, child birth, and infant development. In summary, the following are the specific recommendations:

1) The pregnant woman who abuses drugs must be designated as high risk and warrants specialized care in a perinatal center where she should be provided with comprehensive services including pharmacotherapy for her addiction, when indicated, obstetrical care and psychosocial counselling.
    a) Pharmacotherapy for the addicted pregnant woman may involve voluntary drug-free therapeutic communities, methadone detoxification (depending on the time in pregnancy when it is requested or methadone maintenance.
    b) The pregnant drug-dependent woman should be admitted to a hospital setting where a complete history and physical examination may be

accomplished, including laboratory testing to evaluate her overall health status.

c) Psychosocial guidance should be provided by experienced counselors who are aware of the medical as well as the social and psychological needs of this population.

2) Careful attention must be given to the assessment and management of the newborn with regard to potential morbidity because of perinatal stresses as well as the onset, progression, and pharmacologic treatment of abstinence.

a) Mother / infant attachment should be encouraged prenatally and postpartum. To decrease the possibility of child neglect, special emphasis should be on enhancing parenting skills of these women.

b) The continued ability of the mother to care for the infant after discharge from the hospital must be assessed by frequent observations in the home and in clinic settings (Finnegan 1989).

With the burgeoning problem of cocaine abuse in pregnant women and the continuing prevalence of women using many drugs, adequate residential treatment facilities for pregnant women and their children must be developed in order to prevent the drastic increase in perinatal morbidity and mortality that this population brings to our country.

If physical, psychological and sociological issues of pregnant opiate dependent women and their children are appropriately addressed, the potential physical and behavioral effects of psychoactive drugs on the mother, her fetus, the newborn and the child may be markedly reduced. The task for clinicians is enormous when contemplating the rehabilitation of such populations, but must be addressed if we are to decrease the intergenerational transmission of the many problems surrounding drug abuse in pregnancy. As the population of abusers and trends of drug use change, treatment services should be reassessed and revised.

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AFFILIATION

Departments of Pediatrics, Psychiatry and Human Behavior, Thomas Jefferson University, Philadelphia, PA 19107

Update on Behavioral Treatments for Substance Abuse

A. Childress, R. Ehrman, A. McLellan and C. O'Brien

INTRODUCTION

Behavioral treatments for substance abuse have a long history, even pre-dating the frequently cited example of Kantorovich's treatment of alcohol abusers with aversive stimuli in the early 20th century (Kantorovich, 1930). The ancient Romans are said to have put spiders and other foul stimuli in the bottom of wine glasses in an attempt to discourage over-imbibers (Smith 1982). Several extensive historical reviews of behavioral therapies in substance abuse are available (Droppa, 1973; Callner, 1975; Nathan and Goldman, 1979; Ross and Callner, 1981; Sobell et. al. 1982; Elkins, 1988), including one by the current authors (Childress et al., 1985a).

The first part of this paper highlights several current applications of behavioral therapies in substance abuse. The final section of the paper updates our own work combining classical **extinction** (repeated, non-reinforced exposure to stimuli previously paired with drug use.) with **psychotherapy** and other interventions in the treatment of abstinent opiate and cocaine abusers (Childress et al., 1986, 1987).

BACKGROUND

Abused drugs and, by association, drug-related stimuli have **reinforcing, discriminative,** and **eliciting** properties. These stimulus properties are not easy to dissociate: a *single* stimulus--for example, the sight of a drug dealer, can *simultaneously:* 1) attract the patient because of the close association between the dealer and the powerful primary

reinforcing properties of the drug, 2) signal the patient that a *response contingency* is in effect; i.e., drug-seeking behavior is now very likely to be reinforced **(discriminative** properties), and 3) trigger strong physiological arousal, drug craving and other drug-related responses in the patient **(eliciting** properties) because of the dealer's *repeated association with drug administration* and drug effects.

Of course, 'real world' situations leading to drug use are even more complex. They often contain not just *one* stimulus (e.g., the dealer) with *multiple* properties, but *many* stimuli (the location itself, the presence of regular drug users, the sight of drug paraphernalia, etc.), *each* with *multiple* properties, all potentially interacting and contributing to the final end-point behavior of drug-seeking and drug-taking.

Appreciating this 'real world' complexity is a useful backdrop to the following discussion, in which behavioral treatments will be discussed in terms of the stimulus properties they are *intended* to address. In many cases, their effects may be broader or different than is assumed.

BEHAVIORAL TREATMENTS ADDRESSING REINFORCING PROPERTIES

Drug-seeking behavior is powerfully reinforced thousands of times over the natural course of the patients' addiction. Current behavioral treatments addressing the **reinforcing** properties of drugs and drug-related stimuli include **operant extinction** and **contingency management.**

Operant Extinction

One way of addressing reinforcing properties is to block or remove them...drug-seeking behavior would no longer be reinforced and should eventually **extinguish.** In animal research, **operant extinction** of 'drug-seeking' is quite straightforward: omitting the reinforcing consequence (either the drug itself or a drug-related stimulus secondarily

reinforcing because of its association with the drug) from the lever-press behavior causes the behavior to eventually cease. In the 'real-world' clinical situation it is difficult to extinguish drug-seeking in a strictly analogous way because drugs are difficult to completely remove from the environment. One 'real-world' tool for operant extinction is a *pharmacologic* treatment that specifically blocks the reinforcing actions of a drug *even if the drug is administered.* **Naltrexone,** of course, provides this opportunity for opiate abusers. Though not popular among most opiate abusers (who often want to retain the option of feeling opiate effects), naltrexone may be a useful tool among subgroups of opiate abusers with a high incentive for abstinence: e.g., medical professionals who will lose their license to practice if they relapse and federal probationers who risk reincarceration if they relapse or return to drug-related crimes (Metzger, Woody, Cornish, McLellan and O'Brien, 1988). With the recent upsurge of cocaine abuse, research is underway to find pharmacologic agents which will specifically and effectively block the reinforcing effects of cocaine, but none is available at present.

Contingency Management

Another way of addressing the reinforcing properties of drugs and their associated stimuli is to find strong positive or negative reinforcers that will encourage competing non-drug behaviors, and/or discourage drug use behaviors. For example, Crowley (Anker and Crowley, 1981; Crowley, 1986) found that cocaine abusers were more likely to maintain abstinence if they knew a signed 'confession' of their lapse would be sent to their medical licensing board or employer. Several variants on these **contingency management** and **contracting** techniques have been tried, including positive reinforcement for clean urines through money or increased methadone dosages (Stitzer et. al, 1980). The most clinically useful of the contingency techniques incorporate reinforcers with a connection to the 'real world community outside the treatment setting (e.g., letters of progress or problems to employer or family). Involvement of these 'real world' reinforcers helps extend the influence of

treatment beyond the immediate treatment setting, into the patient's family and community.

BEHAVIORAL THERAPIES ADDRESSING DISCRIMINATIVE PROPERTIES

Stimuli associated with drug use can act as discriminative stimuli (SDs), signalling the patient that a response contingency is in effect. For example, the sight of a shooting gallery or 'crack' house cues the patient that drug-seeking will likely be reinforced.

Changing the Message of the Discriminative Stimulus

A basic behavioral approach to the discriminative properties of drug-related stimuli is to change an SD for drug-seeking (the sight of a dealer, the onset of craving, etc.) into an SD for some **alternative behavior** which *will* be reinforced, but not by drug use. The alternative behaviors may include avoidance (immediate withdrawal from the 'high risk' situation), assertive refusal of drug offers, engaging in a pleasurable physical activity, deep relaxation, etc. (Zackon et al., 1985). The reinforcers for these behaviors may include an increased sense of mastery, self-control and self-efficacy (Marlatt and Gordon, 1985; Annis et al., 1988). In a promising step, several clinical research teams are now combining the training of response alternatives to drug-related SDs (often referred to as **relapse prevention training)** with other techniques such as cue exposure (see next section). Controlled studies of this combined approach are now ongoing in both alcohol (Cooney et al., 1983; Monti et al., 1988;) and nicotine (Niaura et al., 1988) dependent populations.

BEHAVIORAL TREATMENTS ADDRESSING ELICITING PROPERTIES

Drugs themselves are powerful **eliciting** stimuli. They trigger a wide range of subjective and pharmacologic effects. Stimuli repeatedly *paired* with drug administration (the sight of a

dealer, a particular drug-related location) can acquire the ability through *classical conditioning* to elicit a wide range of conditioned responses, including *drug-like, drug-opposite* or *drug-compensatory* effects, and *drug craving* (Eikelboom and Stewart, 1982; Siegel, 1979; O'Brien et al., 1987, 1988; Childress et al., l986a, 1987). Many of these elicited responses could play a role in the high rate of relapse which characterizes substance abuse disorders. Two basic approaches to the eliciting properties of drug-related stimuli include 1) **counter-conditioning** of *other* less problematic responses to the same drug-related conditioned stimulus (CS), and 2) attempts to weaken the eliciting power of the drug-related CS through **classical extinction** (repeated presentation of the CS *not followed* by drug administration). The latter procedure is also commonly referred to as **'cue exposure'.**

Counterconditioning

Counterconditioning is most often encountered in the form of **classical aversive conditioning,** in which an aversive stimulus such as emetine nausea or shock is repeatedly paired with characteristic stimulus properties of the abused drug or with actual drug administration (McLellan and Childress, 1985). The goal is a conditioned aversion, such that stimuli (sight, taste, smell, etc.) associated with the substance will trigger nausea or repulsion instead of craving/ desire to use.

Though aversive emetine conditioning has been used in the treatment of more than 30,000 alcoholism inpatients over the past four decades, controlled studies of its presumed benefits lagged far behind (Childress et al., 1985a;). More recently, Cannon (Cannon and Baker, 1981) demonstrated a modest benefit of aversive conditioning for alcoholism patients. This research team also demonstrated the actual presence of a conditioned aversion in a proportion of the treated patients with chemically induced nausea, but not with shock. In a follow-up study, patients with post-treatment tachycardia to alcohol-related stimuli (taken as an index of conditioned aversion) were found more likely to be abstinent at an 18 month follow-up interval

than patients without this response (Miller and Dougher, 1984).

Controlled treatment-outcome studies are now underway which should finally provide a clear assessment of the potential benefits of aversive conditioning techniques in both alcoholism and cocaine populations. Elkins (Elkins, 1988) and his research team in Atlanta are conducting a comparison of conditioned aversions produced by chemically-induced nausea, by shock, and by hypnotically-induced nausea. The outcome of groups receiving these treatments (in addition to standard inpatient treatment) will be compared against control groups receiving milieu therapy or milieu therapy plus relaxation training. It seems likely that aversive conditioning based on nausea will be more effective in the alcohol treatment sample: nausea is a consequence of biologic significance for consummatory behaviors, and it can easily become associated with the taste and smell cues related to ingestion (Garcia et al., 1968; Rozin, 1969). It is unclear whether this same response can be attached to cues associated with cocaine administration, which is usually smoked or injected rather than ingested.

Classical Extinction (Cue Exposure)

There is a recent and growing literature which clearly demonstrates a variety of physiological and subjective responses can be elicited in abstinent former drug-dependent persons by initial exposure to both drug-related (O'Brien et al., 1977; Ternes et al., 1979; Childress et al., 1983, 1984, 1985b, 1986a, 1986b, 1987) and alcohol-related (Cooney et al., 1984; Eriksen and Gotestam, 1984; Pomerleau et al., 1984; Newlin, 1985a, 1985b, 1985c, 1986; Kaplan et al., 1985; Mann et al., 1987 Corty et al., 1988) cues. Though the nature and direction of the *physiological* responses vary across studies, the *subjective* reports often have one response in common: patients experience increased *craving* during initial exposure to cues associated with drug or alcohol administration.

With *repeated,* non-reinforced **cue exposure** (also called classical or Pavlovian **extinction),** both physiogical and

subjective response to these conditioned cues tends to diminish. Several groups have used this principle to reduce the physiological and subjective responses to alcohol-related cues. In an early study, Blakey and Baker (1980) showed that in vivo exposure to 'pub' cues reduced alcohol craving, and improved outcomes in 5 of 6 patients at a 6-9 month follow-up. Laberg and Ellertsen (1987) found repeated exposure to alcohol primes (small amounts of alcohol) reduced craving to the same test cues. As previously mentioned, cue exposure is now combined with relapse prevention techniques (Monti et al., 1987) or skills training (Cooney et al., 1988) in the treatment of alcohol patients. Niaura (1988) is also combining cue exposure with cognitive and behavioral strategies in the treatment of nicotine addiction.

Our own work focused initially on the use of extinction (cue exposure) procedures in treating opiate abuse patients, the results of which have been reported at several prior CPDD meetings (Childress et. al 1983, 1984, 1985b, 1986). In general, this early work showed that repeated exposure to opiate-related stimuli (drug-related audiotapes, videotapes, paraphernalia and rituals) significantly reduced the conditioned craving and conditioned withdrawal symptoms which occurred upon initial presentation. Problems encountered in this early work included the persistence of conditioned physiological responses (even after 20 hour-long sessions of extinction), and incomplete generalization of extinction to 'real world' cue situations.

In our most recent work, we have been developing cue exposure procedures for cocaine abusers, a population which now constitutes the majority of new admissions for substance abuse treatment at the Philadelphia VA Medical Center. We now have **physiological** and **subjective** data from both **laboratory pre-test** and **extinction sessions** from 10 pilots and 33 additional patients who were randomly assigned to extinction (plus other clinical treatments) or control conditions. These treatments were initiated in an inpatient setting (post-detoxification) and continued on an outpatient basis post-discharge. The design, procedures and methods of data

analysis for this ongoing work were detailed in a preliminary report at the 1987 CPDD Proceedings (Childress et al., 1987).

Physiological Responses

Data from the **laboratory pre-test** prior to treatment confirm our preliminary finding that cocaine abusers respond differentially to Neutral vs. Cocaine-related stimuli in terms of both peripheral skin temperature and galvanic skin resistance (both indices of arousal). Temperature data are presented graphically in **Figure 1,** showing reductions in peripheral skin temperature which were significantly greater to **Cocaine-related** stimuli than to **Neutral** stimuli.

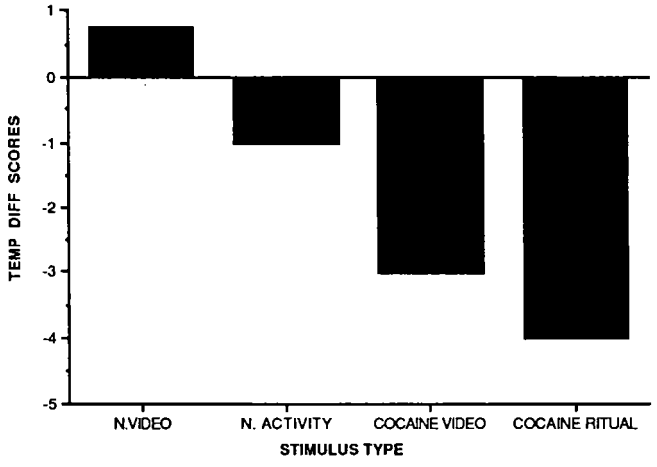

Figure 1. *Skin temperature response in cocaine abusers to Neutral vs. Cocaine-related stimuli (N=30)*

The physiological variable of galvanic skin resistance **(GSR)** revealed a similar pattern of results, with responsivity significantly greater to **Cocaine-related** than to **Neutral** stimuli. Analyses of **heart rate** data showed trends similar to those for GSR, but just missed statistical significance.

Data from **extinction** sessions (featuring repeated exposure to

cocaine-related audiotapes, vidoetapes and paraphernalia) show the skin temperature response to Cocaine-related stimuli diminished significantly as a function of extinction trials. However, responsivity was still in evidence for many patients even after 15, hour-long extinction sessions. GSR responsivity was also reduced, but still apparent, in the 15th hour of extinction.

Subjective Responses

Cocaine craving was the most intense and most frequently reported subjective response in both **laboratory** and **extinction** settings. Extinction sessions were effective in significantly reducing conditioned cocaine Craving, High, and Withdrawal over the course of 15 extinction sessions. **Figure 2** illustrates the reduction in reports of subjective high, craving and withdrawal as a function of extinction sessions.

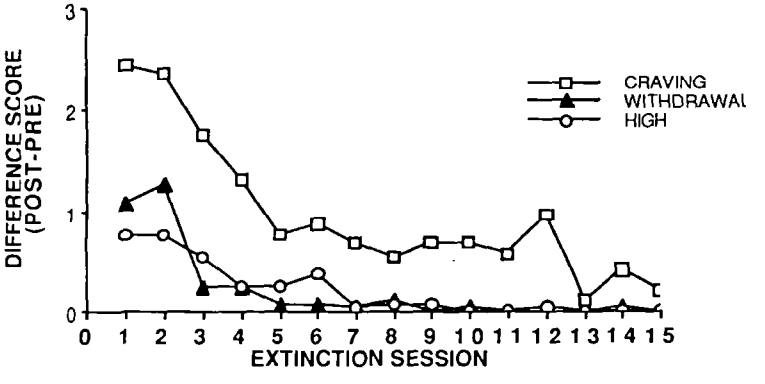

Figure 2. *Reduction in subjective responding to Cocaine -related stimuli as a function of extinction (N=25).*

Clinical Outcome

Early indicators of **clinical outcome** suggest that patients who receive the treatment combination of extinction plus psychotherapy (another adjunctive treatment found useful in

our substance abuse populations, Woody et al., 1983) tend to have better retention in the outpatient treatment phase and fewer cocaine positive urines than patients in the other three comparison treatment packages. Though these relative differences are encouraging, we hope to increase the absolute magnitude of the benefits by teaching patients to actively combat the conditioned craving and arousal which they. experience in response to cocaine- related stimuli. We have piloted several 'coping with craving' techniques and feel they will significantly enhance the effectiveness of passive cue exposure.

SUMMARY

In the past five years there has been an encouraging increase in the amount and quality of research on-behavioral treatments for substance abuse. Some of the most promising approaches combine behavioral treatments with each other (e.g., relapse prevention and cue exposure) or with other forms of treatment (e.g., psychotherapy), attempting to maximize impact on the multiple determinants of drug use. We are fast approaching the time when a patient may be systematically evaluated to develop a profile of vulnerabilities (cue responsivity, psychiatric symptoms, etc.) to rationally determine the treatment or combination of treatments of greatest potential benefit.

REFERENCES are available from the first author upon request.

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AUTHORS

Anna Rose Childress, Ph.D., Ronald Ehrman, Ph.D., A. Thomas McLellan, Ph.D. and Charles P. O'Brien, M.D., Ph.D. are affiliated with the Veteran's Administration Medical Center and the Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA.

Designing Substance Abuse Preventive Interventions Within a Developmental Framework

R. Lorion

A decade ago, proponents of funding for the development of interventions to reduce the prevalence of emotional and behavioral disorders based their arguments on the potential rather than documented benefits of such strategies (e.g. Albee, 1980; Cowen, 1973). Frequently, they referred to the long-held public health axiom that prevention rather than treatment is essential to the control of serious diseases (Bloom, 1977). They also correctly pointed out that available mental health treatments were neither sufficiently effective nor accessible to represent adequate responses to mental health (e.g. Lorion, 1983) or alcohol and drug involvement (Richmond, 1979).

In the intervening years two important trends have occurred. First, increasingly sophisticated epidemiological studies have been conducted to monitor the nation's mental health (e.g. Eaton et al., 1984) and substance related (e.g. Johnston et al., 1986) problems. These efforts establish convincingly the critical need for preventive approaches to both sets of disorders. For example, such disorders are found in between one-sixth and one-fifth of the general population. These levels are significantly higher among the disadvantaged. Moreover, although some decrease may be occurring in the overall level of substance use among the nation's youth, this trend does not apply to members of same minority groups. Finally, use of cocaine and its derivative "crack" continues to rise, especially among youth.

Fortunately, a second trend has also occurred during this period. Significant progress has been made in the development of viably effective preventive interventions designed to respond to such varied problems as familial retardation, adolescent substance abuse, and reactive depression (Price et al., 1988). A range of preventive alternatives are now available for avoiding or minimizing childhood disorders (Lorion, in press). A scientifically valid evidential basis now exists for national policies to fund the continued development and dissemination of interventions to reduce the prevalence of targeted emotional and behavioral disorders (Lorion and Allen,

1988). As will be reported by colleagues in their presentations, that evidential base extends to interventions to reduce alcohol and drug abuse. The purposes of my comments are to present a conceptual framework within which to examine those results and, more generally, to think about subsequent steps in the development of a program of research to expand our understanding of how to prevent substance related problems.

THE MEANING OF DEVELOPMENTAL: "Development" is used herein in two ways. In the broadest sense, it refers to a paradigm for understanding the evolution of human behavior as a consequence of a synergistic transaction between individual characteristics and environmental factors. As explained below, this paradigm, labeled the "transactional perspective" by Sameroff (Sameroff and Chandler, 1975; Sameroff and Fiese, 1988), applies an ecological approach to explaining the genesis of functional and dysfunctional behavior. In a narrow, problem specific sense, "developmental" reflects the fact that patterns of use or nonuse represent components of ongoing processes which have identifiable histories and potentially predictable and alterable futures. I would propose that the continuing enhancement of our capacity to design and apply effective interventions to prevent alcohol and drug involvement depends on the incorporation of both senses of "developmental" in the design and evaluation of preventive strategies.

Reflecting that position, I offer the following definition of prevention research:

> prevention research can be conceptualized as applied developmental analyses involving the identification and Systematic manipulation of processes related to the development of adaptive/maladaptive behavioral constellations in order to increase or decrease respectively, the rate or level at which those behavioral constellations occur in the general population or some part thereof.

Underlying this definition are the assumptions summarized in Figure 1. Presented sequentially, these assumptions concretize the underlying temporal nature of preventive intervention research. Thus, the potential that a strategy will successfully reduce the prevalence of a disorder depends on the field's accuracy in predicting the occurence of that disorder. In turn, predictive accuracy presumes the existence of psychometrically sound diagnostic, risk identification and monitoring procedures. For this criterion to be met, one needs to understand the temporal characteristics of the problems to be prevented. Thus, information is gained about when and how onset occurs (e.g. does it represent a sudden or prolonged process?), the nature and timing of its evolutionary stages, and the anticipated "half-life" of efforts to prevent its occurence or exacerbation.

DISTINCTIONS AMONG PREVENTIVE INTERVENTIONS: It should be noted that a developmental perspective applies to both primary and secondary preventive interventions. In the former case, the premorbid period and etiological factors associated with the onset of a problem are emphasized. As discussed below, this point is easily operationalized if "onset" refers to the distinction between "never used" and "ever used" for each substance. It becomes less clear if "onset" is defined as the point at which use becomes "misuse" or "abuse". With respect to secondary prevention programs, the focus is on the pathogenic sequence following onset. Of concern to such programs is the rapid dissolution of symptoms and the avoidance of their future exacerbation.

Figure 1

Assumptions underlying prevention

Prevention assumes prediction
Prediction assumes:

- reliable diagnostic criteria
- epidemiologically derived base rates for
  - population at large
  - identified subgroups
- knowledge of relevant risk factors which predispose individual to disorder e.g. - genetic
  - environmental
  - experiential
- consideration of issues relevant to time
  - onset
  - incubation
  - rate and invariance of the pathogenic sequences
  - "critical periods" for intervention
  - "half-life" of intervention

Time presumes developmental process at multiple levels:

Macro-level
- Adoption and adaptation of "preventive" social policies (e.g. measles vaccination)

Micro-level
a) facilitation/resumption of expected maturational sequences
b) - termination/interruption of ongoing pathogenic behavioral sequences
   - initiation of positive behavioral sequences

Developmental processes presume:

- measurement at multiple points in time (e.g. repeated measurement probes)
- analysis of sequential vs. episodic states
- analysis of developmental trajectories along predefined pathways (normative or pathogenic)

As explained elsewhere (Lorion and Allen, 1988), legitimate questions have been raised about the distinction between primary and secondary approaches. At the very least, it is evident that all possible forms of early preventive interventions should be developed and field tested at this point in time. Once a number of demonstrably effective interventions exist, the reasonable next step would be to compare their respective benefits and costs in order to determine which should be disseminated on a national basis. Unquestionably, such decisions will involve the complex consideration of economic as well as "quality of life" factors.

THE EVOLUTION OF A DISORDER: In the narrowest sense, alcohol and drug problems must be appreciated as representing a range of behavior far more complex than the simple distinction between "use" and "nonuse". As reported by Johnston et al. (1986), Baumrind (1986), and Kandel and Yamaguchi (1986), use is neither statistically unusual among adolescents nor necessarily pathological. In fact, Baumrind questions whether experimental involvement should even be considered developmentally inappropriate. What is also clear, is that a rather lengthy decisional process precedes the initiation of use and continues for an indeterminate period of time thereafter. Thus, for each substance, decisions are made about the initiation of use, the repetition of that use, its cessation or continuation and subsequent level. We have yet to understand prospectively the implications of each of these decisions for personality and for their health consequences.

We do know, however, that the decision to use one substance appears to influence subsequent decisions about others. At one pint it was assumed that a distinct "gateway" sequence could be identified which represented a Guttman-type scale of involvement. Thus, Kandel (1980) had proposed that there was a predictable sequence of beer and wine: cigarettes and hard liquor; marijuana: and other illicit drugs. Unable to support this sequence with her subjects, Baumrind (1986) proposed that if a sequence did exist, it could best be understood as reflecting initial involvement with substances perceived as "socially acceptable" followed by increasingly unacceptable substances.

The individual nature of the consequences of substance involvement represents a genuine challenge for substance researchers. We need to understand how the majority of those who initiate use subsequently reduce or eliminate further involvement without intervention. Thus, it appears that the majority of drug users decide on their own to control or discontinue that use. Is that true for all substances? If so, how does it occur naturally and to what extent can that process be incorporated within preventive interventions?

A TRANSACTIONAL ECOLOGICAL MODEL: As noted, alcohol and drug abuse may be understood within a transactional perspective.

This framework provides, I believe, a useful model within which to integrate existing information about disparate risk factors and insights into the mechanisms by by which these factors operate. The basic elements of this model are described by Sameroff (Sameroff and Chandler, 1975: Sameroff and Fiese, 1988). In a recent paper, my colleagues and I have attempted to apply it to the prevention of wild and adolescent disorders (Lorion et al. 1988).

Sameroff explains that his model evolved out of the struggle prospectively to confirm apparent causes of early childhood disorders which had been suggested through retrospective analyses. Repeatedly, such risk factors did not result in symptoms. conversely, apparently healthy infants developed disorders for which the assumed risk factors did not occur in their histories. In effect, the prediction of Which infants, on the basis of "reproductive risk", would develop disorder could not be accurately accomplished. Careful examination of the histories of numerous cases of childhood disorder revealed that a second dimension, i.e. "a continuum of caretaking casualty", also needed to be considered. These dimensions were not, however, independent contributors to etiology. Rather, Sameroff and his colleagues demonstrated that each influences the other in a continuously synergistic manner.

For example, a child born with colic may have a relatively problem-free development outcome if the primary caretaker (usually the mother) has a high tolerance for stress and a self-concept not threatened by an infant in distress. Alternately, the child's outcome could be much more negative if the caretaker responds to the baby's distress with feelings of inadequacy and anger and, in turn, rejected the infant. one could easily project the long-term consequences of such an interactional pattern.

The complexity of the transactional system, however, is reflected in its prediction that the child's level of distress is, in part, mediated by the caretaker's response which, in turn is mediated by the child's response to caretaking. Viewing these two dimensions as ongoing dynamic sequences which are in a continuous evolution based on prior history and the immediate state of the other provides the elements of the framework.

How do we know, however, that such a model applies to alcohol and drug use among children and adolescents? Evidence of individual differences in responsiveness to alcohol and drugs has long been known. Gradually we are developing an understanding of the immediate and long-term implications of fetal alcohol syndrome, fetal marijuana syndrome, and perinatal withdrawal from cocaine and heroin addiction. We know that the children of alcoholic and substance abusing parents are at increased risk of substance involvement as well as for other forms of cognitive and emotional dysfunction. It would seem

reasonable to consider all of these as reflective of what Sameroff labeled "the continuum of reproductive casualty".

Evidence reported by Baumrind (1986), Bry (1983), Hawkins et &al. (1986), and Murray and Perry (1986) strongly supports the conclusion that a "continuum of caretaking casualty" may also be identifiable in the lives of substance-involved youth. Baumrind, for example, argues strongly for the primary contribution of parental influences on the development of pro-substance knowledge and attitudes. In her view, parental indifference to or acceptance of the use of socially acceptable" substances is a key risk for early onset. If one assumes that "caretaking" includes experiences in both the home and external environment, the relevance of this dimens ion to, adolescence involvement appears even stronger. For example, Hawkins et al. (1986) point out:

> "It appears reasonable from the evidence reviewed on childhood predictors of early initiation and abuse that adolescent drug abuse should be viewed from a developmental perspective. Early initiation as well as patterns of abuse can be considered responses to or results of experiences from birth through adolescence. Early antisocial behaviors, early experiences in the family, later experiences in school, and finally, interaction with peers all appear to be implicated in the etiology of drug use and abuse. From a developmental perspective, it can be argued that early experiences in the family are likely to influence social boding to the family (Hirschi, 1969), social and self-control (Reckless, 1961), and subsequent experiences in school, as well as the likelihood that social bonds of attachment and commitment to education will develop (Bahr, 1979). Similarly, experiences at school are likely to influence the extent to which a youth will develop social bonds of attachment and commitment to prosocial activities and prosocial others (Schafer and Polk, 1967; Hirschi, 1969)".

Thus, one can presently identify both individual and environmental characteristics consistent with Sameroff's risk dimensions of reproductive casualty and caretaking casualty. In my view, a careful analysis of the substance related literature should produce additional support for the relevance of these dimensions to predicting involvement with alcohol and drugs. That step alone, however would be insufficient basis on which to argue that the transactional perspective is a valuable heuristic for understanding and ultimately preventing this category of disorder. What is needed are data which substantiate prospectively the aforementioned synergistic relationship.

Consider the predictions which must be confirmed to validate that perspective. In the-first place, one must demonstrate that neither individual nor environmental factors alone are

sufficient to cause alcohol or drug abuse. The relatively low predictive, albeit statistically significant; relationships reported thus far for the individual risk factors identified to date (e.g. Hawkins et al., 1986) support this assumption. Second, one would anticipate that the inclusion of factors from both dimensions results in higher predictive accuracy than found with predictors from either dimension alone. Available evidence appears to support this tenet (e.g. Baumrind, 1986; Bry, 1983; Hawkins et al., 1986; Murray & Perry, 1986).

These findings, in my view, represent a foundation on which can be built the next step in etiological research. Specifically, we must begin to identify the mechanisms underlying the development of the constellation of factors which must be present for an individual to initiate involvement with substances, and to proceed from limited experimentation to problemmatic dependence and addiction. If Sameroff's model is to serve as a heuristic for understanding that mechanism, we must design methodologies for elucidating the process by which factors within either dimension synergistically influence each other in a continuous manner. That task represents a major challenge for the behavioral sciences for it requires that multiple factors from each dimension be monitored simultaneously and longitudinally. It requires the precise measure of causal influences that are bi-directionally sequential.

In his recent discussion of the model, Samaroff (Sameroff and Fiese, 1988) provides some clues about how to think about its applicability to emotional and behavioral states. For example, he suggests that careful examination of the environmental dimension would identify the "developmental agenda", i.e., the culture's timetable for the achievement of developmental milestones. The components of that agenda, i.e., "statutes" (direct cultural influences), and "styles" (social interactive patterns through which the agenda occurs) can be distinguished, quantified, and potentially altered. Immediately, one must become involved in the careful articulation of the "cultural code". With respect to substance use, for example, one must determine individual assumptions about the extent and meaning of such use in adolescence and its consequent attributional implications for the adolescent subculture.

sameroff also proposes that the dynamism which defines the transactional sequence reflects the operation of a complex set of regulations occuring at the macro (i.e., the cultural), the mini (i.e., the family or peer group), and the micro (i.e., directly between the two dimensions) levels which determine each dimension's permeability respective to the other. If we can identify these regulatory principles, we may begin to understand and eventually control the relevant transactional processes.

FINAL COMMENTS: One must question whether the degree of complexity represented by the transactional mode is needed to prevent alcohol and drug abuse. Given our record thus far of predicting use, misuse and, abuse, I would argue that a complex process certainly exists. I would also argue that we need to change dramatically how we approach these problem. Unquestionably, the inclusion of parameters such as "cultural codeine" and regulatory system mandates that sociologists and cultural anthropologists collaborate in our efforts. To understand the effects of substances on those who take them we must consult with psycho-pharmacologists and clinicians who treat those who are dependent. I might add that we must also involve our potential subjects within such research. Only then, will we insure that relevant cohort influences are appreciated and reflected in our designs.

Would that we could easily solve the drug problem. Realistically, however, its complexity is unlikely to be much less than found for other major health care problems. It should, therefore, receive parallel support and serious attention from policymakers.

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AFFILIATION

University of Maryland
Department of Psychiatry
College park, Maryland 20742-4411

Defining “Success” in Drug Abuse Prevention

G. Botvin

INTRODUCTION

For well over twenty years now health professionals, teachers, and community leaders have developed and implemented a variety of programs designed to prevent or reduce drug abuse. These efforts have been plagued by a number of problems including a lack of clarity with respect to program goals, limited resources, little or no evaluation, and, where programs have been evaluated, an inability to prevent or reduce drug use/abuse.

In recent years, we have witnessed the development and testing of what many experts have characterized as a "new generation" of prevention programs. These approaches have differed from traditional approaches in several important ways. First, they have been based on a more comprehensive understanding of the complex array of factors promoting and maintaining drug abuse. Second, they have been theory-based, relying to a large extent on social psychological theories such as social learning theory (Bandura, 1977), persuasive communication theory (MGuire 1964), and problem behavior theory (Jessor and Jessor, 1977). Third, a major distinguishing feature of these approaches is the emphasis on skills training, rather than on increasing knowledge or changing attitudes. Finally, they have been research driven, with a primary focus on evaluation.

Unlike traditional approaches to drug education-which rely on the provision of knowledge about the adverse consequences of use and frequently employ fear arousal techniques in an effort to scare individuals into not using drugs--these newer approaches have focused primarily on the social and psychological factors believed to promote substance abuse. Moreover, they have adopted teaching methods based on the behavior change literature in an effort to teach a variety of skills related either empirically or theoretically to resisting pro-drug use social influences.

These approaches can be conceptualized falling along a continuum, with some approaches focusing very specifically and directly on teaching social resistance skills for effectively refusing offers to smoke, drink, or use drugs: and with other approaches utilizing a somewhat more comprehensive strategy including the teaching of an array of general life skills.

Significant progress has been made in recent years with the advent of these newer primary prevention strategies. Recent reviews of the growing literature evaluating these approaches (e.g., Botvin, 1986; Flay, 1985) indicate that, for the first time in the history of the field, evidence of changes in drug use behavior has been obtained rather consistently in a number of studies by a number of different investigators. However, despite the positive results of these studies and the demonstrated efficacy of these approaches with respect to drug-taking behavior, the early optimism produced by these findings has abated somewhat and given way--at least in some quarters--to a more skeptical perspective.

Questionshave arisen concerning the durability of the effects produces by these new approaches; the extent to which these approaches generalize from drug use to drug abuse; and, since most of these studies have been conducted with cigarette smoking, the extent to which these approaches are effective with other forms of drug abuse. Questions have also been raised concerning the extent to which positive effects demonstrated with white middle class populations are likely to generalize to other populations, particularly urban minority populations and other high risk populations.

Finally, questions have been raised concerning the efficacy of these prevention approaches when they are removed from the nurturing and arguably somewhat artificial environment of research, and are applied in the "real world." Because of these and other questions concerning the effectiveness of current prevention approaches and their implications for policy, it is important to take a step back and consider how to define "success" in the area of drug abuse prevention. What are the legitimate goals of prevention? How do we know when we have achieved those goals? What are reasonable expectations in a field that is still in its infancy? How do we keep in perspective the occasional failures which come when we are attempting to push back the boundaries of prevention? And, since progress is likely to come in small increments, how do we know when its time to stand up and cheer?

In this context, then, the focus of this paper does not relate specifically to defining evaluation criteria for prevention studies (although implications for establishing such criteria could be derived from this material). Rather, the central focus of this paper is considerably broader: how to define success with respect to the entire field. More specifically, this paper will focus on clarifying the goals of prevention, identifying the milestones or benchmarks of success, appraising the current status of prevention research, and identifying areas where more work is needed.

CONCEPTUALIZING PREVENTION

Prevention has traditionally been divided up into three levels or types: primary prevention, secondary prevention, and tertiary prevention. The objective of primary prevention is to prevent a disorder or disease from occurring before any manifestations of that disorder or disease are evident. The objective of secondary prevention is to identify and treat individuals as early as possible

in order to reduce the length and severity of the disorder or disease. The objective of tertiary prevention is to reduce the degree of impairment once a disorder or disease has been developed in order to minimize the long-term consequences.

Only the first type of prevention, primary prevention, actually refers to prevention in its truest sense. The other two types, although frequently conceptualized as prevention, are in actuality treatment. It has frequently been argued that this tripartite conceptualization of prevention should be eliminated because it tends to blur the lines seperating prevention and treatment, and creates ambiguity concern ing what is meant by prevention. This is an important issue with implications for the delineation of prevention goals and the criteria for defining success, particularly in the area of drug abuse prevention.

THE GOALS OF PREVENTION

The major overarching goal of national drug abuse policy is defined in terms of reducing the prevalence of drug abuse throughout the United states. This could be accomplished through the development and implementation of effective treatment and prevention strategies. Once drug abuse prevention is conceptualized in terms of primary prevention, it becomes somewhat easier to define prevention goals. Although national drug abuse policy goals correctly concern reducing the prevalence of drug abuse, the appropriate goal of prevention efforts should be defined in terms of reducing the incidence of drug abuse (i.e., the number/percentage of new cases) rather than in terms of overall prevalence (i.e., the total number/percentage of cases, combining both old and new cases). The implication for the development, implementation, and evaluation of individual preventive interventions is that they should focus on preventing the onset or initiation of drug abuse.

BENCHMARKS OF SUCCESS

In attempting to assess the status of either the field of prevention as a whole or the efficacy of particular interventions, it is helpful to have criteria defining success. The most important point to be made relative to defining success in drug abuse prevention is that, like other areas of science, the development of effective prevention models is a process involving the gradual, and sometimes torturously slow, accumulation of knowledge. Success should be conceptualized as incremental progress through a series of smaller successes, a series of smaller accomplishments, which lead us step by step to the ultimate goal-success with a capital "S." Success in the larger sense for drug abuse prevention must be defined in terms of reducing the incidence of drug abuse in our society. Since it is a long and difficult journey, it is necessary not only to keep our research compass pointing in the right direction, but also to identify accomplishments which can be used as benchmarks or milestones for judging progress along the way.

For the purpose of this discussion, eight major benchmarks have been identified. These concern the accumulation of acceptable evidence indicating that preventive interventions have been developed which

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(1) are feasible and acceptable to the target populations; (2) can impact on variables associated with drug abuse or drug abuse risk: (3) can impact on the use/abuse of at least one drug: (4) can impact on the use/abuse of multiple drugs: (5) can produce durable effects: (6) generalize across populations; (7) are adaptable to different conditions, providers, delivery methods; and (8) are exportable and easy to disseminate. The benchmarks have been ordered in a more or less hierarchical fashion which can be used as a logical and sequential approach to prevention research.

Feasibility and Acceptability

The first benchmark for the field of prevention (or for a particular prevention approach) concerns whether promising prevention models exist which are feasible and acceptable to both the target population and the program providers. Interventions which are too complex, require training that program providers are unlikely to possess, have goals which are inconsistent with the norms of the community, or are mandated by overly zealous administrators, may either not be implemented altogether or may be implemented with an inadequate degree of fidelity. Under these circumstances, even the most effective prevention approach will fail.

Impact on Drug Abuse Risk Variables

A second benchmark to be used in assessing the progress made toward developing effective prevention approaches is whether or not they impact on variables associated with drug use/abuse. These variables might include knowledge of the deleterious effects of drug use, attitudes toward drug use, perceptions of the prevalence of drug use by peers or adults, behavioral intentions, self efficacy, self esteem, locus of control, etc. However, it is important to point out that many studies have been published which have produced significant knowledge or attitude changes, for example, but which have not produced changes on drug use behavior. Consequently, evidence of the ability to impact on variables associated with drug use/abuse must be regarded as a low level of evidence for the effectiveness of a particular prevention approach

Although the only real test of the effectiveness of a prevention program is the extent to which it impacts on drug-taking behavior, it may not be possible to collect behavioral data in some circumstances or with certain populations. Some school administrators or parents may refuse to permit the collection of data on drug use, particularly illicit drug use. As a result, the only means of evaluating program outcome may be with respect to variables associated with drug use/abuse. Similarly, studies evaluating preventive interventions conducted with elementary school children cannot utilize behavioral variables because the prevalence of drug use is too low in most instances to permit meaningful statistical comparisons among individuals this young.

Interventions which can demonstrate an impact on variables which might be conceptualized either as risk factors for drug use/abuse or as proxy measures of drug use behavior (e.g., behavior intention) can at least be viewed as demonstrating the potential of being an

effective prevention approach. Clearly this kind of evidence alone does not indicate that a particular approach is effective, but it is a step in the right direction and does provide at least some basis for claiming a modicum of success.

Impacting on a Single Type of Drug Behavior

At third benchmark of success in drug abuse prevention is evidence indicating that a given prevention approach can prevent or delay at least one form of drug-taking behavior. Typically, drug involvement is measured by items assessing frequency/amount of use, patterns of use, and use associated with negative consequences. Outcome evaluation can be conceived of in terms of drug use status (categorical variables) or scales of drug involvement (continuous variables). At the extreme end of both kinds of measures should be the inclusion of behavior that would be defined as drug abuse.

Although the overall goal of drug abuse prevention is, by definition, to prevent (or at least delay) the initiation of drug abuse, in actuality the goals of preventive interventions are generally operationalized in terms of the wider continuum of drug use. Indeed, for both ideological and research reasons, the goals of prevention programs targeted at youth tend to be defined in terms of the prevention of relatively low levels of drug use rather than in terms of drug abuse as it might be defined clinically.

A reasonable expectation of prevention programs targeted at students during the beginning of junior high school would be that significant and meaningful reductions in the incidence of new drug use (i.e., the transition from non-use to use) could be demonstrated using a standard 30-day current use variable. With somewhat older populations, where overall drug rates are typically higher, a reasonable expectation would be that prevention program be able to demonstrate an impact on levels of drug involvement (e.g., weekly or daily use) that begin to approach what might be defined as abuse.

Since primary prevention programs must be implemented before individuals are likely to become drug abusers, this generally means conducting preventive interventions during or before the junior high school years (the beginning of adolescence). However, among individuals in this age group, the base rates of drug abuse are so low that unless sample sizes are extremely large, statistical power will generally be too low to identify real program effects. Hence, although the ultimate goal of drug abuse prevention efforts is to decrease the incidence of drug abuse, the most appropriate outcome variable for most preventive interventions targeted at individuals during the early adolescent years is use rather than abuse. Impact on drug use is, then, not only viewed as a legitimate goal of prevention approaches, but also as providing presumptive evidence of the efficacy of an intervention for preventing drug abuse. However, if drug use is employed as the outcome variable, then the burden must fall on researchers to demonstrate that preventive interventions found to effectively reduce drug use do eventuate in reductions of drug abuse incidence.

Furthermore, the efficacy of preventive interventions targeted at adolescents can most reasonably be judged in terms of their impact on the use of tobacco (nicotine), alcohol, or marijuana because their use occurs toward the beginning of the developmental progression of drug abuse (Kandel, 1978). In addition, since these are the three most prevalent forms of drug use in our society, the base rates are higher than for other substances. Consequently, statistical power for data analyses will be higher for these substances than for substances with lower base rates, increasing the potential for detecting program effects. Thus, one of the most significant benchmarks of progress in the field of drug abuse prevention would be the presence of interventions which can produce measurable effects on at least one of the three "gateway" drugs.

Impacting on Multiple Types of Drug Behavior

Assuming that it is possible to demonstrate an impact on the use/abuse of one drug, a problem which needs to be confronted when attempting to develop effective preventive interventions is the extent to which prevention programs designed to impact on one substance can also impact on others--i.e., the issue of generalization. If a particular intervention, designed to prevent substance "X" and subsequently found through evaluation research to be effective with "X," what kinds of intervention modifications might be necessary to render it generalizable not only to "Y" but to other substances? Thus, a fourth benchmark of success is the presence of evidence demonstrating that a given intervention can impact on more than one substance. For example, interventions may be demonstrated to impact on all three gateway substances.

A potential problem confronting prevention researchers is that specific interventions may need to be developed for different drugs or at least for different classes of drugs. If this turned out to be the case, then, it would not auger well for the future of prevention in the real world. Interventionists would be overwhelmed by the complexity of a multitude of different interventions, each tailored for a specific drug or class of drugs. For school-based interventions, the school schedule would buckle under the sheer weight of the number of interventions which would need to fit into an already crowded academic calendar. The best hope is that the causes of the various forms of drug use/abuse are similar enough so that a generic intervention can be developed which is reasonably effective with all--or at least most-forms of drug abuse.

Durability of Effects

Demonstrating that prevention approaches can impact on any degree of drug use has to be regarded as a significant accomplishment, particularly in view of the fact that few prevention programs have been able to impact on drug-taking behavior. Still, while it may certainly be of theoretical significance that a particular prevention approach is capable of reducing drug use based on comparisons conducted at the conclusion of the intervention, it will certainly be of limited practical significance. If preventive interventions are to ultimately result in reductions in the incidence of drug abuse, they must be able to produce effects which are reasonably durable.

Thus, the next major benchmark to be used in defining success in drug abuse prevention is the extent to which evidence exists supporting the durability of effects.

Determining the durability of any observed program effects requires longitudinal evaluation studies. Such studies are fraught with difficulties relating to tracking individual participants over the course of the study, differential attrition, the flagging enthusiasm of institutional support personnel (e.g., administrators and teachers in school-based studies), and resistance on the part of participants who quickly lose interest in answering the same questions year after year.

Beyond the arduous task of conducting longitudinal drug abuse prevention research, it may be unrealistic to expect that any intervention will produce a lasting impact on behavior--i.e., that drug abuse can be prevented indefinitely by a single short-term intervention. Rather, in order to achieve long-term effects, it may be necessary to implement long-term preventive interventions. For example, in school-based prevention, it may be unrealistic to expect that a single prevention unit, conducted during the beginning of junior high school, will produce lasting reductions in drug abuse incidence without ongoing interventions throughout junior and senior high school. If it is necessary to conduct interventions over an extended period of time, with different age groups as they move through different developmental stages, it will be necessary to determine the most effective interventions for each of these age groups/developmental stages as well as the most effective combination of intervention components.

Generalizability Across Populations

Another important issue concerns the generalizability of prevention effects from one population to another. Thus, a major prevention benchmark is the presence of evidence indicating that interventions exist which are effective with a broad range of individuals. Since interventions are likely to be developed and initially tested on a particular population, a basic question which must be answered concerns the extent to which interventions developed and proven effective with one population will work with other populations. These different populations can be defined in various ways--for example, in terms of race/ethnicity, socioeconomic status, age group, or drug abuse risk.

Based on the fact that these populations are different, it might be argued that different preventive interventions are necessary. Different interventions might be necessary because the causes of drug abuse might differ in some fundamental way. Alternatively, the causes of drug abuse may be similar enough to warrant the application of a given prevention approach to several populations, but aspects of the intervention my need modification to render it more suitable to other populations. For example, interventions targeted at minority populations must be designed to be culturally sensitive. Some modifications might simply involve the kind of "translation" that can be accomplished by a skilled provider familiar with the population being targeted.

Adaptability

To be effective in the real world, preventive interventions will need to be adaptable to a variety of intervention conditions, providers, and delivery systems. Different conditions may require interventions which can be effective when implemented according to different scheduling formats, with large or small groups, in health or science class, etc. These interventions will need to be flexible and adaptable in order to be utilized. They will need to be capable of being effective when implemented by more than one type of provider (e.g., by health professionals, teachers, or older peers). Moreover, they will need to be adaptable to different delivery system such as those targeting individual students, schools, and the larger community. Another benchmark assessing the progress of drug abuse prevention, therefore, is the presence of evidence implicating that interventions which have been demonstrated to be effective under certain conditions, with certain providers, or using certain delivery systems are effective when adapted to other conditions, providers, and delivery systems.

Exportability and Ease of Dissemination

Related to this are the issues of exportability and ease of dissemination. It may be quite difficult to transplant interventions found to be effective in one kind of environment to another. For example, interventions found to be effective in a nurturing research environment may be ineffective in a less nurturing environment where there is tremendous competition for time and resources. Some interventions may require specific intervention conditions that rarely exists or that are relatively unique to a particular situation. Such interventions, even if highly effective, will be difficult to disseminate and consequently will be of minimal value in achieving national reductions in the incidence of drug abuse. Thus, important considerations are the nature and structure of particular interventions as well as the resources needed to successfully implement them. For these reasons, a final benchmark to be used in defining success in drug abuse prevention concerns not only having interventions which are effective, but also concerns having interventions which are exporatable and have a high potential for widespread dissemination.

THE CURRENT STATUS OF PREVENTION RESEARCH

Although it is beyond the scope of this paper to provide a detailed review of the prevention literature, it would be worthwhile to briefly assess the current status of prevention from the perspective of the criteria for success delineated above. The findings of evaluation studies conducted over roughly the past decade provide support for the efficacy of psychosocial prevention approaches based on the social influence model and on the broader life skills model. These approaches have been demonstrated to significantly reduce the use of at least one substance (typically cigarette smoking) with junior high school students. Evidence also exists demonstrating that initial reductions in experimental use can result in later reductions in abuse.

Although the focus of most school-based prevention studies has been on cigarette smoking, some evidence exists demonstrating the efficacy of these approaches on several types of drug use. For example, in addition to demonstrating effects on cigarette smoking, these approaches have also been demonstrated to impact on both the use and abuse of alcohol and marijuana. However, these data come from only a small number of studies at this point, and no studies have demonstrated effects with illicit drugs other than marijuana. Data also exist demonstrating the presence of program effects for up to three years, although virtually all of the longer-term follow-up has been limited to studies focusing on cigarette smoking. Additionally, because these effects appear to erode overtime, ongoing interventions may be necessary during the high risk adolescent years in order to maintain any preventive gains.

A major criticism of the field has been that studies demonstrating the efficacy of these newer prevention approaches have been conducted almost exclusively with white, middle class populations. Evidence available from the few studies conducted with other populations, as well as preliminary data from studies currently underway, suggests that these approaches are effective with several different populations including urban minority populations and rural populations. Moreover, some studies have found that these approaches are effective with high risk adolescents (where risk is defined in terms of peer use).

These interventions have been found to work when implemented by peer leaders, teachers, and health professionals. In school-based studies, interventions appear to work equally well when implemented in different subject areas and in different intervention formats. Sane effort has been made recently to adapt these intervention approaches to different delivery systems by developing components targeting parents, schools, or the larger community. Furthermore, different intervention methods have been used including small group discussion, behavioral training, video taped instruction, and mass media. While these approaches generally appear to be readily exportable and should be relatively easy to disseminate, these issues have not yet been studied in a systematic fashion.

All this would indicate that by the benchmarks of success delineated above, significant progress has been made in the quest for effective prevention approaches. Approaches exist which are feasible and acceptable to the target populations, which have impacted on variables associated with drug use, and more importantly which have impacted on drug use behavior. Many studies have demonstrated prevention effects with a single type of drug behavior (tobacco use): only a few studies have demonstrated effects with multiple types of drug behavior (tobacco, alcohol and/or marijuana use).

Prevention effects have been found to be reasonably durable over the intermediate term, and limited evidence exists supporting the effectiveness of these approaches with several different populations. Limited evidence also exists concerning the extent to which these interventions are adaptable to different conditions, providers, and delivery system. Although no systematic data have been collected,

there is every implication that these preventive interventions are readily exportable and easy to disseminate.

SUMMARY AND CONCLUSIONS

The ultimate measure of success in drug abuse prevention obviously and unequivocally must be to reduce the incidence of drug abuse among our nation's youth. However, the problem of drug abuse in our society is complex, and does not lend itself to facile solutions or "quick fixes." Because of the distance between where we are now and where we have yet to go, success should be viewed as a process involving a series of mostly minor victories. Benchmarks, such as the ones suggested in this paper, can provide a means of charting and assessing our progress. When viewed from this perspective, it becomes clear that we have made considerable progress over the past decade--progress that has been painfully slow but steady. Yet, much remains to be done. In view of the importance and magnitude of the task involved in reducing drug abuse incidence in our society and the failures of Past prevention efforts, it is important that we recognize the accomplishments of the field--that we stand up and cheer in celebration of each victory, however small, as we move forward in the development of effective prevention approaches.

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AUTHOR

Gilbert J. Botvin, Ph.D.
Associate professor
Cornell University Medical College
411 East 69th Street, KB-201
New York, NY 10021

Neurophysiological Consequences of Amphetamine Administration

P. Groves, L. Ryan, M. Diana and R. Gariano

The psychomotor stimulants, including the amphetamines, cocaine and methylphenidate, act by altering monoamine neurotransmission. Perhaps the greatest challenge facing neuropharmacologists is to understand how altered monoamine neurotransmission modifies information processing in the brain. It is clear that many non-monoamine neurons are ultimately responsible for the organization., generation and execution of psychostimulant-induced behaviors and, indeed, the firing rate and firing pattern of neurons throughout the brain are altered by psychostimulants. To understand the neurophysiological consequences of psychostimulant administration one necessary step is to understand how psychostimulants alter the activity of monoamine neurons. In this report, we will examine the actions of amphetamine on monoamine neurons in the brain, emphasizing the effects of released monoamines on monoamine autoreceptors.

EFFECTS OF AMPHETAMINE ON MONOAMINE NEURON FIRING RATE

In anesthetized animals, the acute systemic administration of amphetamine inhibits the firing of noradrenergic, dopaminergic and serotonergic neurons (Groves and Rebec, 1976). In rats, noradrenergic locus coeruleus neurons show 50% inhibition at an effective dose of approximately 0.25 mg/kg, iv (Engberg and Svensson, 1979; Ryan et al., 1985), whereas substantia nigra pars compacta dopamine neurons have an ED50 of approximately 1.6 mg/kg, iv (Bunney et al., 1973). Serotonin neurons of the dorsal raphe nucleus have a biphasic response pattern: mild excitation at low doses, inhibition at higher doses with an ED50 of 3.0 mg/kg (Rebec et al. 1982).

The inhibition of monoamine neurons by amphetamine is mediated, in part, by local release of monoamines. This release may occur from dendrodendritic synapses, which have been identified and described in the substantia nigra (Groves and Linder, 1983). Amphetamine has been shown, for instance, to cause dopamine release in the substantia nigra (Cheramy et al., 1981). Local monoamine release may also occur from recurrent axon collaterals, as occurs in the locus coeruleus (Aghajanian et

al., 1977; Groves and Wilson, 1980) and in the raphe nuclei (Chazal and Ralston, 1987). Additionally, amphetamine's actions on monoaminergic target cells may change neuronal firing elsewhere which may then secondarily alter monoamine neuron firing. For instance, projections from the forebrain may contribute to the amphetamine-induced inhibition of some substantia nigra dopamine neurons (Bunney and Aghajanian, 1978), but not of ventral tegmental area (VTA) dopamine neurons (Wang, 1981). Dorsal raphe neurons, as another example, may be excited at low doses as a consequence of the reduction in locus coeruleus firing, but inhibited at higher doses by autoreceptor activation (Rebec et al. 1982).

The local amphetamine-induced release of monoamines inhibits neuronal firing by acting on somatodendritic autoreceptors. Activation of noradrenergic alpha-2 autoreceptors hyperpolarizes the somatic membrane of locus coeruleus neurons by increasing a K+ conductance (Aghajanien and VanDerMaelen, 1982; Williams et al., 1985), similar to the increase in K+ conductance reported to follow autoreceptor activation on serotonin neurons of the raphe (Aghajanian and Lokoski, 1984). Autoreceptor mediated inhibition of dopamine neurons may result from a reduction in the constant depolarizing current thought to mediate pacemaker-like firing (Grace and Bunney, 1984). In all three monoamine systems, somatic autoreceptor activation by amphetamine inhibits neuronal firing and reduces the impulse dependent release of transmitter onto target neurons.

AMPHETAMINE EFFECTS ON TERMINAL AUTORECEPTORS: TERMINAL EXCITABILITY

Terminal autoreceptors are believed to modulate calcium-dependent neurotransmitter release. In *in vitro* preparations, activation of autoreceptors by exogenously applied autoreceptor agonists typically inhibits release, whereas autoreceptor blockade by antagonists typically enhances release (Langer, 1981; Starke, 1980). Thus, terminal autoreceptors work in concert with somatic autoreceptors to reduce monoamine release. Because amphetamine increases the extracellular concentration of monoamines as a consequence of increasing non-calcium dependent monoamine release and blocking monoamine reuptake (Kuczenski, 1983), it may, indirectly, activate both somatic and presynaptic terminal autoreceptors. This autoreceptor activation may compensate, to some extent, for the neurotransmitter release provoked by amphetamine.

Terminal autoreceptors may modify neurotransmitter release by causing ionic changes similar to those observed at the soma, but clearly they must affect release by mechanisms other than altering firing rate. To investigate the neurophysiological consequences of terminal autoreceptor activation, we have used the technique of terminal excitability modified from the one developed by Wall (1958) to study presynaptic inhibition in the spinal cord. In Wall's experiments, increases in excitability were

interpreted as indicating presynaptic terminal depolarization, an interpretation later confirmed by experiments showing that primary afferent terminals were depolarized during the presynaptic inhibition (Kocsis and Waxman, 1982). In our experiments we examine the electrical excitability of single presynaptic axons in the central nervous system (Groves et al., 1981). Axonal terminal excitability is determined by measuring the current necessary to initiate an action potential in the axon when the current is applied across a stimulating electrode positioned in the parenchyma near the axon. This action potential will travel antidromically to the soma where it may be detected using extracellular recording electrodes. Excitability curves are created by plotting the percentage of stimulus trials that evoke an antidromic response versus the stimulating current. The current just sufficient to evoke a response on every trial defines the threshold. Changes in terminal autoreceptor activation induced by amphetamine are detected as changes in the threshold and as shifts to the left or right of the excitability curves.

EFFECTS OF AMPHETAMINE ON DOPAMINE TERMINAL EXCITABILITY

Considerable evidence suggests that d-amphetamine enhances the release of dopamine in the neostriatum and other target structures (Zettestrom et al., 1986). Local infusions of d-amphetamine (1-50 uM) into the dopamine terminal fields decreases terminal excitability of nigrostriatal neurons (Tepper et al., 1984), mesoaccumbens VTA neurons (Mereu et al. 1985) and mesoprefrontal VTA neurons (Gariano et al. 1986). In Figure 1A, the response of a mesocortical neuron to direct infusion of 10 uM amphetamine into the frontal cortex parenchyma is illustrated. The first infusion shifted the excitability profile to the right, the second infusion further shifted the curve. We interpret the decreased electrical excitability as indicating that the terminals become hyperpolarized or that transmembrane conductance increased or both. Intravenous administration of d-amphetamine (0.5-1.0 mg/kg) produced, qualitatively, the same effect as shown here for a nigrostriatal neuron (Figure 1B; Groves et al. 1981). These effects of amphetamine are mediated by dopamine acting upon presynaptic terminal receptors. Various dopamine antagonists, including haloperidol, fluphenazine and (-)sulpiride (Tepper et al. 1984; Mereu et al. 1985) all increase terminal excitability and can reverse the effects of amphetamine. For example, in Figure 1A a systemic injection of a low dose of haloperidol partially reverses the effect of two earlier infusions of amphetamine suggesting the effect is mediated through dopamine receptors. Blockade of dopamine synthesis by alpha-methyl-p-tyrosine also prevented amphetamine's effects on terminal excitability.

The dopamine receptors at which amphetamine acts to change terminal excitability are located on the presynaptic axon. The effect is not mediated through post-synaptic feedback because

chemical lesion of the post-synaptic site does not alter the effects of amphetamine on terminal excitability (Tepper et al. 1984; Mereu

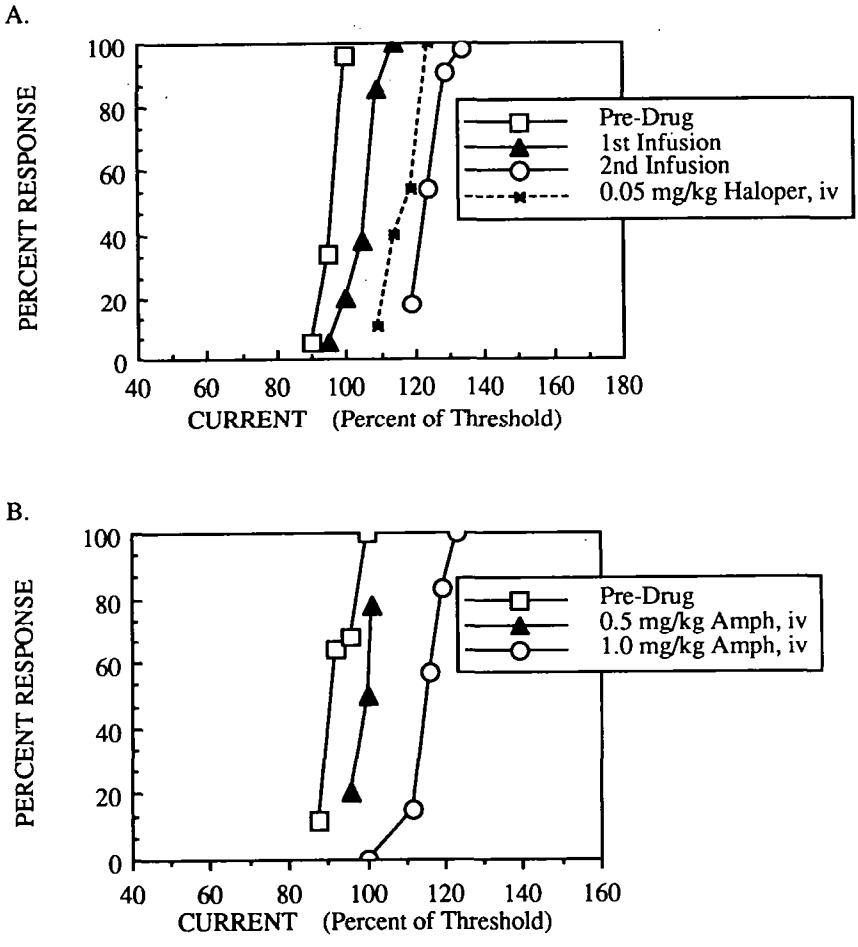

FIGURE 1. *Amphetamine decreases excitability (shifts curves to the right of mesocortical (A.) and nigrostriatal (B.) dopamine neurons when infused directly (10 uM) into the terminal fields (A.) or when given systemically (B.).*

Note: 1B is redrawn from Groves et al., 1981.

et al. 1985). Similarly, the decreased excitability obtained with the direct dopaminergic agonist apomorphine (1-10 uM) is not affected by kainic-acid pretreatment (Tepper et al., 1984). Control experiments demonstrating the lack of effect of physiological saline (0.9% NaCl) and of substances, such as clonidine, that

affect receptors not known to exist on dopaminergic striatal terminals, have strengthened this view.

The dopamine terminal autoreceptor is the so-called release-modulating and/or synthesis-modulating autoreceptor. Based on much biochemical, pharmacological and behavioral evidence (see Arnt 1987 for a recent review) this receptor appears to be of the D2 type. Terminal excitability should be therefore modulated by this entity. Recently the presence of two different terminal receptors, one modulating synthesis and the other modulating release, has been proposed (Roth et al., 1987). This proposed dichotomy awaits confirmation, but preliminary experiments in our laboratory with D1 selective agents support the presence of two different presynaptic dopamine autoreceptors located on dopaminergic nigrostriatal neurons (Diana et al., submitted).

EFFECTS OF AMPHETAMINE ON NORADRENERGIC TERMINAL EXCITABILITY

In a manner that parallels the effects of amphetamine on dopamine terminal excitability, local infusions of amphetamine (5-50 uM) directly into the frontal cortex terminal field of noradrenergic locus coeruleus neurons reliably decreased terminal excitability (Figure 2A). Local infusions of the direct acting alpha agonist, clonidine, also decreased terminal excitability. Amphetamine's effect could be blocked by prior local infusion of the alpha noradrenergic antagonist, phentolamine, and by pretreatment with the noradrenergic synthesis blocker, alpha-methyl-p-tyrosine (Nakamura et al., 1982). Thus, this decrease in terminal excitability produced by local infusions of amphetamine indicates that amphetamine increased norepinephrine release which in turn activated terminal autoreceptors.

In contrast, systemic administration of amphetamine increased excitability of frontal cortex terminals of locus coeruleus neurons (Nakamura et al., 1982). This change was not observed in non-terminal regions of the axon indicating it was probably an autoreceptor mediated effect and not a change in other properties of the axon. This paradoxical antagonist-like effects of amphetamine was dose dependent: low doses increased excitability whereas high doses decreased excitability (Figure 2B; Ryan et al., 1985). In contrast, somatic autoreceptors were consistently activated, with all doses causing an inhibition of firing, ranging from 50% at 0.25 mg/kg, iv, to nearly 100% at doses above 1.0 mg/kg, iv.

This paradoxical result was explained as a competitive interaction between several of amphetamine's different effects. The net effect of amphetamine on monoamine neurotransmission results from the interaction of mechanisms that tend to increase extracellular levels of monoamines (facilitation of non-impulse dependent release, blockade of degradative pathways, blockade of reuptake) and mechanisms

that tend to decrease extracellular levels (inhibition of neuronal firing by activation of somatodendritic autoreceptors, inhibition of calcium dependent

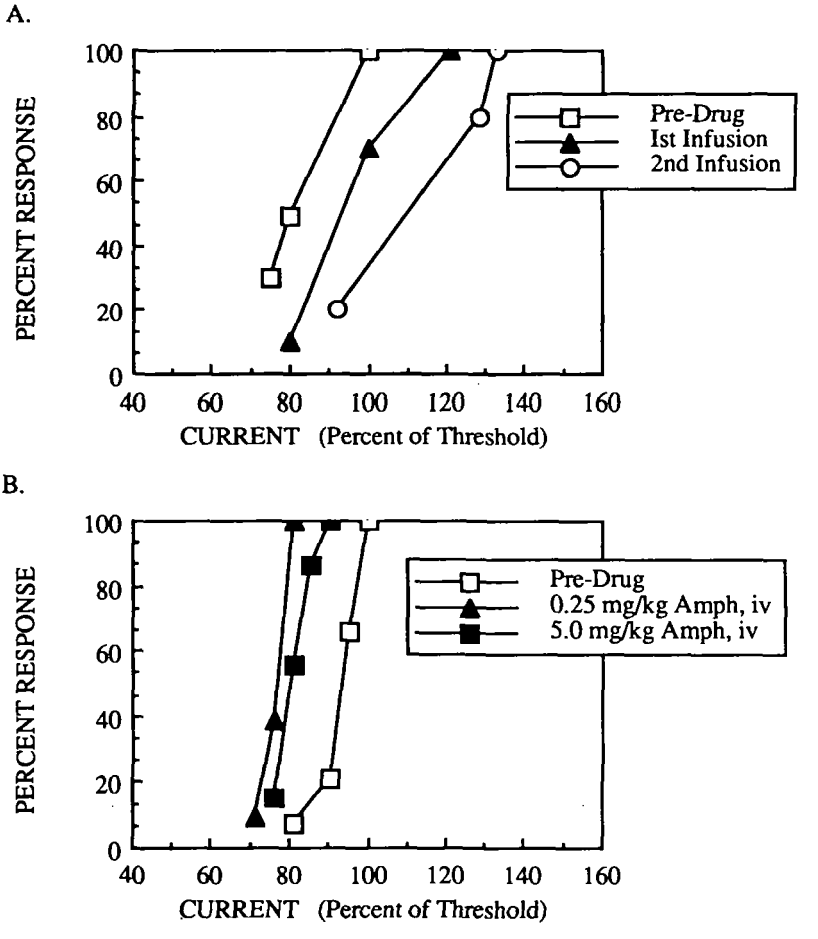

FIGURE 2. *Amphetamine decreased the excitability of noradrenergic locus coeruleus neurons when infused (10 uM) into the frontal cortex terminal fields (A.). In contrast, the effect of systemic administration was dose dependent, a low dose exerted an antagonist-like effect, increasing excitability, whereas a subsequent higher dose decreased excitability (B.).*
Note: 2A redrawn from Nakamura et al., 1982.

release by stimulation of terminal autoreceptors). For dopamine neurons, the net effect of all doses of amphetamine appears to be increased extracellular concentration of dopamine. Recent *in vivo* dialysis studies show that amphetamine increases striatal

extracellular concentrations of dopamine as much as 12-20 fold (Zettestrom et al., 1986). In contrast, for noradrenergic neurons, which are deeply inhibited at much lower doses than are dopamine neurons and which have much smaller cytoplasmic pools of neurotransmitter available for exchange diffusion mediated release (Kuczenski, 1983), the net effect of low doses of amphetamine may be to decrease neurotransmitter release (Ryan et al., 1985). Higher doses may lead to increased extracellular concentrations of norepinephrine. Thus the antagonist-like effect of amphetamine on noradrenergic terminal excitability may indicate decreased release of norepinephrine in the cortical terminal fields following systemic administration. Huang and Maas (1981) reached a similar conclusion on the basis of other evidence. Verification of this inference awaits direct in vivo measurements of norepinephrine release from these regions.

EFFECT OF AMPHETAMINE ON NON-MONOAMINE TERMINALS: THE STRIATONIGRAL PROJECTION

Neostriatal neurons projecting to the substantia nigra possess D1 dopamine receptors on their terminals (Altar et al. 1987). Direct stimulation of these receptors with the D1 selective agonist SKF 38393 causes a decrease in terminal excitability (Ryan et al., unpublished results). In contrast, amphetamine has no effect on the electrical excitability of these terminals (Figure 3).

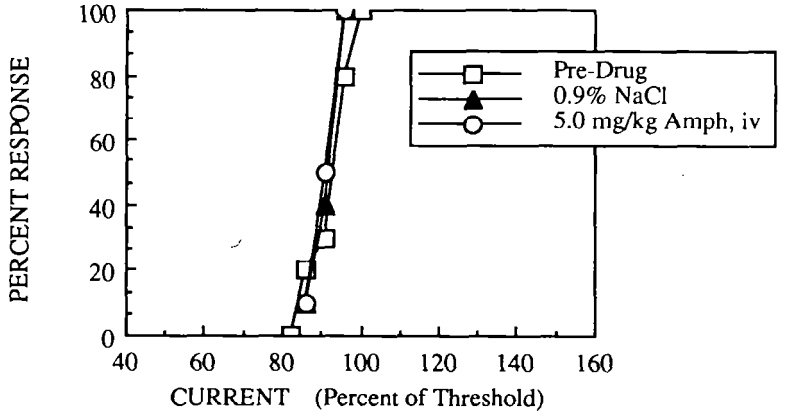

FIGURE 3. *Amphetamine does not alter the excitability of non-dopaminergic neostriatal neurons that project to the substantia nigra in chronically implanted, behaving rats (shown here) or in urethane anesthetized rats.*

This lack of effect was observed in both chronically implanted, freely moving rats given amphetamine subcutaneously (0.25-5.0 mg/kg, sc) and in rats anesthetized with urethane and given

amphetamine intravenously (1.0-5.0 mg/kg, iv; Ryan et al., 1988). Even under conditions optimal for activating these receptors, in which amphetamine is blocking reuptake and facilitating release to produce extracellular dopamine levels beyond normal for the substantia nigra, these receptors do not appear to be activated. Thus the D1 heteroreceptors found on non-monoamine neurons arising from the neostriatum appear not to play a role in the pharmacology of amphetamine and, indeed, may not be important in the normal functioning of this projection.

CONCLUDING REMARKS

Amphetamine has widespread actions in the central nervous system and influences a wide range of animal and human behavior. Many of the actions of this and related psychostimulant drugs are believed to occur by virtue of the ability of the agents to increase the extracellular accumulation of monoamines. When amphetamine is applied in the vicinity of monoaminergic somata and dendrites it releases monoamines and causes an inhibition of neuronal firing indirectly by stimulating somatodendritic autoreceptors. In those cases where intracellular recordings have been used, it is clear that this inhibition occurs because the membrane potential at the level of the cell body becomes hyperpolarized. Application of amphetamine in the region of the monoamine terminal field has an apparently similar effect on the cell, causing a release of monoamine and activation of autoreceptors at this site and agonist-induced decreased terminal excitability. Thus amphetamine indirectly activates somatodendritic and terminal monoamine autoreceptors, both of which tend to decrease monoamine release, and compensate, to some extent, for amphetamine-induced monoamine release. Thus, the dose dependent effects of amphetamine on monoamine release and upon somatic and terminal monoamine autoreceptors have important implications for the actions of psychomotor stimulants on the brain and behavior.

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AFFILIATION:

Department of Psychiatry, M-003, University of California San Diego, La Jolla, California 92093

Behavioral and Electrophysiological Effects of Delta-9-THC in Rats

S. Deadwyler, C. Heyser, R. Michaelis, R. Hampson, and C. Breese

INTRODUCTION

The need to understand the effects of drug action on brain mechanisms has been an important and increasing area of interest in drug abuse and dependence. Rapidly expanding information on the neurobiological basis of the actions of drugs of abuse has provided important links between the potential for use dependence of substances and their effects on brain processes (Sharp 1984). Perhaps even more importantly such areas of research will provide eventual understanding of how drugs of abuse change neurobiological mechanisms and subsequently gain control over behavioral processes. Unfortunately, major areas of progress in research do not always coincide with society's needs for understanding actions of the particular substances currently abused within the community. For instance, the remarkable advancement in the analysis of the neurobiological changes following acute and chronic exposure to exogenous opiates (cf Mansour et al. 1988) comes at a time when cocaine abuse is at an all-time high in this country.

Such inconsistencies are not surprising given the enormous effort and time required to determine the neural underpinnings of substance abuse at the many levels of investigation now available. In addition, the relationship between behavioral and neural aspects of marijuana and cocaine addiction probably involve several mechanisms at each level of analysis including molecular, cellular, and systemic, as well as behavioral. One of the more important goals of investigation of brain-behavior relations in drug abuse is the identification of processes operative in animal models of drug addiction that are representative of human behavioral and cognitive endpoints but can be explored at each of the above levels. Two major requirements in this regard are 1) the identification of neural models and processes which are affected by chronic and acute drug administration, i.e., processes that change in ways that are consistent with increased and maintained drug levels in the brain and 2) documentation of how those neural changes are manifested through alterations in behavior and cognition. One of the most important factors in determining the functional consequences of substance abuse therefore is the characterization of the manner in which these drug-induced neural changes alter behavior and cognition.

Any effective animal model of drug dependency and abuse must establish an interaction between the sites of action of the drug in the CNS and behavioral correlates of increased occupancy of those sites of action. For some substances,

there may be a relatively straightforward relation between degree of receptor occupancy by the ligand and behavioral response, for instance, "aggression" induced in rats during opiate withdrawal (Boshka *et al.* 1971). However, drug effects on mechanisms of learning, memory, and cognition are not likely to be so simply expressed or related. Therefore one of the first tasks in understanding this relationship is to determine whether a dose-relationship exists between the behavioral processes under investigation and the effects of the drug and to establish if there is a relationship between the amount of substance in the system and performance. Secondly, such a relationship between drug action and behavior is more firmly established if the same dose effect curve exists for the suspected neural correlates and the behavior in which they are observed (Deadwyler 1986).

STUDIES ON THE EFFECTS OF DELTA-9-THC ON HIPPOCAMPAL AND BEHAVIORAL PROCESSES

In previous studies we have examined the dose-dependent effects of delta-9-THC, the psychoactive ingredient in marijuana, on behavior and hippocampal neural mechanisms (Campbell et al. 1986a&b; Deadwyler et al. 1985). Recently we have extended these investigations to other behavioral tasks. There has been a remarkable consistency between the dose effects of this compound across different behavioral tasks which explored separate behavioral processes. By examining effects in several different behavioral tasks, and on similar neural mechanisms within each task, we have been able to designate some performance features affected by delta-9-THC which are dose related and appear to have hippocampal neural correlates.

Tone-Discrimination Task

Rats trained to discriminate one of two successively presented tones exhibit identified and differentiated sensory evoked potentials and neuronal discharges in the hippocampus following the presentation of conditioned tone stimuli (Deadwyler *et al.* 1985, Foster et al. 1987). In animals treated with delta-9-THC, changes in the amplitudes of these identified components of hippocampal tone-evoked potentials corresponded to decreases in performance accuracy and increased response latency (Fig. 1) (Campbell *et al.* 1986). The 0.5 mg/kg dose was without significant effect on tone discrimination behavior while the 2.0 mg/kg dose decreased performance accuracy to 60% of control levels. These changes were associated with a significant decrease in amplitude of the evoked potential component reflective of synaptic input from the entorhinal cortex to the dentate gyrus (N_1 in Fig. 2), and an increase in the component associated with the afferent input to that same dentate area from the medial septum (N_2 in Fig. 2). Thus, THC differentially altered the tone evoked response reflective of sensory input to the hippocampus when performance accuracy on the tone discrimination task was also disrupted.

Concomitant recordings of tone evoked discharges of the granule cells in the dentate gyrus showed a similar dose dependent disruption by THC in the same behaving task (Campbell et al. 1986b). Tone evoked extracellular unitary discharges from identified granule cells were moderately suppressed at dose levels of THC of 1.0 mg/kg and severely reduced at 2.0 mg/kg. The 0.5 mg/kg dose was again without significant effect (Fig. 3). Recovery from the acute effects of delta-9-THC occurred within 2-4 hrs as shown in Figures 1-3. An important control for nonspecific effects of delta-9-THC was the fact that recordings from

cells in the inferior colliculus, a primary auditory pathway, were unchanged at any dose level, indicating that the suppression of tone-evoked hippocampal responses and behavioral performance was not related to a generalized suppression of neural activity in primary auditory pathways.

In the above studies, it was determined that delta-9-THC had powerful influences on the sensory evoked activity in the dentate gyrus associated in dose-dependent fashion with THC induced decreases in tone discrimination performance. However, it was not known whether this effect on behavior might be mediated by changes in 1) the ability to detect the tone stimulus or 2) the animal's ability to register or retrieve the meaning of the tone stimuli once detected. Further behavioral investigations of these possibilities provided additional information.

Signal Detection Task

In order to determine whether decreased tone discrimination behavior was the result of a decreased ability to detect the tone stimulus, rats were trained to respond to very short (250 msec) tone pulses of varying intensities. Threshold tone intensity was set prior to each daily session employing a 50% behavioral detection criterion. This intensity then constituted the midpoint of a seven tone stimulus scale. Each tone was presented randomly to the animal at an average rate of 1/60 sec. Under these circumstances it was determined that the N2 component of the tone-evoked hippocampal potential occurred only when auditory stimuli were detected behaviorally (i.e., responded to) and not in those instances when tone pulses were not detected behaviorally (Fig. 4). This is similar to a result previously reported by Kettner and Thompson (1982). The tone intensity curve reflects the change in behavioral sensitivity across the seven randomly presented tone levels. It was then determined that delta-9-THC caused a systematic change in behavioral detection of the tone stimuli across the same dose range which was effective in the tone discrimination task. Figure 5 shows a dose-dependent change in detection. Again the 0.5 mg/kg dose had no effect on tone detection which is consistent with the results obtained in the tone discrimination task. However higher doses 1.0-1.5 mg/kg were increasingly disruptive of tone detection and at 2.0mg/kg tone detection was eliminated at all intensities. Recovery on this task were similar to that on the tone discrimination task (Campbell *et al.* 1986) and also proved to be dose-dependent (Fig. 6). The nonpsychoactive cannabinoid derivative, cannabidiol, produced no change in tone detection behavior even though doses as high as 4.0 mg/kg were utilized (Fig. 7).

These results indicate that delta-9-THC produced a significant disruption in ability to detect short duration tone stimuli. The disruption occurred across all tone levels, suggesting a generalized depression in trained attentiveness to the brief tone cue. The fact that the higher doses produced more disruption in the detection task than in the tone discrimination task suggests that some of the effect on discrimination capacity resulted from a decrease in ability to detect the CS+ trials. Since hippocampal potentials were only observed on detect trials in this task, a massive effect of delta-9-THC undoubtedly included disruption of sensory information processing in hippocampal structures.

Delayed Matching to Sample Task

Another factor which could have influenced tone discrimination behavior under the influence of delta-9-THC was "memory" retention of the significance of either the CS+ or CS- tone stimulus. To assess whether memory was affected by delta-9-THC a delayed-matching-to-sample (DMTS) task was employed in which one of two spatially distinct levers was pressed and then a matching response required following random delay intervals consisting of 1-30 seconds (Dunnett 1985). Normal animals show a progressive decrease of 30-40% in performance accuracy on this task at delays of 30 sec. Animals with hippocampal lesions show deficits in this task at longer (5-30 sec) but not shorter (1-5 sec) delay intervals (Dunnett 1985). Figure 8 indicates the dose-dependent decrease in performance on the DMTS task over the same dose ranges previously found to be effective in the tone-discrimination and tone-detection tasks. Animals injected with THC in the DMTS task showed a significant dose-dependent decrement in retention across delay intervals greater than 1-5 sec., and there was a statistically significant dose x delay interaction where increasing doses of THC produced more pronounced retention deficits. At delays less than 5 sec there was no significant effect on performance at any dose level, indicating that effect of delta-9-THC has very little effect on performance when retrieval of sample information is immediate (less than 5 sec). However when the delay interval is longer, the effect of the compound is more dramatic and this effect increased significantly with dose level. These data indicate a possible selective effect of delta-9-THC on processes of retention and retrieval which could be dissociated from the performance aspects of the task. Since results of this nature have been reported for animals with hippocampal lesions (Dunnett 1985), this suggests that the action of delta-9-THC was to specifically impair hippocampal information processing that was demonstrated in the tone detection and tone discrimination tasks to be related to the storage or retrieval of sensory information over a long delay interval.

CONCLUSIONS

The above findings support the following conclusions: 1) delta-9-THC disrupts sensory and other type of information processing in the hippocampus during the performance of 3 different types of behavioral tasks; 2) the deficits in some tasks appear to be nonspecific (tone detection) while in others (DMTS) the effects of delta-9-THC were quite specific to only the mnemonic demands of the task; 3) the types of behavioral disruption correspond to altered hippocampal neural activity evoked by the sensory stimuli. In addition, the decrease in tone detection capacity could not be explained by a generalized inability to perform the motor aspects of the task under the influence of delta-9-THC, since performance in the DMTS task at the shorter delay intervals was unaffected at even the highest (2.0 mg/kg) dose. In each of the three experimental paradigms, there was a marked consistency in the dose-dependency across the dose range of THC employed even though the behavioral and cognitive requirements of the tasks varied to a large degree. The most severely disrupted performance at the highest dose level (2.0mg/kg) was in tone detection task, while performance was essentially spared at this same dose level in the DMTS task for shorter (1-5 sec) delay intervals. These data support previous notions of the action of delta-9-THC in humans in that both perceptual and memory effects of the drug were demonstrated (Miller and Branconnier 1983).

The above findings suggest that abused substances may affect behavioral performance in different ways. However, depending upon the dose range employed, such effects may only become manifested during situations requiring specific behavioral responses. It is therefore difficult to assess the exact nature of the neurobiological disruption associated with the administration of compounds such as delta-9-THC unless similar dose levels result in similar effects on both the behavior and its neural correlates.

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AUTHORS

Sam A. Deadwyler, Ph.D.
Charles J. Heyser
Ron C. Michaelis, Ph.D.
Robert E. Hampson, Ph.D.
Charles R. Breese
Department of Physiology and Pharmacology
Bowman Gray School of Medicine of
Wake Forest University
300 S. Hawthorne Road
Winston-Salem, North Carolina 27103

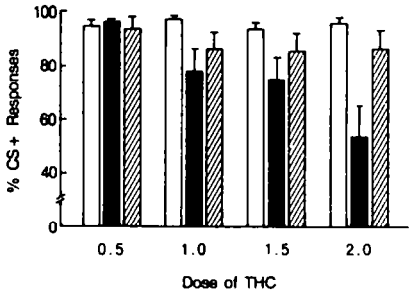

Figure 1. Behavioral effects of delta-9-THC. Mean percentage of responses to the CS+ tone in the discrimination task for each dose level. The group mean is indicated for the 100-trial preinjection session (open bars), the postinjection session (solid bars) and the recovery session (striped bars). Error bars denote ± S.E.M. (Figure reproduced with the permission of Williams and Wilkins, publishers of *The Journal of Pharmacology and Experimental Therapeutics,* from Campbell *et al,* 1986, 239:936-940).

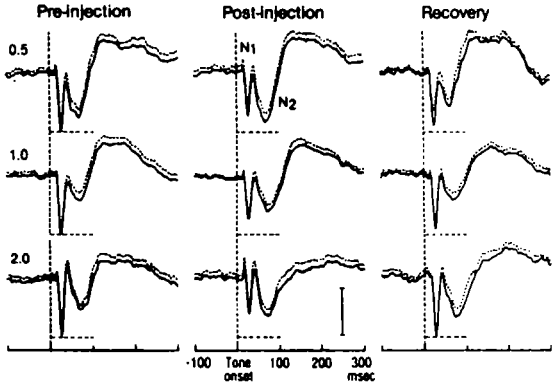

Figure 2. Effects of delta-9-THC on OM AEPs evoked by tone stimuli during the discrimination task. Each row of traces presents the composite average OM AEPs for each of the three consecutive sessions preinjection, postinjection and recovery corresponding to the dose of delta-9-THC indicated at the left. Solid lines represent the OM AEP average over all animals and sessions; dotted lines, the S.E.; horizontal dashed lines denote preinjection N1 amplitude levels at each dose level. Calibration: 200 uV. (Figure reproduced with the permission of Williams and Wilkins, publishers of *The Journal of Pharmacology and Experimental. Therapeutics, from Campbell et al,* 1986, 239:936-940).

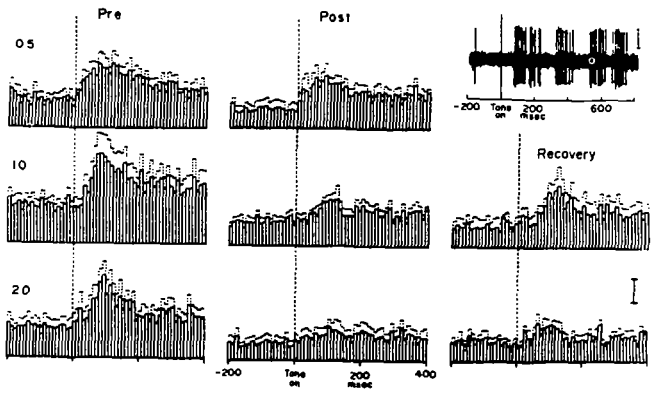

Figure 3. Effect of delta-9-THC on G-cell discharges evoked by the CS+ tone. Each row of PEHs corresponds to the dose of THC (milligrams per kilogram) indicated at left. Pre, preinjection; post, session 0 to 2 hr after THC injection; Recovery, recovery session (2-4 hr after THC). Dotted traces reflect ± S.E.M. across animals and sessions. Inset at upper right shows illustration of isolated single G-cell action potentials to CS+ tone on a single trial. Callibration: PEH = 0.2 spikes/10 msec bin/trial; upper right = 0.2 mV. (Figure reproduced with the permission of Williams and Wilkins, publishers of *The Journal of Pharmacology and Experimental Therapeutics,* from Campbell *et al.,* 1986, 239:941-945).

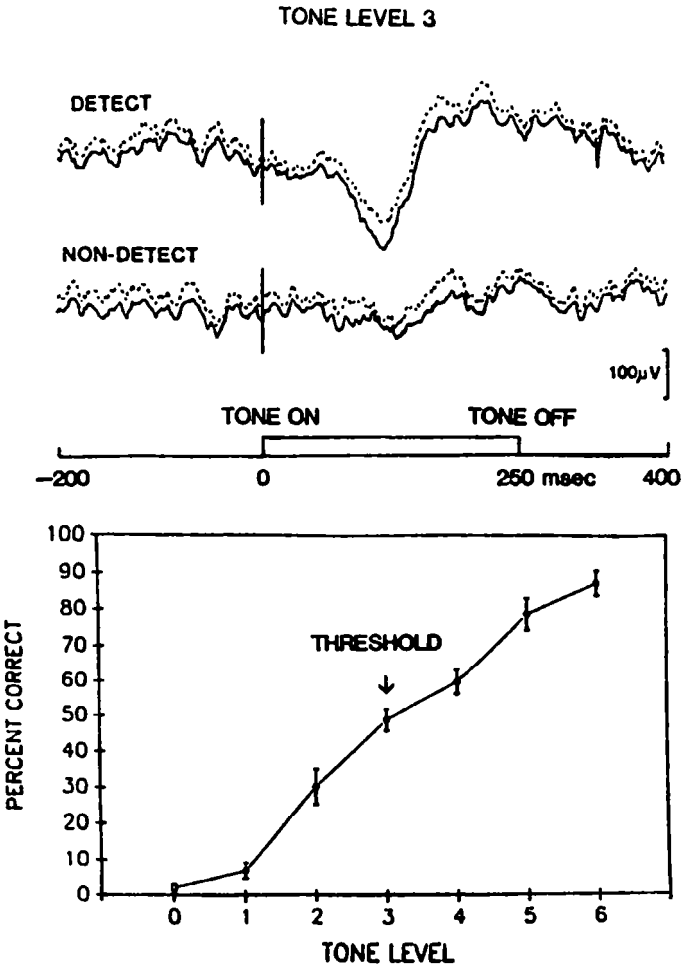

Figure 4. Averaged tone evoked potentials recorded from dentate gyrus (Fig. 2) during signal detection task for level 3 tone (threshold) only. Potentials were sorted into two categories: detect (in which a behavioral response occurred) trials vs nondetect (no behavioral response) trials. A time-locked evoked potential (N2) is present only on detect trials (upper trace). The N2 component is present as reported previously when the single tone conditioning paradigm is utilized. Onset and offset of the tone stimulus is indicated. Graph at bottom indicates individual animal performance at the various tone intensity levels with threshold (tone level 3) defined as that intensity at which 50% responding occurred. Tone level 3 was set daily for each rat, all other intensities were proportionately increased or decreased in intensity relative to that tone level. AU tone levels except "0" were in the detection range of cells recorded from the inferior colliculus under similar conditions (Campbell et al. 1986b).

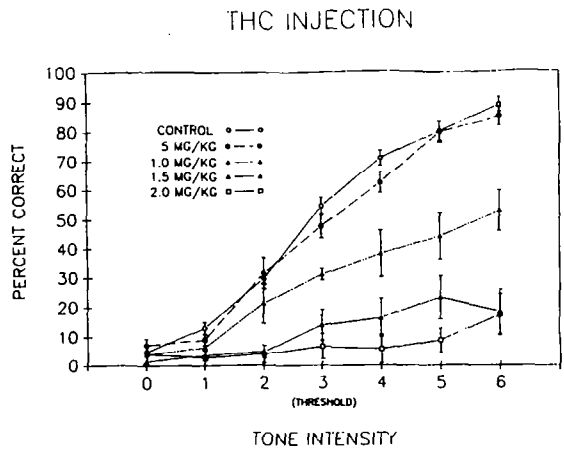

Figure 5. Effects of delta-9-THC on signal detection task. Mean behavioral response for each tone level averaged across eight separate animals. Delta-P THC systematically depressed responding to all tone intensities in a dose-dependent fashion. The 0.5 mg/kg dose was without effect while the 2.0 mg/kg dose suppressed responding at all intensities.

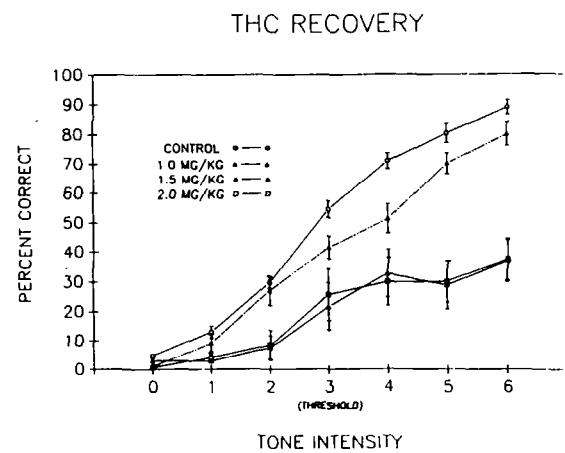

Figure 6. Recovery from the effects of delta-9-THC on signal detection performance. Each curve represents performance two-four hours after delta-9-THC injection at the indicated dose levels. Note that recovery was also dose-dependent suggesting residual effects of delta-9-THC on neural systems which were both temporally as well as magnitude related.

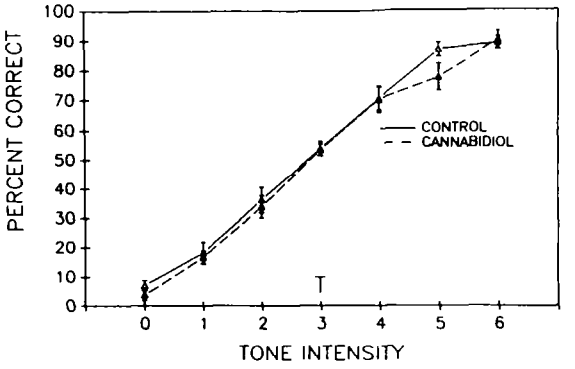

Figure 7. Lack of effect of nonpsychoactive derivative of delta-9-THC, cannabidiol (2.0 mg/kg) on signal detection performance. Preliminary evidence indicates that even at dose levels twice as high cannabidiol had no effect on the signal detection behavior.

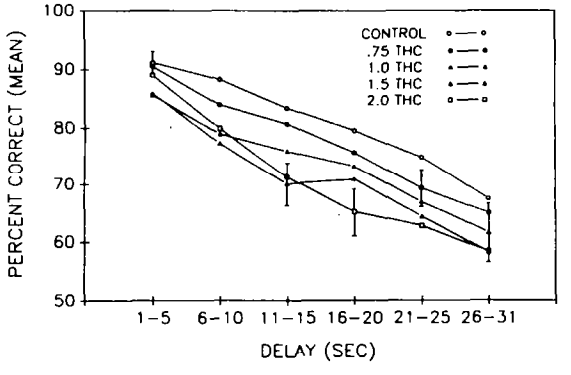

Figure 8. Effects of delta-9-THC (dose range 0.5-2.0 mg/kg) on DMTS performance. Higher doses of delta-9-THC were associated with more pronounced deficits in performance as a function of longer delay intervals. There were no significant differences between any of the doses at the 1-5 sec delay interval indicating that performance aspects of the task were not disrupted, while deficits in the memory requirement of the task increased with increasing doses of delta-9-THC as well as increasing length of delay. Only the largest standard errors in each group are plotted so as not to obscure visual comparisons between curves at each of the delay intervals.

Actions of Cocaine on Central Monoamine Neurons: Intracellular Recordings *In Vitro*

J. Williams and M. Lacey

SUMMARY

Intracellular recordings from monoaminergic neurons in brain slice preparations have been used to study the action of cocaine. The firing rate of noradrenergic neurons in the locus coeruleus, serotonergic neurons of dorsal raphe and dopaminergic neurons from substantia nigra zona compacta is reduced or inhibited by the transmitter substance that they produce. This process, which can be brought about by release of the transmitter from either dendrites or axon collaterals in the vicinity of the cell bodies, has been referred to as 'autoinhibition'. In each case, the inhibition results from a membrane hyperpolarization caused by an increased conductance to potassium ions. In each of the nuclei, cocaine caused a decrease in spontaneous firing through a membrane hyperpolarization. This action of cocaine was blocked by the respective 'autoreceptor' antagonist. In the locus coeruleus and dorsal raphe cocaine prolonged an inhibitory postsynaptic potential caused by the release of noradrenaline and 5-HT respectively. The concentration-response curve for the exogenously applied agonist in each of the three nuclei was shifted to the left in the presence of cocaine. These observations indicate that the activity of these aminergic neurons was affected by cocaine through an inhibition of the reuptake of endogenously release transmitter. The blockade of reuptake increased the extracellular level of noradrenaline, 5-HT and dopamine in the respective nuclei to concentrations which caused electrophysiologically detectable effects. This suggests that the reuptake process is a primary mechanism for termination of the response. In addition, this action of cocaine occurs at behaviorally relevant concentrations. The fact that all monoaminergic transmission can be affected by cocaine may contribute to the complexity of its behavioral actions.

INTRODUCTION

The best known action of cocaine is the inhibition of monoamine reuptake (reviewed by Lakoski & Cunningham, 1988). Such reuptake sites are transmembrane carrier proteins whose function is dependent on a sodium gradient between the outside and inside of the cell. These reuptake sites are found on neurons which produce the monoamine transmitters. For each of the monoamines, noradrenaline, 5-HT and dopamine, the carrier protein on a single cell type is relatively specific for the amine which it takes up. Unlike 'receptor' pharmacology where antagonists can have 100-1,000 fold selectivity, agents that block the reuptake of the monoamines do not show great specificity. Cocaine is one such example. The IC50 of cocaine to block [^{3}H] mazindol binding at noradrenaline and dopamine uptake sites and [^{3}H] paroxetine binding at 5-HT sites ranged from 150 nM to 1.5 µM (Ritz, Lamb, Goldberg & Kuhar, 1987). The concentration of cocaine required to block monoamine reuptake into slices or synaptosomes ranged from 48 nM to almost 10 µM depending on the conditions of the experiment (Calligaro & Eldefrawi, 1987; Reith, Sershen, Allen & Lajtha, 1983; Heikkila, Orlansky & Cohen, 1975). Such an observation indicates a basic similarity in the binding site on which cocaine acts for each of the reuptake protein(s).

Monoamine-containing neurons in each of the locus coeruleus, dorsal raphe and substantia nigra, have receptors ('autoreceptors') for the transmitter that they produce (α_2, 5-HT_{1A}, and D_2 respectively). Neurons in each nucleus are hyperpolarized by an increased conductance to potassium following activation of their respective 'autoreceptor' (Williams, Henderson & North, 1985; Williams, Colmers & Pan, 1988; Lacey, Mercuri & North, 1987; Tepper, Groves & Young, 1985). There is also evidence of dendritic or recurrent collateral release of transmitter from neurons in each nucleus (Egan, Henderson, North & Williams, 1983, Surprenant & Williams, 1987; Yoshimura & Higashi, 1985; Korf, Zieleman & Westernik, 1976; Geffen, Jessel, Cuello & Iversen, 1976). Finally, in the locus coeruleus and dorsal raphe electrical stimulation in the area of the nucleus results in an inhibitory postsynaptic potential (ipsp) that is mediated by noradrenaline and 5-HT, respectively. The presence of a high density of monoamine containing neurons (therefore uptake sites) and a robust response to the respective monoamine transmitter render each of these three nuclei ideally suited to study the action of cocaine using electrophysiological methods.

METHODS

Slices from rat brain containing each of the three nuclei were prepared as has been described previously (Williams, Henderson, Scefner, Nishi & Egan, 1984; Williams, Colmers & Pan, 1988; Lacey, Mercuri & North, 1987). The recording conditions were identical in the three preparations. Slices (300 µm) were cut on a vibratome and placed in a tissue bath, submerged in flowing

(1.5 ml/min) physiological saline at 37 °C. Intracellular recordings were made with KCl (2 M) filled electrodes having resistances of (30-80 MΩ). Drugs were applied in known concentrations by changing the superfusion solution to one which contained the drug and also by pressure ejection. The tip of pressure ejection pipet was placed in the superfusion solution above the surface of the slice. Synaptic potentials were evoked with bipolar tungsten electrodes placed in the area of the nucleus. In some experiments agonist and synaptic currents were measured using a single electrode voltage clamp.

RESULTS

Locus coeruleus.

Neurons of the locus coeruleus fire action potentials repetitively in the slice preparation in the absence of an applied stimulus (Williams, North, Shefner, Nishi & Egan, 1984). Cocaine (1-30 µM) caused a reduction of spontaneous action potential firing and an outward current (Surprenant & Williams, 1987, FIGURES 1-3). This action occurred over a period of 5-10 min following the onset of superfusion with cocaine and reversed 15-30 min following the washout of cocaine. The maximum effect of cocaine by itself was a complete inhibition of firing, a hyperpolarization of 3-10 mV or an outward current of 20-120 pA. The inhibition in firing and outward current caused by cocaine were antagonized by the α_2-receptor antagonist, idazoxan. In the absence of cocaine idazoxan had no effect on firing rate or membrane potential. This observation indicates that the effect of cocaine resulted from α_2-adrenoceptor activation, probably resulting from an increase in the extracellular concentration of noradrenaline released from locus coeruleus neurons.

Application of noradrenaline caused a membrane hyperpolarization by an increased conductance to potassium ions, an action mediated through α_2-adrenoceptors (Williams, Henderson & North, 1985). The dose response curve to noradrenaline applied by superfusion was shifted to the left by cocaine. The shift to the left in the dose response curve was dependent on the concentration of cocaine applied. The maximum shift of the noradrenaline dose response curve by cocaine varied from 10-110 fold among different neurons. This observation was probably dependent on the location within the slice that the recording was made. That is, there were probably a variable number of uptake sites between the neuron under study and the superfusion solution containing a known concentration of noradrenaline. In spite of the variability of the potentiation of the noradrenaline response by cocaine, the EC50 (3-5 µM) and concentration that caused a maximum effect (30 µM) were similar among different cells (Surprenant & Williams, 1987). It was also apparent that the reuptake mechanism in the locus coeruleus had a large capacity. The hyperpolarization or outward current induced by noradrenaline (even at 100 µM) was increased by cocaine, indicating that 100 µM noradrenaline did not saturate

the reuptake process.

FIGURE 1.

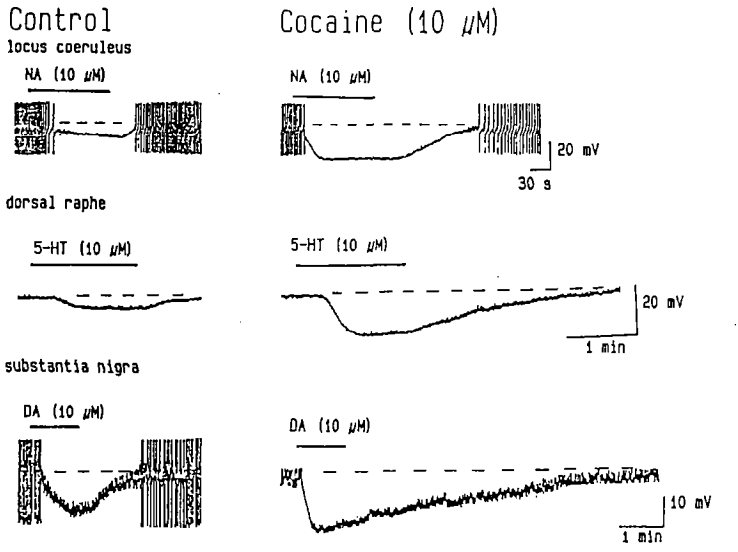

FIGURE 1. *Cocaine potentiates the hyperpolarization caused by exogenously applied noradrenaline (NA) in locus coeruleus. 5-HT in the dorsal raphe and dopamine (DA) in the substantia nigra. All traces are of membrane potential at rest. In the dorsal raphe the hyperpolarization caused by cocaine is not shown.*

The noradrenergic inhibitory postsynaptic potential (ipsp) in locus coeruleus ranged in amplitude from 5-20 mV. The duration at the half-maximum amplitude was about 1s and had a total duration of about 2 s. The amplitude and duration of the ipsp were increased by cocaine (FIGURE 2). Although the increase in amplitude of the ipsp produced by cocaine occurred in all cells, this was not concentration dependent and varied widely among cells. The increase in duration of the ipsp was however dependent on the concentration. The maximum increase in the duration of the ipsp at the half-maximum amplitude was about 8 fold at a cocaine concentration of 30 μM: The EC50 was 4 μM and 1 μM caused a 2 fold increase in duration (Surprenant & Williams, 1987).

Dorsal raphe.

Under conditions where neurons of the locus coeruleus neurons fired spontaneously, dorsal raphe neurons did not. The resting membrane potential of dorsal raphe neurons was near -65 mV in the slice preparation. Cocaine (100 nM - 30 μM) caused a hyperpolarization of the membrane which was slow in onset (15-30

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min) and washout (>30 min). The hyperpolarization induced by cocaine (10-30 µM) was 10-15 mV in amplitude. In cells where cocaine produced a 15 mV hyperpolarization, 5-HT had little or no additional effect. Although 90% of neurons were hyperpolarized by cocaine, the amplitude of the hyperpolarization varied from cell to cell and from slice to slice. Presumably the. hyperpolarization resulted from accumulation of spontaneously released 5-HT in the extracellular space following the blockade of reuptake.

Exogenously applied 5-HT hyperpolarized dorsal raphe neurons by increasing a potassium conductance that is similar to the potassium conductance increased α_2-adrenoceptor agonists in the locus coeruleus. This action of 5-HT is thought to be mediated by the 'autoreceptor' (5-HT_{1A}) and is blocked by spiperone (1 µM). The sensitivity of dorsal raphe neurons to 5-HT was increased in the presence of cocaine (300 nM - 10 µM, FIGURE 1). Quantification of the increase in sensitivity to 5-HT was *not* possible as in many cells cocaine by itself hyperpolarized the cells, sometimes to a potential that was near the maximum effect. Experiments to determine the sensitivity of the cells to both cocaine and to exogenously applied 5-HT required the elimination *of* endogenous 5-HT. Another approach to study the action of cocaine in dorsal raphe was to use endogenous 5-HT that was released synaptically.

FIGURE 2.

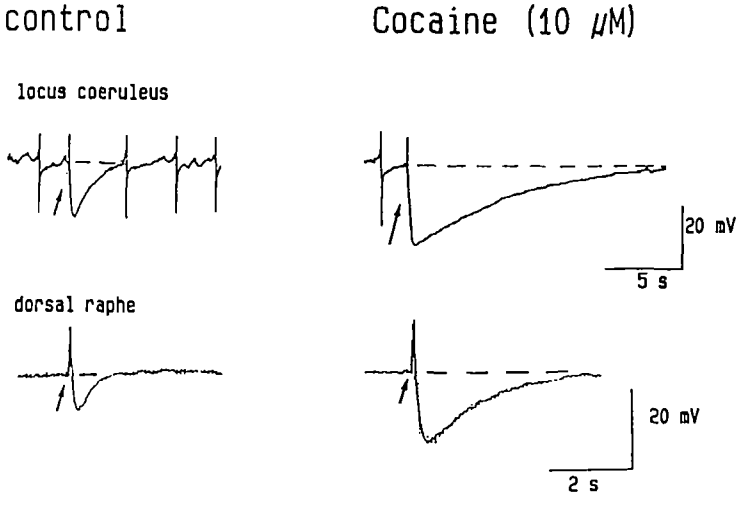

FIGURE 2. Cocaine potentiates the inhibitory postsynaptic potential in locus coeruleus and dorsal raphe.

The 5-HT mediated ipsp in the dorsal raphe was sensitive to cocaine. The duration of the ipsp was increased by cocaine, as was found for the ipsp in locus coeruleus (FIGURE 2). The prolongation of the ipsp in dorsal raphe was more sensitive to

cocaine than that in the locus coeruleus. A two fold increase in the ipsp duration was obtained with 300nM cocaine in the dorsal raphe, (c.f. 1 µM cocaine in the locus coeruleus). Cocaine (300 nM - 10 µM) also increased the amplitude of the ipsp (FIGURE 2). Higher concentrations caused a depression of the amplitude. This effect on the amplitude of the ipsp was similar *to* that found in the locus coeruleus but occurred at about three fold lower concentrations.

Substantia nigra.

Cells in the substantia nigra zona compacta fire action potentials spontaneously at a rate of 1-5 Hz in the slice preparation. These presumed dopaminergic neurons are hyperpolarized by agonists acting on dopamine D_2-receptors through an increase in potassium conductance (Lacey, Mercuri & North, 1987). Cocaine (1-10 µM) abolished or reduced the rate of action potential firing (FIGURE 1). When the cell was either hyperpolarized by passing sufficient current through the electrode to prevent action potential firing or voltage clamped at -60 mV, cocaine (10 µM) caused a hyperpolarization (2-8 mV) or outward current (20-120 pA). The amplitude of the hyperpolarization or outward current caused by cocaine (10 µM) was about one third of the maximum effect that could be evoked by application of dopamine in the presence of cocaine. This action of cocaine occurred within 5-10 min following application and recovered within 30 min of washout. The D_2 receptor antagonist (-)-sulpiride (1 µM) rapidly reversed the effect of cocaine, but did not have any effect in the absence of cocaine.

FIGURE 3.

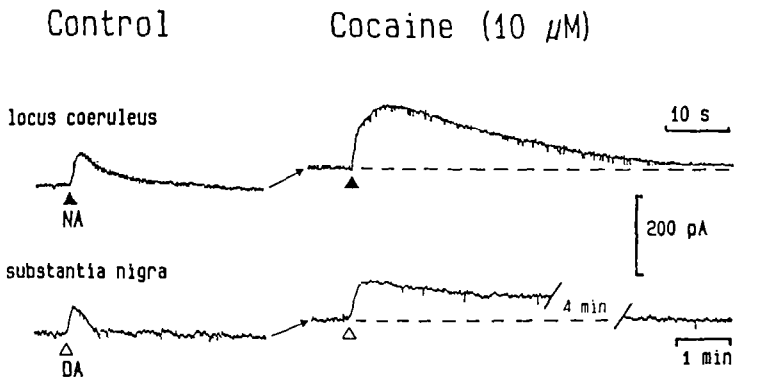

FIGURE 3. *Cocaine increased the amplitude and duration of the outward current caused by pressure ejection of noradrenaline (NA) in the locus coeruleus and dopamine (DA) in the substantia nigra. The holding potential in each was about -60 mV.*

The hyperpolarization or outward current induced by dopamine was increased in amplitude by cocaine (10 μM, FIGURES 1&3). Such an action was observed when dopamine was applied by pressure ejection or by superfusion. When dopamine was applied by pressure ejection the effect of cocaine was particularly dramatic in both time course and amplitude (FIGURE 3). When applied by superfusion the concentration-response curve to dopamine was shifted to the left. Since cocaine already had caused a substantial effect itself (probably by increasing the extracellular concentration of endogenous dopamine), the analysis of the shift in the dose response curve could not be quantitatively interpreted. As was found in the locus coeruleus, the outward current caused by dopamine (100 μM) in the presence of cocaine (10 μM was greater than that in the absence of cocaine.

DISCUSSION

The action of cocaine by itself was similar in each of the three brain nuclei; locus coeruleus, dorsal raphe and substantia nigra. In each case the excitability was decreased in the presence of cocaine and in each the respective 'autoreceptor' antagonist reversed this inhibition by cocaine. Similar results have been obtained using extracellular recording in vivo. Local application of uptake inhibitors have been found to inhibit the firing of cells in each of the three nuclei (see Lakoski & Cunningham, 1988). In vivo, autoreceptor antagonists have been found to increase the rate of spontaneous firing in these nuclei. The results obtained in brain slices suggest that there is a low level of transmitter release that is normally below the level of electrophysiological detection, since autoreceptor antagonists had no excitatory action. When reuptake was blocked, transmitter accumulates even in a superfused slice to a level which was physiologically measurable.

The sensitivity of neurons in each of the three nuclei to monoamine transmitters was increased by cocaine. This increased sensitivity was observed when monoamines were applied exogenously or released synaptically. In each case the duration of monoamine action seemed to be the most sensitive measure of the action of cocaine. Such a result is dependent on the conditions of the experiment. In a situation where diffusion is very limited, as is found in brain (even in brain slices), uptake or metabolism of transmitter is the primary mechanism for termination of response. In these experiments, one might expect diffusion to be similar in each of the slice preparations. In preparations where there are few or no diffusion barriers, such as the submucous plexus of the guinea pig ileum, uptake or metabolism of transmitter has less of an effect on the duration of transmitter action. In the submucous plexus, cocaine prolonged noradrenergic transmission but the increase was less than half that found in the locus coeruleus (Surprenant & Williams, 1987). Similarly in the submucous plexus cocaine caused an increase in the sensitivity to noradrenaline of about

2 to 3 fold compared to 30 to 100 fold in locus coeruleus. Such an observation probably results from differences in diffusion in the two preparations.

In the projection areas of the neurons in these three nuclei the levels of monoamines will be determined by the interaction between impulse activity in the nerve terminal and the dynamics of the monoamine uptake system. Both of these will be affected by cocaine. Depolarization-induced release might be expected to be reduced (due to the inhibition of firing of the cell body), but the effect of released monoamines would be enhanced by the blockade of uptake. One possible consequence of the action of cocaine in the cell body region of monoamine neurons may be to shift the control of their activity from being largely regulated by intrinsic membrane properties to being more dependent on excitatory synaptic input.

The acute action of cocaine in each of the three nuclei is consistent with a blockade of the reuptake of monoamine transmitters. This action, in the absence of any evoked input, has an inhibitory effect in the slice preparation. One might expect that such an inhibitory action would be even greater in vivo where monoaminergic tone is higher. The concentration range of cocaine which was effective in the slice (100 nM to 10 µM) is comparable to that which is found in the plasma following a euphoria-inducing dose of cocaine in human subjects (1 µM, 5 min after 32 mg given intravenously, Javaid, Fishman, Schuster, Dekirmenjian & Davis, 1978). Given the diversity of the projections of monoaminergic neurons, it is likely that cocaine will alter neurotransmission at a muliplicity of sites in the brain. Its effects on any given region or pathway will probably be determined by the density and type of monoaminergic innervation. This implies that the psychotropic effects of cocaine are due to complex modifications of the activity and ineractivity of several neuronal systems.

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AUTHORS

John T. Williams, PhD.
Michael G. Lacey, PhD.

Vollum Institute
Oregon Health Sciences University
3181 SW Sam Jackson Park Road
Portland, OR 97201

Recognition and Activation of a 5-HT Receptor by Hallucinogens and Indole Derivatives

H. Weinstein, R. Osman and G. Mercier, Jr.

INTRODUCTION

Recent developments in identification, isolation, purification and structural. analysis of target proteins have enhanced the capabilities of theoretical approaches to analyze, describe and even predict biological mechanisms. These developments have focused attention on the role of the receptor macromolecule in generating and modulating the recognition and activation steps which are the primary molecular events in ligand mediated receptor functions. It is clear that understanding this role of the receptor macromolecule is crucial for understanding the process of biological signal transduction. Consequently, new theoretical approaches are being developed and applied to study the entire sequence of molecular events leading from ligand recognition to the triggering and modulation of a receptor activation mechanism. Elements of such a comprehensive picture of the molecular mechanisms of action at receptors for the neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) are summarized here, based on a brief review of results from early studies on the molecular determinants for RECOGNITION at these receptors, a short introduction to a proposed model for receptor ACTIVATION, and the insight obtained recently from the simulation of the proposed activation mechanism inside a PROTEIN MODEL of a receptor environment.

Our interest in the elements of molecular recognition at 5-HT receptors and the activation of these receptors by full and partial agonists, stems from our search for molecular determinants for the actions of hallucinogens. The receptors of the neurotransmitter 5-HT in brain constitute a prime target for the action of these drugs (for a recent review see Arvidsson et al., 1986). Hallucinogenic compounds belong to chemically and structurally different classes, including indolylalkylamines, ergolines, and phenylalkylamines. Even small structural changes within each class seem to alter hallucinogenic activity and potency on receptors. Consequently, we first used the methods of theoretical chemistry to examine and compare molecular

properties and reactivity characteristics of structurally dissimilar molecules in these classes, in an approach to structure-activity relations which we pioneered and developed (Weinstein; 1975; Weinstein et al. 1981). Following this approach, the properties of the dissimilar molecules, e.g., their molecular electrostatic potentials (MEP) and electric fields they generate, were calculated and compared for active and inactive compounds in order to identify the common molecular determinants for recognition (Weinstein et al., 1981). Based on these recognition determinants, described below, we then proposed a model for the receptor recognition site that could exhibit the discriminant properties evidenced by the rank order of affinities of congeners and related compounds at 5-HT binding sites. Simulation of the properties of the recognition model yielded the specific receptor activation model that is also briefly described below. To gain insight into the role of the receptor protein environment in the modulation of the putative activation model, we simulated the activation mechanism inside a specific protein. In the absence of a detailed molecular structure for a 5-HT receptor , we adopted a heuristic approach in which the activation mechanism is simulated inside a protein of known structure, selected according to a set of specific criteria and assumptions. The simulation revealed the role that the macromolecular structure can play in the modulation of such a mechanism (Mercier et al., 1988; 1988b), and identified new sets of steric and field constraints that would contribute to the discrimination of agonists and antagonists at the receptor. These new findings are also reviewed briefly.

MOLECULAR DETERMINANTS FOR RECOGNITION

To identify the molecular properties that are essential for recognition at 5-HT receptors, we simultaneously analyzed and compared the reactivity characteristics of compounds that have affinity for 5-HT binding sites, and/or are active as agonists and antagonists at the 5-HT receptors linked to adenylate cyclase (5-HT_1) or on the 5-HT_2 receptor mediating the contraction of the rabbit aorta (see references and details in Weinstein et al., 1987). On that basis we proposed a mechanistic hypothesis to explain the rank order of the affinity of 5-HT congeners that bind to 5-HT sites and receptors (Weinstein et al., 1981). This hypothesis relates the recognition of structurally homologous compounds, expressed in the rank order of their affinity, to the intramolecular rearrangement needed to make a 5-HT congener recognizable at the binding sites. (For a recent review see Weinstein et al., 1987). The molecular reactivity criteria for 5-HT-like recognition (Weinstein et al., 1978) are generalizable to other structural families (Reggio et al., 1981) including LSD and its derivatives (Weinstein et al., 1981; Weinstein et al., 1987). These theoretical findings were confirmed experimentally (Weinstein et al., 1978; Reggio et al., 1981; Mazurek et al., 1984), most recently for a series of compounds designed at Roussel and tested

for activity on 5-HT and histamine receptor systems (Weinstein et al., 1987).

Based on these theoretical and experimental results, we identified the elements of recognition for 5-HT and LSD analogs that interact with the serotonin receptor. These are common to the hallucinogenic derivatives of 5-HT (e.g., psilocin), to LSD and its congeners, and to the Roussel compounds. The elements of recognition were tested by further theoretical work in which we simulated the complexation between 5-HT congeners and a model recognition site. The model was chosen, from considerations of complementarity to the properties of the ligand, to be the imidazolium cation (Weinstein et al., 1981). The complexes between various 5-HT congeners and imidazolium were then simulated computationally (Weinstein et al., 1981; Reggio et al., 1981). The results confirmed the key role of the electrostatic interaction in the formation of these complexes and thereby explained the basis for the success of the electrostatic orientation vectors that we had defined (Weinstein et al., 1978; 1981) as discriminant predictors of recognition requirements.

FROM RECOGNITION TO ACTIVATION

The simulation of interactions of the imidazolium cation with derivatives of 5-HT and hallucinogenic compounds - which served to test the proposed recognition mechanism - yielded information on the consequences of the complexation. This information determined a proposed model for activation. Thus, the analysis of the changes produced in the electron density distribution of imidazolium, chosen as the heuristic receptor model, by its interaction with 5-HT indicated a major rearrangement around the N-H bond. The change in proton affinity of the nitrogen suggested that the interaction may induce a proton transfer from the imidazolium cation to a potential proton acceptor (Osman, et al., 1985, 1987). The construction of the proton transfer model (PTM) followed the heuristic approach we are taking in that it conserved the elements of recognition which characterized the imidazolium cation, and extended the receptor model to contain a responsive function in the form of a proton transfer from imidazolium to an ammonia molecule which could function as the proton acceptor.

Using this heuristic model, our studies showed that in the absence of 5-HT the process of proton transfer from imidazolium to ammonia has a high barrier and a minimal driving force. When 5-HT approaches the PTM, the interaction lowers the barrier and generates an appreciable driving force for the proton transfer. These studies pointed to the combination of molecular determinants for recognition with the primary steps in the triggering of the proton transfer, as a plausible molecular mechanism of activation of a 5-HT receptor (Osman, et al., 1985, 1987). It is noteworthy that such proton transfer systems are ubiquitous in proteins (Scheiner, 1985; Scheiner et

al., 1986) and often serve as the responsive, catalytic, components in enzymes (Fersht, 1985).

Because the proton transfer process takes place in a protein, the energetics of the process will be affected by its environment. It is of interest, therefore, to learn the role that a protein structure can play in such a process. The effect of the protein environment on a proton transfer has been investigated in several systems (Allen, 1981; Tapia, et al., 1985; Thole and van Duijnen, 1983; Umeyama, et al., 1984; Warshel and Russel, 1986) and the primary effect was found to be electrostatic in nature. It is thus likely that the protein environment will modulate the proposed activation process through a similar electrostatic effect.

ROLE OF PROTEIN STRUCTURE IN ACTIVATION

In order to gain insight into the nature of the electrostatic effect that the protein environment may have on the proposed activation process inside a receptor whose structure is not known, we had to continue the heuristic approach. In this context, the heuristic approach consists of evaluating the effect of a protein of known structure, e.g., an enzyme, in terms of the contributions from elements of secondary and tertiary structure of the protein. Structural considerations and the nature of the activation mechanism make it reasonable to assume that the role played by the receptor-macromolecule in the process will be analogous to that of the polypeptide environment in the catalytic activity of enzymes (Weinstein et al., 1985; Liebman and Weinstein, 1985). Notably, the sparse structural data available for receptors as well as the hydrophobicity properties of the protein sequences in the isolated and cloned receptors (Schofield et al., 1987) suggest that structural features identified as important for the activity of enzymes, e.g. alpha helices (Hol et al., 1978) are also major components of the membrane. bound protein systems (Allen at al., 1987; Yeates et al., 1987). It is reasonable, therefore, to seek insight into the structure-function relations in the 5-HT receptor of unknown structure, by using the high resolution structural data of enzymes as heuristic model systems. Because the structure-function relationships in the latter can be defined in terms of structural elements that are expected to be common to both protein systems, such inferences would be based on the assumption that the function of the structural elements indentified in the enzyme is conserved in the receptor protein by virtue of the molecular properties inherent in the structure. Indeed, enzymes have already been used as model systems for studying receptor mechanisms (Goodford, 1980; Weinstein et al., 1985). However, contrary to enzyme catalysis where the polypeptide structure is expected to aid the steps in the reaction coordinate of the catalytic mechanism, the polypeptide structure of a heuristic model for a receptor would be expected to hinder those steps that lead to receptor activation;

otherwise, the polypeptide structure would trigger a response in the absence of the ligands that are responsible for receptor activation.

The choice of a particular enzyme model system to study the effect of the protein structure on the activation mechanism depends on inferences regarding the nature of the specific neurotransmitter binding site, and the available details concerning the putative mechanism for the activation of the receptor. In our heuristic studies we selected-actinidin as a model protein for the receptor environment because it contains an appropriate juxtaposition of groups that can participate in a proton transfer process, and its structure is well characterized in terms of its secondary and tertiary structural components. Such components are identified in all proteins including neurotransmitter receptors. Based on the premise that the structural elements which are conserved across different proteins and have similar properties, also conserve their function, the heuristic approach 'we take represents a new strategy in the study of structure-function relationships in receptors.

Computational Details

Actinidin (E.C. 3.4.22.14) was identified among the proteins in the Brookhaven Protein Data Bank (PDB) (Bernstein et al., 1977), as a protein that fulfills the requirement of containing a putative proton transfer model. The proton transfer system consists of His 162 hydrogen bonded to Cys 25. Trp 184 is in close proximity to this system and is aligned in a stacking geometry with respect to the imidazole ring of His 162.

The potential energy curve for proton transfer between ND1 of His 162 and SG of Cys 25 was obtained from ab initio quantum mechanical calculations on a model system consisting of the side chains modeled by imidazolium and by methanethiol (Mercier et al., 1988). Thus, the proton transfer is in a direction opposite to that assumed in the catalytic process of actinidin, and the system represents an inactive form of the enzyme that exists at low pH. The relative geometry of the components of the proton transfer system were taken from the crystallographic coordinates of the enzyme. The critical points on the potential energy curve for proton transfer were obtained by a full optimization of the positions of the hydrogen involved in the proton transfer process and the hydrogens bound to the methanethiol; all other internal coordinates were kept frozen. To test the basis set dependence of the calculations, several were used ranging from a minimal STO-3G to an extended 6-31G** basis set. The qualitative nature of the results was found to be basis set independent; we present below some of the results obtained from calculations with the minimal basis set.

The electrostatic effect of the protein environment was calculated as $ES = \sum V(R_j)\, Q_j$, where $V(R_j)$ is the electrostatic

potential generated by the proton transfer system at the position of the point charge Q_j, representing an atom of the protein. The electrostatic potential was calculated from the wave function of the proton transfer system. The point charges, Q_j, were obtained from a dipole conserving population analysis (Thole and Van Duijnen, 1983a) of the wave-functions of the amino acids of the protein, obtained from Hartree-Fock calculations with the Mehler-Paul basis set (Mehler and Paul, 1979). The incorporation of the point charges from the environment, Q_j, into the quantum mechanical Hamiltonian gives a small polarization of the wave function that is nearly constant as a function of the proton movement (Mercier et al., 1988 b). A useful approximation of the electrostatic interaction energy can be obtained by representing the quantum motif by a collection of point charges obtained from a Mulliken population analysis of its wave function (Weinstein et al., 1981b).

EFFECTS OF THE PROTEIN STRUCTURE ON THE PROTON TRANSFER

The potential energy curve of the proton transfer in the imidazolium/methanethiol complex calculated in vacuum, shown in Figure 1, reflects the basic difference in the proton affinities of the neutral molecules. In the complex, the form with the proton on the sulfur (M2) is 35.6 Kcal/mole higher than that with the proton on imidazole (Ml), and the barrier to the transition state (TS) for the proton transfer from imidazolium to methanethiol is 39.0 Kcal/mole. Calculated in the presence of the protein (QxQ FULL), the potential energy curve changes and loses the double-well character which is typical of hydrogen bonded systems (Fig.1). This is due to a differential effect of the protein on the three extrema of the curve. The electrostatic interaction energy between the proton transfer system in the Ml geometry and the protein is -315.5 Kcal/mol, it is only -299.7 Kcal/mole in geometry M2, and -304.6 in the TS geometry. Consequently, the energy of M2 relative to that of Ml is raised by 15.5 kcal/mole. This effect of the protein on the proton transfer system is consistent with the proposed catalytic role of the protein (Thole and van Duijnen, 1983; Kollman and Hayes, 1981), which stabilizes the zwitterionic state in which a proton has been transferred from Cys 25 to His 162, i.e., in a direction opposite to the one considered here. As in the native enzyme, in the proton transfer system the protein prefers the geometry with the proton on imidazole (M1) over that with the proton on the sulfur (M2). However, there is an important difference between the effect of the protein on the proton transfer system and its original effect on the catalytic site. In the enzyme the protein is stabilizing a. charge separated, zwitterionic, state which differs very significantly from the neutral state-primarily by a large dipole moment generated by the charge separation (see Dijkman et al., 1988 - and references therein). On the other hand, in the proton transfer system studied here, from imidazolium to the thiol

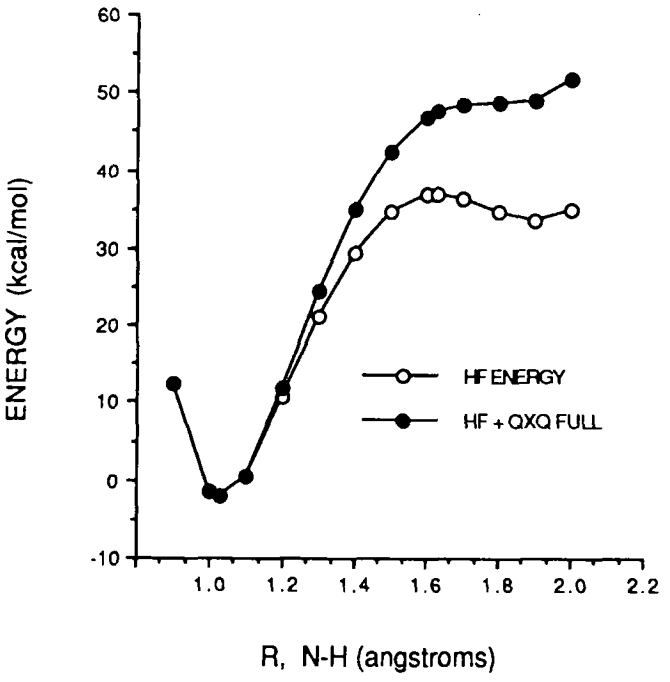

Figure 1. Potential energy curve for proton transfer in the imidazoliun/methanethiol system in vacuum and in the presence of the full protein. Energies are relative to separated imidazolium and methanethiol.

of Cys 25, the protein exhibits a sensitivity to the position of a proton that moves across a distance of approximately 1A between two neutral ligands. An important question is whether this sensitivity is due to the strong inhomogeneity of the potential generated by the protein in the limited space between the proton donor and acceptor, or due to the sensitivity of the protein to the changes in the components of the proton transfer system. This question can be answered within the electrostatic approach used here, because the effect of the protein on the individual components of the proton transfer system can be evaluated; the total effect is the sum of the effects on the individual components. Such a decomposition is shown in Figure 2 as a function of the position of the proton.

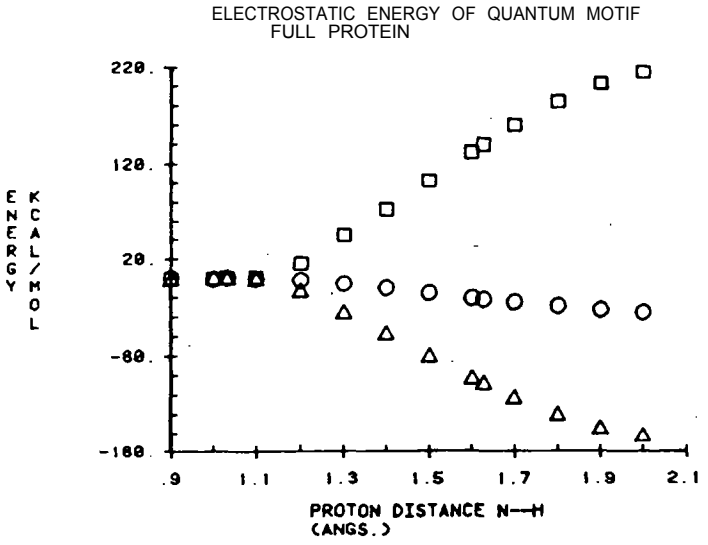

Figure 2. The effect of the protein on the components of the proton transfer system as a function of the position of the proton: The energy of protein interaction with (□) imidazole, (△) methanethiol, (○) the proton.

The results clearly demonstrate that the electrostatic potential generated by the protein is not strongly inhomogeneous and is not the source of the observed effect. First, the electrostatic interaction energy changes with the moving proton only by 33 kcal/mole over a distance of 1A. Secondly, the protein stabilizes the proton as it moves from imidazole towards the methanethiol. This effect is opposite to what is expected if the effect of the protein on the movement of the proton were due to an inhomogeneity in the electrostatic potential. In fact, the effect of the protein on the methanethiol and the imidazole is much larger and ultimately determines the total electrostatic effect. As the proton moves from the imidazole towards the methanethiol, the protein stabilizes the methanethiol by as much as 154 kcal/mole and destabilizes the imidazole by as much as 202 kcal/mole. Since the changes in the proton donor and the proton acceptor are primarily due to charge redistribution upon the movement of the proton, these results demonstrate that the protein is more sensitive to the changes in the charge distributions of the proton transfer complex than to the position of the proton.

THE ROLE OF LIGAND BINDING

The conclusion regarding the mode in which the protein modulates the proposed activation mechanism has important implications for the suitability of the proton transfer system as a heuristic model of a receptor. As was previously demonstrated

(Osman et al., 1985, 1987) the driving force for the proton transfer in our earlier model (Osman et al., 1987) was due to the charge redistribution induced in imidazolium by the interaction with 5-HT. Similarly, in the present proton transfer system the interaction between an incoming ligand (e.g., 5-HT) and the proton transfer system is expected to induce a charge redistribution which will be further stabilized by the protein. This double effect, triggered by the binding of the ligand, should facilitate the proton transfer and produce a change that propagates through the protein structure. As shown in Table 1, the calculated barrier to proton transfer (TS-M1) and the driving force (M2-M1) in a proton transfer model taken from actinidin, are sensitive to the simulated binding of the ligand. The simulation was carried out by mutating the indole portions of the compounds listed in the table into the protein at the position of Trp 184, and calculating the proton transfer energies (His 162 to Asn 182) in the presence and absence of the simulated ligands. The ligands include tryptamine (TRYP) and single substituted 5-HT congeners as well as methylenedioxy derivatives (MDOX) and 5-carboxamidotyptamine. At this preliminary stage no attempt was made to optimize the orientation of the ligand towards the proton transfer complex. The results show that even in this non-optimal position, the various compounds are found to affect the proton transfer energy curve in the direction expected from activating ligands - i.e., lowering the barrier and increasing the driving force for the movement of the proton.

TABLE 1. Simulation of the effect of 5-HT agonists on the proton transfer energy (Orientation of drugs = Trp 184)

	E Barrier[a] (TS-M1)	E Minima[a] (M2-M1)
5-OH TRYP	-2.5	-4.4
TRYP	-2.4	-4.2
4-OH TRYP	-2.1	-3.6
6-OH TRYP	-1.4	-2.5
7-OH TRYP	-1.0	-1.8
4,5-MDOX TRYP	-1.7	-3.4
5,6-MDOX TRYP	-1.6	-2.8
5-$CONH_2$ TRYP	-1.5	-2.5

[a] In Kcal/mol.

CONCLUSIONS FROM THE HEURISTIC MODEL

Insight into the role of protein structures in the mechanisms of drug receptors for which no high resolution structural data are available (such as is the *case* with the membrane bound 5-HT receptors), was gained from the electrostatic effects of the polypeptide structure of actinidin in terms of the contributions from elements of protein structure common to all the proteins for which structural data are available (Richardson, 1981). Recent evidence suggests that such elements of secondary structure are also prominent in the structure of membrane bound neurotransmitter receptors (Schofield et al., 1987). The electrostatic effects computed from such elements of protein structure should be generalizable from proteins of known structure to those for which high resolution structural data is not yet available, if it is assumed that those structural elements which are conserved across protein structures, also conserve their properties and hence their function.

The choice of actinidin as the heuristic model was guided by the main features of the imidazolium/proton acceptor complex that had been previously proposed as a model for the activation mechanism of one of the 5-HT receptors (Osman et al., 1987). The proton transfer mechanism investigated here is therefore different from the imidazole/methanethiol complex that served in the analysis of the catalytic mechanism of actinidin (Thole and van Duijen, 1983; Kollman and Hayes, 1981). Not surprisingly, this proton transfer is opposed by electrostatic interaction with the protein. This is appropriate for a receptor model because the putative proton transfer in a receptor should be triggered only upon binding of the ligand.

The protein was found to be much more sensitive to the charge redistribution in the proton donor and the proton acceptor than to the moving proton. Since the interaction of 5-HT with a receptor model induces a charge redistribution in its components, the protein may affect the proton transfer by interacting favorably with the charge polarized groups (Mercier et al., 1988; 1988 b) and thus facilitating the proton transfer. Molecular mechanisms can be revealed only by quantum mechanical descriptions of the catalytic groups or groups involved in receptor activation because the electronic properties and, hence, the electrostatic properties of the donor and acceptor fragments change upon proton transfer. However, to complete the picture the computations must include not only a description of the electrostatic properties of the surrounding polypeptide structure and the solvent, but also their dynamical properties. The need to include the dynamical properties of the environment is particularly clear in the case of receptor activation, a process expected to produce conformational changes in the macromolecule triggered by the elementary reaction (e.g. proton transfer) elicited by the binding of agonists. Consequently, we are currently investigating the ability of a variety of agonists to trigger the proton transfer within the framework of

the actinidin model of a 5-HT receptor, considering both the proton transfer and the conformational changes induced by it.

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AUTHORS

Harel Weinstein, D.Sc.
Roman Osman, Ph.D.
Gustavo A. Mercier, Jr.

Departments of Physiology and Biophysics
and of Pharmacology
Mount Sinai School of Medicine
of The City University of New York
New York, New York 10029

Effects of Morphine Treatment on Endogenous Opioid Biosynthesis

D. Bronstein, K. Trujillio and H. Akil

Advances in our understanding of endogenous peptide biochemistry and anatomy now provide a framework in which important regulatory questions can be raised. We now know that all peptides containing the opioid amino acid core, Tyr-Gly-Gly-Phe-Leu or -Met (Leu- or Met-Enk), are derived by proteolytic cleavage from one of three opioid precursor proteins (proopiomelanocortin, POMC; prodynorphin, Prodyn; and proenkephalin, Proenk). While POMC processing gives rise to only one opioid peptide domain (i.e., Beta-endorphin; ßE), the opioid core is repeated 7 times in the Proenk sequence (4 copies of Met-Enk and single copies of Met-Enk-Arg^6-Gly^7-Leu^8 (MERGL), Met-Enk-Arg^6-Phe^7, and Leu-Enk), and there are 3 copies of the Leu-Enk sequence in Prodyn (Dyn A, Dyn B, and **α**-neo-endorphin). The number of endogenous opioid peptides may be much greater than those described above since chemically modified (e.g., acetylated or amidated), carboxy-terminal shortened (e.g., $ßE_{1\text{-}27}$ or $Dyn_{1\text{-}8}$), or combined forms (e.g., Peptides E and F) of these peptides have also been detected (see Akil et al., 1988, for review).

Events involved in peptide biosynthesis are similar for the three opioid families. There are unique genes which code for the specific mRNAs corresponding to the POMC, Prodyn, and Proenk proteins. As the mRNA is translated, the precursor molecule is packaged in vesicles where it is cleaved and chemically modified to produce opioid (as well as non-opioid) peptide products. The difficulties involved in studying the regulation of opioid peptide systems becomes apparent when it is realized that regulatory mechanisms may exist at each level of the biosynthetic pathway, i.e., transcription of the gene into heteronuclear RNA (hnRNA), the processing of hnRNA into mRNA and its transport from the nucleus to the cytoplasm, the translation of mRNA into precursor proteins, the processing of the precursor molecules into peptide fragments, the chemical modification of these peptides into forms with altered physiological activity.

Much of what is now known about the regulation of opioid peptide biosynthesis has been derived from studies of endocrine or neuroendocrine systems (e.g., POMC regulation in the anterior and intermediate pituitary gland (Schacter et al., 1982; Akil et al., 1985; Shiomi et al., 1986), hypoglycemic effects on Proenk in the adrenal (Wilson et al., 1984), and osmotic control of Prodyn in the paraventricular/neurohypophyseal system (Sherman et al., 1986)). Results from these systems demonstrate that regulation of opioid peptide synthesis occurs at several levels in the biosynthetic pathway (e.g., transcription, translation, processing) and that different regulatory mechanisms may be engaged, depending on whether cells are acutely or chronically stimulated. Perhaps the most important lesson learned from these studies is that, in general, biosynthetic activity is tightly coupled to secretion, i.e., increased secretion leads to increased peptide biosynthesis and vice versa.

At present, we do not know whether principles of regulation derived from endocrine/neuroendocrine studies are applicable to neural systems. The paucity of knowledge about endogenous opioid regulation in the CNS is attributable to several factors: 1) relatively small concentrations of opioid peptides and mRNAs are present in neural systems; 2) precursor proteins are synthesized in cell bodies while most peptide products are stored and released distally in axon terminals; and 3) it has been difficult to define experimental manipulations which alter the activity of opiate-containing neurons. The development of techniques which were sensitive enough to measure low levels of specific opioid peptides and RNAs at least partially overcame the first 2 problems and permitted the study of whether various experimental treatments might affect endogenous opioid biosynthesis in the CNS.

One of the first CNS regulatory questions addressed in this laboratory was whether exogenous opiate administration would alter endogenous opiate biosynthesis. Early on, Kosterlitz and Hughes (1975) had postulated that morphine tolerance/dependence might result from agonist-induced feedback inhibition of enkephalin biosynthesis. Initial studies did not support this hypothesis, as no changes in ßE-ir or enkephalin-ir were found following short-term (3-10 days) morphine treatment (eg., Fratta et al., 1977; Hollt et al., 1978). However, at the time of these studies, it was not known that total ßE- or enkephalin-immunoreactivity may have reflected immunoreactivity from several different peptides. Conceivably, one effect of chronic morphine treatment might be to alter the processing patterns of the opioid precursors. Such changes may not affect total immunoreactivity, but may instead cause a shift in the relative amounts of the different peptide products. For example, long-term morphine exposure might affect POMC-containing neurons by increasing the normal rate of conversion of $ßE_{1-31}$ to $ßE_{1-27}$. Possible shifts in the relative amounts of $ßE_{1-31}$ and $ßE_{1-27}$ would be physiologically relevant since carboxy-terminal shortened forms of

βE_{1-31} are much less potent opiate agonists compared to βE_{1-31} (Akil et al., 1981). In contrast to the earlier studies on BE and enkephalins, Weissman and Zamir (1987) recently reported that Dyn B concentrations in the globus pallidus and ventral tegmental areas increased following 3 days of heroin treatment. They also found a significant heroin-induced rise in levels of the Proenk peptide, MERGL, in the substantia nigra. However, it is not possible to know if the increases in the steady-state levels of these opioids are the result of increased or decreased activity in Prodyn and Proenk neurons.

In this paper, we describe the initial findings of studies examining the effects of chronic morphine treatment on the three opioid peptide families. The intent in these studies was to measure multiple parameters of opioid biosynthetic activity so that inferences could be made about whether specific opioid systems had been up- or down-regulated. The morphine administration paradigm consisted of implanting rats with either 75 mg morphine or placebo pellets (one pellet on Day 1 and 3 pellets on Day 4) and sacrificing animals on Day 7.

EFFECTS OF MORPHINE TREATMENT ON POMC SYSTEMS

There are two groups of POMC-containing cell bodies in the brain, the major group localized in the arcuate nucleus of the hypothalamus and the smaller group situated in the caudal medulla in the nucleus tractus solitarius (NTS). The arcuate neurons project to diverse regions of the brain including the septum, amygdala, periaqueductal gray (PAG), and thalamus. The projection regions of the NTS cell group are not presently known. In the present studies, we determined the concentrations of various ßE-ir peptides in 2 cell body regions (the hypothalamus and NTS) and in 1 terminal field region (the midbrain PAG). Total ßE-ir in tissue was determined by radioimmunoassay using an antibody directed primarily against the mid-portion of βE_{1-31}. At the dilution used (1:40,000), this antibody reacts equally well with βE_{1-31} and ß-lipotropin (ß-LPH), and is approximately 25-50% cross-reactive with βE_{1-27}, ßE1-26, N-acetylated (NAc) ßE1-31, NAc ßE1-27 and NAc ßEl-26. There is no cross-reactivity at 1 micromolar concentrations. with α-endorphin, γ-endorphin, Met-Enk, Leu-Enk, Dyn A, Dyn B, alpha-neo-endorphin or a number of non-opioid POMC-derived peptides (ACTH, α-MSH, γ-MSH).

Confirming earlier reports, we (Bronstein et al., 1987) found that 7 days of continuous morphine exposure had no effect on concentrations of total ßE-ir in the hypothalamus, PAG or NTS (Table 1). Morphine and placebo treated samples were then pooled, developed on Sephadex gel chromatography columns, and collected fractions were assayed for ßE-ir. This procedure permitted us to quantify ßE-ir peptides having different molecular weights, in peaks

representing POMC, ß-LPH, $ßE_{1-31}$ and $ßE_{1-27}/ßE_{1-26}$ (the latter two BE forms could not be resolved from each other on this column). In the three brain regions examined, we found that concentrations of the different POMC-derived peptides were roughly the same in morphine and placebo treated animals. These results indicate that the processing of POMC protein to ßE-ir products is apparently not affected by 7 days of continuous morphine exposure.

TABLE 1

EFFECTS OF MORPHINE TREATMENT ON ß-ENDORPHIN CONTENT IN BRAIN[1]

	1 DAY	3 DAY	7 DAY
Hypothalamus	100 ± 8	247 ± 28*	104 ± 20
Midbrain	154 ± 18*	70 ± 19	104 ± 17
NTS	75±10	94 ± 21	105 ± 10

[1]Numbers represent means ± SEM of morphine treated animals expressed as a percentage of mean placebo values.

*Differs significantly from placebo group. $p < .05$.

However, recent data (Mochetti et al., 1984; Mochetti and Costa, 1986), showing that 5 days of morphine pelleting caused a 50% decrease in levels of POMC mRNA in the hypothalamus, suggest that morphine may inhibit neural activity and ßE secretion in POMC-containing cells. (We are presently attempting to quantitate POMC mRNA in morphine and placebo treated rats to verify whether morphine does down-regulate POMC message levels). If morphine does inhibit BE release, ßE-ir concentrations may transiently rise since POMC would continue to be translated and processed into peptide products, but these peptides would not be released. In a study to test this hypothesis, we (Bronstein et al., in preparation) found that ßE-ir levels in the midbrain were elevated in morphine-treated animals 24 hr after pelleting but had returned to placebo levels after 72 hr (Table 1). Conversely, morphine treatment did not alter ßE-ir concentrations in the hypothalamus 24 hr following pellet implantation but caused a 2-3 fold increase in POMC-derived peptide levels after 72 hr (Table 1). Gel chromatographic analysis revealed that the increase in hypothalamic ßE-ir at 72 hr was due to a selective accumulation of $ßE_{1-27}/ßE_{1-26}$-size material. These results may be interpreted to support the hypothesis that morphine inhibits the secretion of, ßE-related peptides from POMC neurons. The midbrain PAG increase in ßE-ir 24 hr following morphine pelleting may reflect

an accumulation of peptides axonally transported from cell bodies in the arcuate but not released from nerve terminals. The slower time course for the rise in ßE-ir levels in the hypothalami of morphine-treated animals may result from continued translation and processing of POMC protein in cell bodies without further transport to terminal fields. Following 7 days of morphine treatment, we saw no differences in brain levels of ßE-ir between placebo and morphine treated animals, suggesting that the biosynthetic rate of POMC peptides may have declined (as a result of the reduction in POMC mRNA levels) to match the rate of ßE release.

EFFECTS OF MORPHINE TREATMENT ON PRODYNORPHIN AND PROENKEPHALIN PEPTIDES

The striatum (STR) and substantia nigra (SN) contain among the highest concentrations of Prodyn peptides found in the brain. Prodyn cell bodies in the STR send descending projections which terminate heavily in the SN pars reticulata (Vincent et al., 1982; Fallon et al., 1984). In addition to dynorphins, the STR and SN contain high concentrations of Proenk peptides. While the enkephalins in these two brain regions probably do not arise from a single system, extensive knowledge about the striatonigral-nigrostriatal loop make this an excellent system for studying regulation of both Proenk and Prodyn neurons.

In the present study, following the morphine treatment described above, STR and SN were extracted in 100% methanol:0.1N hydrochloric acid (1:1; v:v), and the extract radioimmunoassayed with antisera highly selective for the Prodyn peptides Dyn A1-8, Dyn A_{1-17}, Dyn B, and a neo-endorphin (α-Neo), and the Proenk peptide MERGL. As seen in Figure 1, morphine-treated animals showed increases in all four Prodyn peptides in the STR compared to placebo animals, with increases ranging from 18 to 27% for the different peptides. No changes in Prodyn peptides were observed in the SN. In contrast to the morphine-induced increases in Prodyn peptides in the STR, there was no difference between placebo and morphine treated animals in MERGL levels in this brain region. While there appeared to be a slight decrease in MERGL in the SN of morphine-treated animals, this effect was not statistically significant.

Analogous to what was observed in the POMC system, the increases in Prodyn peptides observed in the present experiment may have resulted from an inhibition of peptide release from striatal neurons. If this hypothesis is correct, by following Prodyn peptides over a time-course of morphine treatment, one should see an initial increase in peptide concentrations as release is inhibited, followed later by a decrease in peptide concentrations as synthesis is shut down. We have begun to study this possibility, examining Prodyn peptide levels following 1, 3 and 7 days of morphine treatment (Trujillo et al., 1988). Results thus far are inconclusive; it may be necessary for us to

examine morphine treatment over a period longer than 7 days in order to observe the predicted results. In addition, we are quantitating prodynorphin mRNA levels in our various treatment group. A recent report (Ryan at al., 1987) that Prodyn mRNA levels in the striatum declined by 30-40% following 5 days of morphine pelleting supports the hypothesis that morphine inhibits secretion of Prodyn peptides from striatal neurons.

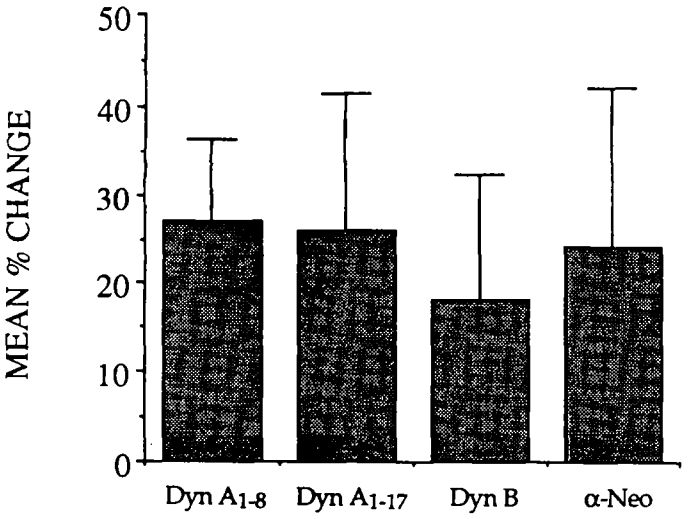

FIGURE 1. Increase in Prodynorphin peptides in the striatum following 7 days of morphine pelleting. Data are expressed as mean percent increase (± SEM) over placebo treated control values.

GENERAL DISCUSSION

At first glance the morphine-induced increases in brain POMC and Prodyn peptides appear to be inconsistent with the feedback inhibition hypothesis of morphine action. According to a simplistic interpretation of this hypothesis, chronic morphine treatment should, by inhibiting synthesis of opioid peptides, cause decreases in peptide concentrations in affected neurons. However, it is possible that feedback inhibition of endogenous opioid neurons could produce increases in peptide concentrations identical to those seen in the present experiments. An initial inhibition of peptide release by morphine might cause a buildup of peptides in opioid neurons, prior to the inhibition of peptide synthesis. This appears to be the case in POMC neurons as ßE-ir levels in the midbrain and hypothalamus increase following 24 and 72 hr of morphine exposure, respectively. The increase in hypothalamic ßE-ir appears to result from a selective accumulation of $ßE_{1\text{-}27}/ßE_{1\text{-}26}$ with little change in ßE1-31. Previous results from the intermediate pituitary gland (Akil et al., 1985)

indicate that when POMC biosynthesis is activated, processing of the precursor to βE_{1-31} keeps pace with the increased rate of synthesis, but the ßE1-31 conversion to βE_{1-27} does not. Thus, under conditions of chronic stimulation, there is less complete peptide processing, and βE_{1-31} accumulates relative to $\beta E_{1-27}/\beta E_{1-26}$. The fact that morphine selectively elevated hypothalamic $\beta E_{1-27}/\beta E_{1-26}$ levels in the present experiments would suggest that the converse may be taking place; that is, when rates of biosynthesis and secretion are decreased, there is increased conversion of βE_{1-31} to βE_{1-27}. The present data, together with the finding that POMC mRNA levels were reduced in morphine-treated animals (Mochetti and Costa, 1986), suggest that morphine inhibits the activity of POMC neurons, eventually causing a decrease in POMC biosynthesis.

Similar to POMC neurons, the morphine-induced increases in various Prodyn-derived peptides in the striatum may have resulted from an inhibition of peptide secretion. Chronic morphine exposure apparently causes decreased Prodyn biosynthesis, as evidenced by the reductions in Prodyn mRNA levels following 5 days of morphine pelleting (Ryan et al., 1987). At this time, it is unclear why POMC peptides become elevated within 72 hr after morphine pelleting whereas increases in Prodyn peptide levels take longer to develop. The effects of morphine on Proenk secretion and biosynthesis are less clear. No changes in Proenk peptides were observed in this, and previous, studies whereas decreases in Proenk mRNA have been reported by some (Ryan et al., 1987) but not all (Mochetti et al., 1984) investigators.

Despite the intuitive simplicity of the feedback inhibition hypothesis, it may be that no such mechanism is operating on endogenous opioid systems. It is also possible that feedback inhibition may regulate some endogenous opioid systems (e.g., POMC, and perhaps, Prodyn), but not others (e.g., Proenk). However, it is not necessary to invoke a unitary hypothesis for the effects of opiate drugs on endogenous opioid neurons; these neurons exist in distinct brain systems and may be regulated by very different mechanisms. It is currently unknown whether regulatory opioid autoreceptors exist, and if they do, on which opioid neurons they might be present. Likewise, it is unknown whether feedback pathways for the regulation of specific endogenous opioid systems are present in the brain. Thus, the effects of opiate drugs on endogenous opioid systems may be mediated by mechanisms other than feedback inhibition. If this is indeed the case, then an alternative explanation must be found for the observed increases in POMC and Prodyn peptides. One possibility is that morphine causes increased peptide synthesis in one or both of these systems. In the case of striatal Prodyn, a putative pathway can easily be postulated for such an effect. The striatonigral-nigrostriatal loop is a complex brain system with numerous inputs, outputs and intrinsic relationships (see Watson et al., in press). The effects of morphine on Prodyn peptides could

therefore be secondary to its effects on other neurons in this system. For example, morphine is known to increase activity of nigrostriatal dopamine neurons (see Pert et al., 1979); increased activity of nigrostriatal dopamine neurons is known to increase concentrations of Prodyn peptides in the STR and SN (Peterson and Robertson, 1984; Trujillo et al., 1987; Hanson et al., 1988).

The observed changes in Prodyn and POMC peptides may have important implications in terms of opioid dependence and withdrawal. For example, Prodyn peptides are known to activate kappa receptors; activation of kappa receptors is aversive to laboratory animals and produces dysphoria in human subjects (Pfeiffer et al., 1986). It is intriguing to speculate that the increases in Prodyn peptides seen following chronic morphine might be involved in the dysphoria of opiate withdrawal (see Watson et al., in press, for further discussion of this possibility). In addition, due to the different receptor actions of βE_{1-31} and βE_{1-27}, changes in the relative concentrations of these peptides could be important in withdrawal. In order to understand the precise role of these changes, however, it will be necessary to know not only the character of the stored peptides, but also to determine whether these peptides are released in the same proportions following opiate treatment and/or during withdrawal. It must also be considered that chronic opiate treatment alters opiate receptor properties. Coordinate changes in peptide release and receptor efficacy could dramatically alter the tone of the opioid system in question. Thus, in order to describe at a molecular level the alterations in opioid systems produced by chronic opiate treatment, we need to determine not only the steady-state levels of peptides in the opioid neurons, but also the releasability of these peptides, and their effect on receptors in untreated, opiate-treated, and withdrawn animals.

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AUTHORS

David M. Bronstein, Ph.D.
Keith A. Trujillo, Ph.D.
Huda Akil, Ph.D.

Mental Health Research Institute
University of Michigan
205 Washtenaw Place
Ann Arbor, MI 48109-0720.

Molecular Mechanisms of Drug Reinforcement—Current Status

S. Dworkin and J. Smith

Elucidation of the molecular biology of the reinforcing functions of drugs is an extremely complex research endeavor. Experiments designed to investigate such processes must include not only the sophisticated procedures of molecular biology, but also must consider the intricate interrelationships between behavior and molecular events. Current knowledge of the central nervous system (CNS) components of complex behavioral processes is not at the same level of resolution as the events that occur at single receptor sites. For example, a question of similar complexity challenge for molecular biology would be the integration of processes involved for events occurring at all of the heterogeneous receptor sites or channels on a neuron at any given moment. The myriad of factors and outcomes increases exponentially with the number of interrelated systems involved. At what point would the level of molecular analysis exceed the limits that obviate a behavioral analysis? An explanation for a simple single lever press response likely involves thousands of molecular events in each of several thousands of nerve endings. It is difficult to conceive how the complexity of processes observed at the behavioral level of analysis can be evaluated and understood using these molecular procedures. It may well be that the most molecular level of analysis of the neurobiological components of a behavioral process will be the identification and characterization of the neuronal networks and circuits involved in the process. Thus, the identification of the neurons, critical receptors and connections between these component network neurons may be the highest resolution for complex behavioral processes.

Investigations of the neurobiological components of drug reinforcement have been limited by an ambiguous interpretation of the conditions necessary for assessing the reinforcing effects of drugs. This is exemplified by the assumption that a drug possesses some intrinsic reinforcing property that is not dependent upon an interaction of the organism with the environment. Drugs do not have immutable behavioral

properties and investigations that do not consider this factor will be incomplete. The potential reinforcing effects of a drug depend on a number of behavioral variables. For example, cocaine can function as an extremely efficacious reinforcer, maintaining extensive self-administration patterns in a number of situations (Balster and Schuster. 1973; Spealman and Goldberg, 1978) and yet function as an aversive stimulus in a conditioned taste aversion paradigm (D'Mello et al., 1981; Goudie et al., 1978; Hunt et al., 1985). Moreover, cocaine can function as both a positive reinforcer and a negative reinforcer in the same animal during the same session (Spealman, 1979, 1985). Monkeys will emit both a response that results in cocaine presentation and a second response that terminates the opportunity to receive cocaine during the same experimental session. If cocaine had immutable reinforcing properties, then an animal would not be expected to terminate the opportunity to, inject the drug.

Investigations of the molecular biology of drug reinforcement must include an analysis of the mutability of drug effects by behavioral variables. Thus, appropriate identification of the neuronal networks and circuits that mediate the reinforcing effects of drugs would be a major step toward understanding the neurobiological mechanisms involved. Such information would then permit an analysis of the molecular mechanisms at receptor sites that are specific to these processes. Several behavioral procedures that either directly or indirectly measure the reinforcing effects of drugs have been developed and can be utilized to provide a molecular analysis of drug action.

Indirect procedures for assessing the reinforcing effects of drugs involve the non-contingent administration of the substance. Several of these procedures have been used to determine the behavioral. pharmacological and neurobiological mechanisms that may be involved in the reinforcing functions of abused drugs. Brain electrical stimulation procedures (ICSS) can be used to evaluate the effects of drugs on responding maintained by the response contingent electrical stimulation of specific CNS sites. ICSS procedures have provided important information concerning reinforcement mechanisms in general and the potential neurobiologic components of the reinforcing functions of drugs. These procedures have shown most drugs of abuse lower thresholds for direct electrical stimulation of discrete brain regions and this results has been suggested to indicate the reinforcing effects of these drugs (Reid, 1987). For example, most opiates that have abuse potential lower thresholds while those with little or no abuse potential (i.e. pentazocine, cyclazocine, phencyclidine, naloxone, naltrexone) do not effect thresholds (Kornetsky, 1985). However, the importance of behavioral variables was demonstrated in the early development of this procedure (Steiner et al., 1969). Rats

were shown to escape from the noncontingent stimulation of a brain region that had previously maintained self-stimulation, this occurred even though the noncontingent stimulation occurred at rates previously supporting self-stimulation. Contemporary methodologies using 2-deoxyglucose auto-radiography, as a measure of glucose utilization, have used the ICSS paradigm to provide important information concerning the neurobiologic components of contingent and noncontingent stimulation of discrete brain regions (Esposito et al, 1987; Porrino et al, 1984a, 1984b). If the neuronal networks mediating the reinforcing function of brain stimulation are the same as those that mediate the reinforcing function of drugs, then the data collected with this procedure will be valuable. However, there is an absence of direct data to substantiate this premise. At this time ICSS is still at best, an indirect measure of the reinforcing function of drugs.

The conditioned place preference procedure (CPP) assesses the ability of environmental stimuli paired with a drug to become conditioned reinforcing or punishing stimuli. Most drugs of abuse can result in the development of a place preference while drugs that are typically reported to be dysphoric result in conditioned place aversions. Most abused drugs will produce a conditioned place preference under appropriate conditions. Moreover, a CPP can be produced to the direct injection of amphetamine (Carr and White, 1983) and morphine (Vander Kooy et al., 1982) into the nucleus accumbens. Neurotoxin lesions of dopamine innervations of the nucleus accumbens attenuate CPP to systemic opiates and amphetamine but not to i.p. cocaine (for a recent review see Phillips and Fibiger, 1987). More recently, it has been shown that C-OHDA lesions of the nucleus accumbens blocks the CPP to intravenous cocaine which indicates that the route of drug administration can be an important determinant of the neurobiological components of CPP. Glucose utilization studies of i.p. (London et al., 1986) and i.v. (Porrino et al., 1988) cocaine administration and dose-related effects of amphetamine (Porrino et al., 1984) have further elucidated the role of pharmacological variables including the route of administration and dose in determining the neurobiological effects of abused drugs. The i.v. administration of cocaine as well as the administration of a low dose of amphetamine resulted in the activation of brain areas thought to be involved in reinforcement while the i.p. administration of cocaine and the high dose of amphetamine activated regions that are related to motor activity and stress.

Two additional procedures can provide indirect measures of the reinforcing effects of drugs. These procedures included taste pairing paradigms and tests of the discriminative stimulus effects of drugs. Conditioned taste aversion procedures (CTA) can also measure the potential noxious effects of a drug.

Whereas, drug discrimination paradigms assesses the ability of the interoceptive stimuli produced by the drug to serve as a discriminative stimulus that occasions a specific response. Pharmacologic manipulations which disrupt the self-administration of morphine. cocaine and amphetamine also block the formation of a conditioned taste aversion to these drugs (Grupp, 1977, Hunt et al., 1985: Sklar and Amit, 1977). Direct assessments of the involvement of discrete brain regions in the discriminative stimulus functions have been reported using the drug discrimination paradigm. The direct microinjection of amphetamine into the nucleus accumbens generalized to a stimulus produced by the systemic administration of the drug, while, injections into the striatum did not (Nielsen and Scheel-Kruger, 1986). Moreover, 6-OHDA lesions of the nucleus accumbens attenuated the stimulus effects of both amphetamine (Dworkin and Bimle, 1988) and cocaine (Dworkin and Smith, 1988)

There are potential problems with all of the indirect procedures with regards to studying the molecular components of drug reinforcement. As previously stated it is often assumed that the administration of an abused drug has intrinsic hedonic value in the absence of the behaving organism. Thus, noncontingent presentation is thought to have the same effect (i.e., pleasing, euphoric, response strengthening) as contingent presentation. Thus, reward neurons are activated independently of any controlling relationship between the organism and the environment. To conclude that response independent presentation of any environmental event is reinforcing most likely is in error. The potential fallacy of such conclusions is particularly obvious after considering the major differences between contingent and not contingent environmental events noted in the literature. For example, noncontingent presentation of previously self-administered brain stimulation is aversive (Steiner et al, 1969). Noncontingent intracranial stimulation at sites that were previously contingently delivered also activates different brain regions than those activated by self-administered electrical stimulation (Porrino et al. , 1984). Yoked noncontingent infusions of morphine into physically dependent rats effect different neuronal circuits than those modulated in self-administering littermates (Smith et al, 1982). The response contingent intravenous presentation of cocaine has greater toxicity compared to same pattern of infusions that are self-administered (Dworkin et al., 1988). An increasing body of evidence suggests contingent presentation of a reinforcer to be different than noncontingent presentation of the same event. Such data questions the use of CPP as a behavioral model for the investigation of reinforcement. The drug discrimination procedure is subject to the same criticisms since noncontingent drug presentation also occurs. The drug cues are used to occasion a particular response that results in the

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presentation of a non-drug reinforcer. This procedure utilizes the entire stimulus properties of the drug, but the response independent presentation precludes conclusions about reinforcing effects.

The only procedures that directly addresses the reinforcing effects of a drug is self-administration. It can be assumed that the reinforcing effects of the drug are present with self-administration. However, the self-administration environment is a complex ambience with all of the potential unconditioned behavioral effects of a drug present. These include response contingent presentation as well as the stimulus properties, rate effects, effects on ingestive behaviors and all other potential behavioral actions and interactions of the drug. Thus, the self-administered drug has stimulus properties that can set the occasion for the emission of other responses, can increase or decrease rates of behaviors including subsequent responding maintained by the drug, can modulate the intake of food or water which in turn can further increase or decrease drug intake or can modulate other unspecified physiological functions in either a direct or indirect manner. Separation and identification of neuronal activity changes specific to the reinforcing effects of a self-administered drug is a challenge, because of this complexity. Appropriate control groups are necessary to isolate and identify the neuronal correlates of the reinforcing effects of abused drugs. The response contingency must be isolated yet potential response differences must be minimize so that motor activity is not responsible for any observed changes. It is also necessary to control for unconditioned drug effects not related to reinforcement. If these requirements can be fulfilled, then-the observed changes will likely represent events involved in reinforcement processes. It is possible that unique and separate systems evolved to mediate the reinforcing properties of drugs especially those that have endogenous counterparts. It is more likely, however. that these substances activate neuronal systems that mediate these processes for non-drug reinforcers in much the same way as ICSS.

To describe and investigate the neurons that mediate reinforcement in terms of neuronal networks or systems has distinct advantages over attributing such complex processes to one or two pathways or neurotransmitter releasing neuronal systems. First of all, it is more parsimonious with the data that indicate a number of pathways and neurotransmitters are involved in reinforcement processes. Secondly, it is more consistent with what is known about complex brain function. No neuronal system in the CNS is independent of influences from other neurons. It is unlikely that any intricate process can be conveyed by a single pathway. Reinforcers are also not immutable or constant. The state of the organism (deprivation. satiation) can modulate the saliency of a

putative reinforcer. Many reinforcers are not universal but develop reinforcing efficacy through conditioning, especially in primates. All of these factors are important to the processes that comprise reinforcement. It is not likely that one or two neuronal pathways could control and integrate these complex processes. Neuronal networks or circuits involving a number of pathways and neurotransmitters are necessary. One may potentially attribute the pleasing or "rewarding" effect of the direct electrical stimulation of a brain region to the activation of a specific pathway or group of pathways, since this is exactly what is occurring. However, theories based solely on such phenomena will not likely account for the complex processes of reinforcement. Reward theories may potentially explain intracranial electrical self-stimulation but may generalize to little more. Reinforcement should not be considered to occur in the absence of a three term contingency (Skinner, 1938) which includes the conditions that occasion a response, the response itself and the consequences of responding. Molecular theories of drug reinforcement must include an analysis of these terms along with the potential modification of the resulting processes by additional factors. These additional influences include pharmacological factors (ie. the drug class, dose and route of administration) and behavioral variables (ie. the organisms behavioral and previous drug history, the current context in which the behavior is occurring) and the interaction of all of these factors with the molecular components of reinforcement. A neurobiologic theory of reinforcement must be able to explain complex behavioral processes. It is likely that the neuronal network model will be a useful framework within which to develop such a theory.

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AUTHORS

Steven I. Dworkin, Ph.D.
James E. Smith. Ph.D.

Department of Psychiatry
Louisiana State University School of Medicine in Shreveport
P.O. Box 33932
Shreveport, LA 71130-3932

Cannabinoid Action in the Central Nervous System

B. Martin, A. Bloom, A. Howlett and S. Welch

INTRODUCTION

There has been considerable effort directed toward understanding the mechanism by which cannabinoids produce their psychotomimetic effects; yet, our understanding of cannabinoid action in the brain remains relatively limited. Certainly, one of the major impediments in establishing a biological correlate for psychotomimetic activity is the complexity of the cannabinoid behavioral syndrome. The behavioral effects of cannabinoids are dependent upon numerous variables which include species differences, dose, experimental conditions, etc. There has always been a keen interest in developing cannabinoids that exert a unique spectrum of action which would enhance their clinical potential and at the same time serve as important research tools (see review on cannabinoid structure-activity relationships by Razdan 1986). The neurochemical and biochemical effects of cannabinoids have been reviewed recently by Dewey (1986) and Martin (1986). The possibility that cannabinoids act to alter membrane integrity has been actively pursued since Paton *et al.* (1972) likened the cannabinoids to steroid anesthetics. They proposed that the lipophilicity of the cannabinoids played an important role in their psychotomimetic actions. This notion remains attractive given the plethora of actions that cannabinoids have on biological systems and the fact that there are not specific cannabinoid antagonists. However, there is the bothersome aspect of structure-activity relationships that appears to defy the membrane disruption postulate. In particular, recent synthesis of analogs such as 11-OH-dimethylheptyl-Δ^8-THC (Mechoulam *et al.,* 1987) and the cannabinoid analgesics (Johnson and Melvin 1986) have clearly shown that cannabinoids can be extremely potent and exhibit great stereoselectivity. Despite the lack of a specific antagonist, evidence is emerging from behavioral studies and from in vitro binding experiments that there may be specific receptors that are responsible for mediating some of the actions of the cannabinoids. Intuitively, it would seem reasonable that the cannabinoid behavioral syndrome results from multiple actions of these agents on the central nervous system. It is for this reason that we have chosen to coordinate the research efforts of several laboratories in evaluating the biochemical effects of cannabinoids.

CANNABINOID REGULATION OF CYCLIC AMP METABOLISM

In neuronal cells in culture — Studies of cannabinoid effects on neuronal cells were initially performed on a cloned line (N18TG2) of neuroblastoma cells in culture. The advantages of such a preparation over CNS preparations are that the

effects can be studied in a homogeneous cell population of genetically and phenotypically identical cells, and that the environment of these cells can be controlled. Initial studies determined that cannabinoids which exhibit cannabimimetic activity decrease cyclic AMP content of neuronal cells (Howlett 1984). The adenylate cyclase in plasma membranes from these cells was inhibited by centrally active cannabinoid drugs (Howlett and Fleming 1984). Several lines of evidence suggested that a cannabinoid receptor was mediating the effects on adenylate cyclase. The response was rapid and reversible both in intact cells and in membrane preparations, suggesting that an irreversible cytopathological change was not responsible for the effects (Howlett 1985; Dill and Howlett 1988). The concentration that was required was in a range that would be expected to be present at the site of action in vivo. The inhibition of cyclic AMP synthesis occurred at nanomolar concentrations of drugs in intact cells (Howlett *et al.,* 1986) and with a Ki of 360 nM for adenylate cyclase inhibition in membrane prepartions (Howlett 1987). The inhibition of adenylate cyclase by a series of constituents of marijuana and of metabolites of cannabinoid drugs paralleled the ability of these compounds to produce a psychological high in humans and to alter behavior in animals (Howlett and Fleming 1984; Howlett 1987). Δ^9-THC was more potent than Δ^8-THC, and cannabinol was only partially active at its maximally effective concentration. Cannabidiol, cannabigerol, cannabichromene and olivetol were inactive. Hydroxylation at the C11 position resulted in compounds having approximately one order of magnitude greater potency. Hydroxylation at other positions reduced the potency or eliminated activity. Not only did the inhibition of adenylate cyclase follow a strict structure-activity relationship, the response was demonstrated to be stereoselective (Howlett and Fleming 1984). The inhibition of adenylate cyclase was exhibited by only certain cell types, indicating that the effects on the enzyme are not universally observed. This cellular selectivity would argue for a requirement for a phenotypically distinct difference between responding and non-responding cells, such as would be expected for the presence of a cannabinoid receptor. The final argument favoring a cannabinoid receptor is that the regulation of adenylate cyclase is via G_i (Howlett 1985; Howlett *et al.,* 1986), a G-protein that mediates the adenylate cyclase response of hormone and neuromodulator receptors.

If the cannabinoids regulate adenylate cyclase in neuroblastoma cells via a receptor, the nature of the receptor can be described pharmacologically. It was demonstrated that the response was not a result of modification of the eicosanoid or peptide receptors that stimulate adenylate cyclase in the neuronal cells (Howlett 1984). Furthermore, the cannabinoid effects were neither synergistic nor additive at maximally effective concentrations with agonists to the muscarinic, α_2-adrenergic or δ-opioid receptors that act via G_i in the neuroblastoma cells (Howlett 1984). Antagonists at these receptors failed to block the cannabinoid effects, providing further evidence that these pharmacologically distinct receptors were not mediating the cannabinoid effects (Howlett 1984; Devane *et al.,* 1986). These data provide arguments that the regulation of adenylate cyclase by cannabinoids results from an interaction with a pharmacologically distinct receptor on neuronal cells.

In the CNS — Evidence now exists that cannabinoids regulate cyclic AMP production in the brain similar to that described in the neuronal cell model systems. Rat brain slices were prepared and the cyclic AMP accumulation was determined in vitro. The nantradol analog, desacetyl-levonantradol, inhibited forskolin-stimulated cyclic AMP accumulation by approximately 50% in the striatum and 45% in the hippocampus. The cannabinoid response was demonstrated to involve cells that respond to vasoactive intestinal peptide and to D_1 dopaminergic agonist in the striatum (Bidaut-Russell and Howlett 1988). A similar inhibition of cyclic AMP

accumulation was demonstrated for morphine which could be blocked by naloxone. However, the inhibition of cyclic AMP accumulation by cannabinoids could not be blocked by naloxone and was neither synergistic nor additive with the response to morphine. These data indicate that the opioid and the cannabinoid receptors may produce a similar inhibition of adenylate cyclase in neurons; however, the receptors for these drugs are pharmacologically distinct, as they are in the neuroblastoma cell model.

Desensitization of the cannabinoid response — Tolerance to the effects of marijuana has been documented in humans and in a number of animal models; however, a cellular mechanism for such an effect has not been previously reported. The tolerance that develops to opioids may be in part due to a modification or sequestration of receptors (see Harden 1983 for review). Using the model neuronal system, it has been demonstrated that the response to cannabinoid drugs is significantly reduced after 4 to 24 hr exposure to potent cannabinoids (Dill and Howlett 1988). The desensitization occurred in the absence of any detectable cytopathology, and was dose-dependent and specific for the cannabinoid response. This phenomenon of homologous desensitization may represent at least one cellular mechanism for the development of tolerance by cells in the CNS.

MODULATION OF FREE INTRACELLULAR CALCIUM CONCENTRATIONS BY Δ^9-THC IN SYNAPTOSOMES

In neurons the concentration of free intracellular calcium plays a pivotal role in the mechanism of stimulus-secretion coupling. Calcium entry through the "slow" calcium channels which occurs as a late part of the action potential has been shown to be required for neurosecretion (Katz and Miledi 1969; Baker *et al.,* 1973; Baker 1978). The entry of calcium through the slow calcium channels has been proposed to increase intracellular calcium concentrations in the cytosol which in turn stimulates a chain of intracellular events leading to the release of neurotransmitters (Katz and Miledi 1968; Miledi and Thies 1971; Zucker and Estrella 1983). Until recently, changes in intracellular calcium were often extrapolated from experiments measuring either the uptake or the efflux of calcium. Although these measures are valid in themselves, they do not always reflect changes in the concentration of intracellular calcium. That is, if enhanced efflux of calcium accompanies enhanced uptake, the net change in intracellular calcium will be minimal. If sequestration of calcium is enhanced in selected pools, uptake measures will not accurately reflect changes in intracellular calcium. The difficulty in determining calcium uptake at time points less than 5 seconds after depolarization is great, yet it is within this time frame that calcium channels activate, open and close following depolarization. Thus, it is important to monitor alterations in intracellular calcium within seconds after the administration of a depolarizing stimulus. New methodology using the calcium indicator FURA-2/AM and a spectrofluorometric system (SPEX Inc.) has eliminated many of the aforementioned problems inherent with uptake methodologies. FURA-2/AM, an ester, readily passes into cells and synaptosomes where it is cleaved to the free acid, FURA-2. The FURA-2 is trapped in the cells and binds to free intracellular calcium. The binding of calcium to the FURA-2 produces a shift in the wavelength of excitation of the FURA-2 from 380 nM to 340 nM. The ratio of the intensity of the signal at 340/380 nM can be converted into calcium concentrations based upon the known K_D of the FURA-2 as well as other parameters as described by Grynkiewicz et al. (1985) and Tsien *et al.* (1985). The SPEX computer system records changes in calcium at times less than one second after the addition of a drug or a depolarizing stimulus which enables events to be recorded immediately following depolarization.

Cannabinoids, due to their lipophilic nature, may produce profound changes in membranes and the calcium channels contained therein. It has been shown that cannabinoids inhibit calcium uptake following depolarization of synaptosomes both at concentrations which disrupt the synaptosomal membranes (10^{-5}M) as well as at concentrations which do not produce damage to the membranes (Harris and Stokes 1982). The concentrations of cannabinoids which alter calcium uptake have also been shown to depress release of synaptic neurotransmitters (Hershkowitz *et al.,* 1977). However, changes in neurotransmitter release induced by cannabinoids, although correlated to the effects of the drugs on calcium uptake, do not always correlate to the psychoactivity of the drugs [for reviews see Pertwee (1988) and Dewey (1986)].

Studies have been undertaken in our laboratory to investigate the effects of the cannabinoids on free intracellular calcium concentrations using FURA-2/AM as the calcium indicator in order to determine whether changes in intracellular calcium correlate with the psychoactivity of these drugs. The effects of Δ^9-THC on both basal and depolarized levels of intracellular calcium were studied in synaptosomes which were prepared from whole mouse brain according to the method of McGovern *et al.,* (1973) and loaded with 2 µM FURA-2/AM for 45 min prior to 3 washes. Two-ml samples of the synaptosomes (1.5 mg/ml protein) in a well oxygenated Krebs/ bicarbonate buffer (1 mM calcium, pH 7.4) were placed in a cuvette at 37 °C with stirring and allowed to equilibrate for 5 min prior to the addition of the drug or vehicle. The Δ^9-THC was prepared in 1% DMSO so that the final concentration of DMSO in the cuvette was 0.01%. Synaptosomes were depolarized at 1 min after the addition of the drugs or vehicle using 50 mM KC1 prepared in Krebs/bicarbonate buffer, pH 7.4. Conversion of the 340/380 nM ratios to intracellular calcium concentrations was done according to the formula of Grynkiewicz *et al.* (1985). All changes in calcium concentration were expressed as the percent change from the baseline present when drugs or depolarizing stimuli were administered.

The results of preliminary studies with Δ^9-THC and morphine are shown in Figure 1. The basal level of free intracellular calcium $[Ca]_i$ was 384 ± 30 nM. The vehicle had no significant effect on either the basal levels of $[Ca]_i$ or on levels of $[Ca]_i$ after depolarization. Δ^9-THC produced a very small rise (<10%) in basal calcium. However, Δ^9-THC appeared to produce a dose-related attenuation of depolarization-induced increases in $[Ca]_i$, Δ^9-THC produced significant effects only at a concentration of 10^{-5}M. Since it has been shown previously that this concentration of Δ^9-THC produces morphological changes in synaptosomal membranes (Harris and Stokes, 1982), it is possible that the attenuation of depolarization-induced increases in $[Ca]_i$ by Δ^9-THC (10^{-5}M) represents a toxic membrane effect. Morphine produced a significant dose-related attenuation of depolarization-induced $[Ca]_i$. The attenuation of KCl-stimulated changes in $[Ca]_i$ by Δ^9-THC (10^{-5}M) was comparable to that observed using 100 fold less morphine (10^{-7}M). However, the data show a trend toward modulation of $[Ca]_i$ by THC at doses which are not toxic to synaptosomal membranes. Future research in our laboratory will be directed toward evaluation of potent THC analogs on $[Ca]_i$ in order to clarify the role of calcium in the mechanism of action of the cannabinoids.

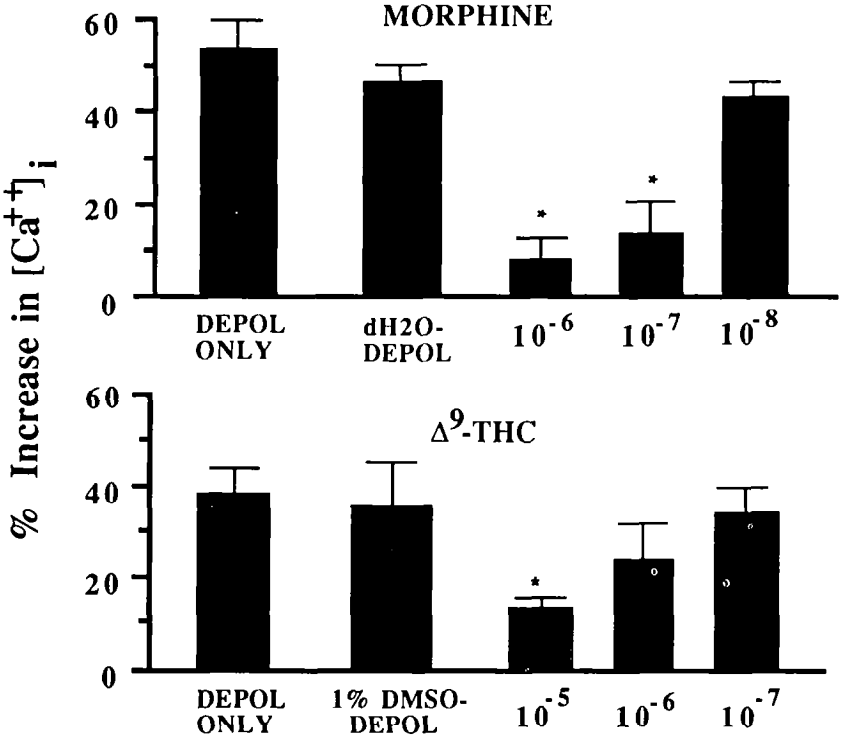

FIGURE 1. Effects of morphine and Δ^9-THC on depolarization-induced changes in intracellular calcium levels.

EFFECTS OF CANNABINOIDS ON THE FLUIDITY OF BRAIN MEMBRANES

Two general mechanisms of action have been proposed for the cannabinoids. The first is that they act through a specific receptor as do the opioids and many other drugs. Evidence for this has been discussed previously in this report. A second mechanism of action that has been proposed for the cannabinoids is that they alter the fluidity or ordering of biological membranes. This possibility was first investigated because of the highly lipophilic nature of most cannabinoids and their preferential association with biological membranes (Martin *et al.,* 1976). The membrane perturbation hypothesis of cannabinoid action is supported by studies demonstrating changes in the order or fluidity of phospholipid vesicles and other model membrane systems. Techniques such as electron spin resonance, proton NMR and differential scanning calorimetry have been used. We have used fluorescence polarization techniques (Hillard *et al.,* 1985) to demonstrate that Δ^9-THC and other cannabinoids alter the fluidity of rat brain synaptic plasma membranes (SPM). Low concentrations of the psychoactive cannabinoids Δ^9-THC and 11-OH $\cdot\Delta^9$-THC decreased the polarization of 1,6-diphenylhexatriene (DPH) fluorescence emission in SPM. The same concentrations of cannabidiol (CBD) and cannabinol (CBN), compounds with little or no marijuana-like psychoactivity, did not affect DPH polarization. This study indicated that the structure-activity

relationship for fluidizing brain membranes was consistent with that for psychoactivity. Time-resolved fluorescence polarization studies indicated that the change in polarization was primarily the result of a decrease in lipid order (Bloom *et al.,* 1986). The increase in fluidity produced by the psychoactive cannabinoids was unique to brain SPM since all cannabinoids tested decreased the fluidity of heart and liver plasma membranes at all concentrations examined (Bloom *et al.,* 1987).

We have now examined the effects of six cannabinoid analogs on the fluidity of brain SPM. These compounds vary significantly in structure, aqueous solubility and in their spectrums of pharmacological activity. This study should aid us in understanding the relationship between the aforementioned properties of the cannabinoids and their membrane effects.

SPM were prepared from whole rat brain homogenates using Ficoll and sucrose density gradients. Membrane fluidity was determined using DPH and trimethylammonium-DPH (TMA-DPH) as probes. DPH is thought to locate throughout the membrane and report on the hydrophobic core of the membrane while TMA-DPH remains near the surface of the membrane and reports on that region. The fluorescence polarization of DPH and TMA-DPH in membranes was determined using a SLM - 8000C T-format fluorometer. Cuvette temperature was maintained at 35°C by a circulating water bath and monitored with a thermocouple placed in the cuvette just above the light path. Cannabinoids were added using a DMSO vehicle which was without significant effect over the concentration range studied.

The effects of Δ^9-THC and the six cannabinoid analogs are shown in table 1. All of the compounds were tested over a concentration range 1 to 50 mM. Δ^9-THC produced a biphasic increase in membrane fluidity as measured by DPH. Significant increases in fluidity were seen with concentrations as low as 3 mM and the effect was maximal at 10 mM. Higher concentrations were less effective. The

TABLE 1. Cannabinoid effects on brain synaptic plasma membrane fluidity.

COMPOUND	DPH	TMA-DPH
Δ^9-THC	⇑⇓	⇑
$\Delta^{9,11}$-THC	⇑	⇑
TMA-Δ^8-THC	⇑	⇓
MB-Δ^8-THC	⇓	⇑
11-COOH-Δ^8-THC	⇑	NC
12-ß-AMINO-D^8-THC	⇑	NC
12-ß-S-ACETYL-Δ^8-THC	⇓	⇑

Fluidity increased (⇑), decreased (⇓) or biphasic effect (⇑⇓) or no change (NC)

polarization of TMA-DPH fluorescence was decreased (fluidity increased) only at concentrations of 30 mM and higher. This difference indicates that the core of the membrane is more sensitive to the fluidizing action of THC than are regions near the surfaces of the membrane. Fluidity as measured using DPH was increased by

all of the other compounds tested except morpholinobutyryl -Δ^8-THC (MB -Δ^8-THC) and 12ß-S-acetyl -Δ^8-THC. Those two compounds both decreased the fluidity at the membrane core. Δ^9-THC was the most potent of the compounds tested and the only one whose effects were biphasic over the concentration range examined. The effects of these compounds when measured using the TMA-DPH probe were more variable. Fluidity of the more superficial membrane regions was increased by Δ^9-THC, $\Delta^{9,11}$-THC, MB-Δ^8-THC and 12ß-S-acetyl-Δ^8-THC but was decreased by TMA-Δ^8-THC. The water-soluble morpholinobutyryl compound was the most potent and effective of these compounds. The other two compounds tested did not significantly affect TMA-DPH fluorescence polarization.

The compounds tested all have somewhat unique pharmacological profiles. Similarly, it can be seen that there are qualitative differences among their effects on the fluidity of brain synaptic plasma membranes. Thus it is possible that the differences in the membrane interactions of these compounds are involved in the differentiation of their pharmacological actions.

CONCLUSIONS

The results presented in these investigations support our contention that numerous mechanisms may be involved in the production of cannabinoid behavioral effects. Our understanding of these mechanisms will come as new probes continue to be synthesized, as evidence continues to emerge regarding a cannabinoid receptor, and as a better understanding of the actions of cannabinoids on biological membranes is formulated.

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AUTHORS

Billy R. Martin, Ph.D., and Sandra P. Welch, Ph.D.
Department of Pharmacology and Toxicology, Box 613
Virginia Commonwealth University, Richmond, VA 23298-0613

Alan S. Bloom, Ph.D.
Department of Pharmacology and Toxicology
Medical College of Wisconsin
8701 Watertown Plank Road, Milwaukee, WI 53226

Allyn C. Howlett, Ph.D.
Department of Pharmacology
St Louis University Medical Center
1402 South Grand Boulevard, St. Louis, MO 63104

Receptor-Transductive Mechanisms for Drugs of Abuse

L. Abood

INTRODUCTION

There are two distinct problems with respect to the molecular mechanisms associated with drugs of abuse: one pertaining to the acute mechanisms involved in the pharmacologic actions of the drugs and the second to the mechanisms underlying the chronic effects associated with dependence and withdrawal. There is, however, a link between the two mechanisms, insofar as drugs are abused because of the euphoregenic and other desirable effects associated, at least with their initial use. Among the acute reinforcing effects of the drugs are euphoria, tranquilization or stimulation, analgesia or diminished awareness, increased libido, and psychodelic phenomena. For the most part, a tolerance develops to the peripheral pharmacologic actions of the drug, including cardiovascular, autonomic, and hormonal; and since such effects are generally undesirable, their attenuation with prolonged drug use is in itself reinforcing. To varying degrees, depending on the nature of the drug, tolerance also develops to the desirable neuropharmacologic effects of the drugs; a tolerance which is overcome by increasing the dosage.

Drugs of abuse appear to be acting both acutely and chronically on the nervous system either by acting on receptors and their transductive systems associated with the action of neurotransmitters and neuromodulators or by directly modifying membrane properties involved in neuronal excitation. Although there have been great strides made in our knowledge of the receptors involved in drug action, attempts to demonstrate an *in vivo* relationship between the density and affinity of receptors and drug dependence has not been very successful. In recent years more effort has been devoted to the examination of receptor-linked second messenger or transductive systems for changes associated with tolerance and dependence.

Regulatory mechanisms for controlling neuronal function

In recent years considerable effort has been devoted to possible regulatory mechanisms associated with both the acute and chronic action of drugs of abuse. Neuronal regulatory mechanisms control

neuronal excitability by a) directly affecting ionic conductance, b) influencing a rate-limiting enzyme involved in the synthesis of a neurotransmitter, and c) controlling the responsivity of the receptor itself. Bioelectric excitation and conductance involves the opening and closing of specific ionic channels which control the movement of Na, K, Cl, and Ca. Neuronal excitability is mediated by various neurotransmitter receptors which function in one or three ways: 1) the receptor itself may comprise the ionic channel, as in the case of the nicotinic cholinergic; 2) the receptor may be coupled to the guanosine nucleotide binding (G) protein controlling ionic channels via a CAMP-dependent protein kinase, as in the case of the dopaminergic or opioid receptors; in cardiac muscle a CAMP-dependent kinase is presumed to be involved in the phosphorylation of the voltage dependent Ca channel (Osterrieder et al., 1982); or 3) the receptor may be directly linked to the phosphorylation of channel proteins.

The regulation of neuronal function can also be achieved by controlling the responsiveness of the receptor to its agonists or antagonists. One known mechanism for the phenomenon of receptor desensitization, which follows chronic exposure to a neurotransmitter or a receptor agonist, involves phosphorylation of the receptor (nicotinic cholinergic) itself by a CAMP-dependent protein kinase (Huganir et al., 1984). In the case of the ß-adrenergic receptor, there is also a suggestion that receptor phosphorylation may not require cAMP (Stasser et al., 1986).

Still another regulatory mechanism is achieved through control of the rate-limiting enzyme in the synthesis of the neurotransmitter or neuromodulator, a mechanism that generally involves a cAMP-dependent phosphorylation of the enzyme (Krebs and Beavo, 1979).

Regulatory transductive systems for neurotransmitters and hormones

Neurotransmitters, neuromodulators and hormones act by perturbing regulatory mechanisms which control such functions as ionic conductance, metabolism, or release of a neurotransmitter or hormone. Among the transductive or second messenger systems which perform this regulatory function are adenylate cyclase, guanylate cyclase, and a specific phospholipase C which results in the production of diacylglyceride (DAG) and inositide polyphosphates. The first three act in turn by directly activating specific protein kinases, while the latter controls Ca flux, which can activate a calmodulin-dependent protein kinase as well as initiate excitation or neurotransmitter release.

Adenylate cyclase and regulatory G proteins

A number of neurotransmitter receptors are coupled to adenylate cyclase (AC) to either stimulate or inhibit the production of cAMP. Among the neurotransmitter receptors which stimulate production are dopamine, ß-adrenergic, and serotonin, while those that inhibit include α-adrenergic, muscarinic cholinergic, and opioid. The interaction of a ligand with a receptor produces a signal which is transmitted into the cellular interior by guanine nucleotide-binding (G) proteins, which either stimulates (G_s) or

inhibits (G_i) AC.

There is increasing evidence that opioid receptors in some mammalian neuronal systems are negatively coupled via a G protein to adenylate cyclase, an enzyme leading to the production of cyclic AMP (cAMP) which in turn regulates a number of enzymic systems involved in neurotransmitter and hormonal synthesis, ionic conductance, and DNA activation. The regulatory G protein system consists of either an inhibitory component G. which inhibits cAMP production, or a stimulatory component G_s which stimulates cAMP production. Activation of the G protein occurs upon its combination with GTP, while its inactivation results by its ability to hydrolyze GTP to GDP. In photoreceptors there exists another type of G protein, transducin, which, upon light activation, stimulates a membranous phosphodiesterase that degrades cAMP, the second messenger believed to be associated with the opening of excitatory Na channels. The activation of Na channels in turn is thought to be under control of cGMP, another second messenger system.

Some opioid receptor systems inhibit adenylate cyclase by causing its interaction with an inhibitory GTP binding protein, G. which then, upon hydrolysis of GTP, dissociates from the catalytic unit to remove the inhibition. Evidence for the coupling of the receptor to G. derives from the observation that pertussis toxin, which acts by dissociating G. from AC via ADP-ribosylation (Bokoch et al., 1983), is able to prevent the opioid-induced inhibition of AC (Kurose et al., 1983). One of the complicating factors in examining receptor-mediated responses of the opioid system is the heterogeneity of the receptor itself, where subtypes would be expected to have different second messenger or transductive sys terns. Tumor cell lines with a preponderance of one receptor subtype have been used to investigate receptor-linked transductive systems. Included among these are the NG 108-15 neuroblastoma-glioma hybrid with a δ-receptor linked to AC via an inhibitory G_i to control prolactin secretion. The concentrations of opioid agonists needed for an effect on AC are in the µM range; whereas the K_d values for opioids in general are in the nM range, but in the absence of Na. Since at physiologic concentration of Na the number of binding sites and the affinity of opioids are considerably reduced, µM concentrations of the opioids may be required for some of their pharmacologic effects. Insofar as the decrement of receptor affinity by Na is potentiated after pertussis toxin-induced ADP ribosylation of G_i, it has been suggested that the Na effect on affinity is somehow associated with the G protein. The inhibitory effect of GTP on opioid binding (Blume, 1978; Childers and Snyder, 1980; Rosenbaum and Sadee, 1983) has also been cited as evidence for an association of the receptor with a G protein. Further evidence for such an association derives from the observation that the inactivation of G_i by pertussis toxin abolishes the opioid inhibition of AC in NG 108-15 cells (Bokoch et al., 1983) rat striatum (Abood et al., 1985), and spinal cord-ganglion cultures (Crain et al., 1987). Pertussis toxin is believed to act by dissociating G_i from AC via ADP-ribosylation.

DADLE has been reported to inhibit Ca conductance in NG 108-15 cells; and the fact that the effect could be prevented by prior intracellular application of G_o was interpreted as evidence for the involvement of G_o in voltage-dependent Ca channels (Heschler et al., 1987). In dorsal root and sympathetic ganglia Ca channels are inhibited by such neurotransmitters as dopamine, norepinephrine, and GABA, an effect which can be mimicked by the intracellular administration of GTP and and blocked by pertussis toxin (Crain et al., 1987).

Other drugs of abuse which also directly or indirectly involve transductive systems linked to G proteins are amphetamine and cocaine, which appear to exert their action by increasing the postsynaptic action of dopamine and norepinephrine, both exerting a stimulatory action on AC.

The involvement of norepinephrine in opioid action

Opioids and other agents indirectly involve second messenger systems by virtue of their ability to modify the synaptic levels of neurotransmitters, such as catecholamines, serotonin, and ace tylcholine, which in turn are coupled to second messenger systems.

There have been a number of studies in animals to suggest that opiates inhibit the stimulated release of NE (Jackish et al., 1986). Opiates inhibit the electrical activity of noradrenergic neurons of the locus coeruleus via μ, but not δ or κ receptors (Cox and Werling, 1987). Opiates appear to inhibit the K-stimulated release of NE from slices of cortex, hippocampus, and cerebellum, structures which receive projections from the locus coerlueus (Christie et al., 1986). In rats the effect appears to involve only u-receptors (Werling et al., 1987), while in guinea pig μ, δ, and κ receptors appear to play a role. It has also been reported that the electrically stimulated release of NE from rat hippocampal slices is regulated by μ, but not δ or κ receptors; whereas, in rabbits the effect is mediated by κ receptors (Jackish et al., 1986).

The inhibition of the K-stimulated release of norepinephrine from brain slices by morphine and DADLE appears to be dependent upon the degree of activation of α_2-adrenoceptors (pre-synaptic), insofar as the opioid inhibition was abolished following the suppression of release by clonidine (α_2-agonist), but unaffected following a doubling of release by the α_2-antagonist, phentolamine (Schoffelmeer et al., 1986).

Based on the finding that clonidine, a partial agonist at α_2-adrenergic receptors, alleviated the withdrawal signs of opiates in rats (Sparber and Meyer, 1979; Charney et al., 1984a), it has been suggested that elevated withdrawal is associated with elevated NE activity. It has also been proposed that the increased NE activity is responsible for withdrawal symptoms (Charney et al., 1984b). Clonidine has even been claimed to replace methadone in opiate withdrawal with a minimum of withdrawal symptoms (Charney et al., 1984a). Such observations

would tend to support an earlier finding of an increased firing rate in locus coeruleus neurons in morphine tolerant rats following the administration of naloxone (Aghajanian, 1978); however, the validity of this finding is questionable since residual morphine may have been present in the preparation.

Phosphatidyl inositol turnover as a second messenger system

The involvement of PI turnover as a transductive system has been linked to a number of receptors in the peripheral nervous system and end organs. Included among the neurotransmitters and neuropeptides demonstrated to be linked to PI are acetylcholine, serotonin, vasopressim, norepinephrine, and bradykinin (see (Fisher and Agranoff, 1987) for review). With the exception of acetylcholine there is little data linking most of these substances to PI turnover. There are a number of studies demonstrating that the muscarinic cholinergic receptor is linked to PI hydrolysis via a GTP-binding protein which regulates the hydrolysis of a specific phospholipase C that converts PI into inositol triphosphate (IP_3) and a diacylglyceride (DAG). The IP_3 appears to be involved in the mobilization of Ca and the DAG in the activation of a specific protein kinase C, an enzyme which has a regulatory function in the synthesis of neurotransmitters, secretion, ionic conductance, and receptor sensitivity. Upon completion of their regulatory function, IP_3 and DAG are reincorporated into PI for resumption of the cycle. Although GTP appears to be involved in the receptor-mediated activation of PI hydrolysis, the specific G protein has yet to be identified.

There is evidence that desensitization occurs in the brain muscarinic receptor with repeated exposure to carbachol, a muscarinic agonist, while exposure to muscarinic antagonists, such as atropine, results in upregulation of the receptor (Kurose et al., 1983). It has also been demonstrated in human neuroblastoma cell lines that the pharmacologic activation of protein kinase C by phorbol esters results in a desensitization of the receptor to both antagonist binding and PI turnover, simulating the prolonged exposure to carbachol (Liles et al., 1986). It would appear that a reciprocal relationship exists between the muscarinic receptor recognition site and the transductive system possibly involving a regulatory G protein. More direct evidence for such a link between the receptor and a G protein derives from studies demonstrating GTP-sensitive high affinity binding of acetylcholine with phospholipid vesicles reconstituted with purified brain muscarinic receptors and GTP binding proteins (Haga et al., 1988).

The mechanism of action of cocaine and d-amphetamine

By virtue of the ability of cocaine and δ-amphetamine to elevate synaptic concentrations of catecholamines, they indirectly involve cAMP as a second messenger. At ß-adrenergic and D_1 -dopaminergic receptors the actin of both drugs would enhance an a at α_2-adrenergic (pre-synaptic) would inhibit cAMP production.

In addition to its ability to interfere with the synaptic reuptake of catecholamines, cocaine, like other local anesthetics, is able

to block excitability by reacting directly with ion channels. It has been demonstrated that the nicotinic acetylcholine receptor-mediated conduction block associated with cocaine and other local anesthetics involves blockade of the open ionic channels resulting in depolarization. From measurements of the kinetics of ion fluxes in PC12 cells, it was shown that cocaine inhibited the initial phase of the receptor-mediated ion flux by binding to the open channel (Karpen et al., 1985). Phencyclidine also blocks the initial phase of ion flux and, in addition, increases the rate of receptor inactivation (desensitization) by shifting the equilibrium of the acetylcholine receptor from the active to the inactive form. It can be inferred from such findings that while cocaine binds equally as well to the open (active) and closed (inactive or desensitized) channels, PCP has a higher affinity for the closed configuration of the ionic component of the acetylcholine receptor.

Mechanism of action of phencyclidine (PCP)

PCP appears to be exerting its action at diverse receptors, including cholinergic (muscarinic and nicotinic), σ-opioid, and an allosterically linked N-methyl δ-aspartate (NMDA) receptor-ion channel complex. It has been demonstrated that PCP and other dissociative anesthetics non-competitively antagonize the excitatory effects of NMDA. Since glutamate and NMDA markedly enhance the binding of PCP, as well as σ-κopioids, to rat brain membranes, it was inferred that PCP and related agent preferentially interact with the activated configuration of the NMDA receptor-ion channel complex (Fagg, 1987). Since K channel blockers, such as 4-aminopyridine, antagonized the binding of ^{3}H-PCP analogues to rat brain membranes, it was inferred that the PCP receptor is a κ channel (Sorensen and Blaustein, 1986).

Neuromodulation via the hypothalamic magnocellular system

One of the major neuromodulatory systems in mammals is the neuroendocrine magnocellular system of the hypothalamus which acts as a central mechanism for the regulation of hormonal activity and, conceivable, the function of a number of neurpeptides originating in the hypothalamic cells. The magnocellular neurons of the supraoptic (SON) and paraventricular (PVN) synthesize and secrete hormone and neuropeptides into the systemic circulation for action at peripheral end organs. In addition the neurons of the PVN send projections to various regions of the central nervous system to secrete such neuropeptides as vasopressin and somatostatin. The release of vasopressin from magnocellular neurons is stimulated by such neurotransmitters as acetylcholine and norepinephrine (Sladek and Knigge, 1977); while the release of oxytocin was decreased by opioids which appear to be blocking the excitation-coupling mechanism (Gribkoff and Dudek, 1987).

Opioid receptors are believed to be involved in the release of LHRH from hypothalamic neurons, since the blood levels of LH and testosterone are reduced upon the acute administration of morphine and markedly increased after the administration of naloxone (Cicero et al., 1983). Furthermore, after either acute or chronic

administration of morphine to rats, the release of LH is markedly increased following a single dose of morphine, an observation which is indicative of supersensitivity.

CONCLUSION

Although second messenger systems have been shown to be involved in the action of a number of drugs of abuse, there is little information concerning either their extent of involvement or the functional link regulating neuronal excitability. Drugs such as the opiates, LSD, and scopolamine, by interacting directly with neurotransmitter receptors coupled to G-proteins, can directly affect second messenger systems; whereas, cocaine, δ-amphetamine, and opiates, by modifying synaptic levels of neurotransmitters, indirectly involve second messenger systems. It is likely that modifications in transductive systems are associated with chronic use of drugs of abuse; however, to date, progress in this area has been slow.

Table 1

	Receptor	Transducer	Comments
Opioids	μ δ κ σ	G_i-AC G_o	(-) AC Ca conductance
Cocaine	DA reuptake system	G -AC (8ia DA)	(+) AC
	Conductance	ion channel	(-)
Amphetamines	CA reuptake CA release	G_s-AC	(+) AC
Barbiturates	$Gaba_A$	Cl channels	(+) cl channels allosteric site
Phencyclidine	NMA (glutamate receptor σ- opioid)	ion channels (K, Na, Ca)	(-) NMA excitation allosteric site
THA			
LSD	5-HT	PL C-PI AC (?)	(-) PI (-) AC
Scopolamine (anti-muscarinic)	ACh	PL C-PI	(-) ACh stim. PI turnover

Transductive systems associated with various drugs of abuse.

Abbreviations: AC = adenylate cyclase; DA = dopamine; CA = catecholamines; NMA = N-methyl δ- aspartate; PLc = phospholipase c, PI = phosphoinositides; ACh = acetycholine.

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Public Health Management of AIDS and Drugs in Amsterdam

G. van Brussel and E. Buning

Introduction

Amsterdam is a small city, 700,000 inhabitants. It is the capital of The Netherlands. The drug population consists of 7,000 addicts. In this presentation, I will try to clarify our method of dealing with the drug problem and the need of containment of the HIV-epidemic within this population.

In order to receive the development of our present public health drug management approach, I will briefly comment on the current situation on drugs in Amsterdam. Before this expose, some opening remarks are made on various elementary aspects of a successful drug management policy.

Opening Remarks

Drug abuse of heroin and cocaine is a threat to individual and public health.

Despite great efforts, current treatment modalities offer effective solutions for a relatively small percentage of drug abusers. This being so, the best aim for those who are not willing or capable to kick the habit, is to reduce the harm done to themselves and to society.

To cope with drug problems it is necessary to integrate planning, law enforcement and public health policies.

In order to be effective, public health services for drug abusers have to be available for and aimed at all active drug abusers in a certain area.

In an escalating (deregulated) drug epidemic, it is essential to make distinctions between drugs with acceptable risks; soft drugs; cannabis products; and drugs with unacceptable risks; hard drugs; heroin; cocaine; amphetamine; LSD.

If changes in use from soft to hard drugs occur, this is not the result of a physical but of a sociological stepping-stone effect.

Effective public health drug policy incorporates AIDS prevention and care for drug addicts with ARC/AIDS.

The present situation on drugs in Amsterdam

At this moment, the drug population in the city consists of about 7,000 drug addicts; of them, 3,250 are of Dutch; 1,750 of ethnic; and 2,000 of foreign origin. Of the Dutch origination drug abusers, some 40% "shoots up", the rest and practically all of the ethnic drugs users "chase the dragon" (i.e., smoking). Of the foreign originating drug abusers about 70% "shoot up", the rest and practically all of the ethnic drug abusers "chase the dragon."

The mean age of the drug population in 1987 is 30.1 years. The cohort under 22 years makes up only 4.8% of the total drug population.

Figure 1

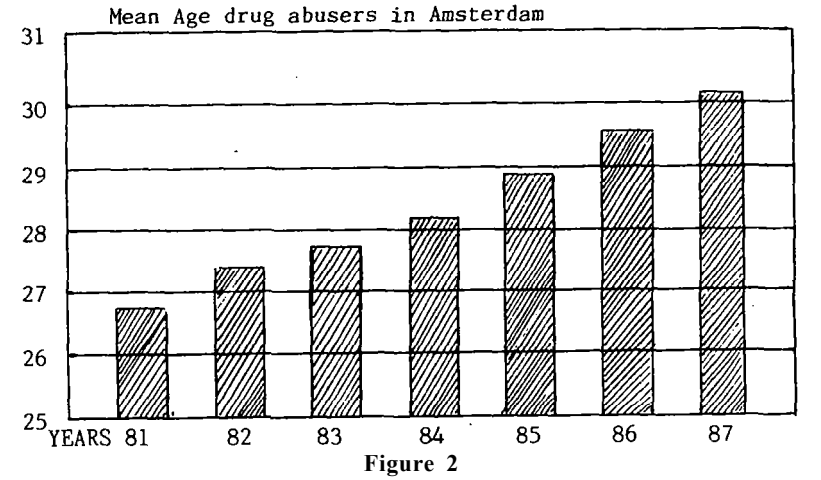
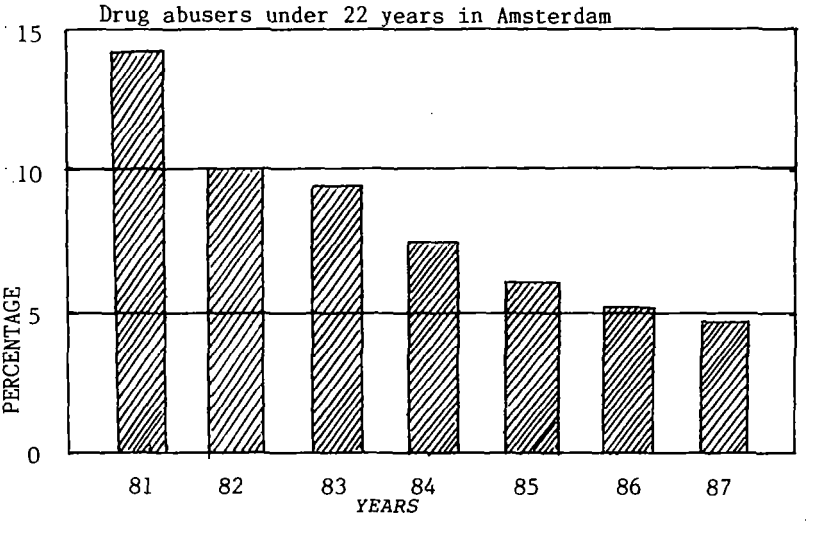


Figure 2



These figures are part of a constant age trend since 198 1. The drug problem in Amsterdam is heroin-dominated. This is probably due to a constant supply of high-grade (30-40%) street heroin with a constant price (about $100 per gram). Cocaine and benzodiazepines are widely used among these heroin addicts. Seventy percent of all heroin addicts take cocaine as well as heroin. Those addicts shooting up heroin also shoot up cocaine. Those "chasing the dragon" with heroin, prepare cocaine base with bicarbonate heated on a spoon. then smoke the free base. Crack preparations of cocaine have not yet been found in Amsterdam. Approximately 50% of the drug addicts take. beside heroin and cocaine, benzodiazepines. Barbiturates and methaqualone preparations have become rare since these preparations have been incorporated in the opium law.

To our surprise, notwithstanding the trend in the U.S.A., our drug problem is still opiate-dominated As a result of this, methadone programs still have a great attraction for drug addicts in Amsterdam. The mortality rate in the indigenous Amsterdam drug population is low, measured in the quantity of lethal overdosages.

Table 1

Over-Dosage deaths in Amsterdam

	1983	1984	1985	1986	1987
Dutch ethnic origin	21	21	22	19	21
Foreign origin	32	52	20	41	40
Total	53	73	42	60	61

Remarkable is the fairly constant number of overdosage deaths in the Dutch and ethnic drug addicts in 1987, 21 people; and the large variations in numbers among foreign drug addicts in 1987.40 persons. A death-provoking factor in this last segment probably is the absence of tolerance for the relatively strong street heroin.

The mentioned basic assumptions and facts concerning the Amsterdam drug population prompted us to specific measures on the field of Drugs and Drugs-AIDS-public health policies.

Drugs - public health policies

In Amsterdam, a helping system has been developed in which emphasis is put on the services to active drug abusers. A main instrument is the dispensing of methadone. This is being done on three levels:

1. In two rebuilt city buses about 600 drug abusers are being treated with individual daily, liquid methadone dosages at different locations in the city.

2. In three out-patient clinics about 300 drug addicts are being treated with individual methadone dosages. These are dispensed in daily or contingency take home form. In these clinics clients are monitored with urine tests and motivated to abstain from heroin and cocaine.

3. Two hundred General Practitioners dispense methadone on a weekly take home basis to 900 clients per week. Yearly, the total members in G.P. practice is 1,500. These clients are structured drug addicts, for a large part working and with a regulated drug addiction. G.P.'s are supported by b.i.j. M.D's from our department.

The total number of different patients seen in this methadone system is 5,000 clients yearly. To be allowed to participate in the bus program, a client has to be identified and medically registered. He or she has to be examined by a physician. A social worker offers specific counseling on drugs or HIV-related matters and primary social care, housing, welfare. Special attention is given to care for addicted prostitutes. drug addicts with severe psychiatric pathology, to children of addicted parents and to illegal foreign addicts.

Apart from all these client-based aid facilities we offer specific outreaching support to the city police, the Amsterdam hospitals and general practitioners. In the police-project all arrested drug addicts are visited daily by a doctor of the drug-department of the Municipal Health Service. In this way, in 1987, 2,117 drug addicts were seen. Of this number, 28% was originating from ethnic minority groups, notably Surinam; 26% was foreign originating, mainly German. About half of them had no contact with helping agencies in 1987. This police-support activity gives us an insight in current drug trends, especially in these drug abusers not seeking voluntary aid. Apart from being a source of information, visiting and helping addicts in police cells gives opportunity for a strong motivation to seek voluntary help, in the form of methadone treatment.

Table 2

Police-project

Year	Number of drug patients seen
1977	653
1978	1,091
1979	1,053
1980	1,243
1981	1,549
1982	1,656
1983	1,787
1984	2,456
1985	2,046
1986	1,981
1987	2,117

The hospital support system originated in the observation that hospitals were not able to cope with the disruptive behavior associated with active drug abusers. As a result of this situation, many seriously ill drug abusers were discharged too early from the hospital or even not hospitalized at all. For this

reason we offer consultative support in the person of psychiatric nurses, who are drug experts.

This hospital project is an important factor in future case management of AIDS-drugs patients.

Table 3

Hospital Support system

Number of hospital admissions

1984 - 362
1985 - 326
1986 - 357
1987 - 375

General practitioners are important in the Dutch medical system, because they are regarded as being primarily responsible for all health system interventions to a patient. Apart from defining our aim in harm-reduction, we made the observation that specific help offered in a drug-oriented environment (waiting areas of drugs-clinics) can be stigmatizing. This led us to the hypothesis that it might be beneficial for selected drug addicts to receive help in the form of methadone-week-prescriptions from their general practitioners.

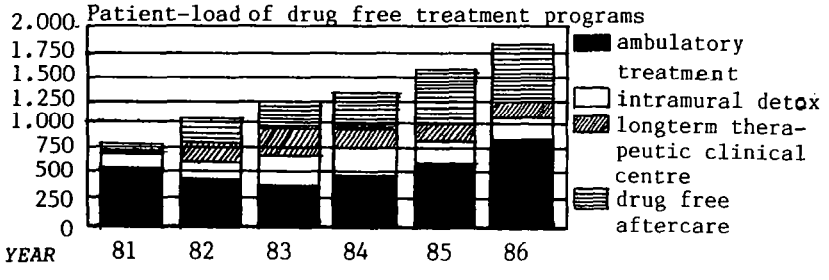
At this moment about 200 of Amsterdam 400 G.P.'s are willing to prescribe methadone to patients selected by the Municipal Health Service.

In order to give the general physician necessary support, we offer consultative service in the form of drug expert out-reaching physicians.

The cooperation with therapeutic facilities

When the policy of large scale. low threshold methadone programs was adapted, it was expected that the patient load in therapeutic institutions would fall drastically. Contrary to the expectation, we found that these treatment facilities could function better and more efficiently in the new situation. Drug patients admitted for drug free treatment programs were in better shape physically and socially when they participated in the methadone program.

Figure 3



Drug containment policy

Amsterdam has an integrated drug policy. The policy is carried out by the Municipal Health Service where public health is concerned, by the Metropolitan police when public order, crime dealing is concerned. The public health approach is oriented on monitoring the drug epidemic by medical registration, by name and birthdate of all drug addicts. In order to control the harmful medical efforts of the drug epidemic, our efforts in the form of methadone and syringe exchange are aimed at the active drug addicts.

The police approach is aimed at maintenance of public order. In correspondence with the opening remarks, distinction is made between soft (cannabis) and hard drugs (heroin, cocaine, amphetamine). High priority is being given to hard drug dealing, low priority to the possession of soft drugs. As a result of this distinction, the metropolitan police chooses not to concentrate on enforcing the law if cannabis consumption or small scale soft drug dealing is involved. This has given rise to a more or less controlled situation in soft drugs. A beneficial effect has been the fact that there exists a complete dissociation between soft and hard drug scenes. In our opinion, this is a major cause of young people experimenting with soft drugs; not enrolling in hard-drug experimenting; because the dealer where they buy soft drugs doesn't sell hard drugs. Another result is the possibility to give the police the opportunity of concentrating on important hard drug crime.

AIDS-Drugs-policy

In this policy two aspects can be differentiated: prevention of HIV infection and care for ARC/AIDS patients.

Prevention of HIV infection. This is done by general and individual information campaigns, leaflets, etc. In Amsterdam, a major activity in this field is the syringe-exchange system. In this system a used syringe is exchanged free for a sterile one. The ratio behind the exchange mode is the assumption that drug addicts who insist on using drugs have a right to sterile syringes; one the other hand, other citizens have a right to streets free of HIV-infected syringe

Table 4.

Year	Number of exchanged syringes
1984	25,000
1985	100,000
1986	400,000
1987	700,000

Condoms are handed out; for addicted prostitutes. There is a barrier for use in the clients, however.

Care for ARC/AIDS drug-patients

The situation of AIDS in the Netherlands per 1-1-1988 is as follows:

Total patients - 420

Of these 21 iv drug users (5% of the total)
5 are homosexual

HIV-infection among drug abusers.

In a recently published research project, in 582 drug patients, (25% of the total), were HIV-infected. In the group of i.v. drug users, 29% were infected. In the group of non-drug users, five persons were seropositive; of these, four are homosexuals and one is a prostitute.

In the present phase of the HIV epidemic among drug addicts in Amsterdam, special attention is given the growing number of TBC among HIV-infected drug addicts. The combination of high dosage methadone programs (liver induction by tuberculostatic medicaments, notably rifamipicine) and TBC monitoring seems very effective.

For those addicts in our population who contract ARC/AIDS, we have set our aim at enabling the patients and the medical specialists in the hospitals to provide a medical care of the same quality that non-addicted people with ARC/AIDS get, notably AZT treatment.

AFFILIATION:

Municipal Health Services
1018 WT Amsterdam
The Netherlands

229-045 0 - 89 - 11 QL3

Preventing the Spread of HIV in Injecting Drug Users—the Experience of Syringe-Exchange Schemes in England and Scotland

G. Stimson, L. Alldritt, K. Dolan and M. Donoghoe

ABSTRACT

In 1987 the UK government launched experimental schemes for distributing injecting equipment to injecting drug users in order to help prevent the spread of Human Immunodeficiency Virus. This paper reports on the first few months of the schemes, and provides some initial evidence of changes in clients' risk behaviours.

The schemes have been reasonably successful in attracting clients, but are less successful in retaining them. Schemes have demonstrated that equipment can be distributed to clients on an exchange basis. Schemes have reached new groups of clients, including many without previous or current treatment contact or other help for their drug problems.

The baseline assessment of clients found that most had accurate knowledge of the risk of infection from sharing injecting equipment. Most thought that they were at low risk of infection. Syringe sharing in the last four weeks was reported by 36%. A minority engaged in multiple sharing. Most clients were sexually active and many had partners who did not inject drugs. The main reason for attendance was worry about AIDS, and a majority of clients reported that they had already made changes in their injecting practices because of AIDS. Some reported changes in sexual behaviour.

A sample of clients followed-up at 2-4 months indicates a reduction in syringe sharing.

POLICY BACKGROUND, PROGRAMME REQUIREMENTS AND RESEARCH DESIGN

The schemes were launched as part of the UK government strategy to prevent the spread of HIV. The government decision to launch the schemes was announced in December 1986. This followed reports of high levels of HIV infection among some groups of injecting drug users. High rates of HIV antibody seropositivity in injecting drug users were reported in Edinburgh (in one group

tested 1983-5, 51% were seropositive (Robertson et al 1986a,b). More recently rates of between 0 and 10 % have been reported in different parts of the UK (Advisory Council on the Misuse of Drugs 1988). Whilst the potential for a serious HIV problem exists, in many areas there is time for preventive action.

Programme requirements

In England the Department of Health and Social Security invited drug agencies to establish schemes on a pilot one-year basis, with offers of some financial support. In Scotland, the Scottish Home and Health Department asked Health Boards to set up schemes. Agencies were asked to provide (a) injecting equipment on an exchange basis to drug misusers already injecting and unable or unwilling to stop: and (b) counselling for clients' drug problems. advice on safer sex and counselling on HIV testing. Scottish schemes included a medical input.

Fifteen agencies participated in the programme, 12 in England and one in Scotland (locations listed in the Acknowledgements). The offical start was April 1987, and 8 agencies were running schemes by June 1987 and all were operational by August 1987.

The total number of clients attending from June 1987 until the end of March 1988 was approximately 2500. There was a wide variation in caseload, with four agencies with less than 30 clients, and three with more than 300. The largest schemes are seeing between 20 and 50 clients each day.

Research design and fieldwork

Our research brief was to monitor the <u>implementation</u> of the schemes, and their <u>impact</u> on clients' risk behaviour. Information is collected on the agencies, including location, staffing, costs, working methods and philosophy. needles and syringes distributed and returned, and number and frequency of client visits. Information on clients is collected (a) on intake to schemes using a brief Intake Sheet (IS). (b) During the first month of attendance baseline information on clients' behaviour, attitudes and knowledge relevant to injecting drug use and HIV transmission is collected by the First Client Questionnaire (FCQ). (c) A similar questionnaire is repeated at 2 - 4 months (SCQ) to measure changes in behaviour, attitudes and knowledge. All client information is based on self-report and interviews with clients are conducted by agency staff.

Data sets

The data sets used in this report comprise: (a) IS for clients taken into the schemes by 30 October 1987 (n=769) (b) FCQ for clients taken into the schemes by 30 October 1987 and received by us by 30 November 1987 (n=182) (c) FCQ and SCQ compared for first 106 cases to end March 1988. Percentages are based on the above returns or valid responses adjusted for missing data. Missing data range from 0 to 10%.

IMPLEMENTATION AND OPERATION

On first attendance (intake) most clients (56%) reported worries about AIDS as a reason for attendance, followed by scarcity of equipment (38%).

Client characteristics

As assessed at intake, the schemes attract older drug injectors (mean age 26.8 years. range 17 to 52), with a long elapsed drug use (mean injecting use = 7.7 yrs.) and a higher proportion of men (78%) than women (but there are large variations between agencies in the male/female ratio of clients). Most clients are opiate users (heroin 57%, methadone 13%). However, there are also some primary amphetamine injectors (17%). which is significant in the UK context since amphetamine users rarely present to drug agencies.

Most clients were not receiving treatment elsewhere: 31% had no previous treatment, 35% had previous treatment but not currently, and 34% were in current treatment.

When interviewed (FCQ) a majority had never had help for their drug use from: social worker (74%): probation officer (51%): drug dependence unit (53%): out-patient department (62%): in-patient detox. (68%) or other IP treatment unit (57%): therapeutic or residential community (68%): private clinic (89%) or private doctor (75%): self help group (75%): accident and emergency department (54%).

Many clients attended for only a few visits, with 34% making one visit only, and 53% making two visits only. Clients who attended once only were: slightly younger (25.9 yrs, sd 5.5 against 27.3, sd 6.5 $p = 0.031$), were more likely not to have had previous treatment, (41.4% against 28.2%, $p = 0.001$) and tended (ns) to have slightly shorter elapsed time since their first injection (7.1 yrs, sd 5.3 against 8.0, sd 5.7. $p = 0.152$). The pattern of attendance is accounted for by diversion to other help and treatment, the fact that syringe-exchange schemes are not monopoly suppliers (in the UK pharmacists are allowed to sell syringes), accessibility (most clients [54%] who continue attending travel two miles or less to their scheme), and by other client outcomes such as imprisonment, hopitalisation and other interuptions to drug use.

Supply and exchange of injecting equipment

All agencies offer a choice of syringe and needle sizes for clients. All (except one) supply swabs and condoms. Four supply sterile water. Supplies are free to clients except in the pharmacy scheme. Clients were given an average of 7 syringes per visit, with a range from 1 to over 100. Cost of equipment issued per client visit is approximately £0.70. Returned equipment is subject to a visual check and clients deposit loose equipment in a safe container for later destruction. Staff then issue new

equipment, on approximately a one-to-one basis. The average rate of return of used syringes was 78%. including equipment issued to clients who have not returned.

The staff cost (wages) per client reached by agencies is in the range of approximately £36 to £100.

BASELINE HIV RISK BEHAVIOUR OF CLIENTS

The baseline risk behaviour is assessed by FCQ in the first month of attendance (reported here in the interviewed sample n=182).

Baseline syringe-sharing is presented in Figure 1. Sexual behaviour is presented in Figure 2.

FIGURE 1. Sharing needles and syringes. Baseline behaviour reported at FCQ. All percentages are based on FCQ, total sample base of approx. 182

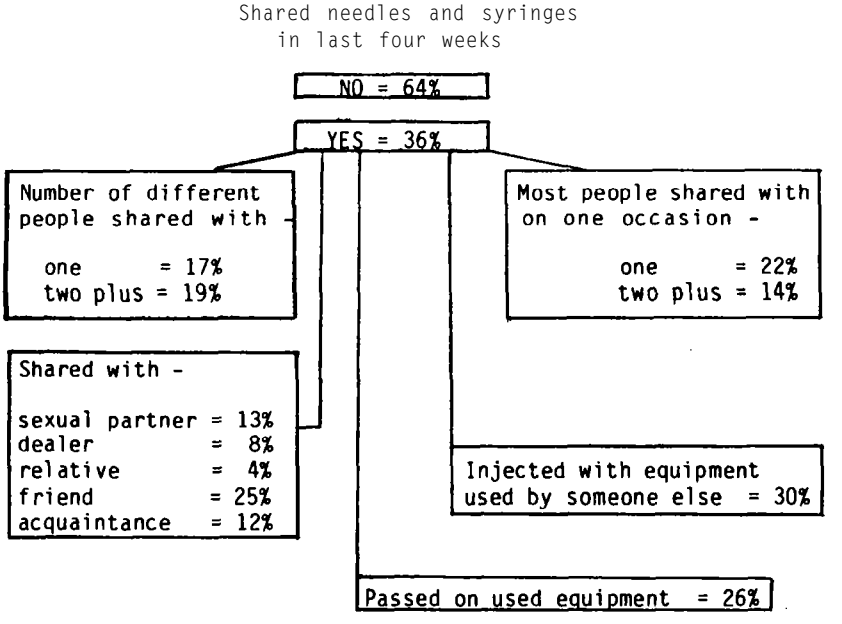
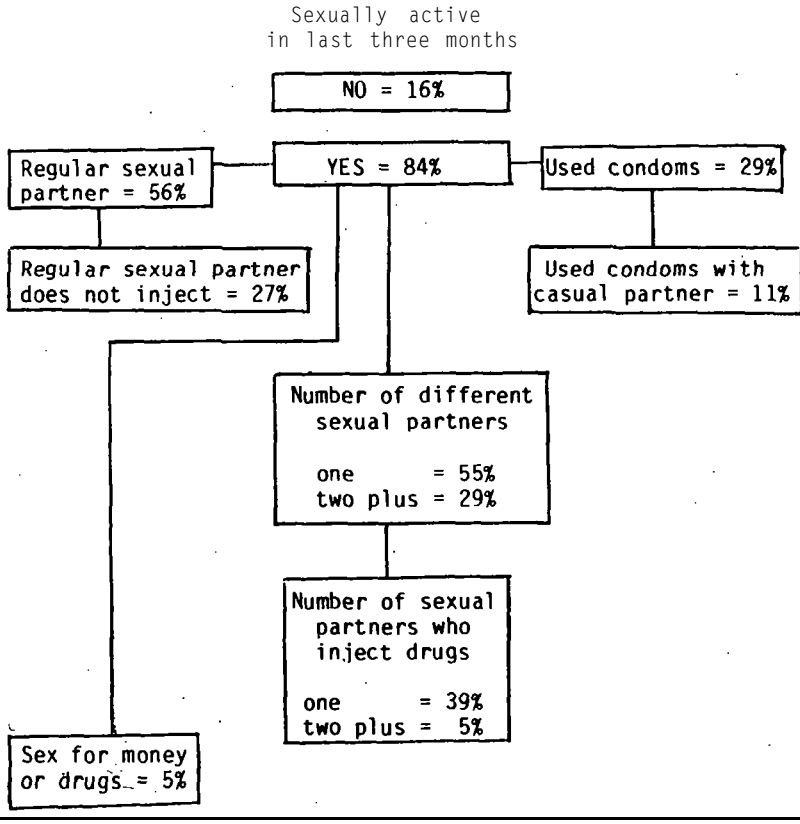


FIGURE 2 Sexual behaviour. Baseline behaviour reported at FCQ. All percentages are based on FCQ, total sample base of approx. 182



Only a minority of clients (8%) believed that they were at high risk of HIV infection. A further quarter (24%) believed that they had "some" risk. The majority of clients believed they had a "low" (43%) or "no" (24%) risk of contracting HIV.

There was a ceiling effect in terms of knowledge of many aspects of HIV transmission. Over 94% of clients agreed that (a) rinsing a syringe in water is not a safe method of cleaning (b) that it is unsafe to share syringes even occasionally. There was uncertainty about the interpretation of HIV test results with 39% agreeing that HIV infection always shows up in a test.

CHANGES IN SYRINGE SHARING BEHAVIOUR

There is provisional evidence of change in risk behaviour amongst those who continue attending schemes. There is a reported decline in sharing equipment, measured across a number of variables. This has been assessed by a comparison of data for the first 106 cases who were interviewed with the first questionnaire (FCQ) and followed-up with second questionnaire (SCQ) at 2-4 months (to end March 1988). Table 1 gives group comparisons on several variables which measure syringe sharing. The overall rate of sharing needles and syringes has declined from 35% to 25% (note that the percent sharing at FCQ differs from Figure 1 because the sample sizes are different). There is a reported decline in needle and syringe sharing across all measures, which gives reasonable confidence in the reliability of the measures.

TABLE 1 Changes in syringe sharing between first and second interview: group comparisons for first 106 cases to end March 1988

In last 4 weeks:	FCQ	SCQ
Not shared equipment	65%	75%
Shared equipment	35%	25%
Used others' equipment	26%	18%
Passed on used equipment	32%	24%
Shared with 2+ people	18%	13%
Shared with 2+ people, one occasion	15%	10%
Shared with sexual partner	12%	5%
dealer	9%	6%
relative	5%	5%
friend	23%	17%
acquaintance	14%	9%

A comparison of individual changes in reported behaviour is shown in Table 2. Whilst there is an overall reduction in syringe sharing, the pattern of changes exhibited by individuals show that the majority of clients have been able to sustain their ability not to share syringes (52%), and a further group

has stopped sharing (23%) or reduced sharing (4%). However, 14% who reported that they did not share at FCQ, reported sharing at SCQ.

TABLE 2 Changes in syringe sharing between first and second interview: individual comparisons for first 106 cases to end March 1988

Not sharing T1, not sharing T2	52%
Sharing T1, not sharing T2	23%
Reduced sharing T1 - T2	4%
Sharing at same level T1 - T2	3%
Increased sharing T1 - T2	5%
Not sharing T1, sharing T2	14%

Further evidence for the impact of the schemes in enabling clients to change behaviour is that at the first interview 55% gave as a reason for sharing that they found syringes hard to obtain, whereas by the second interview only 10% gave this as a reason.

CONCLUSIONS

Preliminary assessment of the implementation of the schemes

Many schemes have been operating for only a short time and are still establishing reputations and working practices. The picture is dynamic and assessment will alter as they develop. A provisional view is that (a) schemes reach clients who are not reached by other services for drug users (b) many clients lack primary medical and social care (c) schemes miss some groups: younger users, and women (d) client dropout is high: schemes need to increase retention maximise the benefits of first contact (e) syringes are distributed with acceptable exchange rates (f) counselling: staff and clients find it easier to discuss drug use than sexual behaviour (e) staff aim for a non-judgemental approach (g) working philosophies: all staff engage in HIV risk reduction and some have a general harm minimisation approach.

Preliminary assessment of risk behaviour

The significance of the national baseline data here is that the characteristics of the task of helping drug injectors to change their behaviour are identified.

Syringe sharing levels are lower than reported in earlier studies (Robertson et al., 1986 a b; Mulleady and Green 1985:

Brettle 1986, Brettle et al 1986) but despite this the number of sharing occasions is high. On our data, for every 100 injectors there will 30 who use others' equipment, on average 12 times in the last four weeks. A minority engage in high risk sharing.

Sexual activity is slightly higher than reported in a similar age group in a recent national survey Department of Health and Social Security and Welsh Office 1987), many had sexual partners who did not inject drugs, and condom use was uncommon with casual partners.

Obstacles to risk reduction

Despite accurate knowledge of HIV transmission a significant proportion engaged in high risk behaviours at or soon after intake. This suggests that there are obstacles to the adoption of less risky behaviours. The current prevention strategy provides knowledge about the need to change, and provides the means (syringes) to change. A prevention strategy must consider factors that make it difficult for clients to change, and sustain change over a range of situations and circumstances. New approaches might include helping drug users to develop the social skills for adopting safer practices and avoiding situations where risk will occur.

Preliminary assessment of changes in risk behaviour

Based on the small sample who continued in attendance at syringe exchange schemes and who received both first and second questionnaires our assessment is that the schemes appear to have been successful in helping many clients to sustain low risk behaviour, have helped others to reduce their risk by ceasing to share syringes, and helped some to reduce the level at which they share. These are provisional conclusions based on a small sample who to date have been interviewed. We noted above that many clients do not continue attending schemes. There are other outcomes for clients who do not continue attending, but these outcomes have not been assessed at this stage of the research.

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AUTHORS

Gerry V Stimson PhD, Lindsey Alldritt MSc, Kate Dolan BA and Martin Donoghoe BSc

Monitoring Research Group, University of London Goldsmiths' College, London, SE14 6NW, UK

Current Epidemiology of AIDS Among IV Drug Users in New York City

D. Des Jarlais

Through 17 June 1988, there have been 5478 IV drug users (including 684 who also engaged in male homosexual activity) among the 14,660 adult cases of AIDS in New York City. In the cases reported since the beginning of 1988, IV drug use has been the most common risk behavior (NYC Dept. of Health personal communication). HIV has been present among IV drug users in New York City for over a decade.

The first evidence of HIV infection among heterosexual IV drug users in the city comes from the pediatric cases of AIDS. In 1977, three children later developed AIDS, so it must be assumed that they had contacted HIV infection perinatally and that the mothers were infected. The first known HIV seropositive blood sample from an IV drug user was collected in 1978, as part of a study of liver disease conducted at Beth Israel Hospital. Later samples from this same study show the HIV seroprevalence rate rising rapidly from 1979 through 1982 (Novick et al., 1986).

Since 1984, we have been conducting seroprevalence studies of IV drug users who had recently entered treatment in Manhattan. These studies have shown a relatively stable seroprevalence rate of 55% to 6O%. The seroprevalence rate among the different cohorts entering treatment has ranged from 54% to 63%, with no increase overtime (Des Jarlais et al., 1988). The highest and the lowest rates came from different programs, and were collected in 1984. Primm and colleagues have also been conducting seroprevalence studies of IV drug users entering treatment in New York, and also find a stable seroprevalence rate (Brown, Jr., L.S. et al., 1988). Studies of IV drug users not in treatment in New York also find a similar seroprevalence rate (Kleinman, P., et al., 1988)

The relatively stable seroprevalence rate in New York does not mean that there are no new HIV infections occurring among IV drug users in the city. Rather, it should be seen as a balance between a lowered infection rate, loss of seropositives from active IV drug use and entry of new (seronegative) persons into IV drug use. Even at the current lowered rate of new infections (estimated from cohort studies to approximately 7% per year of seronegatives, Des Jarlais, D.C. et al., 1988), there is still

a substantial probability that a currently seronegative IV drug user will become infected with HIV before he or she permanenttly stops injecting drugs. The relatively stable seroprevalence rate should be seen as providing more time to do needed AIDS prevention, rather than suggesting that prevention is not med.

The current HIV situation among IV drug users in New York City suggests that we need to expand our epidemiologic research activities. We need not only to continue our seroprevalence, natural history and behavior change studies, but also to develop a better understanding of the size and dynmics of the IV drug-using population in the city. The current estimate of the number of IV drug users in the city is 200,000 (NYS DSAS State Plan Update). This estimate is based on a procedure that was developed in the late 197Os and is now becoming very dated. As a check on this estimate, we did a simple capture/recapture estimate in 1984. One hundred consecutive deaths among known narcotic users were matched against the methadone registry, which at that time contained 68,000 persons known to have entered methadone treatment since the fall of 1978. There was a 30% match of the deaths and the methadone registry, giving a very rough estimate of 227,000 IV drug users in the city from 1978 through 1984.

Assuming that some of these drug users had permanently stopped using drugs during this time, this capture/recapture check suggests that the current estimate of 200,000 IV drug users for the city is likely to be correct to the nearest 50,000. It also emphasized the need for better knowledge of the rate at which persons enter and leave IV drug use in New York City.

We are now past the periods of introduction and rapid spread of HIV among IV drug users in New York. The current phase of the epidemic is one of a lowered rate of new infections, with a substantial risk for both current and new IV drug users. Understanding the future spread of HIV among IV drug users in New York will require not only studies of risk behavior of current Iv drug users, but also studies of the dynamics of IV drug using population.

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AFFILIATION:

New York state Division of Substance Abuse Services
New York, New York 10013

The Role of Intravenous Drug Use in Cases of AIDS in Adolescents and Young Adults

H. Gayle

As of June 30, 1988, more than 65,000 cases of the acquired immunodeficiency syndrome (AIDS) had been reported to the Centers for Disease Control (COC). Of these, 743 (1%) were adolescents, 13-21 years old. Another 2,353 (4%) were young adults, 22-24 years old, most of whom were probably infected with the human immunodeficiency virus (HIV) as adolescents. Most AIDS cases of AIDS in adolescents and young adults can be attributed to sexual contact, 59% of cases to male homosexual or bisexual contact and 8% to heterosexual contact. Another 10% of cases are in homosexual men who also use intravenous (IV) drugs and may have been infected through sexual contact or sharing needles. Intravenous drug use (IVDU) accounts for up to 23% of cases in this age group with 13% attributed to IVDU alone. However, if all cases related to IVDU are considered, including those of persons infected by a sexual partner who uses IV drugs, then IVDU accounts for 26% of all cases in this age group.

The proportion of adolescent and young adult AIDS cases related to IVDU varies by age, race, sex, and geographic location. The proportion of IVDU-related cases increases steadily with age: 8% of all AIDS cases in 13-15 year olds, 20% in 16-18 year olds, 24% in 19-21 year olds, and 28% in 22-24 year olds.

A greater proportion of AIDS cases are related to IVDU in black and Hispanic adolescents than in young adults whites. Nineteen percent of AIDS cases in white patients are related to IVDU. However, in blacks and Hispanics 27% and 48% of AIDS cases, respectively, are IVDU-related.

Most AIDS cases in adolescent and young adult women are related to IVDU. Sixty-two percent of cases in these women are related to IVDU, with 39% of cases in female IV-drug users and 23% in sexual partners of IV-drug users. For adolescent and young adult men, 21% of cases are related to IVDU, with 10% in male IV-drug users, 11% in homosexual male

IV-drug users, and <1% in male sexual partners of IV-drug users.

Twenty-seven percent of all adolescents and young adults with IVDU-related AIDS reside in New York State, wnile 19% of all persons with AIDS in this age group reside there. New Jersey and Puerto Rico both have 10% of the IVDU-related cases and 6% and 3% of the total adolescent and young adult cases respectively. Therefore, in relation to their overall proportion of adolescent and young adult cases, New York, New Jersey, and Puerto Rico are relatively overrepresented.

When adults are compared with adolescents and young adults, the proportion of cases attributed to IVDU directly is greater for adults, but the proportion of cases in the sexual partners of IVDU is greater in the younger age group. Additional comparisons between these aye groups did not show substantial differences.

The number of IVDU-related cases in adolescents and young adults over time increased steadily, with 3 cases reported in 1981 and 747 cases reported by 1987, the last full reporting year. This increase is proportional to the increase in IVDU-related cases in the adult population.

Although most AIDS cases in adolescents and young adults are attributed to sexual contact, over 20% are related to IVDU. Adolescents and young adults who use IV drugs may not be adequately reached by existing programs to prevent HIV infection among IV-drug users. Many adolescents and young adults who are sexual partners of IV-drug users may not consider themselves at risk for HIV infection. To be effective prevention efforts must consider the developmental and sociodemographic features that characterize at-risk adolescent populations.

Helene D. Gayle, M.D., M.P.H.
AIDS Program
Center for Infectious Diseases
Centers for Disease Control

Synthesis and Binding Studies of Affinity Ligands Based on Ditolylguanidine (DTG). A Study of Their Interactions with the Sigma Binding Site

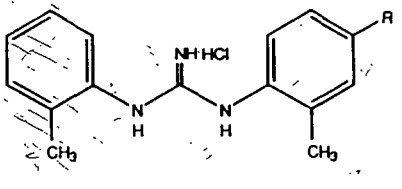
C. Kim, A. Reid, R. Rothman, A. Jacobson and K. Rice

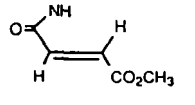
We have previously described the synthesis and the biochemical and pharmacological effects of a number of irreversible ligands for opioid and PCP receptors. These compounds have proven to be invaluable tools for the characterization of the structure and function of receptor subtypes. We have also reported that variation of the relatively small electrophilic group at C_7 in the endoethanooripavine family of opioids markedly altered the opioid receptor subtype selectivity (Jacobson et al., Life Sci. 33: 159, 1983).

Recently, Weber et al. (Eur. J. Pharmacol. 142: 61, 1987), described the synthesis of isothiocyanate 2, an irreversible ligand based on DTG (1) which specifically labels sigma binding sites but does not interact with PCP receptors..

In order to determine the effect of changes in the size and type of the electrophilic group in DTG on the sigma binding sites, we have resynthesized 2, and have synthesized three novel compounds 3, 4, and 5 which contain various electrophilic groups.

Compound 4 (at 1 µM) produced a significant irreversible decrease in [^{3}H]DTG binding of 31%, but compounds 3 and 5 were neither active nor selective up to 10 µM.



R = H;	DTG (1)
R = NCS	DIGIT (2)
R = $NHCOCH_2Br$;	DIGBA (3)
R = $O(CH_2)_2O(CH_2)_2NCS$;	DIGIE (4)
R = 	DIGMF (5)

AFFILIATION
Section on Drug Design and Synthesis, LN, NIDDK, NIH (C.H.K, A.E.J, K.C.R), and Unit on Receptor Studies, LCS, NIMH (A.R., R.B.R), Bethesda, Maryland 20892.

Cerebral Sonographic Characteristics and Maternal and Neonatal Risk Factors in Infants of Opiate Dependent Mothers

M. Pasto, S. Ehrlich, L. Graziani, A. Kurtz, B. Goldberg, K. Kaltenbach and L. Finnegan

The effect of in-utero narcotics on the developing central nervous system are still not fully known. To analyze associated neonatal factors and further expand the data base of sonographic parameters (i.e. ventricular configuration and cerebral measurements) 139 infants were prospectively studied over a 36 month period. Subjects were matched against a control group born to drug free mothers comparable in race, age and relevant socioeconomic factors. Data were analyzed on 47 drug exposed and 31 control infants who had examinations within 72 hours of birth and again at 1 and 6 months of age. Drug exposed infants were smaller in both birth weight ($p = .05$) and head circumferences ($p = .03$). Gestational age and incidence of intrauterine growth retardation were similar to both groups. For the drug exposed infant factors studied, meconium staining was higher ($p = .01$) and 11% had nonsurgical heart conditions and another 11% had transient anemia or bilirubinemia (0% in control). At birth, drug exposed infants had more slit like ventricles ($p < .001$), smaller lateral ventricles (right $p = .02$, left $p = .06$), and intracranial hemidiameters ($p = .01$) than the control infants. When comparing all infants with slit-like ventricles ($n = 55$) to all those without ($n = 22$), the slit group: 1) was lower in weight, 2) had smaller head circumferences, 3) had a slightly higher complication rate and 4) showed a higher percentage of slits in whites (88%) versus slits in blacks (64%).

AFFILIATION

Departments of Radiology, Neurology and Pediatrics, Thomas Jefferson University, Philadelphia, PA 19107.

Effects of Triazolam and Buspirone on the Acquisition and Performance of Response Chains in Humans

W. Bickel, S. Higgins and J. Hughes

An assessment of a drug's abuse liability includes the degree of behavioral impairment it produces. For example, triazolam is reported to produce behavioral impairment to a greater extent then other similar drugs, while buspirone is reported to produce little behavioral impairment. Previous studies, however, have not examined the effect of these drugs on human learning. In this study, the effects of triazolam (0, 0.25, 0.5, 0.75 mg/ 70 kg body-weight, p.o.) and buspirone (0, 10, 20, and 30 mg/70 kg body-weight, p.o.) were examined on human learning (acquisition) and performance of response sequences.

Subjects were normal adults. The experimental task was the repeated acquisition of behavioral chains procedure. The task was composed of two components. In the first component, acquisition, subjects were required to learn via trial and error to advance a digit located in the center of the video screen from zero through 0 using three keys. A new response sequence had to be learned each time the task was done. In the second component, performance, subjects were presented with the same sequence each time the task was done. Sessions were conducted pre, 30, 60, 90 and 120 min post drug administration.

Triazolam generally produced a dose-related increase in percent errors in the acquisition component, but only increased errors at higher doses in the performance component. Buspirone generally produced an increase in percent errors at the highest dose on acquisition, but generally had minimal effects on performance. Responses rates did not show selective effects across the components Triazolam produced a greater magnitude increase in percent errors then buspirone and consequently appear to have a greater abuse potential.

AFFILLIATION: Departments of Psychiatry and Psychology, University of Vermont, 1 South Prospect, Burlington, VT 05401

Rapid Eye Test to Detect Drug Influence

F. Tennant

The author has formalized a rapid eye test (RET) comprised of five common physical signs known to occur with drug use: (1) abnormal pupil size: (2) retarded pupil reaction to light: (3) nystagmus; (4) non-convergence: and (5) general observation for redness, glazing, watering, droopy eyelid, retracted upper eyelid, and swollen eyelids. Screening can be conducted in less than two minutes, and can be taught to allied health and non-medical persons. A screen is considered positive when two of the five signs are present, and this is reason to confirm findings with urine or plasma assay.

To help determine the validity of eye screening, two nurses were trained in the procedures. Between March and July, 1987, 79 consecutive patients were admitted to an outpatient drug treatment program. One of the nurses performed the RET with no knowledge of the patient's history. Based solely on eye signs, the nurse picked one drug, cocaine, marijuana, phencyclidine, amphetamines, or heroin that she believed most likely to produce the positive RET. Confirmatory urinalysis was done by Polarized Flourescent Immunoassay (Abbott TDX^{R}). Twenty-nine (29;36.7%) patients had a positive eye screen, and the nurse correctly predicted the drug of influence in 24 (82.7%) tests. Forty-eight (48) of the 79 patients had one or more drugs in their urine, 24 (50.0%) of these had a positive eye test. Of the 31 patients who had no drugs in their urine, 26 (83.9%) had a negative eye screen. Even though the nurses could pick only one suspected drug, they demonstrated a predictability of 82.7% and the test proved to have a specificity of 83.9%.

It is concluded that a positive eye screen, under the criteria established here, is very suggestive of drug use and warrants confirmatory body fluid confirmation.

AFFILIATION
Community Health Projects Medical Group
336 So. Glendora, West Covina, California 91790

Conditioned Tolerance in Opiate Addicts

Ft. Ehrman, J. Ternes, J. Holahan and C. O'Brien

Using a rat model, Siegel has demonstrated that tolerance to opiates is in part situationally specific. He has suggested that situationally specific or conditioned tolerance (CT) is a learned phenomenon mediated by environmentally elicited conditioned responses (CRs) which oppose or compensate for drug induced effects. In support of this explanation, drug related environments where CT is observed have been shown to elicit withdrawal like CRs in the absence of drug administration. Further, Siegel has hypothesized that accidental overdose deaths may in part be the the consequence of drug use in a nondrug related environment.

CT has previously been observed only in animals. In the present investigation this phenomenon was studied in detoxified opiate addicts. Both physiological and subjective dependent variables were utilized. Each addict participated in four experimental sessions. Two trials entailed the drug cook up ritual followed by hydromorphone or saline self-injection and two trials consisted of the infusion of either saline or hydromorphone.

The results from this experiment indicate that: 1) drug ritual stimuli elicit drug opposite (withdrawal like) responses in skin temperature, skin resistance and heart rate, 2) drug ritual stimuli increase the subjective report of craving but do not substantially influence feelings of withdrawal or high, 3) low correlations between physiological responsivity and subjective state following drug stimulus presentation, 4) evidence of conditioned tolerance was obtained for the skin temperature and heart rate but not for skin resistance or any of the subjective measures.

Given the results of this study and the potential relationship between accidental overdose and CT, further investigation to determine whether this laboratory phenomenon exists in the natural environment of the active opiate abuser and the environmental circumstances surrounding accidental overdose is clearly warranted.

AFFILIATION

Department of Psychiatry, University of Pennsylvania Medical School and Psychiatry Service, Philadelphia VAMC.

Effects of Sigma Ligands on Rubral Neurons

R. Matsumoto and J. Walker

Sigma receptor ligands induce postural and locomotor effects when microinjected into the red nucleus and substantia nigra of rats. To further investigate the physiological role of sigma receptors in vivo and their involvement in motor regulation, the responses of rubral neurons to iontophoretic applications of 1,3-di-o-tolylguanidine (DTG), (+)-3PPP and (+)-pentazocine were examined in this study.

Extracellular recordings were made in halothane-anesthetized rats. Single units were recorded from for 5 minutes before 50 mM DTG, (+)-3PPP or (+)-pentazocine were iontophoretically applied. DTG was applied on 16 neurons in the red nucleus. Inhibition was produced in 14 cells (88%), no effect was observed in 2 neurons (12%) and no excitations were seen. Fewer effects were produced by DTG in the surrounding reticular formation, corresponding to the lower density of sigma receptors.

(+)-3PPP had varied effects on rubral neurons. Of the 13 neurons tested, 2 showed inhibition (15%). 5 responded with excitation (38%) and 6 showed no effect (46%). Although (+)-3PPP produced few inhibitions, the effects were dramatic and clearly linked to applications of the drug. The excitations, on the other hand, were weak and occurred primarily at high ejection currents. Preliminary studies with (+)-pentazocine suggests that it mainly has inhibitory effects.

It is noteworthy that all of the rubral units recorded in this study were located in the magnocellular division of the red nucleus and displayed the large amplitudes typical of neurons in the rubrospinal tract. It is possible that sigma receptors in this pathway are associated with some of the postural and motor effects produced by sigma ligands.

Affiliation: Department of Psychology, Brown University, Providence, RI 02912

Effect of Repeated Administration of a Combination of U-50,488H and CPZ on Body Temperature of Rats

R. Martinez, E. Geller and M. Adler

The combination of the kappa opioid receptor agonist U-50,488H(trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamide methane-sulfonate hydrate; 80.0 mg/kg, s.c.) and the neuroleptic chlorpromazine hydrochloride (CPZ; 5.0 mg/kg, s.c.), produces a profound hypothermia ($\leq$ S11°C at 20°C ambient) in rats that is partially reversible by naloxone. When administered separately, each drug decreases body temperature, but the combination results in a potentiated (superadditive) effect. In the present study, rats were kept in individual cages in a Hotpack environmental room at 20±0.3°C ambient and 50±5% relative humidity. Temperatures were recorded by means of a digital thermometer at 1-h intervals for 6 h post-injection. The drugs were dissolved in 0.9% saline on the test day. Administering the combination of CPZ and U-50,488H once a day for 6 days resulted in what appears to be a development of tolerance to the hypothermic effects of the drugs. After 6 daily administrations of the drug combination, the peak effect occurred sooner (240 min compared to 360 min) and the maximum effect was reduced from -8.03±0.77°C below control to -3.58±0.77°C. Comparison with the results from animals administered CPZ or U-50,488H alone showed that the combination still appeared to have a greater than additive hypothermic effect on the final day of testing, although this effect was much smaller than that observed after the acute administration. Both pharmacokinetic and pharmacodynamic factors may be involved in the development of hypothermia with this combination of drugs.

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AFFILIATION

Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140

Morphine-induced Immunosuppression: Involvement of Glucocorticoids and Prolactin

H. Bryant, E. Bernton and J. Holaday

Morphine pellet implantation produces a number of immunosuppressive effects in mice. Chronic morphine administration also affects a number of neuroendocrine responses which are known to affect immune function (e.g. corticoserone is immunosuppressive, and prolactin is immunopermissive. Therefore, these studies were conducted to examine possible neuroendocrine based mechanisms for immunosuppressive effects of morphine pellet implantation.

Morphine implants (75 mg; NIDA) in sham ADX C3H/HeN mice elevated serum corticosterone levels over a 6 to 72 hr window following implantation. Within 48 hr, morphine pelleted mice exhibited marked reductions in spleen and thymus size relative to placebo pelleted controls. Adrenal hypertrophy was noted, and lymphocyte proliferative responses to a T-cell mitogen, concanavalin A (Con A) and a B-cell mitogen, lipopolysaccharide (LPS) were reduced in morphine pelleted mice. Splenic and thymic atrophy were less pronounced in ADX-morphine pelleted mice. The magnitude of the attenuation of LPS-induced lymphocyte proliferation was reduced in ADX-morphine pelleted mice and the Con-A response was essentially normal in ADX-morphine mice. Effects similar to adrenalectomy were found in morphine pelleted mice given 3 to 10 mg/kg RU-486 twice/day.

Twice daily injections of either prolactin (1 mg/kg) or metoclopramide (25 mg/kg) did not alter morphine-induced splenic or thymic atrophy, although these regimens did reverse morphine-induced suppression of Con A-induced blastogenesis. Metoclopramide alone had a mild stimulatory effect on Con A-induced proliferation in placebo pelleted mice. However, morphine pellet implantation had no effect on circulating prolactin levels over a 6 to 120 hr time period, suggesting the pharmacologic reversal of the suppressed Con A response in morphine pelleted mice does not necessarily imply a role For prolactin in the immunosuppressive effects of chronic morphine.

In conclusion, activation of the hypothalamo-pituitary-adrenal axis contributes to morphine-induced immunosuppression. while a role for prolactin in the effect is unlikely. (RU-486 kindly supplied by Rousell Uclaf).

AFFILIATION:

Department of Medical Neuroscience, Division of Neuropsychiatry. Walter Reed Army Institute of Research, Washington, D.C. 20307-5100

Women at Risk for AIDS and the Effect of Educational Efforts

C. Arenson and L. Finnegan

In order to evaluate educational efforts in the prevention of HIV infection in at risk women, 42 drug dependent pregnant and post-partum women receiving comprehensive treatment were surveyed. Drug use history revealed that *95%* are current or past IV drug users with an average of 12 years of needle sharing; 82% of the subjects reported cessation of needle sharing. The women completed questionnaires detailing their sexual and drug-using behaviors followed by a 30 minute factual workshop on AIDS. Questionnaires were repeated two to three weeks after the workshop. Results revealed that 79% are currently engaged in some risk behavior for HIV infection, such as prostitution, lack of condom use and sexual partners who are IV drug users. Nearly all women (97%) knew that AIDS is spread by sharing needles and 88% knew how to effectively clean needles. All women knew that condoms provide protection during intercourse. The women showed concern about AIDS and 71% feared infection, but did not believe they would catch the disease in spite of their past and/or current high risk behaviors. AIDS workshops had little influence on the subjects' knowledge and behavior since differences in pre and post tests were insignificant. These women are clearly at high risk for exposure to HIV infection, both through sexual and needle sharing behaviors. Although our survey revealed some cessation of needle sharing practices, condom use and other safe-sex practices were negligible. Conventional teaching efforts had a limited effect on increasing knowledge or changing behaviors. These women clearly remain at high risk for future infection in them and their children until IV drug use ceases and sexual behaviors change.

AFFILIATION

Department of Pediatrics, Thomas Jefferson University, Philadelphia, PA 19107.

Plasma Neurochemical Markers in Drug Dependence to Select Treatment Agents and Predict Outcome

F. Tennant

Two pilot studies to determine if a plasma neurochemical marker may be useful to select treatment agents and/or predict short-term treatment outcome were done. Of eight cocaine-dependent subjects, three had a good short-term treatment outcome as judged by remaining in out-patient treatment for 28 days and converting urine from cocaine-present to cocaine-absent. Treatment consisted of amantadine and desipramine. Prior to beginning treatment, two of the three (66.7%) good-outcome compared to zero of five (0%) poor-outcome subjects raised their plasma dopamine two hours after a 200 mg oral amantadine challenge by more than 5% (P=.107; Fischer's Exact Test.

Six long-term, relapsing nicotine-dependent subjects were assessed by a variety of neurochemical measures just prior to a six-week medical withdrawal regimen consisting of the dopaminergic agents, amantadine and levodopa. The four subjects who were able to cease smoking as documented by plasma cotinine and urine-nicotine analysis, had a significantly lower plasma dopamine level (mean=21.8 pg/ml; Range 20-27) than the two unsuccessful smokers (mean=245.5 pg/ml: Range 205-286). (P< .05).

Although the results of these two, pilot studies are very preliminary, they suggest that plasma dopamine and perhaps other plasma markers may help select treatment agents and predict outcome.

AFFILIATION

Community Health Projects Medical Group
336 South Glendora Avenue, West Covina, California 91790

Tolerance and Cross-tolerance to 3, 4-Methylenedioxymethamphetamine (MDMA), Methamphetamine and Methylenedioxyamphetamine

J. Zacny, R. Virus, M. Kleven, L. Seiden and W. Woolverton

The effects of (±)-3,4-methylenedioxymethamphetamine (MDMA) (0.62-20.0 mgkg), (+)-methamphetamine (MA) (0.62-5.0 mg/kg) and (±)-3,4-methylenedioxyamphetamine (MDA) (0.62-5.0 mg/kg) on milk intake in rats were determined before and during a period of repeated daily administration of MDMA. Experimental sessions consisted of 15-min access to a sweetened milk solution each day, 5 days a week. After determination of the acute effects of MDMA, MA and MDA on milk intake, rats were injected 15 min before or after each session with either MDMA (2.5-5.0 mg/kg) or saline. During this chronic administration period the acute effects of MDMA, MA and MDA (injected 15 min prior to the session) were redetermined. In rats that had been injected with MDMA on a chronic basis either before or after the milk-drinking sessions, the dose-response function of MDMA was shifted to the right, indicating that tolerance had developed. A greater degree of tolerance had developed in the group of rats injected with MDMA before, rather than after, the sessions. Cross-tolerance to MA developed only in the group of rats that had been injected with MDMA on a chronic basis before the milk-drinking sessions. Cross-tolerance to MDA did not develop in any of the 4 groups of rats. The rats were sacrificed 50 days after completion of the redetermined dose-response functions and their brains were dissected for regional analyses of monoamines. Levels of the serotonin metabolite, 5-hydroxyindoleacetic acid, in striatum were higher in the two groups that had been injected with MDMA on a daily basis; otherwise, serotonin, dopamine, and dopamine metabolite levels did not differ across the groups. Our results suggest that tolerance develops to the behavioral effects of MDMA and that cross-tolerance develops to MA but not MDA. Further, the fact that tolerance to MDMA developed in groups of rats injected before or after the milk-drinking session suggests that a pharmacologic component is involved in tolerance to MDMA, but probably does not involve long-term monoamine changes. Environmental variables may play a role in determining the extent of tolerance development to MDMA and whether cross-tolerance develops to MA.

AFFILIATION

Drug Abuse Research Center, Pritzker School of Medicine, The University of Chicago, Chicago, Illinois 60637

The Prevalence of HTLV-1 and HIV-1 Infection in Intravenous Drug Abusers in an AIDS Endemic Area

L. Brown, Jr., H. Lee., M. Cerney, J. Allain, B. Primm, A. Chu and K. Foster

While human immunodeficiency virus (HIV-1) infection has legitimately been the target of many investigators, the significance of infection by other related retroviruses remains unclear. We examined the seroprevalence of HIV-1 and human T-cell lymphotropic virus type-I (HTLV-1) infection in 731 intravenous drug abusers (IVDAs) in New York City methadone maintenance clinics.

Under a human subject approved protocol, patients recently admitted to drug treatment were recruited for voluntary participation. Following informed consent, a standardized questionnaire was administered about health history and patterns of drug use. Medical examinations were performed and blood specimens were collected, screened and confirmed for the Presence of HTLV-1 and HIV-1 antibodies and HIV antigen.

The overall HIV-1 and HTLV-1 antibody prevalence was 56.6% and 17.6%, respectively. The HIV antigen prevalence was 3.7%. Sixty two percent of the study population was infected by either retrovirus; once infected by either retrovirus, infection by the other was greater ($p<0.0001$). The prevalence of dual infection was 12.4%. No differences in drug use patterns, medical history or physical examination were appreciated between IVDAs infected with both retroviruses and those with infection by either. Dual infection seen In this population supports similar routes of transmission for both retrovlruses with lesser infectivity for HTLV-1. HTLV-1 does not appear to affect the clinical status of HIV-1 infection.

AFFILIATIONS: Addiction Research and Treatment Corporation, Brooklyn, NY and Harlem Hospital, College of Physicians and Surgeons and Health Administration Division, School of Public Health, Columbia University, New York, NY, and Abbott Laboratories, Chicago, Ill.

Cocaine and Pregnancy: Maternal and Infant Outcome

S. Livesay, S. Ehrlich, L. Ryan and L. Finnegan

The number of infants born to women who abuse cocaine is rapidly increasing. Although the effects on the adult user are known, little has been reported regarding the neonate. Subjects included 239 women: 93 cocaine-using drug dependent women, 83 non-cocaine-using drug dependent women and 63 non-drug dependent comparison women. The drug dependent women were enrolled in a program providing pre and postnatal services. The groups were similar in maternal age, socioeconomic status, nicotine use and parity, but differed in race. Emergency cesarean sections, meconium staining and small for gestational age infants occurred more often in the cocaine group. Birth weight, length, head circumference, gestational age, one minute Apgar scores, mean neonatal abstinence scores and incidence of nuchal cords were significantly lower in the infants of the cocaine-using drug dependent women. No differences were found in the incidence of intracranial hemorrhage or congenital anomalies. The occurrence of Sudden Infant Death Syndrome and need for cardiorespiratory monitors was similar for cocaine and non-cocaine exposed infants of drug dependent women, but greater than the figures reported in the general population. Abruption placentae occurred in 9% of cocaine drug dependent women, 4% of non-cocaine drug dependent women and 2% of drug free women,. More premature deliveries were seen in the cocaine group (20%) than in the non-cocaine (11%) and drug free (3%) groups. These findings suggest that cocaine use in pregnancy adversely affects maternal and infant outcome.

AFFILIATION

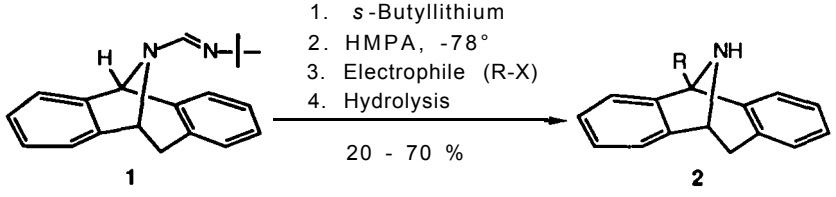
Departments of Radiology, Neurology and Pediatrics, Thomas Jefferson University, Philadelphia, PA 19107.

Synthesis and Biochemical Evaluation of MK-801 Analogues: C5-Substituted Derivatives of (±)-10, 11-Dihydro-5H-Dibenzo[a,d]cyclohepten-5,10-imine

J. Monn, A. Jacobson, M. Mattson, K. Rice, D. Kiesewetter and R. Finn

(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine (MK-801) is a highly potent and selective ligand for the phencyclidine (PCP) receptor which possesses anticonvulsant and neuroprotective properties. As part of our studies aimed at further characterizing the pharmacology of the PCP receptor, we sought an efficient synthetic route to C5-substituted analogues of MK-801 so as to a. explore the effect of various groups in this position on PCP receptor binding, and b. prepare a positron emitting (^{11}C) analogue of MK-801 for imaging brain PCP receptors in conscious mammals. We now report the realization of these goals for racemic dibenzo[a,d]cyclohepten-5,10-imines.

C5-Substituted analogues **2** were prepared by a novel lithiation-alkylation sequence employing racemic N-*tert*-butylformamidine derivative **1**, and examined *in vitro* for their ability to bind to the PCP receptor. From the preliminary biochemical data, it appears that the region of the PCP receptor adjacent to the C5-position of MK-801 preferentially interacts with small, lipophilic substituents. Utilizing the lithiation-alkyation sequence, a positron emitting isotope of (±) MK-801 -- (^{11}C) MK-801 -- has been prepared and purified to radiochemical homogeneity.



1 **2**

Affiliations

Section of Drug Design and Synthesis, Laboratory of Neuroscience, NIDDK (J.A.M., A.E.J., M.V.M., K.C.R.); Department of Nuclear Medicine, Clinical Center (D.O.K., R.D.F). National Institutes of Health, Bethesda, Maryland 20892

Effects of Nicotine on Physiological Responses to a Repeated Arithmetic Task

L. Epstein, K. Perkins, J. Jennings and S. Pastor

This study was designed to assess the effects of smoking and the conditioned effects of omission of nicotine and nicotine plus behavioral cues on electrodermal and neuromuscular (EMG) activity related to affect. Males smokers (19.7±4.4 cigarettes/day, .75±.45 mg) engaged in six arithmetic task trials. Prior to the first five trials, three groups smoked thelr usual cigarette brand while subjects In a fourth group did not smoke. Before the sixth trial, subjects either smoked their usual brand cigarette as in trials 1-5 (S/S), smoked a non-nicotine cigarette (S/O), or did not smoke (S/NS). Subjects in a fourth group remained non-smoking (NS/NS). Carbon monoxide and heart rate responses documented differences In smoke and nicotine delivery between smoking and non-smoking subjects during the first five trials. During trials 1-5 smoking was related to a significant decrease in the rate of non-specific electrodermal responses, and differential effects on facial EMG. Corrugator (frown muscle) activity was reduced non-significantly, while a significant increase in task zygomatic (smile muscle) activity was observed. These results are consistent with the data showing smoking is pleasurable, while it can decrease arousal. On trial 6, subjects in Group S/NS showed a significant increase in electrodermal responding and corrugator EMG compared with the S/S group, but not compared with the S/O group, suggesting that novel omission of nicotine plus behavioral cues but not nicotine alone was responsible for the electrodermal and neuromuscular changes.

AFFILIATION

Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

Sexual Behaviors of Intravenous Drug Abusers: Implications for Heterosexual HIV Transmission

L. Brown, Jr., B. Primm, A. Chu and K. Foster

Sexual contact with intravenous drug abusers (IVDAs) is the predominant mode of transmission for U.S. born heterosexual cases of AIDS; yet, little information is available about the sexual behaviors of the drug addicted. To further explore the pivotal role of IVDAs in heterosexually acquired HIV infection, we examined the sexual behaviors of patients enrolled in drug treatment clinics in Brooklyn and Manhattan of New York City during the Fall of 1966.

One hundred IVDAs were randomly recruited to anonymously complete a questionnaire regarding their drug use patterns and sexual behaviors during two time periods: 1977-1985 (interval one) and 19851986 (interval two). This study population reported 776 sex partners (65% of whom were non-IV drug users) during interval one and 378 sex partners (67% of whom were non-IV drug users) during interval two. Males averaged 11.1 and 5.5 sex partners respectively during interval one and interval two, while females averaged 2.7 and 1.2 sex partners during interval one and interval two, respectively. Males, in comparison to females, were more likely to have a non-IV drug using sex partner during interval one (p=.0001) and interval two (p=.01). Condoms were used only 18.6% of the time by the 12.2% of the study population who reported their use.

Given the previously reported 61% HIV infection rate in this population, the frequency of unprotected sex and the frequency of non-drug using sex partners, the IVDAs in this study represent a significant source for sexually transmitted HIV infection.

AFFILIATIONS: Addiction Research and Treatment Corporation, Brooklyn, NY and Department of Medicine, Harlem Hospital Medical Center, College of Physicians and Surgeons and Division of Health Administration, School of Public Health, Columbia University, New York, NY

Effects of Daily Versus Alternate-Day Dosing of Buprenorphine in Heroin-Dependent Volunteers

R. Johnson, P. Fudala and N. Krieter

Eighteen male subjects (heroin dependent at the time of admission) volunteered for a 12-week study. Using a parallel-group design, subjects were randomly assigned to one of two groups (A or B per group). All subjects underwent a 3-day dose induction procedure and were maintained on 8 mg of buprenorphine hydrochloride (BUP) administered sublingually through day 18. On days 19 through 30, Group A continued on an everyday dosing schedule while Group B continued on an every other day dosing schedule. Baseline physiolgic and behavioral measurements for each subject at each hourly observation time (6.5, 11.5, 14.5, and 23 hrs following BUP) were calculated as the mean of the measurements made on days 14 and 15. Observations made on days 19 through 30 were transformed to observation minus baseline values before performing AMOVAs. BUP or placebo were administered sublingually under double-blind conditions in a 30% (v/v)ethanol solution.

Pupillary constriction followed BUP administered; however, on non-drug days, Group B's pupils dilated. subscales of the Addiction Research center Inventory (ARCI), Observer and Subject Drug Effect Questionnaires, and a withdrawal symptom questionnaire were used to rate signs and symptom of acute opioid effects and withdrawal. On the days subjects in Group B received no drug, LSD scale scores (measure of dysphoria) and syptoms including muscle cramps/backaches, painful joints/weak knees, and runny nose all increased. There were significant differences between groups for "drug liking", "drug effect", and "good effect" on the days both groups received BUP, with Group B's responses being greater than Group A's. On the days all subjects received BLIP, subjects' rating of "high", "drug liking", "drug effect, and "good drug effect" all increased. Subject-rated withdrawal remained constant between treatment and baseline for Group B and decreased from baseline for Group A. Observer-rated withdrawal was generally higher for Group B and lower for Group A when treatment was compared to baseline. There were no differences between groups across days for any physiologic measures except pupil diameter; there were significant differences between groups across hours for all

physiologic measures except supine pulse and systolic blood pressure.

Although several behavioral and physiologic measures normally sensitive to opioid withdrawal indicated statistically significant differences between the two groups, the actual changes observed were not clinically significant as evidenced by small changes reported by both subjects and observers, and by the Phase of the study. Similarly, differences in agonist effects reported by subjects in both groups were also minimal.

Results from this study indicate that buprenorphine can be dosed on an every-other-day schedule with only minimal symptoms of withdrawal. However, it is not known whether the blockade afforded for other opioids by this dose of BUP would also be maintained for 48 hours. Thus, although the clinical use of buprenorphine on an every-other-day dosage schedule may be possible, the duration of both agonist and blockade effects must be considered.

AFFILIATION

NIDA, Addiction Research Center, Baltimore, MD 21224

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Trends in HIV Infection Among Intravenous Drug Abusers in an Economically Impoverished Area

L. Brown, Jr., R. Battjes, B. Primm, A. Chu and K. Foster

While intravenous drug use represents the second most frequent behavior associated with AIDS, the future of this epidemic can not be fully appreciated in discussions limited to persons meeting the CDC surveillance definition. Those persons, who can not be diagnosed as having AIDS but are infected with the human immunodeficiency virus (HIV), represent an important reservoir of infection. We have been monitoring trends in HIV seroprevalence in intravenous drug abusers (IVDAs) in New York City.

Following Informed consent, a standardized questionnaire of drug use, and sexual behaviors was administered to IVDAs recently admitted to drug treatment. Medical histories and blood specimens for HIV serological testing (ELISA and Western blot confirmation) were obtained. The HIV seroprevalence for 1985, 1986, and 1987 was 54%, 61%, and 59%, respectively. In each cohort, HIV antibodies were generally more prevalent in males, as compared to females, and among black and Hispanic IVDAs, as compared to whites. The greatest differences between these cohorts were appreciated in HIV seropositivity in black, white and male IVDAs. While the prevalence of tuberculosis and hepatitis remained stable, the HIV seroprevalence increased between 1985 and 1986 for those subjects who admitted to a history of clinical TB (from 50% to 64% HIV-Ab+) or hepatitis (from 46% to 70% HIV-Ab+). HIV infection in this sample may suggest that this population may be reaching its saturation point.

AFFILIATIONS: Addiction Research and Treatment Corporation, Brooklyn, NY and Department of Medicine, Harlem Hospital Medical Center, College of Physicians and Surgeons and Division of Health Administration, School of Public Health, Columbia University, New York, NY, and Division of Clinical Research, National Institute on Drug Abuse, Rockvllle, Md.

Use of the Formalin Test in Rats as a Tonic Pain Model

H. Wheeler, F. Porreca and A. Cowan

Historically, pharmacologists have evaluated analgesics in animal models of acute, rather than tonic, pain. The rat paw formalin procedure is a test giving continuous pain that is qualitatively different from the transient pain associated with conventional tests for analgesics. Widespread use of the formalin test in drug screening has probably been hindered by the subjective rating scales advocated in the literature. Here, we describe objective, spontaneous behavioral responses of rats towards formalin. Additionally, the influence of aspirin, morphine and PD 117302 (a selective agonist at kappa opioid recognition sites; (±)-trans-N-methyl-N[2-(l-pyrrolidinyl)-cyclohexyl]benzo[b]-thiophene-4-acetamide) on these responses is quantitated and compared.

Male S.D. albino rats (70-100 g; n=6-10) were each injected with 50 µl of 50% formalin under the skin of the dorsal surface of the right hindpaw. The rats displayed (a) flinching/shaking of the paw and hindquarters and (b) licking of the injected paw. The time course of these responses was biphasic with an initial peak at 0-10 m and a more enduring response at 20-50 m post-formalin. $A_{50's}$ for morphine against both early and late phases of flinching and licking were comparable (0.5-1 mg/kg, s.c.). PD 117302 was 45 times less potent against acute flinching than it was against tonic flinching (A_{50}=0.4 mg/kg, s.c.). In contrast, this agent was more potent (15x) against early phase licking (A_{50}=0.03 mg/kg) than against late phase licking. Aspirin (125-500 mg/kg, p.o.) was not active against late phase responding but (weakly) attenuated both behaviors during the early phase in a manner unrelated to dose.

It is concluded that (a) the formalin test may be used to investigate both phasic and tonic pain and (b) analgesics show differential activities depending upon the nature of the endpoint under study. (Supported by Grant DA 03945 from NIDA).

AFFILIATIONS

Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140 (HW and AC) and University of Arizona College of Medicine, Tucson, AZ 85724 (FP).

The Effectiveness of Methadone Maintenance Treatment in Reducing Intravenous Drug Use and Needle Sharing Among Heroin Addicts at Risk for AIDS

J. Ball, W. Lange, C. Myers, S. Friedman and B. Brown

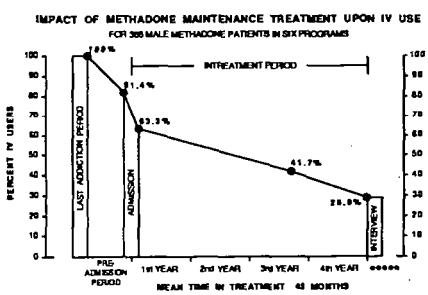
In a three-year field study 633 male addict patients at methadone maintenance programs in New York City, Philadelphia and Baltimore, treatment was found to be effective in reducing IV drug use and needle sharing practices. Of 388 patients who remained in treatment for one or more years, 71 percent had ceased IV usage; most had completely stopped for one or more years. Still, 29 percent had used drugs intravenously in the preceding month, and of these 30 percent had shares needles during this period.

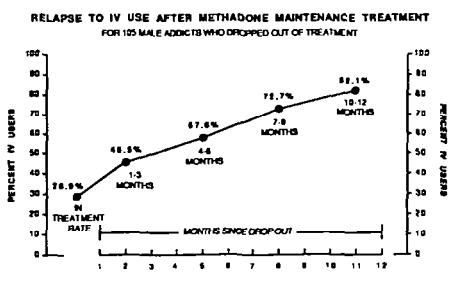
Marked differences in the effectiveness of particular programs were observed: current IV use varied from over 56 percent of patients in treatment at some clinics to less than 10 percent at another. These differences in treatment effectiveness were related to both length of patient stay and the quality of treatment provided. Long-term stay was associated with successful outcome as was treatment in programs which provided dedicated medical and counseling services.

A third of the 633 patients had dropped out of treatment during the year following their first interview. It was found that most patients rapidly relapsed to IV use - 82 percent relapsed in 10-12 months and 48 percent of these were engaged in needle sharing.

AFFILIATION

National Institute on Drug Abuse, Addiction Research Center, P.O. Box 5180, Baltimore, MD 21224



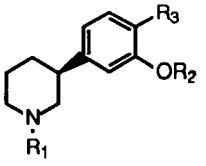


Synthesis and Evaluation of Potential Selective Sigma Ligands

N. Grayson, A. Jacobson, W. Bowen, L. Bluth and K. Rice

Selective ligands are necessary tools for studying the effects mediated by brain phencyclidine (PCP) and sigma binding sites. The sigma site can be selectively labelled with [^{3}H]di-o-tolylguanidine ([^{3}H]-DTG) or (+)-1[^{3}H]-3(3-hydroxyphenyl)-N-(1-propyl)piperidine ((+)-[^{3}H]-3-PPP). A potentially irreversibly sigma ligand based on di-o-tolylguanidine, di-o-tolylguanidine isothiocyanate, has been been synthesized by Weber, et. al. (*Eur. J. Pharmacol.,* **1987,** 142, 61).

Recent evidence suggests that specific sigma binding may consist of drug interactions with different subsites and that di-o-tolylguanidine and (+)-3-PPP may interact differently with these subsites. We have therefore initiated a project to synthesize compounds that are structurally similar to (+)-3-PPP as affinity ligands for the sigma sites. To date, two different , novel ligands (**1, 2**) containing the reactive isothiocyanate group have been synthesized. Compound **2** exhibits an approximate IC_{50} of 10 μM and is selective for sigma over PCP binding sites in guinea pig brain membranes.



Compound	R_1	R_2	R_3
3-PPP	*n*-C_3H_7	H	H
1	C_2H_4NCS	CH_3	H
2	*n*-C_3H_7	CH_3	NCS

Affiliations

Section of Drug Design and Synthesis, Laboratory of Neuroscience, NIDDK, National Institutes of Health, Bethesda, Maryland 20892 (N.G., A.E.J., K.C.R.); Division of Biology and Medicine, Section of Biochemistry, Brown University, Providence, Rhode Island 02912 (W.B., L.B.)

Analogs of the Phencyclidine Agonist Dexoxadrol. Synthesis and Structure-Activity Relationships

A. Thurkauf, M. Mattson, D. Goodwin, A. Jacobson and K. Rice

Dexoxadrol (1) was developed in the 1960's as an analgesic agent. In man, dexoxadrol was found to have analgesic potency equal to that of aspirin and to be free of respiratory depression. Analgesic potency is greatly increased by replacing one of the phenyl groups in dexoxadrol with an ethyl group. One of these ethyl isomers, etoxadrol, showed some promise as an anaesthetic agent. Etoxadrol and dexoxadrol were eventually shown to have limited clinical usefulness after undesirable post-anaesthetic effects were noted. The psychological state initiated by these dioxolane analgesics resembled that of phencyclidine intoxication. Binding studies later demonstrated that dexoxadrol, but not its enantiomer, had considerable affinity for phencyclidine receptors in rat brain. As part of our program to examine the structural requirements for phencyclidinelike activity, we prepared a variety of analogs of these dioxolanes and tested them for their ability to inhibit ^{3}H-TCP binding at the phencyclidine receptor. The structural variants included changing one of the phenyl groups of dexoxadrol to hydrogen (IC_{50} > 7000 nM), methyl (880 nM), ethyl (etoxadrol, 128 nM), propyl (76 nM), butyl (216 nM) and decyl (1000 nM). In addition, meta-phenyl substitutions were included on etoxadrol and compared to comparable changes on PCP. The electron withdrawing groups nitro and isothiocyanate were deleterious to activity in both structural classes. In contrast, 3-amino substitution increased receptor binding affinity for PCP but greatly decreased it for etoxadrol (IC_{50} > 5000 nM). Similarly changing phenyl to 2-thienyl increased receptor affinity in the PCP series but the 2-thienyl analog of etoxadrol was only about half as active (230 nM) as its parent. Finally the pyrrollidine analog of dexoxadrol, 2,2-diphenyl-4-(2-pyrrolidinyl)-1,3-dioxolane was essentially inactive at the PCP receptor (IC_{50} = 3300 nM).

AFFILIATION

Section on Drug Design and Synthesis, Laboratory of Neuroscience, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892.

Amphetamine and Cocaine Self-Administration are Mediated by Different Mechanisms: The Influence of Dopamine and Serotonin Transporters

M. Ritz and M. Kuhar

We have reported that the reinforcing effects of cocaine can be correlated with drug binding to the mazindol site on the dopamine transporter. Amphetamine and cocaine produce similar physiologic and behavioral effects in both animals and humans. It has been shown that amphetamines generalize to cocaine in drug discrimination studies and brain dopamine may be involved in mediating their reinforcing effects. However, our previous data suggested that amphetamine, relative to cocaine, is a more potent reinforcer than would be expected based on its affinity for the mazindol site on the dopamine transporter.

The goal of this study was to identify receptors which mediate reinforcing effects of amphetamine and related phenylethylamines. Thus, we have compared the published potencies of these compounds in studies of drug reinforced behavior with their binding potencies at both monoamine uptake sites and neurotransmitter receptor sites. The affinity of each drug was examined at each site using standard *in vitro* binding techniques. Inhibition of ligand binding to these sites was determined by analysis of competition curves and calculation of K_i values.

The results of these experiments indicate that amphetamine exhibits a pharmacologically relevant affinity for dopamine, norepinephrine and serotonin uptake sites as well as for $alpha_2$ receptor sites. A multiple regression indicates that inhibition of ^3H mazindol binding to the dopamine uptake site is significantly positively correlated with the reinforcing effects of cocaine-related drugs, but not of amphetamine and other phenylethylamines. The reinforcing effects of phenylethylamines were not positively correlated with inhibition of ligand binding to any of the sites tested. However, self-aihinistration of amphetamine, but not cocaine, appears to be inversely related to the inhibition of ^3H paroxetine binding to the serotonin transporter.

AFFILIATION

Molecular Pharmacology Laboratory, Neuroscience Branch, NIDA, Addiction Research Center, Baltimore, Maryland 21224

Problem Severity in Cocaine Versus Opiate Addiction

M. Droba, L. Goehi, F. DeJesus and T. McLellan

The majority of research on cocaine abuse has focused on persons of high socioeconomic status. We used the Beck Depression Inventory and the Addiction Severity Index to evaluate 50 male veterans applying for cocaine abuse treatment to gain a wider perspective on the cause and treatment of cocaine addiction. We compared the descriptive features of the cocaine group with 50 male veterans requesting mathadone treatment for primary opiate abuse. All subjects were of low socioeconomic status.

The cocaine and opiate groups were similar in the severity of psychiatric and social/family problems except the cocaine subjects were more likely to be married. There were extreme differences in race, employment problems, legal problems, number of years abusing drugs, alcohol use and medical problems. The opiate group was slightly more depressed that the cocaine group.

A treatment approach to the cocaine addict recognizing the high rate of alcohol abuse, intact family structure and high rates of employment is likely to incorporate traditional approaches to alcoholism and early family intervention. Employment during early treatment may contribute to early relapse by reinforcing conditioned cues, particularly payday cocaine use.

AFFILIATION

Department of Psychiatry, University of Pennsylvania and Philadelphia V.A Medical Center, Philadelphia, Pa. 19104

Toxicology Screening in Acute Spinal Cord Injury

S. Schnoll, A. Heinemann, M. Brandt, R. Maltz and M. Keen

The validity of self-reported intoxication at time of spinal cord injury (SCI) was examined by comparing self-reports with the results of blood serum and urine analysis for 88 cases at admission to an acute SCI center. Serum ethanol greater than 50 mg/dl was the most frequently found substance (observed in 40% of the cases) followed by urine analysis evidence of cocaine (14%), cannabinoids (8%), benzodiazepine (5%), and opiates (4%). Evidence of substances with abuse potential was found in urine for 35% of the sample. While 62% of the sample had either serum ethanol greater than 50 mg/dl or a positive urine analysis, only 42% of the sample reported being under the influence of some substance at the time of SCI. Although the relationship between these two measures was statistically significant, self-report and toxicology results were discordant in 34% of the cases. These results suggest that routine drug testing at admission to an SCI center will produce false negative and false positive results if substance presence alone is interpreted as evidence of intoxication.

AFFILIATION

[1]Department of Medicine and Psychiatry, Medical College of Virginia/ Virginia Commonwealth University, Richmond, VA 23298-0109.

[2]Department of Rehabilitative Medicine, Northwestern Medical School, Chicago, IL 60611.

[3]Department of Psychology, Illinois Institute of Technology, Chicago, IL 60611.

Chronic Opioid Antagonist Treatment Produces Supersensitivity to the Analgesic Effects of Morphine in Opioid Sensitive and Insensitive Mice

B. Yoburn, S. Kruescher and V. Sierra

Chronic treatment with opioid antagonists such as naltrexone (NTX) increases both CNS opioid binding sites (upregulation) and morphine's analgesic and lethal potency (supersensitivity). In the present studies, we have examined supersensitivity in Swiss-Webster mice obtained from two different suppliers. These mice differ in baseline sensitivity to the analgesic (tailflick) and lethal actions of morphine. The potency of morphine in untreated mice of the Swiss-Webster strain from Taconic Farms (TSW) and Charles River Labs (CRSW) were found to differ by a factor of 2.4 for lethality 24hr following morphine (LD_{50} for TSW=313mg/kg; CRSW=745mg/kg) and 1.7 for analgesia 30min following morphine (ED_{50} for TSW=3.7mg/kg; CRSW= 6.2mg/kg) ($p<.05$ for both effects). Groups of mice were implanted with subcutaneous NTX (15mg) or placebo pellets for 8 days, the pellets then removed and mice tested 24hr later with analgesic doses of morphine (0.5-6mg/kg). There were no significant differences ($p>.O5$) in baseline tailflick latency prior to morphine for either the TSW or CRSW mice in NTX or placebo-treated groups. NTX increased the analgesic potency of morphine by 1.9 ($p<.O5$) in both the TSW strain and in the CRSW strain. NTX treatment also increased specific [^{3}H]naloxone (1nM) binding in brain by 45-50% in both strains even though binding in placebo-treated TSW mice was 40% greater than placebo-treated CRSW mice.

Chronic opioid antagonist treatment produces approximately equal receptor upregulation and supersensitivity to the analgesic effects of morphine in CRSW and TSW mice even though the TSW mice are more sensitive to morphine. These results indicate that baseline morphine sensitivity and baseline [^{3}H]naloxone binding sites may not affect the degree of upregulation and supersensitivity and raise the possibility that increases in morphine's potency are related to relative, rather than absolute, increases in binding. (supported by NIDA Grant #DA04185)

AFFILIATION

College of Pharmacy, St. John's University, Grand Central & Utopia Parkways, Queens, NY 11439

Methadone Maintenance for AIDS-affected IV Drug Users: Treatment Outcome and Psychiatric Factors After Three Months

S. Batki, J. Sorensen, C. Coates and D. Gibson

"AIDS-affected" IV drug users were enrolled in a prospective study of methadone maintenance treatment (MMT) effects. IV heroin addicts with AIDS, ARC, HIV infection, or their sexual/ partners were evaluated at admission and after 3 months of MMT. Measures of drug use included self-report via the Addiction Severity Index (ASI), weekly urine testing, and skin examination for puncture marks. The Beck Depression Inventory (BDI), the Beck Hopelessness Scale, and questions about AIDS knowledge, attitudes, and high-risk sexual or drug-related behaviors were also given. The first 29 subjects showed significant decreases in overall IV drug use, heroin use, cocaine use, and money spent on drugs. Alcohol use did not decline in the first three months of MMT.

TABLE: Mean Days of Drug use in Past Days (from ASI)

	Intake	After 3 Months MMTP
Total IV Drug USE	28.6	5.9 (p<.0001)
Heroin Use	28.8	3.8 (p<.0001)
cocaine use	8.1	2.9 (p<.04)

There was a small but significant ($p<0.04$) increase in knowledge about AIDS after three months. Sexual activity declined, while the rate of condom use did not change. Subjects displayed high levels of psychological distress. At entry into treatment, 67% showed evidence of depression on the BDI, 59% had high Beck Hopelessness scores, and 18% reported suicide attempts in the month prior to admission. ASI-reported anxiety and cognitive problems were present in 79% and 64% of subjects, respectively. Psychological distress remained high even after three months of MMT. Distress at entry appeared to correlate with poor treatment outcome. Subjects who entered MMT with high BDI scores were over-represented among those who still had significant heroin use after three months of MMT.

UCSF School of Medicine, Dept. of Psychiatry, Substance Abuse Services, S F General Hospital, San Francisco, CA 94110.

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Antagonist Actions of the Opioid Analgesic Nalbuphine in Humans

G. Bigelow, K. Preston and I. Liebson

To assess the extent to which the agonist-antagonist analgesic nalbuphine would display agonist actions versus antagonist actions when administered to opioid-dependent human volunteers the effects of nalbuphine were compared to hydromorphone, a protoypic mu agonist, and naloxone. an opioid antagonist. Subjects were 5 adult males enrolled in methadone maintenance treatment (30 mg/day, po). Drug conditions, given IM, included saline placebo, nalbuphine HCl 0.375, 0.75, 1.5, 3 and 6 mg. naloxone HCl 0.1 and 0.2 mg and hydromorphone HCl 4 and 8 mg and were tested under double-blind conditions in 2.5 hour experimental sessions. Physiologic measures were heart rate, skin temperature, blood pressure, respiration rate and pupil diameter. Both opioid agonist-like and antagonist-like subjective effect and observer-rated measures were collected.

Hydromorphone significantly increased ratings on the subjective measures consistent with morphine-like effects such as ratings of opioid agonist symptoms and liking of the drug effect. Naloxone precipitated opioid abstinence as indicated by increased subject-rated opioid withdrawal symptoms and Sick and Bad Effects visual analog scales and by increased observer-rated withdrawal signs and Himmelsbach scale scores. Naloxone also significantly decreased skin temperature and increased heart rate and systolic and diastolic blood pressure. Nalbuphine produced significant effects on many of the same subjective, behavioral and physiological measures as naloxone including increased subject-rated opioid withdrawal symptoms and Sick and Bad Effects visual analog scales, increased observer-rated withdrawal signs and Himmelbach scale scores, and decreased skin temperature and increased blood pressure and pupil diameter. The effects of nalbuphine and naloxone on individual items on both the subjects' withdrawal symptoms scale and observer's Himmelsbach withdrawal scale were similar in profile. None of the effects of nalbuphine were similar to those produced by hydromorphone.

Nalbuphine did not appear to have any opioid agonist effects at tested doses in these subjects maintained at relatively low levels of physical dependence. It did have clear antagonist effects. and precipitated characteristic opioid withdrawal signs and symptoms in these methadone-dependent subjects. The profile of these withdrawal effects was qualitatively similar to, and indistinguishable from that of naloxone.

AFFILIATION

The Johns Hopkins University School of Medicine, Baltimore, Maryland 21224

Mediation of Ethanol Self-administration by Prostaglandin Synthetase Inhibitors

F. George

Prostaglandin synthetase inhibitors (PGSIs) antagonize acute responses to ethanol. This study examined the effects of pretreatment with indomethacin (INDO), a potent PGSI, on ethanol self-administration. Rats were pretreated daily via i.p. injection with one of several doses of INDO or vehicle. Rats were given daily injections with each dose for five consecutive days, then given two days recovery time. This was repeated once for each condition, providing a control for possible cumulative effects of INDO. Blood ethanol levels were measured on the final day of certain INDO conditions, and on the final vehicle retest day. There were no significant differences found between any vehicle conditions. INDO produced significant increases in ethanol intake at 1.9 mg/kg, and significant decreases at 2.5 and 5.0 mg/kg. Patterns of responding indicate that while INDO produced an extinction of ethanol self-administration, cessation of INDO treatment produced no long lasting effects on responding, as determined by the return to baseline levels of operant behavior and stability of baseline retests. The pattern of changes in ethanol self-administration suggests that INDO altered responding by decreasing the reinforcing effects of ethanol and not by producing a conditioned aversion to ethanol. Blood ethanol levels were reduced by a percentage similar to the reductions seen in responding, suggesting that INDO did not affect ethanol intake by interfering with ethanol metabolism or distribution. In a subsequent experiment, rats were trained to self-administer a saccharin solution. INDO was administered as described above. INDO had no effect on oral self-administration of saccharin, suggesting that INDO did not reduce ethanol drinking via some nonspecific effect on performance or motivation. The results suggest a common prostaglandin related mechanism is important in mediating both acute sensitivity to and the reinforcing properties of ethanol.

AFFILIATION: Behavior Genetics Laboratory, Preclinical Pharmacology Branch, Addiction Research Center, National Institute on Drug Abuse Box 5180, Baltimore MD 21224 USA

Opioids Regulate Neurotumor Cell Growth in Tissue Culture

P. McLaughlin and I. Zagon

Previous investigations utilizing opioid antagonists to perturb interaction of endogenous opioids and opioid receptors have demonstrated that tumorigenic events are regulated by a delicate equilibrium between opioids and receptors, and suggest that endogenous opioids exert a tonic inhibitory effect on neoplasia. To determine if these mechanisms act at the cellular level, the role of endogenous opioid systems *on* growth of murine neuroblastoma (S2OY) (=NB) in culture was examined. NB cells were plated for 24 hr and various concentrations ranging from 10^{-4} M to 10^{-12} M of various opioids and peptides were then added daily; 48 hr later, cells were harvested and live cells counted by trypan blue exclusion tests. Peptides representative of the three opioid precursor molecules, prodynorphin, proenkephalin A, and POMC, were included. The pentapeptide, methionine enkephalin, was the most active growth inhibitory agent, followed by peptides slightly modified in structure. Further examination of growth curves indicated a dose-response effect of methionine enkephalin that was naloxone reversible. Studies of thymidine incorporation and mitosis revealed a reduction of 25% in labeling index and *a* 72% decrease in mitotic index relative to control levels. In order to determine whether cells in culture had opioid receptors, binding assays were performed using prototypic ligands and appropriate methodology for mu (^{3}H-DAGO), delta (^{3}H-DADLE), and kappa (^{3}H-EKC) receptors, as well as for methionine enkephalin. Specific and saturable binding was detected for radiolabeled DADLE, EKC, and methionine enkephalin in NB cultures. Radioimmunoassays of media and cells revealed that log phase cultures possessed detectable levels of two endogenous opioid peptides: beta endorphin and methionine enkephalin. These studies indicate that endogenous opioids and opioid receptors are associated with neurotumor cells in culture, and that endogenous opioid systems - particularly methionine enkephalin - are involved in the regulation of cell proliferation in such a manner as to serve as inhibitory factors.

AFFILIATION

Department of Anatomy, The Milton S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033

Desipramine Treatment of Cocaine Abuse in Methadone Maintained Out-Patients

I. Arndt, L. Dorozynsky, G. Woody, A. McLellan and C. O'Brien

Methadone maintained male patients meeting the DSM-III criteria for cocaine abuse were randomly assigned to either desipramine (DMI) or placebo. Patients were observed taking their medication daily at the pharmacy window. While maintaining "blind" conditions DMI was adjusted toward a plasma level of active medication of 150-300ug. 72 patients were randomly assigned and 51 have completed the 12-week medication phase. We have a 22% early termination rate. Three patients stopped medication because of adverse medical reactions. Patients were assessed weekly during the medication phase and at 1-, 3-. and 6-month follow-up points. Assessments included the Addiction Severity Index Factor Scores, individual items from the ASI, the Beck Depression Inventory, scores on 20-point "craving" scales, and scores on "control over cocaine use" subjective rating scales. Drug and alcohol use inventories and urine toxicology results were collected. Baseline to 12-week improvements were seen in both groups, but there were no significant differences between the groups in ASI data or craving scales. Urine toxicologies were discouraging, with high rates of cocaine positive urines. The mean for the DMI group was 78% and 74% for the placebo group. Also, 52% of the DMI group's and 53% of the placebo group's urines were positive for other drugs of abuse. Marijuana was not included. Patients in both groups reported fewer days of cocaine use and increased "control" over cocaine urges. There were no significant differences between the groups and in the group across week interactions other than in the positive urine for other drugs. Unexpectedly, the DMI group's urines were 80%, 78% and 80% positive for cocaine at the 1-, 3- and 6-month follow-up, while the placebo group's were 79% 46% and 38%. DMI does not appear to be beneficial in this population for cocaine addiction. Patients treated with it may do less well in the long run. Numbers in our 6-month follow-up groups are small (8 in the 6-month placebo group), so this must be considered tentative.

AFFILIATION: Philadelphia VA Medical Center and University of Pennsylvania Dept. of Psychiatry, Philadelphia, Pa.

Dispositional Interactions of Cocaine (COC) With Delta-9-Tetrahydrocannabinol in the Rat

S. Husain

Recreational use of marijuana is common among individuals who also abuse other drugs like cocaine. This seemingly innocent combination could cause dispositional drug interactions which may prove to be fatal. At present, no studies exist to substantiate or retute this possibility. For this purpose, we studied the effects of THC on the plasma pharmacokinetics of COC in the rat. Control male rats (200-250g) were given ip injections of 20 mg/kg and 40µCi/kg ^{3}H-COC. Plasma radioactivity was followed at 0.25, 0.5, 1, 2.5, 6.5 and 22.5 hr by periocular puncture technique. Similarly, experimental animals were given THC (10 mg/kg, po in sesame oil) 90 min before receiving ^{3}H-COC. In control rats, plasma radioactivity peaked at 0.25 hr and declined rapidly until 2.5 hr after which this decline was gradual. As oppose to this, the experimental animals showed a higher level of plasma radioactivity. Instead of 0.25 hr, the radioactivity peaked at 0.5 hr in rats receiving THC and COC and continued to remain higher than controls throughout its disposition in plasma. These animals also showed longer lasting and more pronounced behavioral responses such as muscular incoordination, ataxia, circular movements, head rotation and including piloerection and shallow breathing when compared to controls. These results suggest that the behavioral changes and these other functional modifications may be due to a dispositional interaction between COC and THC. (This research is supported in part by NIDA Grant DA 03595).

AFFILIATION

Department of Pharmacology, School of Medicine, University of North Dakota, Grand Forks, ND 58201.

Tolerance to Cardiovascular Effects of Nicotine in Humans

K. Perkins, L. Epstein, R. Stiller, J. Jennings, B. Marks and R. Jacob

Acute cardiovascular tolerance to nicotine in humans was examined in two studies employing a nasal spray device which produces rapid, dose-dependent boosts in plasma nicotine. In Study I, acute tolerance within a single dose administration was determined by examining responses at comparable plasma nicotine concentrations on the ascending vs. descending limbs of the plasma concentration curve, with smaller responses on the descending limb indicating acute tolerance (i.e. hysteresis loops). Subjects (n=11 smokers) received 3 fixed doses of nicotine (0.5, 1.0, 2.0 mg) or a placebo (0 mg) in a series of 5 boluses (1 every 60 sec), with each dose presented on a separate day. Changes from baseline in heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure, pulse amplitude (PA), and, in a subsample, plasma nicotine were assessed intermittently during and after dose administration. Uniform boosts in plasma nicotine were observed within doses across subjects. For each dose, results of t-test analyses indicated clear tolerance to SBP and DBP, some tolerance to HR, but no tolerance to PA. In Study II, acute tolerance between dose administrations was examined. Subjects (n=18 smokers) received 4 administrations (1 every 20 mins) of 2 doses corrected for body weight (7.5 ug/kg and 15 ug/kg--approx. 1.1 mg for average subject), with each dose presented on a separate day. Only peak HR response was assessed, determined by HR change from initial baseline to the first 2 mins after each administration. The decline in HR response between administrations 1 and 4 was significant for the 7.5 ug/kg dose (6.4 vs. 3.9 BPM, $p<.05$) and marginally significant for the 15 ug/kg dose (9.6 vs. 7.1 BPM, $p<.10$), suggesting acute tolerance. The response-dependency of acute tolerance in Study I indicates the importance of employing multiple responses in examining acute tolerance to nicotine. Further study of acute tolerance may help clarify physiological adaptation to nicotine.

Affiliation: Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh PA 15213. Supported by National Institute on Drug Abuse grant DA/HL 04174.

Effects of Chronic Buprenorphine Administration on the Human Electroencephalogram

W. Pickworth, B. Koeppl, H. Lee and P. Fudala

Buprenorphine (BUP) is an orally and sublingually active partial opiate agonist with a long duration of action. During chronic BUP administration, the euphoriagenic effects of concomitantly administered opiates are attenuated, and minimal withdrawal symptoms follow the discontinuation of BUP. These properties, along with mu-opiate agonist effects which make it acceptable to the addict, have led to the proposal that BUP may be an effective maintenance drug in the treatment of opiate addiction.

Sixteen volunteer subjects, addicted to IV heroin prior to their admission to a residential research ward, participated in a 56-day study. Subjects received 1 ml of a sublingual ethanol solution (30% v/v) containing BUP or placebo. BUP dosage was rapidly increased to 8 mg daily and maintained until day 18. On day 19, one group (n=8) began receiving placebo or active drug on alternate days: the other group (n=8) continued to receive BUP daily. On days 37 through 56, both groups received placebo. Spontaneous EEG recordings were obtained on days 15, 17, 20, 30, 34, 37, 39, 44, 47, and 56 in subjects relaxed with eyes open or closed. The EEG was recorded from F_z, C_3, C_4, and P_2 and resolved into EEG spectra by means of a computer-based EEG analysis system.

On most EEG measures, the maximal response occurred on days 44, 47, or 56 (8 to 20 days following the last dose of BUP). Alpha power decreased and alpha frequency increased, whereas theta frequency and power decreased. Beta power also decreased during this period. The decrease in alpha power is a characteristic of the opiate withdrawal response. EEG effects were similar in the eyes open and closed conditions. There were no significant differences in the EEG measures of the subjects given BUP daily or on alternate days. These data are consistent with the concept that BUP is a long-acting partial opiate agonist of potential use in maintenance therapy for opiate addiction.

AFFILIATION

NIDA, Addiction Research Center, Baltimore, MD 21224

Waiting for Treatment: Behaviors of Cocaine Users on a Waiting List

B. Brown, J. Hickey, A. Chung, R. Craig and J. Jaffe

The need to maintain often lengthy waiting lists to accommodate drug users requesting treatment has been widely reported (U.S Conference of Mayors, 1987; Community Epidemiology Group, 1987) Nonetheless, there is a virtual absence of study with regard to the behaviors of individuals on treatment waiting lists. The importance of such study is newly emphasized by the emergence of HIV infection as a major issue for drug abuse treatment

Structured interviews and the SCL-90R were employed with 29 applicants to a residential treatment program for cocaine abuse who had been placed on that program's waiting list for periods of 1-6 months Nearly half of the subjects (48.3 %) reported a diminution in use of their primary drug since applying for treatment In response to separate questions subjects reported themselves to be maintaining significantly lower frequencies of drug use currently as compared to the period prior to applying for treatment ($T=18.5$, $p < .01$). Nonetheless, the majority of subjects (58.6%) expected "serious trouble" if they were not accepted for treatment and defined that serious trouble in terms of overdose and/or death (11 of 17 respondents), or deepening crime and arrest (9 of 17 respondents). Scores for subjects on the SCL-90R were consistent with a diagnosis of major depressive syndrome (Derogatis, 1983). However, a majority of subjects (51.7%) reported themselves less interested in obtaining treatment at time of interview than had been the case at the time they applied for treatment and only 17.2 % reported them selves more interested in obtaining treatment

An overwhelming majority (87.0%) of IV drug users (n=23) reported having made changes in their behaviors to avoid the risk of AIDS, most typically naming safer needle use (19 of 20) Moreover, 69.6% of the IV users reported having gone for HIV testing

Study findings suggest the limited utility of a waiting list and the need to explore alternatives to provide support for drug users' efforts to reduce drug taking behaviors

National Institute on Drug Abuse, Addiction Research Center
4940 Eastern Avenue, Bldg. C, Baltimore, MD 21224

Birthweight as a Predictor of Cognitive Vulnerability to Alcohol's Teratogenic Effects

C, Coles, I. Smith, K. Platzman, and A. Falek

Not all prenatally alcohol-exposed infants show Fetal Alcohol Syndrome (FAS) or other effects and no strong dose/response relationship has been established. Effects occur in non-FAS exposed children but their extent is unclear. Some infants, or mother/infant systems are more vulnerable to alcohol's effects than others and identification of these and factors contributing to their vulnerability is vital.

We hypothesized that birthweight is a physical marker for vulnerability to alcohol's cognitive teratogenesis and lowered birthweight could identify alcohol-exposed infants at highest developmental risk. Therefore, 293 nondysmorphic, full-term, low SES black infants whose mothers: a) never drank; b) drank throughout pregnancy (x=10 oz AA per week); or c) stopped during the 2nd trimester (x=11 oz AA) were classified into 3 birthweight categories: 1) Low (1500 to 2500 grams); 2) Questionable (2500-2999 grams); and 3) Normal (3000+). Although, an equivalent percentage (26 to 30%) of all *groups* fell into the "questionable" category, drinkers' children were under represented in the "normal" category.

To examine the relationship between weight, alcohol use and development, these categories were used to classify two subsamples of infants whose developmental status was tested with the Bayley Scales of Infant Development: 1) 132 6-month olds, and 2) 71 12-month olds. In general, at both 6 and 12 months, Bayley scores were: 1) directly related to birthweight and 2) lower in alcohol exposed infants. Of all birthweight-exposure subgroups, the lowest scoring infants at both 6 and 12 months were the low birthweight and continuously exposed infants. These scores ranged from 83 to 86. In this population, Bayley MDI scores <85 are usually associated with mild to moderate retardation at school age and the earlier in infancy such deficits are noted the more serious the deficits can be unless there is a therapeutic intervention. Thus, birthweight is useful in identifying alcohol-exposed neonates who merit follow-up and develomental screening as well as women whose physical and social characteristics merit further study.

AFFILIATION
Human and Behavior Genetics Laboratory, Georgia Mental Health Institute and Department of Psychiatry, Emory University School of Medicine, Atlanta, GA 30306.

Drug Use Patterns by FHP and FHN College Males

M. McCaul, D. Svikis, J. Turkkan and G. Bigelow

The present study compared drug use patterns in college males that differed in family history for alcoholism. Previous studies have shown that these two groups differ on a number of characteristics, including self-reported alcohol intoxication (Schuckit, 1980) and behavioral effects of alcohol (Schuckit, 1985). Questionnaires were mailed to 5,000 male students (aged 18-25) at three local colleges. Based on 888 (17.8%) completed questionnaires, 16.1% of the respondents reported having at least one first-degree relative with one or more alcohol-related life problems (FHP). There were no significant differences between FHP and FHN respondents for age, race or education. FHP respondents reported significantly higher rates of alcohol consumption in the 30 days prior to questionnaire completion (44.9 drinks/month) than did FHN respondents (28.2 drinks/month) ($p < .0005$).

For lifetime use, FHP males were more likely than FHN males to report using a variety of illicit drugs. Specifically, FHP respondents were more likely than FHN respondents to report using marijuana (74% vs. 58%, $p < .001$). benzodiazepines (23% vs. 9%, $p < (.001)$. cocaine (46% vs. 26%. $p<.001$). hallucinogens (29% vs. 16%, $p < .001$), and opiates (17% vs. 8%,$p<.01$). There were no significant differences in the quantities consumed by the two groups.

For recent drug use (i.e., within the past 6 months), FHP respondents were more likely than FHN respondents to report using marijuana (50% vs. 38%, $p<.01$) and cocaine (17% vs. 10%, $p<.05$). Of those respondents who reported any use, FHP respondents reported 26.0 episodes of marijuana use as compared with 16.0 episodes for FHN respondents ($p<.025$). The number of episodes of cocaine use did not differ for the two groups. These results suggest that in addition to alcohol, children of alcoholics show higher rates of other drug use as well.

AFFILIATION
Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine and Department of Psychiatry, The Francis Scott Key Medical Center, Baltimore, Maryland 21224

Sigma Receptors Negatively Modulate Agonist-Stimulated Phosphoinositide Metabolism in Rat Brain

W. Bowen, B. Kirschner, S. Hellewell, A. Newman and K. Rice

The ability of sigma receptors to affect phosphoinositide (PI) turnover was investigated. Rat brain synaptoneurosomes were prepared, labelled with [^{3}H]myo-inositol, and assayed for production of [^{3}H]]linositol-1-phosphate (IP_1) as described by Gusovsky and Daly (Neuropharmacol. 27: 95, 1988). All sigma ligands tested, with the exception of (+)-3-PPP, depressed the basal level of phosphoinositide (PI) turnover at concentrations above 100 uM. The ability of 100 uM carbachol or norepinephrine to stimulate IP production was also inhibited by sigma ligands in a dose-dependent manner. Much lower concentrations were required than to affect basal activity. The rank order of potency to block carbachol-stimulated PI turnover was: (+)-pentazocine > 1,3-di-o-tolylguanidine = dextrallorphan > haloperidol > levallorphan. This rank order of potency correlated well with affinity at sigma receptors (as determined in guinea pig brain), r=0.86. Related (+)-opiates and antipsychotic drugs which lack affinity for sigma receptors failed to affect agonist-stimulated IP_1 production. Interestingly, (+)-3-PPP produced only partial inhibition of the carbachol effect, suggesting partial agonist properties of this compound in this system.

The finding that sigma ligands block agonist-stimulated PI turnover suggests that sigma receptors are members of a novel family of receptors coupled to an intracellular mechanism(s) which negatively regulates components of the phosphoinositide signalling system. Agonists at sigma receptors may therefore modulate the efficacy of other transmitters.

Affiliation: Division of Biology and Medicine, Brown University Providence, RI 02912 and Laboratory of Neuroscience, NIDDK, Bethesda, MD 20892.

Behavioral Effects in the Mouse During and Following Withdrawal From Repeated Ethanol and Nicotine Administration

E. Onaivi and B. Martin

Ethanol alters the normal functioning of neurotransmitters and neuromodulatory systems consequently producing alcohol-related problems. Nicotine is considered to be the major factor in the development and continuation of the smoking habit. The mechanism by which ethanol or nicotine produce dependency and withdrawal symptoms during abstinence is poorly understood which is compounded by the observation that a high correlation exists between ethanol intake and smoking. Therefore, changes in anxiety and locomotor activity responding during and following abrupt cessation from 14 day ethanol ingestion and/or nicotine treatment were investigated.

Naive ICR male mice maintained on a reversed 12:12h 1ight:dark cycle in four groups of six received either no treatment, a gavage of 20 % ethanol/water equivalent to 2.8g/kg ethanol, lmg/kg nicotine twice daily (s.c.) or a combination of the ethanol and nicotine treatments. The animals receiving the ethanol treatments were allowed access to food and 20% ethanol in water ad libitum. Mice were assessed for 20 mins in photocell activity chambers for spontaneous locomotor activity and measures of anxiolysis and anxiogenesis of abstinence withdrawal were obtained using the elevated plus-maze in a 5 min test session. The entries and time spent on the open arms is considered a measure of anxiety.

Control animals spent approximately 60% of the time in the closed arm. The individual treatments of ethanol and nicotine as well as the combination produced a significant increase in the time, spent on the open arm at days 2, 7, 10 and 14. Twenty four hr after the last treatment on day 14, the time spent on the open arm for the ethanol and nicotine treatments were not significantly different from the control mice. However, the mice that had received the combination of ethanol and nicotine spent a significantly decreased amount of time in the open arm. This effect persisted for 48 hr after withdrawal but behavior returned to control values by 5 days after withdrawal. Little changes was observed with locomotor activity during treatment. However, the spontaneous activity of animals that had received the combination of ethanol and nicotine was significantly reduced up to 5 days after the time of withdrawal.

It is therefore concluded that the combined use of ethanol and nicotine may lead to anxiolysis, and the abstinence withdrawal anxiogenesis may be a basis for the continued intake. Supported by the Virginia, Commonwealth Center for Drug Abuse and NIDA Grant DA02384.

AFFILIATION: Medical College of Virginia, Virginia Commonwealth University, MCV Station Box 613, Richmond, VA 23298-0613

Signs and Symptoms of Nicotine Gum Withdrawal

D. Hatsukami

The current study examined the signs and symptoms subsequent to nicotine gum deprivation. In addition, a comparison was made between signs and symptoms of withdrawal from cigarette and nicotine gum employing a within-subject design. Finally, the relationship between severity of nicotine gum withdrawal and relapse to smoking was examined. Smokers were involved in the study for a total of 6 weeks. In week 1, subjects were administered a battery of withdrawal measures during 2 days of ad libitum smoking and 3 of 4 days of cigarette deprivation. In weeks 2-5, subjects were asked to chew ad libitum at least six pieces of one of three doses of nicotine gum (0, 2 or 4 mg). In week 6, subjects discontinued chewing the gum and were tested for signs and symptoms from nicotine gum abstinence. Subjects were administered a battery of measures during 2 days of ad libitum gum chewing and 3 of 4 days of nicotine gum deprivation.

The results showed the following: (1) There were no significant changes after 0 mg gum deprivation; a significant increase in difficulty concentrating after 2 mg gum deprivation; and a significant decrease in heart rate, poorer performance on the reaction time task, and increases in difficulty concentrating, restlessness, and drowsiness after 4 mg gum. (2) The changes found from 4 mg gum deprivation were similar to those found from cigarette smoking. However, signs and symptoms from 4 mg gum tended to be fewer in number and less severe than from cigarettes in spite of similar levels of saliva cotinine. (3) There were very few significant positive relationships between severity of cigarette and nicotine gum withdrawal. (4) There was no relationship between severity of symptoms of nicotine gum withdrawal with relapse to smoking.

AFFILIATION

Department of Psychiatry, University of Minnesota, Minneapolis, MN 55455.

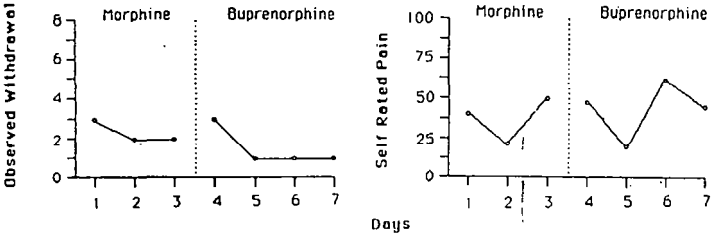
Successful Transfer to Buprenorphine in a Patient on Morphine

J. Hughes, W. Bickel, and S. Higgins

Buprenorphine is a mixed opioid agonist/antagonist which has advantages that suggest its use among drug abusers who require opioids for pain; e.g., it has minimal physical dependence and abuse liability. Presently, it is unclear how long to wait after the last dose of a mu opioid to avoid precipitated withdrawal from buprenorphine.

A 30-year-old man with a history of opioid and other drug abuse and severe Crohn's disease was on morphine sulfate (MS) 3.6 mg iv every 2 h for pain. The patient agreed to be kept blind for the following experiment. We first assessed physical dependence with saline, 0.2 and 0.6 mg of naloxone iv. With the last dose he had classic symptoms of opioid withdrawal. After 3 days of MS, 9 mg iv q 6 h, we substituted saline for the patient's 0300 dose of MS. The next morning (12 hours after the last MS), he began buprenorphine 0.6 mg iv q 6 h. Measures 0.5 hours after the first dose and then daily showed no withdrawal and continued pain relief (Figure).

We conclude 12 h is adequate to prevent withdrawal yet not increase pain. Further studies may indicate less than 12 h is adequate.



AFFILIATION

Human Behavioral Pharmacology Laboratory, Departments of Psychiatry and Psychology, University of Vermont, Burlington, VT 05401

A Survey of How Urinalysis Results Are Utilized By Methadone Maintenance Clinics

D. Calsyn, A. Saxon and D. Bamdt

Little is reported about how urinalysis results are utilized by methadone maintenance clinics. A survey on this topic was sent to all clinics listed in the 1984 Program Directory. Useable surveys were returned by 324 clinics (66%). A "Positive Urine" counseling session is required by 98% of clinics after each positive result. An additional increase in individual counseling is required by 69% of clinics with smaller clinics less likely to require this. Positive urines mandate revocation of take home privileges in 98% of clinics. Take homes are lost from one to 26, weeks most typically for 30 days (43%), 60 days (13%) or 90 days (31%). Attendance at group therapy as a consequence for positive urines is required by 25% of the clinics, more commonly in medium sized clinics and less commonly in proprietary and VA clinics. An increase in frequency of urine screening is a response of 54% of clinics. This action happens more frequently in clinics in the East and less in VA clinics. Contingency contracting is used by 74% of clinics in response to positives. Methadone dose increases are provided in response to positives, especially for opiates, by 77% of clinics. Smaller sized clinics and clinics in smaller cities are less likely to increase dose. Dose decreases are instigated as a response less often (44%). Clinics from smaller cities are less likely to decease dose. Detoxification and discharge are identified as an eventual consequence by 78% of programs. Proprietary programs are less likely to detox and discharge, while programs in smaller cities are more likely to detox. Most programs view discharge as a last resort after continuous positives. Clearly, clinical responses to positive urine screens differ between individual clinics. Since different clinics may achieve different treatment results, urine screening practices represent an important variable to be considered in outcome assessments.

Affiliations: VA Medical Center, Seattle and Dept. of Psychiatry and Behavioral Sciences, University of Washington School of Medicine.

Eighteen Month Outcomes of School- and Combined School- and Family-Based Preventive Interventions

B. Bry, D. Greene, C. Schutte and E. Peckman

Previously we have shown that a school-based preventive intervention for high risk seventh and eighth graders reduces substance use, but the effects are only modest. We have also shown that family-based early intervention reduces substance use, but the effects are often delayed. In an attempt to increase preventive effects and improve their immediacy, the present study examined the impact of supplementing school-based intervention with family-based intervention.

Since other research indicates that families with more adolescent problems exhibit less problem-solving, more blaming, evaluation, and preaching than do families with fewer adolescent problems, our family-based intervention targets these behaviors. Four seventh and eighth graders who exhibited the substance abuse risk factors of poor academic performance, discipline referrals, and distant relationships with parents were assigned for one academic year to teacher-monitors, who saw them several times a week to discuss remedying their problems in school. Then a randomly chosen half (two) of their families were invited to school for weekly family sessions to increase problem-solving and decrease blaming, evaluating, and preaching. The families attended 5 and 8 sessions, respectively. The adolescents' substance use was assessed confidentially, independently, and monthly during two academic years.

Substance use decreased quickly and substantially for the two teens whose school intervention was supplemented with family communication and problem-solving training; whereas substance use stayed the same for the two teens who received only school-based intervention. These findings suggest that further study is warranted into the impact and processes of combined school- and family-based prevention interventions. (Supported by DA 05112)

AFFILIATIONS

Graduate School of Applied and Professional Psychology and the Department of Psychology, Rutgers--the State University, Piscataway, New Jersey 08855-0819

Enhancement of the Rate-altering Effects of Morphine Following Chronic Naltrexone Treatment

A. Young and S. Mattox

Termination of repeated naltrexone treatment can be followed by both a prolonged supersensitivity to direct effects of naltrexone itself and a brief enhancement of sensitivity to the analgesic and lethal effects of morphine. The present study evaluated the impact of continuous infusions of naltrexone (NLTX) on the behaviorally disruptive effects of morphine (MS). Male Sprague-Dawley rats weighing 250-275 g at the start of training were sequentially assigned to one of six experimental groups. For behavioral tests, lever press responses were established and maintained by a schedule under which every 30th response in the presence of illuminated stimulus lamps resulted in delivery of one food pellet. Each experimental session consisted of 4 components in which a 10-min timeout period, during which lamps were off and responses had no programmed consequences, preceded a 5-min presentation of the schedule of food reinforcement. The rate-altering effects of MS were assessed before, during, and after osmotic infusions of saline or a nominal dose of 3, 10, or 18 mg/kg/day NLTX in separate groups of rats. The analgesic effects of MS were assessed in a tail-flick assay before and during infusion of saline or a nominal dose of 10 mg/kg/day NLTX in other rats. Continuous infusion of saline or 3 to 18 mg/kg/day NLTX did not alter the rates or patterns of food-reinforced behavior or baseline tail-flick latencies. During the infusion, 10 mg/kg/day NLTX produced an insurmountable antagonism of the rate-altering and analgesic effects of MS. In contrast, 24 hours after termination of a 10 or 18 mg/kg/day NLTX infusion, the dose of MS required to decrease rates by 2 50% was decreased 2- to 3-fold. Infusion of saline or 3 mg/kg/day NLTX produced no change in MS potency. The change in the rate-altering potency of MS after high doses of NLTX was short-lived, as the dose of MS required to alter rates returned to initial values within 8 days. Thus, exposure to high doses of NLTX produces brief changes in the behavioral potency of MS that parallel changes in its analgesic and lethal potency. (Supported by DA 03796 and RR 08167).

AFFILIATION

Dept. of Psychology, Wayne State University, Detroit, MI 48202

Ethanol Self-administration is not Related to Behavioral or Biochemical Sensitivity to Ethanol

M. Ritz, G. Elmer, T. Suzuki, R. Meisch and F. George

Using genetic methods, these studies investigated biochemical mechanisms underlying ethanol sensitivity and self-administration and the relationship between these ethanol-related phenotypes. We studied ethanol self-administration in several genetically distinct stocks of mice (C57, BALB, LS, SS) and rats (LEWIS, F344, ALKO and ANA). Results indicated that inherited traits strongly influenced ethanol self-administration, and that ethanol preference but not sensitivity is related to self-administratian. Biochemical studies using these same genotypes tested whether animals may inherit different synaptosomal membrane traits which mediate ethanol effects. Genotypes were ranked for self-administration of and sensitivity to ethanol. Synaptosomal membrane preparations were assayed for various traits. Electron paramagnetic resonance experiments indicate innate membrane fluidity is significantly related to ethanol neuro-sensitivity, but not to ethanol preference or self-administration. Reinforcement from ethanol appears to be significantly determined by inherited traits, but these traits are apparently not related to those behavioral or membrane biochemical factors which mediate neurosensitivity to ethanol.

AFFILIATIONS

Molecular Pharmacology Laboratory, Neuroscience Branch, NIDA Addiction Research Center, Baltimore, Maryland 21224

Comparison of the Effects of Buspirone and Chlordiazepoxide on Comparable Rates of Punished and Unpunished Responding

S. Izenwasser, C. Boley and S. Dworkin

Benzodiazepines and other drugs with anxiolytic properties increase responding which is suppressed by electric footshock. Chlordiazepoxide has been shown to increase punished responding while having no effect on comparable rates of unpunished responding. Further, electric footshock appears to lead to changes in benzodiazepine birding in the striatum and the cerebellum, possibly accounting for the punishment-specific effects of chlordiazepoxide on food maintained responding.

Buspirone is a nonbenzodiazepine antianxiety agent which lacks the sedative-hypnotic and muscle relaxant properties of benzodiazepines. Pharmacologically it appears to interact with serotonergic and dopaminergic systems and not with benzodiazepines or GABA. Since it is clinically efficacious as an anxiolytic agent the effects of this drug on comparable rates of punished and unpunished responding by rats was evaluated.

Littermate pairs of male F-344 rats responded under a procedure which generated comparable rates of punished and unpunished responding. The effects of buspirone (0.56-10.0 mg/kg, i.p.) and of chlordiazepoxide (3.0-30.0 mg/kg, i.p.) were determined after stablebaselines of responding had been observed.

Both buspirone and chlordiazepoxide increased punished responding while having little effect on unpunished responding. Although the rate-increasing effects of both drugs were comparable in drug naive animals, buspirone was 10 times more potent with its maximal effect occurring at a dose of 1.0 mg/kg as opposed to 10.0 mg/kg for chlordiazepoxide. In animals previously exposed to chlordiazepoxide, rate increasing effects of buspirone occured maximally at 1.7 mg/kg and were not as great in magnitude as they had been in drug naive animals. The rate increasing effects of chlordiazepoxide were also attenuated in rats previously treated with buspirone.

AFFILIATION
Dept. of Psych., LSUMC, Shreveport, LA 71130-3932

Sex Differences in Hospitalized Cocaine Abusers

R. Weiss, U. Lange, S. Mirin and M. Griffin

Little has been written about the differences between male and female cocaine abusers. We therefore compared sociodemographic characteristics, reasons for cocaine use, drug effects, depressive symptoms, and psychiatric diagnoses in 95 men and 34 women hospitalized for cocaine abuse. Men were more likely to be employed, hold higher status jobs, and be self-supporting. Women are more likely to cite specific reasons for drug use, while men tended to use cocaine as part of a larger pattern of antisocial behavior. Women were diagnosed more often as having major depression, their depressive symptoms improved much more slowly than men's when drug-free. These findings suggest that women cocaine abusers may initially experience more residual problems, e.g., depression and job dissatisfaction, than men after becoming drug-free. Drug treatment centers should therefore design their programs accordingly.

AFFILIATION:

Alcohol and Drug Abuse Treatment Center, McLean Hospital, Belmont, MA 02178

Harvard Medical School, Belmont, MA 02178

Drug-seeking Behavior and Morphine-induced EEG Responses in Morphine Post-addict Rats Chronically Treated with Ethylketocyclazocine (EKC)

G. Young and N. Khazan

We have found that morphine post-addict rats exhibited long-lived protracted abstinence (Psychopharmacology 29: 271-276, 1973), while EKC post-tolerant rats did not (Eur. J. Pharmacol. 125: 265-271, 1986). Therefore, we hypothesized that kappa opioid maintenance in morphine-tolerant rats might lead to a protracted abstinence syndrome of lower severity and to a lower tendency for relapse to opioid self-administration.

Rats were made dependent on morphine by a series of automatic injections and trained to self-administer 10 mg/kg i.v. morphine injections (FR-10). Group I was then placed on morphine maintenance (10 mg/kg, i.v./2 hr) for ten days: Group II on EKC maintenance (4 mg/kg, i.v./2 hr). After two weeks of withdrawal, rats were returned to the experimental cages and given the opportunity to self-administer i.v. saline injections for three days. On the fourth day, rats were challenged with morphine (10 mg/kg, i.v.).

Group I self-administered 19.4 ± 2.3 (mean ± s.d.), 1.4 ± 1.1 and 3.2 ± 0.8 saline injections during three consecutive days. When Group I received morphine challenges, a short-lived behavioral stupor (4.1 ± 1.9 min) was produced. Group II displayed a negative correlation ($r = 0.92$) between total number of saline self-injections on Day 1 and total duration of stuporous behavior produced by morphine challenge on the fourth day. This correlation indicates that if EKC maintenance reestablished a "non-tolerant-like" EEG and behavioral response to morphine challenge, then drug-seeking behavior was less intense.
(Supported by NIDA Grant DA-01050.)

AFFILIATION

Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

Circling Produced by Sigma Ligands via a Dopaminergic Mechanism

R. Matsumoto, S. Goldstein, T. Thompson, R. Patrick and J. Walker

Sigma receptors are found in many brainstem structures that are involved in posture and movement. High densities of these receptors are found in the substantia nigra and anatomical findings suggest that sigma ligands may affect motor systems through the dopaminergic pars compacta.

In this study, 1,3-di-o-tolylguanidine (DTG) and (+)-pentazocine were unilaterally microinjected into the substantia nigra pars reticulate of rats. Circling behavior was quantified to assess the role of sigma receptors in motor control. DTG, tested at five doses (0.07, 0.3, 1.2, 4.7 and 18.6 nmol), produced significant dose-dependent contralateral rotations. Likewise, (+)-pentazocine (1.2, 2.4, 4.7, 9.3 and 18.6 nmol) produced contralateral rotations in a dose-dependent manner. The effects of (+)-pentazocine were also significant, but weaker than those of DTG.

In the second set of experiments, unilateral 6-OHDA lesions of the medial forebrain bundle were made prior to behavioral testing to destroy ascending dopaminergic projections from the substantia nigra. The lesions produced a 95% or greater depletion of tyrosine hydroxylase in the striatum on the lesioned side and also significantly attenuated the circling produced by DTG (1.2 nmol) and (+)-pentazocine (9.3 nmol).

These data show that sigma ligands in the substantia nigra can produce motor activation through interactions with the nigrostriatal dopamine system. The findings of this study, thus, support the involvement of sigma receptors in motor control and the regulation of ascending dopaminergic pathways.

Affiliation: Departments of Psychology and Biology and Medicine, Brown University, Providence, RI 02912

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Effects of Alcohol Intoxication and Hangovers on Subsequent Drinking

C. Smith, S. Bookner and F. Dreher

Data from a questionnaire developed to determine the relationship between drinking practices and effects of drinking were obtained from 221 medical and graduate students and staff. Statistical analyses revealed significant inverse correlations between ethanol intake and the frequency of a number of acute adverse effects including headache, nausea and vomiting; these inverse relationships were especially strong for women and for consumption of spirits. Factor analysis of fifteen symptoms revealed five distinct factors.

The most frequently cited reasons for decreasing or discontinuing drinking related to personal and social obligations, with negative physical and subjective effects of drinking cited by more than two-thirds of the respondents. The effects of hangovers on subsequent drinking were reported by 50 to 71% of the respondents. Only a few report severe hangover symptoms that did not influence drinking. The major reasons cited by an abstaining were: to avoid loss of control (82%), unpleasant taste (79%), to avoid intoordination (67%), to avoid loss of concentration (60%) an to avoid hangovers (51%). Susceptibility to hangovers was not correlated with susceptibility to either migraine or common headache, or with guilt about drinking or guilty feelings following intoxication.

Except for a relatively lower consumption of liquor by women, as compared with beer or wine, important reasons for drinking and for stopping or reducing drinking were similar for men and women.

These results are consistent with the hypothesis that negative events associated with drinking, especially adverse physical reactions to alcohol, severe as deterrents or modifiers of subsequent drinking behaviors.

Major items from this questionnaire were used to develop a modified version for use as a structured interview or self-completion form with patients in hospital for detoxification. Preliminary results from 50 patients indicate the feasibility and utility of parallel studies of the perceived aversive factors by patient populations. (Supported in part by a grant from the Alcoholic Beverage Medical Research Foundation).

AFFILIATION: Department of Pharmacology and Therapeutics, School Of Medicine, State University of New York at Buffalo, NY 14214

Chronic Administration of SCH23390 Produces Sensitization to the Discriminative Stimulus Properties of Cocaine

M. Emmett-Oglesby and D. Mathis

This investigation was designed to test the role of dopamine D1-receptor subtypes in detection of, and tolerance to, the discriminative stimulus produced by cocaine. Thirty rats were trained to discriminate cocaine (10 mg/kg, i.p.) from saline, using a food rewarded two-lever choice task. Following training, cocaine (1.25 20.0 mg/kg) substituted for the cocaine training stimulus in a dose-related manner. Subjects were then randomly assigned to two chronic administration groups. In one group, cocaine (20 mg/kg) was administered every 8-h for 10 days, and the cocaine dose-effect curve was redetermined on days 7-10 of this regimen, at 8-h post the morning injection of the chronic cocaine treatment, The cocaine dose-effect curve was shifted approximately two-fold to the right, indicating that tolerance to the discriminative stimulus properties of cocaine had developed. In the other group, tests were conducted for blockade of the cocaine training stimulus by SCH23390 (0.064, 0.125, 0.25 and 0.5 mg/kg, i.p.), a specific dopamine D1 -receptor antagonist. All doses of SCH23390 at least partially blocked the cocaine stimulus, and the 0.5 mg/kg dose fully blocked the cocaine stimulus, although 8 of the 15 subjects tested did not respond. Subsequently, SCH23390 (0.25 mg/kg) was administered every 24-h for 10 days. On days 7-10 of chronic administration, 24-h post the previous chronic injection of SCH23390, the cocaine dose-effect curve was redetermined. The curve was shifted approximately two-fold to the left, suggesting that chronic blockade of D1 receptors produces sensitization to the discriminative stimulus properties of cocaine. These results indicate that D1 receptors are involved in the detection of the cocaine discriminative stimulus and that chronic ocupation of D1 receptors by a D1 antagonist enhances the detection of the cocaine stimulus. These findings support the hypothesis that tolerance and sensitization to the discriminative stimulus properties of cocaine arise as a consequence of chronic dopamine receptor occupation.

Supported by NIDA grant DA04137.

AFFILIATION

Department of Pharmacology, TCOM, Fort Worth, TX 76107-2690, USA

Acute Effects of Diazepam in Rhesus Monkeys as Measured by Performance in a Battery of Complex Operant Tests

M. Paule and G. Schulze

The acute effects of diazepam (Valium) were assessed using a battery of complex food-reinforced operant tasks that included responding in delayed matching to sample (DMTS, n=5), conditioned position response (CPR, n=7) progressive ratio (PR, n=9), temporal response differentiation (TRD, n=4), and incremental repeated acquisition (IRA, n=9) tests. Diazepam (0.25-4.0 mg/kg iv) produced significant dose-dependent decreases in the number of reinforcers obtained in the TRD and IRA tasks only. TRD accuracy was significantly decreased at doses of 0.25, 1.0, 2.0, and 4.0 mg/kg when compared to vehicle injections. Significant decreases in IRA accuracy generally did not occur at doses below 1.0 mg/kg. DMTS accuracy was decreased at 0.5 mg/kg but showed no clear dose-delay interaction. Performance in the CPR and PR tests showed no significant effects of diazepam exposure over the dose range tested. The relative sensitivities of performance in these operant tasks for detecting diazepam's behavioral effects were thus TRD > IRA > DMTS > CPR = PR. These results indicate that diazepam selectively disrupts performance of operant tasks in monkeys designed to model human correlates of time perception, learning ability and short-term memory.

AFFILIATION

Pharmacodynamics Branch, Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, Arkansas 72079 and Department of Pharmacology and Interdisciplinary Toxicology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205

Normal and Abnormal Natural Killer (NK) Activity in Methadone Maintenance Treatment Patients

M. Ochshorn, E. Khuri, L. Fahey, J. Craig, R. Rodriguez, M. Aldana, H. Albeck and M. J. Kreek

Diverse immunological abnormalities have been observed in narcotic addicts and in former narcotic addicts in treatment. Thirty-four methadone maintained subjects in a demonstration-research methadone maintenance treatment program were studied (of a total 70 patient receiving medication in the clinic at that time) (17 M;17 F), age ranges 18-33y, mean age 25.7y (+/-4.8SD). Presence of hepatitis 8 antigenemia was an exclusion criterion (one patient) because viremia has been reported to alter NK activity. All bloods from methadone treatment patients, as well as from the normal subjects in this study were collected between 8:30am -9:30am. Methadone maintained patients were studied prior to methadone medication. Normal levels of NK activity were determined by studying blood specimens collected form 17 normal volunteer subjects (8 M;9 F), age range 22-44y, mean age 30.6y (+/- 9.9SD), on 32 occasions. NK activity is expressed in target: effector ratio of 1:100. The range of levels in normal subjects is 36.2% to 82.8% (mean 61.3% +/-11.6SD). Of the 34 methadone maintained study subjects studied, 18 (10 M;8 F) had NK activity values within normal range. This subgroup of study patients with normal NK activity had a mean level of NK activity 59.2% +/-11.5SD (range 40.9-79.0%), mean age 25.7y (+/-4.8SD (range 18-33y), and mean methadone dose 65.3mg (+/-23.7SD). One patient was bisexual and one homosexual. Seven of the 34 study subjects (3 M;4 F) had NK activity ranging from 2SD-3SD lower than normal. For these subjects the mean level of NK activity was 33.2% (+/-3.5SD)(range 29.3-38.3%), mean age was 25.9y +/-2.2SD (range 19-33y) and the mean methadone dose was 61.9 mg (+/-28.8SD). One patient was homosexual in the second group. Nine of the 34 patients had NK activity which was lower than 3SD below normal (4 males and 5 females). For these subjects the mean level of NK activity was 24.4% (+/-3.1 SD) (range 19.2-28.2%), mean age was 25.3y (+/-4.2 SD) (range 18-31y) and mean methadone dose was 71.1 mg (+/-19.1SD). We considered what factors might affect NK activity like the presence of hepatitis Bs Ab, hepatitis Bc Ab, years of abusing heroin before starting treatment, methadone dose, history of cocaine, amitriptyline of diazapam abuse during last month, clinical impression of being sick and sexual preference. The Spearman rank correlation test did not show any correlation between theses factors and NK activity.

The Rockefeller University, N.Y., N.Y. & Cornell Med. Col., N.Y.

The Effects of Methylephedrine Chlorpheniramine and Caffeine on Intravenous Self-administration of Dihydrocodeine in Rhesus Monkeys

Y. Wakasa and T. Yanagita

A number of abuse cases of an OTC antitussive syrup containing dihydrocodeine (DC). methylephedrine (ME). Chlorpheniramine (CP). and anhydrous caffeine (AC). have been reported in Japan. The influence of the three ingredients (ME. CP. AC) on the reinforcing effect of DC were examined by the progressive ratio test procedure in intravenous self-administration of DC in 4 rhesus monkeys. The monkeys had previous experience with the progressive ratio test procedure for some drugs. In each trial. a FR-100 period for 7 days was followed by a progressive ratio period in which the ratio for self-administration started at 100:1, increasing geometrically by a factor of the 4th root of 2 at every intake, and when the number of Lever press during 48 hrs after the Last intake failed to reach 50 percent of that required for the Last intake. or failed to reach the criterion for the next intake within 72 hrs after the Last intake. the trial was terminated and the ratio achieved for the Last intake was regarded as the final ratio. At first. the final ratios for DC alone at doses of 60 and 240μg/kg/inj. were compared with the ratios for a mixture of the 4 ingredients including the same dose of DC. The proportions of the ingredients in the mixture were the same as in the antitussive syrup (DC:ME:CP:AC =1:2:0.4:2.1). The final ratio of the 4-ingredient mixture was about 1.9 times higher at 60μg/kg/inj., and about 2.3 times higher at 240μg/kg/inj. than the ratio for DC alone on the average. Then. the final ratios for DC alone at 60μg/kg/inj. and for the mixtures of DC plus one other ingredient were compared. The final ratios for DC-ME. DC·CP, and DC·AC were 1.0. 1.1, and 1.3 times higher than for DC alone. respectively. It was thus suggested that the reinforcing effect of DC is increased when combined with ME, CP, and AC. and that this increment of reinforcing effect of DC is not due to the interaction between DC and any single drug from among ME. CP, and AC. but due to the overall interaction of the 4 ingredients.

AFFILIATION

Department of Psychopharmacology, Preclinical Research Laboratories, Central Institute for Experimental Animals, Kawasaki, Japan

Discriminative Stimulus Effects of Butorphanol, Nalbuphine, Naloxone and Hydromorphone in Opioid-Dependent Human Volunteers

K. Preston, G. Bigelow and I. Liebson

To assess the stimulus properties of opioid agonist/antagonist drugs in opioid-dependent humans, 5 volunteers enrolled in methadone maintenance treatment (30 mg/day, po) were trained in a 3-choice drug discrimination procedure to discriminate between the effects of IM saline (2 ml; S). hydromorphone HCl (10 mg/70 kg; H), and naloxone HCl (0.15 mg/70 kg; N). Subjects earned monetary reinforcement by correctly identifying the training drugs by letter code. Other subjective, behavioral and physiological measures were also collected. Following training subjects were tested for their ability to discriminate between the 3 training drugs; generalization curves for hydromorphone (2.5, 3.5, 5, 7, 10 mg/70 kg), naloxone (0.0375, 0.053, 0.075, 0.105, 0.15 mg/70 kg), butorphanol (0.375, 0.53, 0.75, 1.05, 1.5 mg/70 kg) and nalbuphine (1.05, 1.5, 2.1, 3 mg/70 kg) were then determined.

All 5 subjects learned the H-N-S discrimination. In generalization testing both hydromorphone and naloxone produced dose-related increases in drug-appropriate responses and in characteristic subjective effects measures. Naloxone 0.075 mg produced N-appropriate responding in 80% of trials while 0.105 and 0.15 mg produced N-appropriate responding in 100% of trials. Hydromorphone produced 80% or greater H-appropriate responding at doses of 3.5 mg and higher. Butorphanol produced dose-related increases in identifications as N and in those subjective effect measures increased by naloxone; 1.05 and 1.5 mg produced 88% and 100% N-appropriate responding, respectively. Nalbuphine also produced dose-related increases in identifications as N and in those subjective effect measures increased by N; 2.1 and 3.0 mg produced 100% N-appropriate responding. All 4 drugs produced dose-related decreases in S-appropriate responding. There was little or no cross-generalization between N and H. Naloxone and nalbuphine were never identified as H-like, and hydromorphone was never identified as N-like in the discrimination measures. Butorphanol produced primarily N- or S-appropriate responses, but was rarely identified as H-like.

The antagonist properties of nalbuphine and butorphanol were not differentiated from those of N. Neither butorphanol nor nalbuphine showed any consistent opioid agonist-like effects in these subjects maintained at relatively low levels of physical de pendence. The close concordance between the verbal subjective effect data and the behavioral discrimination data suggests that these measures share a common basis.

AFFILIATION

The Johns Hopkins University School of Medicine, Baltimore MD 21224.

Effects of Ethanol on Regional Cerebral Metabolic Rate and Mood

H. de Wit, J. Metz, J. Brunner and M. Cooper

This study used positron emission tomography (PET) to examine the effects of ethanol (ETH) on brain metabolism in normal volunteers, and to study changes in regional cerebral metabolism (CM) in relation to ETH-related mood changes.

Nine male social drinkers (aged 21-29; average ETH consumption 5 drinks/week) were tested twice each, once with a placebo (PLC) beverage and once with ETH (0.5 g/kg). immediately following beverage ingestion, subjects were injected with 6-8 mCi FDG. During the period of FDG uptake, they performed a visual monitoring task (button pushing in response to light presentations). Immediately before, and 20 and 40 min after beverage ingestion they completed a self-report mood questionnaire (Profile of Mood States; POMS). Regional CM was determined using Sokoloff's model with standard rate constants and 14 regions of interest obtained from tomographic metabolic images.

Across all subjects, ETH did not consistently increase or decrease CM either globally (mean PLC: 9.06 mg/100g/min (sd 1.32;) mean ETH: 8.71 (sd 0.79)) or in particular regions. ETH also did not consistently change POMS measures or psychomotor performance. However, individual differences were observed on both CM and mood measures, and certain changes in regional CM, relative to the rest of the brain, were highly correlated with changes in POMS scores after ETH. For example, subjects who reported increases in positive moods (e.g., Vigor, Friendliness, Elation) after ETH also showed more marked changes in regional CM in the temporal and parietal cortices, relative to the changes produced by ETH in the rest of the brain. The changes in positive moods were positively correlated with the relative changes in the parietal cortex and negatively correlated with relative changes in the temporal cortex. The changes in negative moods were negatively correlated with relative changes in the parietal cortex and positively correlated with relative changes in the temporal cortex.

Thus, ETH affected regional CM and mood differently in different individuals, and these measures of physiological and subjective effects were strongly related. The data suggest a possible physiological basis for diffential risk for ETH abuse.

AFFILIATIONS:

Departments of Psychiatry and Radiology, The University of Chicago, Chicago, IL 60637

Rats Rapidly Learn to Self-administer Sufentonil in Aerosol Form

L. Sharpe, J. Jaffe and A. Jaffe

Our objective was to develop an animal model of drug self administration by the intrapulmonary route -- one of the most common routes used by human drug users. While some workers have developed models in which animals self-administer volatile anesthetics, gases, and even tobacco smoke, our interest was in developing a method that would permit the inhalation of non-volatile substances. Here we report the use of an ultrasonic nebulizer to deliver a vapor containing sufentanil (SF) into a small chamber (vol= 5.1 liters) located inside a standard test cage. Lever pressing delivered 5 sec of aerosol mist; after 15 sec, an exhaust fan was turned on (60 sec) to clear the vapor from the chamber (total timeout period = 80 sec). In the first training sessions, male Sprague-Dawley rats were left overnight in the chamber for 13 to 17 hr (provided ad lib access to water). No food deprivation or food enticements on the lever were used.

The schedule on the first night was a FR 1, which was increased progressively. Once a criterion of at least 1 vapor episode/hr was obtained at an FR 5 schedule, the animals were shifted to 2-hr daytime sessions at FR 5. Of 9 rats given access to SF vapor (50 or 75 ug/ml), 8 reached this criterion within a 15-day cutoff period; 5 did so within 3-8 sessions. Of 6 rats given access to water vapor, 3 reached criterion in 11-14 sessions. An SF rat that failed to reach criterion within 15 sessions did so when food deprived; in H_2O rats, food-deprivation was ineffective unless SF was substituted for H_2O. Responding maintained by SF during the daily sessions was dose dependent at concentrations of 25, 50, and 75 ug/ml ($r = -0.85$, regression analysis). Substituting water vapor for all 3 SF concentrations significantly reduced responding within 5-20 sessions. Naloxone (1 mg/kg, ip) significantly reduced responding for all SF concentrations. We conclude that this method provides a good animal model for the reinforcing properties of non-volatile drugs via the pulmonary route.

AFFILIATION:
NIDA Addiction Research Center, Baltimore, MD. 21224

Brain Structural Abnormalities Differentially Correlate with Severity of Alcohol and Opioid Abuse

N. Cascella, D. Wong, G. Pearlson, C. Nagoshi and E. London

Twenty-three male volunteers (32.0 ± 4.47 years, mean ± SD) with histories of polydrug abuse and 23 age-matched normal controls (3 1.6 ± 4.60 years, mean ± SD) were studied by computerized tomography (CT) to explore the effects of abused substances on brain morphology. Histories of drug use were obtained from ail subjects using a structured clinical interview. The stated amount of substance consumed at peak use was used for correlational studies. Because of variability in modes of reporting amounts of substances consumed (weight, money, frequency), the data on consumption were transformed into severity scores between 1 and 5, according to a standardization method. The severity scores were then related to ventricle brain ratio (VBR) and third ventricle width (TVW). Unlike data obtained on heroin and *cocaine,* amount of alcohol consumed per session and amount consumed per day did not correlate highly with each other. Therefore, two categories of alcohol consumption were maintained for correlational studies. VBR values in substance abusers and controls were 6.32 ± 1.64 and 5.46 ± 2.30 (mean ± SD), respectively. TVW values of substance abusers and controls were 5.38 mn ± 1.10 and 4.01 mn ± 1.68, respectively. Drug users had significantly wider third ventricles ($F(1,21) = 7.87$, $p < 0.01$). In the substance abuser group, age was positively correlated with VBR ($r = 0.55$, $p < 0.01$). Assuming no effect of age, only the alcohol use severity score, indexed as amount consumed per session, correlated significantly with VYR ($r = 0.56$, $p < 0.01$). Severity of alcohol use remained a highly significant predictor of VBR even after partialling out the effect of age through multiple regression. Age and alcohol use did not show any interactive effect on CT measures. Severity of heroin use was negatively correlated with VBR after partialling out the effect of age. The findings support the hypothesis that quantity of alcohol consumed is related to brain atrophy. Furthermore, they show that amount of alcohol consumed per session is a better predictor of VBR than amount of alcohol consumed per day. Finally, they indicate that opioids and other commonly abused substances do not necessarily cause brain atrophy, as indicated by CT measures.

AFFILIATION:

* NIDA, Addiction Research Center, Baltimore, Maryland 21224
** Johns Hopkins University, Baltimore, Maryland 21205

Behavioral Effects of Benzodiazepine Antagonists in Rats Treated Chronically with Diazepam

I. Lucki and R. Kucharik

The effect of benzodiazepine (BZ) antagonists on lever pressing behavior in rats was studied following the chronic administration of diazepam at low doses that are associated with its anxiolytic effects. Food-restricted rats (80% of free-feeding weight) were trained to press a lever for food (45 mg pellets) under a FR20 schedule of reinforcement. Separate groups of 9 rats each were injected with either diazepam (5 q/kg IP bid) or saline (0.9% NaCl) twice daily and were tested between 3-32 weeks of treatment. The morning dose of diazepam was administered 1 hour prior to the experimental test sessions.

Rats treated chronically with diazepam were tolerant to the rate-suppressing effects of an acute dose of diazepam administered 1 hour prior to the session, as shown by a 5-fold increase of the dose of diazepam required to reduce FR20 response rates by 50% (25 mg/kg) compared with controls (5.0 mg/kg). The BZ antagonist flumazenil (Ro 15-1788; 1.0 - 56 n-g/kg IP) administered 10 min prior to the session produced a significantly greater suppression of FR20 responding in rats treated chronically with diazepam than in controls (p 0.001). Similarly, the BZ antagonist CGS 8216 (0.3 - 33 mg/kg IP) given 10 min prior to the session produced greater reductions of FR20 responding in rats treated chronically with diazepam than in saline-treated controls (p = 0.02).

The disruption of lever pressing behavior by BZ antagonists in rats treated chronically with diazepam provides an objective behavioral measure of BZ dependence and withdrawal in rats maintained at low treatment doses of diazepam. This research was supported by USPHS grant DA 05186.

AFFILIATION

Department of Psychiatry, University of Pennsylvania School of Medicine, 207 Piersol Building, Philadelphia, PA 19104-4283

Characterizing Future Smokers

R. Coambs, S. Li, L. Kozlowski, J. Robinson and W. Rickert

In future, the demographics of smokers will change, because although smoking prevalence is declining, some sub-populations are not quitting.

We used a random-sample survey of 736 smokers from Kitchener, Ontario, to investigate "die-hard" smokers who are the least likely to quit. The respondents here divided into two groups judged more and less likely to continue smoking, based on their responses to questions concerning cessation attempts, and intentions to smoke in the future. The two groups were compared on questions about smoking behaviour and attitudes.

The reluctant quitters started smoking at a younger age and smoked more of stronger cigarette brands. The health effects of smoking concerned them less, both for themselves and those around them. When logistic regression was used to predict future smoking status, concern for health was the best predictor. In addition, "die-hards" over the age of 40 showed more physical dependence on nicotine than those under 40, as measured by salivary cotinene.

"Die-hard" smokers were evidently less concerned about the health consequences of smoking. This lack of concern may originate in denial rather than ignorance, resembling that of alcoholics and drug abusers. An accurate characterization of such smokers promises to be very useful in defining health education campaigns to reduce smoking.

AFFILIATION

Addiction Research Foundation, 33 Russell St., Toronto, Canada M5S 2S1, and Labstat Inc., 262 Manitou Dr., Kitchener, Ontario, Canada N2C 1L3

Chronic Pain and Substance Abuse: A Pilot Study of Narcotic Maintenance

J. Kennedy and T. Crowley

A pilot study to assess the feasibility of treating chronic pain patients addicted to narcotics and with a history of substance abuse examined: 1) the ability of a methadone maintenance-like treatment to attract and hold patients, and 2) methadology for evaluation of the patients.

Patients stop their current narcotic and receive a daily oral dose of methadone (15-100 mg). They attend daily for the first 1-3 weeks; then take-out doses are considered individually. Written informed consent is obtained, warning of addiction, side effects, and withdrawal symptoms. Before and every three months thereafter, a battery of tests to evaluate pain (McGill Pain Questionnaire, Visual Analog Scale), mood (Profile of Mood States), and function (Sickness Impact Profile). A treatment contract also includes requirements of weekly random urines, weekly individual psychotherapy, and permission to review records and contact treating doctors.

Results were mixed. Two remain in continuous treatment after 12 and 10 months, respectively. One left at two and a half months, and returned after two months of ER visits, and continues now for five months. The other dropped out at four weeks. We conclude that we can attract patients to this type of treatment and some patients will remain. We observe that therapies with these patients are quite tumultuous.

The test battery and urinalyses permit evaluation and reflect some changes. This was useful when verbal reports were discordant from test reports (exaggerated complaints of pain with minimal change in test scales, changes in pain loci on maps unaccompanied by verbal reports).

Our experience leads us to believe that further research is warranted, not only to extend the present study, but also to look closer at the discrepancies found between the verbal and test report. As these patients obtain narcotics on the basis of verbal complaints, it might be useful to examine the changing course of pain as related to the test parameters.

AFFILIATION: Department of Psychiatry, University of Colorado School of Medicine, Denver, Colorado 80262

Physiologic and Behavioral Effects of a Rapid Dose Induction of Buprenorphine in Heroin-Dependent Volunteers

P. Fudala, R. Johnson, E. Cone and A. Dorbert

A rapid dose-induction procedure was used to evaluate the utility of buprenorphine hydrochloride (BUP) in treating opioid dependence. Nineteen male, heroin-dependent, volunteer subjects were given BUP in ascending daily sublingual dosages of 2, 4, and 8 mg. Subjects were maintained on 8 mg daily for another 15 days. Only the results from the first 4 days are reported here. Physiologic and behavioral observations were performed at 0.5, 1, 2, 4, 6.5, 11.5, 14.5, and 23 hrs following BUP administration. Urine samples were collected daily prior to drug administration and assayed using TDx OPIATES (Abbott Diagnostics, Abbott Park, IL).

Subscales of the Addiction Research Center Inventory (ARCI), Observer and Subject Drug Effect Questionnaires, and a withdrawal symptom questionnaire were used to rate signs and symptoms of acute opioid effects and withdrawal. There were significant effects for hour of observation for observer-rated "subject's liking for drug," "signs of drug effect," and "how subject feels." Significant hour effects were also observed for subject-rated "drug liking," "drug effects," "overall well-being," and "overall sickness," as well as for scores on the ARCI subscales (LSD, MBG, and PCAG; measures of dysphoria, euphoria, and apathetic sedation, respectively). The mean values for all measures initially increased following BUP administration, except those for "overall sickness" and the LSD and PCAG subscales. The peak effect for each measure occurred between 2 and 4 hours following BUP administration. The responses at 23 hours post BUP administration were approximately equal to those observed one-half hour following drug.

For both subject-rated "overall sickness" and "level of withdrawal," the mean responses on day 1 were significantly greater than those for any other day. No differences were observed between the other 3 days, and no differences between days were noted for observer-rated "subject withdrawing."

Increased supine and standing pulse rates, which could be suggestive of opioid withdrawal, were greatest on day 4.

Although 11 of 16 behavioral measures normally sensitive to opioid withdrawal indicated statistically significant differences within days following BUP administration, none were significant with respect to changes across days. Thus, BUP did not precipitate withdrawal as the dosage was increased. Pupillary constriction was greatest on days 3 and 4 when the highest doses of BUP were given, consistent with mu-agonist effect. Urinary opiate excretion data indicated that all subjects were below the 300 ng/ml positive cutoff level by day 4. This is consistent with known excretion data and formed the kinetic basis for the dose-induction procedure.

Results from this study indicate that heroin-dependent individuals may be rapidly inducted onto BUP without precipitating withdrawal while being provided maximum mu-agonist effects.

AFFILIATION

NIDA, Addiction Research Center, Baltimore, MD 21224

Psychiatric Disorders in Twin and Non-twin Alcoholics

L. Briskin N. Anderson, D. Svikis, R. Pickens, K. Lykken and M. McGue

To determine if alcoholic twins are representative of alcoholic individuals in general, we compared prevalence of selected psychiatric symptomatology in alcoholics twins to that reported previously for undifferentiated (i.e., non-twin) alcoholics. Our sample consisted of 183 twin probands (126 males, 57 females) in treatment for alcoholism. Their mean age was 36.0 yrs; approximately 93% were Caucasian. The Diagnostic Interview Schedule (DIS) was used to obtain DSM-III diagnoses of Major Depressive Disorder, Antisocial Personality Disorder and Substance Use Disorder excluding alcohol and tobacco. No significant difference was found between twin probands and non-twin alcoholics in five of six diagnostic comparisons. For males, while no significant difference was found in prevalence of Antisocial Personality Disorder or Substance Use Disorder (excluding alcohol and tobacco), twin alcoholic probands had a lower rate of Major Depressive Disorder than alcoholics in general ($p<.02$). For females, no significant difference was found between alcoholic twin probands and alcoholics in general for Major Depressive Disorder, Antisocial Personality Disorder or Substance Use Disorder. These data support the assumption that alcoholic twins are representative of alcoholics in general, and that data obtained in twin studies of alcoholism can be generalized to the larger population of treatment alcoholics.

AFFILIATION
University of Minnesota, Minneapolis, Minnesota; Johns Hopkins University School of Medicine, Baltimore, Maryland; and National Institute on Drug Abuse, Rockville, Maryland.

Clonidine Disrupts Long-term Memory Processing in Mice

A. Church, L. Flexner, J. Flexner and E. Reynolds

In light of the review being conducted on clonidine by the WHO's Expert Committee on Drug Dependence, it is felt that further characterization of clonidine's action on the mammalian nervous system is warranted. The experimental design that was used to study clonidine's action was one that has proved useful in studying the effects of a number of other drugs that affect the noradrenergic system. That design utilizes a brief period of maze training followed sometime later by the intracerebral administration of puromycin. Bitemporal injections of puromycin consistently Induce amnesia of aversive maze-learning in mice when administered within 3 days of training. These same bitemporal puromycin injections lose their amnestic effectiveness if the latency between training and injection is extended beyond 6 days. Consistent with other evidence, we believe that this change in efficacy of bitemporal puromycin is a result of an underlying brain process whereby memory (in our task) "spreads" during the 6 days following training. Since previous experiments have indicated that the central noradrenergic system is involved in this process of "memory spread", we have examined the effect of stimulation or blockade of the α_2-receptor. To this end, we administered a single dose of the α_2-adrenoceptor antagonist, idazoxan, or the α2-agonist, clonidine, two days following training in a low-shock motivated Y-maze. Ten days later, memory of the maze-training was challenged by bitemporal administration of puromycin. Idazoxan (1 mg/kg, s.c.) had no effect on the process of engram spread. In contrast, clonidine (25 µg--125ng/kg, s.c.) suppressed engram spread for at least 30 days after treatment. When mice were tested at 60 and 90 days after treatment, spontaneous recovery (i.e. engram spread) was evident in only about 50% of the clonidine treated mice. Coadministration of idazoxan with clonidine blocked the effects of clonidine on "memory spread", bolstering the supposition that α_2-receptor stimulation was critically involved In clonidine's actions.

AFFILIATION

Drug Control Section, Drug Enforcement Admin., Washington, D.C.

Comparison Between Withdrawal from Chlordiazepoxide and Ipsapirone, a $5HT_{1A}$ A Agonist Anxiolytic

A. Goudie, M. Leathley and J. McNally

Ipsapirone, a $5HT_{1A}$ agonist, is a non-benzodiazepine (BZ) anxiolytic. Withdrawal from ipsapirone was compared with that from chlordiazepoxide (CDP). Rats were treated as shown:

Group	Phase 1 (Chronic Treatment for 21 days)	Phase II ("Withdrawal" for 5 days)	Phase III
1	Saline	Saline	Ro 15 - 1788
2	Ipsapirone	Ipsapirone	Ro 15 - 1788
3	Ipsapirone	Saline	
4	CDP	CDP	
5	CDP	Saline	
6	CDP	Ipsapirone	

Rats were initially treated with saline, ipsapirone or CDP. Drugs were given i.p. at 10 a.m. and 4 p.m. at doses escalating from 10 to 30 mg/kg/injection. In Phase II, animals were withdrawn from ipsapirone (Group 3) or CDP (Groups 5 and 6) and compared with controls (Group 1) or animals maintained on ipsapirone (Group 2) or CDP (Group 4). CDP withdrawal caused weight loss and anorexia. Ipsapirone withdrawal had no such effects. In animals withdrawn from CDP, but maintained on ipsapirone (Group 6), ipsapirone did not ameliorate withdrawal. In Phase III, Ro 15-1788 (10 mg/kg i.p.) failed to precipitate withdrawal in subjects treated with ipsapirone. These data show that: (i) Withdrawal from CDP causes loss of weight and anorexia. (ii) Ipsapirone does not cause such withdrawal. (iii) In animals chronically treated with ipsapirone, Ro 15-1788 failed to precipitate withdrawal. (iv) In animals withdrawn from CDP, ipsapirone failed to alleviate withdrawal.

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AFFILIATION
Department of Psychology, Liverpool University, P.O. Box 147, Liverpool L69 3BX, U.K.

Comparison of the Effects of Secobarbital and Diazepam on the Acquisition of Response Sequences in Humans

S. Higgins and M. Stitzer

The barbiturates and benzodiazepines produce amnestic effects and disrupt learning. However, the benzodiazepines are purported to produce greater disruption than the barbiturates. Roache and Griffiths (1985), for example, compared the effects of triazolam and pentobarbital in sedative abusers using picture recognition tasks. Both drugs decreased the number of items correctly recognized, but the magnitude of triazolam's effects were greater than those observed with pentobarbital. In the present study we examined whether the benzodiazepine diazepam (0,5,15,25 mg) produced greater impairment than the barbiturate secobarbital (O-50,150,250 mg) in a human learning paradigm.

Subjects were 4 normal men. Drug was administered p.o. in a double-blind crossover design. The experimental task was the repeated acquisition of behavioral chains procedure, which has been used previously to characterize the effects of sedative drugs in humans (e.g., Higgins et al, 1987). The task required subjects to learn via trial and error to advance a digit located in the center of a video screen from zero through 9 using three keys of a numeric keypad (i.e., a lo-response sequence). Each session the task was done predrug and 20,50, and 100 min postdrug. A new response sequence had to be learned each time the task was done. Prior to initiating drug testing, subjects were trained on the task until the percent of errors made in learning the sequence and response rates stabilized.

Secobarbital and diazepam produced quite comparable dose-effect curves. At peak effect, both compounds increased percent errors and decreased response rates as an orderly function of dose. The magnitude of effects did not differ across the two compounds. These results indicate that diazepam and secobarbital produce a very comparable profile of effects on human learning. Thus, with regard to learning impairment, the liability associated with the use or abuse of diazepam versus secobarbital appears to be the same.

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AUTHORS

Stephen T. Higgins, Ph.D.
Department of Psychiatry
University of Vermont
1 South Prospect Street
Burlington, Vermont 05401

Maxine L. Stitzer, Ph.D.
Department of Psychiatry
Johns Hopkins University School of Medicine
Baltimore, Maryland 21224

Ethanol-Induced Narcosis Antagonized by Post-treatment with Indomethacin

G. Elmer and F. George

Previous research in our laboratory has implicated the arachidonic acid cascade as an important system in ethanol's CNS effects. The previous studies examined prostaglandin synthetase inhibitors efficacy when given prior to ethanol. The present study investigated the efficacy of indomethacin, a potent prostaglandin synthetase inhibitor, to antagonize ethanol-induced hypnosis when given post-ethanol treatment. Indomethacin (0, 10.0 mg/kg) was administered I.P. contralaterally 15 min before and 15, 30 and 60 minutes following 4.0 g/kg ethanol I.P. Eight male DBA/2J mice were used at each dose at each time point. Following administration of ethanol animals were placed in a V-shaped trough. Duration of loss of the righting reflex was used as the measurement for ethanol's effect. Animals were judged to be awake when they could right themselves three times within 30 sec. Retro-orbital blood ethanol levels were determined at the time of regaining the righting reflex. Indomethacin decreased ethanol-induced loss of the righting reflex in a time dependent manner. The duration of the loss of the righting reflex decreased by 54% when indomethacin was given 15 minutes after ethanol. Indomethacin had successively less effect at 30 and 60 minutes post-ethanol than at 15 minutes pre and post-treatment. The rate of ethanol elimination was not significantly altered by indomethacin; treated animals regained the righting response at significantly higher blood ethanol levels than did saline treated animals. It has been shown that ethanol increases the fluidization of biological membranes. Since the arachidonate cascade is a membrane-bound system activated by disruptions in the cell membrane, it is possible that ethanol-induced fluidization increases the activity of the arachidonic acid cascade. These data provide further evidence that ethanol's CNS effects are mediated to a significant extent by the arachidonic acid cascade.

AFFILIATIONS

Department of Pharmacology and Toxicology, University of Maryland at Baltimore, 21221 and NIDA Addiction Research Center, Baltimore, MD, 21224.

Support for the Dependence Syndrome Concept in Opiate Abuse

T. A. Kosten, L. Jacobsen and T. R. Kosten

The dependence syndrome was proposed by Edwards and Gross (1976), to link a psychological concept of dependence with biological manifestations of tolerance and withdrawal. This concept was used as the basis for the DSM-III-R criteria: but no study has shown that biological addiction is related to dependence as defined by these criteria. The present study tested this hypothesis in 52 opiate addicts. The Naloxone Challenge Test (NCT) was administered to measure severity of opiate withdrawal and the SCID interview was used to establish DSM-III-R criteria for dependence. The severity of withdrawal (NCT Score) was correlated positively with opiate dependence ($r=0.30$, $p<0.04$). Unexpectedly, NCT Score showed an inverse correlation with cocaine dependence ($r=-0.38$, $p<0.005$). Dependence on alcohol, sedatives, or marijuana were not related to NCT Score. Moreover, neither NCT Score nor opiate dependence were correlated with a social problems score, consistent with the postulate that social problems are separate from dependence. Thus, our results support the validity of the dependence syndrome concept in opiate abuse because opiate withdrawal severity is correlated with opiate dependence in a fairly specific way and is not related to social problems. The inverse correlation between opiate withdrawal and cocaine dependence may suggest that opiate addicts use cocaine to alleviate opiate withdrawal symptoms. Alternatively, they may misinterpret the crash from cocaine as opiate withdrawal.

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AFFILIATION

Yale University School of Medicine, Psychiatry Department, Substance Abuse Treatment Unit, New Haven, CT 06519

Urinary Excretion of Cocaine, Benzoylecgonine and Ecgonine Methyl Ester in Humans

J. Ambre, T. Ruo, J. Nelson and S. Belnap

The excretion kinetics of cocaine (C) and its two major metabolites, benzoylecgonine (BZ) and ecgonine methyl ester (EME) were determined by collecting all urine for 30 hours from five cocaine users (Subjects C, D, E, F and G) given bolus doses followed by exponential cocaine infusions that delivered doses of 253 (Subject C), 444 (Subjects D, E, and F) and 700 mg (Subject G). Plasma cocaine and urine cocaine, BZ and EME were measured by gas chromatography with a nitrogen detector.

Elimination halftimes for EME and BZ, estimated from semilog plots of excretion rates vs time, averaged 3.1 and 4.5 hours respectively, in agreement with ourprevious report. Urinary recovery in D, E, and F was 27-41 percent of the dose, with 14-17 percent as BZ, 12-21 percent as EME and two percent as cocaine. Subject C excreted very little EME; 5-6 fold less than the mean for the other subjects, amounting to only three percent of the dose. Cocaine disposition in Subject G, who received the largest dose and attained plasma levels of 3000 ng/ml, showed some characteristics of a nonlinear process.

AUTHORS:

John Ambre, M.D., Ph.D.
Tsuen Ih Ruo, Ph.D.
John Nelson, M.D., Ph.D.
Steven Belknap, M.D.

AFFILIATION:

Clinical Pharmacology Center
Department of Medicine
Northwestern University Medical School
Chicago, Il 60611

Effects of Nifedipine (A Ca ++ Modulator) Pre-treatment on Cardiovascular and Subjective Responses to Intravenous Cocaine Administration in Humans

C. Muntaner, K. Kumor, C. Nagoshi and J. Jaffe

The use of cocaine has been associated with the occurrence of functional and morphological cardiovascular disorders (Cregler & Mark, 1986). Recent animal studies on the interaction between nitrendipine, a Ca++ modulator, and the cardiac effects of cocaine (Nahas et al., 1985; Trouve & Nahas, 1986) suggest that these drugs may antagonize the cardiac toxicity of cocaine in humans. The present study was conducted in order to examine the effect of nifedipine, a Ca++ modulator, pretreatment on the subjective and cardiovascular effects of i.v. cocaine administration in humans. Seven male volunteers each with recent history of intravenous cocaine use participated in a randomized double-blind study of the interaction of nifedipine with cocaine. Subjects received one dose of cocaine (20 mg or 40 mg) or placebo through a preprogrammed infusion pump, 20 min after pretreatment with nifedipine 10 mg or placebo orally. This schedule gave a total of six conditions (placebo/placebo, placebo/cocaine 20 my, placebo/cocaine 40 mg, nifedipine 10 ng/placebo, nifedipine 10 my/cocaine 20 my, nifedipine 10 mg/cocaine 40 mg). We compared subjective responses and measurements of blood pressure and heart rate using a three-way ANOVA for condition and time. Subsequently we performed t-tests to isolate differences between the cocaine responses with and without nifedipine pretreatment. Response to both the cocaine 40 mg and nifedipine 10 mg/cocaine 40 my conditions were increased compared to the placebo/placebo condition. Results on several subjective variables) including General Drug Effect and Feel Good scales at 2 min post-infusion, revealed that means for the nifedipine 10 mg/cocaine 40 mg condition were significantly decreased compared to the placebo/cocaine 40 mg. Nifedipine reduces blood pressure over all drug conditions and tended to reduce cocaine related increases in the heart rate early after injection. Thus, pretreatment with nifedipine 10 my given 20 min prior to cocaine reduced the subjective response to cocaine shortly after infusion, when subjective effects are at their peak.

Authors affiliation: NIDA Addiction Research Center, P.O. Box 5180. Baltimore, Maryland 21224.

Phenobarbital as well as Pentobarbital Enhances 3H-GABA Binding in Rat Forebrain Membranes

J. Richter and L. Curtis

Most barbiturates enhance binding of ligands at GABA and benzodiazepine sites when chloride is present (e.g., Olsen and Snowman, 1982). However several investigators who have described such enhancement with most barbiturates have noted that the enhancement was not seen with phenobarbital (PheB). If this effect on GABA binding is responsible for the physiological actions caused by barbiturates such as pentobarbital (PB), PheB should enhance GABA binding too since it induces all the same actions as PB.

We have approached this question by first re-examining the effect of PheB on ^{3}H-GABA binding. In our membrane preparation and under our assay conditions (which were chosen to be closer to physiological conditions), we have found PheB does enhance ^{3}H-GABA binding. While PB increased the specific binding by 50% at about 5×10^{-4}M, it took about 1×10^{-3} PheB to increase binding 50%. A maximum effect of about 400% of control was achieved at 5×10^{-3} M PB and 2.5×10^{-2}M PheB. The 2 to 5-fold difference in potency is consistent with known potency ratios of the two drugs for several of their actions. Calcium was necessary for the maximum PheB effect in our membrane preparation. PheB also had a greater effect at 37 C than at 0 C.

Even with our assay conditions however, the PheB effect depended on the tissue preparation; In a preparation made essentially as described by Olsen and Snowman (1982) ^{3}H-GABA binding was enhanced by PB but not by PheB just as they reported. Preliminary results suggest that the characteristics of the freeze-thaw step (temperature, volume, number of cycles) are the important differences between the membrane preparations. The reason why the effectiveness of PheB is so much more sensitive than the effectiveness of PB to these manipulations remains to be determined.
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AFFILIATION

Departments of Pharmacology and Psychiatry (Insititute of Psychiatric Research), Indiana University School of Medicine, Indianapolis, IN 46223.

Naloxone-Induced Analgesia and Supersensitivity to Opiates is Contingent Upon Exposure to Hot Plate While Under the Influence of Naloxone

D. Knoke, C. Poulos, A. Le and H. Cappell

Chronic treatment with naloxone induces analgesia (Greeley, Lê, Poulos & Cappell, 1988; Rochford & Stewart, 1987) and augments morphine induced analgesia (Bardo, Miller & Risner, 1984). The present study determined whether naloxone induced analgesia is dependent upon exposure to painful stimulation while under the influence of naloxone. Second, we determined whether chronic naloxone increases susceptibility to opiate induced behaviors in addition to analgesia.

During acquisition, two groups of rats received naloxone (5 mg/kg) and two groups received saline. One drug group and one saline group were given a hot plate test 20 mins. after injection (NAL-CONTINGENT and SAL-CONTINGENT). The other two groups received hot plate tests 24 hours following injection,(NAL-NONCONTINGENT and SAL-NONCONTINGENT). Paw lick latency was used as an index of pain sensitivity. Following acquisition, four tests were given.

On test 1 rats were given naloxone or saline as usual and all rats were tested on a hot plate 20 mins. after injection. The NAL-CONT group showed substantial analgesia ($\tilde{X}$=17.07) while the mean paw lick latencies of the NAL-NONCONT and SAL groups were indistinguishable (ranging from 6.01 to 6.79 seconds).

On the second test morphine (17.5 mg/kg) was administered and susceptibility to catalepsy was assessed. The results indicated that the NAL-CONT group was significantly more cataleptic than the NAL-NONCONT and SAL controls which did not differ (X^2=9.05, $p<.025$). Catalepsy was not induced; however, under conditions of acquisition when naloxone was administered (test 3).

The final test compared two measures of the analgesic effect of morphine (3 mg/kg) in rats trained with naloxone or saline. Hot plate and tail flick measures were used. The NAL-CONT group was significantly more analgesic on both tests of nociception ($p<.01$).

Repeated exposure to a hot plate under-the influence of naloxone induced analgesia and supersensitivity to opiates which was not induced by naloxone administration alone.

AFFILIATION

Departments of Psychology and Pharmacology, University of Toronto; Addiction Research Foundation, 33 Russell St., Toronto M5S 2S1

A Survey of Urinalysis Screening Policies Among Methadone Maintenance Clinics

A. Saxon, D. Calsyn, V. Harver and L. Erickson

A survey requesting information about urinalysis policies was sent to all methadone maintenance clinics listed in the 1984 Program Directory. Useable surveys were obtained from 324 clinics (66%). The frequencies of collecting observed urine specimens were: more than weekly (4%), weekly (32%), biweekly (10%), monthly (41%), less than monthly (2%), unobserved (6%). Frequency was less in the West and South and for proprietary clinics. Smaller, VA, and government supported clinics tested more frequently. Over 96% of the clinics screen routinely for opiates, cocaine and methadone, with slightly less frequent screening for barbiturates (85%), amphetamines (84%) and benzodiazepines (74%). Only 29% routinely screen for cannabis. VA, smaller clinics and those in the Midwest and South were more likely to test for cannabis. Opiates, cocaine, and benzodiazepines were the three drugs most often detected. Clinics testing for cannabis ranked it third. In the West opiates and amphetamines were ranked higher. In the East cocaine and benzodiazepines were ranked higher. Routine confirmation of laboratory results by a different technique was reported by 69% of clinics. The primary preliminary techniques used were: thin layer chromatography (TLC) (64%), enzyme immunoassay (EIA) (48%) and gas chromatography/mass spectrometry (GC-MS) (14%). The main confirmative techniques used were EIA (43%), TLC (31%), GC-MS (35%) and radio immunoassay (9%). Multiple preliminary techniques were used by 19%, and multiple confirmatory techniques by 13%. In the East and South there was more use of TLC with EIA confirm, whereas in the Midwest there was more use of EIA with TLC confirm. Urine screening practices differ considerably among clinics. Since different clinics may achieve different treatment results, urine screening practices represent an important variable to be considered in outcome assessments.

Affiliations: VA Medical Center, Seattle and Department of Psychiatry and Behavioral Sciences, University of Washington School of Medicine.

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A. Jacobson

Involvement in the Opioid Program - The University of Michigan, Medical College of Virginia, and the NIH (NIDDK, LN)

The Committee on Problems of Drug Dependence has been engaged in the testing of analgesics for their physical dependence potential and abuse liability since 1948. The results of the testing of these drugs at the University of Michigan (UM), under the auspices of the CPDD, have been published continuously since 1952, and from 1974 to the present time the reports from UM have been augmented by those from the Medical College of Virginia (MCV). The procedures which are used at the two testing facilities have changed considerably over the years. Presently, MCV (with Drs. Aceto, Bowman, Harris, and May) tests drugs for their antinociceptive and narcotic antagonist activity in mice, runs all of the single dose suppression (SDS) and precipitated withdrawal (NW or PPt-W) studies in the rhesus monkey, and uses rat infusion (RI) techniques by substitution and in primary physical dependence determinations. Primary physical dependence (PPD) studies in the rhesus monkey have been run in both facilities.

The University of Michigan (with Drs. Woods, Smith, Medzhiradsky, and Winger) has, recently, specialized in self-administration (SI) and drug discrimination (DD) assays in the rhesus monkey, in vitro work (displacement of ^{3}H-etorphine from a membrane preparation from rat cerebrum), and tests using the electrically stimulated mouse vas deferens (VD) preparation. Even more recently, they have been engaged in antinociceptive testing in the rhesus monkey, although results from this test have not been generally reported. Thus, there is little or no duplication of testing, as there was with SDS and NW testing several years ago.

Antinociceptive assays are also carried out at the National Institutes of Health (NIDDK, LN). The Eddy hot plate assay (HP) and, less frequently, the Nilsen assay have been utilized (since 1948 and 1971, respectively). These assays complement the work at MCV. Recently, displacement assays have been used at NIH to discern the effect of some of the submitted drugs on the phencyclidine (PCP) binding site, with ^{3}H-TCP as the radioligand.

A few compounds have been examined for their affinity for the sigma binding site at MCV (using the radioligand ^{3}H-(+)-SKF 10,047). Several opioid-like compounds have, in the past, been shown to interact with PCP and sigma (non-opioid) binding sites (e.g., levorphanol, dextrorphan, SKF 10,047 and several other 6,7-benzomorphans).

Funding

Funding for the laboratories involved in the analgesic testing program comes mainly from NIDA (or NIH), with small contributions from the CPDD. The supplementary funding from the CPDD has, however, been found to be important for the work in the various laboratories, and has certainly proven highly useful to the CPDD for retention of one of its major functions.

Changes in Structural Types of "Opioids" Tested

The type of compound which is tested has also changed over the decades (see Jacobson, 1988, and previous years, for comparison). Several of the compounds which we now receive for testing can no longer be conveniently described by comparison with any of the classical opioids. Thus, for the past several years, I have included a list of "Miscellaneous" compounds (see Tables 8 and 9), compounds structurally dissimilar to the classical opioid-like drugs. Several groups have prepared compounds which are, perhaps, meant to be agonists devoid of CNS activity. These could be peripherally acting analgesics. Appetite suppressants, and compounds with other types of actions (e.g., compounds which are meant to have a suppressant effect on “craving” or desire for heroin, cocaine, et al.) are also being sought. However, even some of the compounds which are structurally similar to “normal” opioids may have been modified to serve these other purposes. Our testing procedures are designed to determine antinociceptive activity, physical dependence potential and abuse liability. The results from these assays are still useful for these drugs. Negative results from them may serve to pinpoint a useful, peripherally active analgesic, for example.

Compounds Evaluated

Tables 1 to 9 have been included to enable the reader to relate the structures of the drugs to their biological activities within particular classes of opioids. These classes are the 4,5-epoxymorphinans (tables 1 and 2), fentanyl-related compounds (table 3), 4-phenylpiperidine-related compounds (table 4), compounds related to haloperidol, the 5-phenylmorphans, and the morphinans (table 5), N-substituted 6,7-benzomorphans (tables 6 and 7), and a group of unclassifiable miscellaneous compounds (tables 8 and 9).

In order to summarize the biological work from UM (Woods et al., 1989), and MCV (Aceto et al., 1989), a considerable number of abbreviations have been used. These are listed prior to the tables themselves. Only that work which was actually accomplished this

year has been recorded in the tables. Previously reported (PR) work on compounds is noted with the year in which the report appeared. It should be emphasized that the year noted is the titled year (e.g., Problems of Drug Dependence, 1986), not the year of actual publication (which usually, but not always, occurred during the year following that denoted in the title of the publication).

Thus, from these tables, the reader can see all of the work done this year, or when some of the work on the compound was previously done, across all facilities associated with the opioid program under the auspices of the CPDD. If the reader would like further, or complete information on a particular compound, the original report of Woods et al. (1989) and Aceto et al. (1989) should be perused through use of the assigned NIH number, since all reports are cross-referenced by NIH number.

ABBREVIATIONS USED IN TABLES 1-9.

1) MOUSE ED50 OR AD50: Antinociceetive assays (ED50, sc injection except where noted, mice) [Confidence limits are listed in the MCV and UM reports (Aceto et al. 1989; Woods et al. 1989)]: **HP** = hot plate; **N** = Nilsen; **PPQ** = phenylquinone; **TF** = tail flick; **TFA** = tail flick antagonism vs. morphine. These assays are performed at MCV, except for the HP and N (carried out at NIDDK, NIH).

I = inactive, without a reasonable dose-response relationship, or insufficiently active for statistical analysis.

2) In Vitro Determinations:
These assays are done at UM.
A) **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 [^{3}H]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 previous reports (Jacobson 1984, and preceding years) in which -Na values were quoted. However, the former numbers can be recalculated for comparison with those which are currently utilized through the use of the +Na/-Na ratio.

B) **VD** = electrically stimulated mouse vas deferens EC50 values, rounded to one significant figure. Agonist activity is stated using "E" followed by a negative number: E = 10^{-x} M, where x = the negative number, thus: 1E-3 = 0.001 M (1 mM), 1E-6 = 1 uM, and 1E-9 = 1 nM (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; **SE** = slight effect on twitch; **SA** = slight antagonism by naltrexone]. Compounds which suppress the twitch and are not antagonized by naltrexone or UM 979 [NIH 8859, (-)-5,9-alpha dimethyl-2-(3-furylmethyl)-2'-hydroxya,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. 1989) 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 narcotic antagonist properties. 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. 1989).

3) Data From Monkey Colonies:
These data are presently from MCV, past reports are from MCV or UM.
A) **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.

B) **NW or PPt-W** = 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.

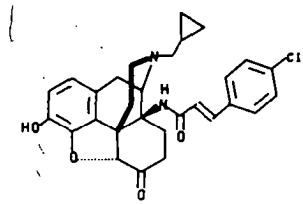
4) Other studies (OTHER):
A) **RI** = rat continuous infusion (from MCV):
a) **SM** = substitution for morphine [**NS** = no substitution for morphine; **CM** = complete substitution; **PS** = partial substitution].
b) **PPD** = primary physical dependence, in rats.
B) **ND** = non-dependent monkeys: **M-like** = morphine-like effect.
C) **PPD** = primary physical dependence (in the rhesus monkey).
D) **SA** or **SI** = self-administrating or self-injection (from UM):
N E = no effect; **High** = codeine-like; **IN** = intermediate between saline and codeine; **SE** = slight effect.
E) **DD** = drug discrimination (from UM).

Previous Reports (**PR**):
Previous work on a compound is noted by year, the year listed in the monograph title (e.g. Problems of Drug Dependence 1986). Note that the date of publication of the monograph generally occurs one year after the titled year of the monograph. The data which have been published in previous reports are shown by a "PR" in the appropriate column (e.g., a PR (1983) in the SDS column would indicate that the SDS work was cited in "Problems of Drug Dependence 1983", which was published in 1984).

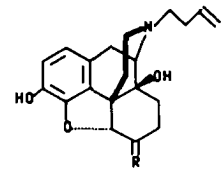
NOTE: The numbers used in the tables may be rounded. For precise values, and details of the procedures, see the MCV and UM reports in these Proceedings (Aceto et al. 1989; Woods et al. 1989).

Abbreviations for structural formulae: **Et**=ethyl

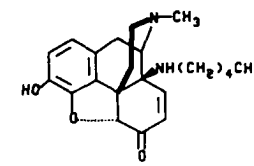
TABLE 1. 4,5-EPOXYMORPHINANS[a]



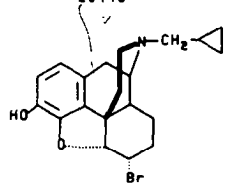
10443



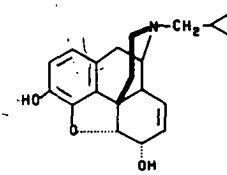
10458: R=O
10459: R=CH_2



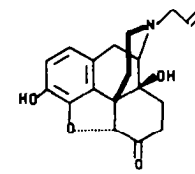
10504



10535



10539

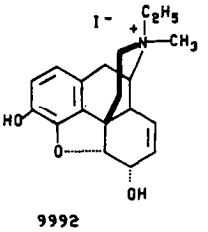


10562
NALOXONE

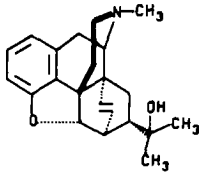
	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES
NIH #	HP	PPQ	TF	TFA	RBH	VD	SDS
10443	--PR (1987)---------				0.65	NE[B]	PR (1987)[C]
10458	I	0.13	I	3.8	7.8	6E-8(48)[SA][D]	NS (10,20)
10459	I	5.8	I	I	8.1	8E-8(76)[A][D]	NS (2,9)
10504	-	0.005	0.008	I	2.86	1E-9(100)[A]	CS (0.008,0.03)[100xM]
10535	I	I	I	0.008	1.92	NE[E]	NS (0.001-0.004)[F]
10539	I	0.1	I	I	1.45	NE[G]	NS (0.05-0.4)[H]
10562	-	1.3	I	0.03	6.26	NE[I]	NS (0.05,0.0125)

A) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS. B) EXTREMELY POTENT IRREVERSIBLE ANTAGONIST OF MU, DELTA, AND KAPPA RECEPTORS. C) OTHER TESTS: NW - PR (1987). D) ANTAGONIZES MU AGONIST (AGONIST-ANTAGONIST ACTIONS, E) EQUIPOTENT TO NALTREXONE AS MU ANTAGONIST, 10x NALTREXONE AS DELTA ANTAGONIST. F) OTHER TESTS: NW - FW (0.08,0.24)[1xN]. G) COMPETITIVE MU, DELTA, AND KAPPA ANTAGONIST (MOSTLY MU). H) OTHER TESTS: NW - PW (0.2,0.8) [4xN]. I) ANTAGONIST OF MU, DELTA, AND KAPPA AGONISTS.

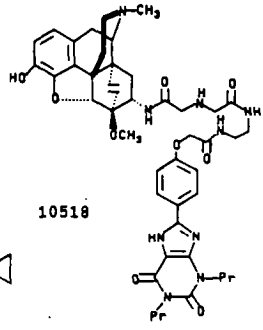
TABLE 2. 4,5-EPOXYMORPHINANS (CONTINUED)[a]



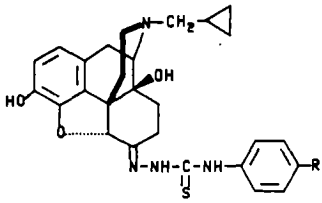
9992



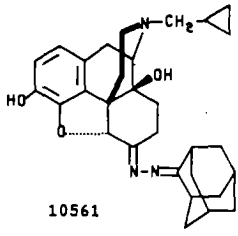
10501



10518



10550: R=Br
10558: R=NO_2



10561

	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES
NIH #	HP	PPQ	TF	TFA	RBH	VD	SDS
9992	----PR (1983)---------				510	3E-6(35)[NA]	-
10501	-	0.2	0.05	I	-	-	-
10518	I	0.22	I	I	-	-	-
10550	I	I	I	0.05	-[B]	-[B]	NS (0.015-0.06)[C]
10558	I	0.003	I	0.05	-[B]	NE[D]	NS (0.05,0.25)[E]
10561	I	I	I	0.03	2.22	2E-6(49)[A][F]	NS (0.025,0.1)[E]

A) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS. B) INSOLUBLE, C) OTHER TESTS: NW - PW (0.1,0.4)[1xN]. D) ANTAGONIZES MU, DELTA AND KAPPA AGONISTS (NOT SIMPLE COMPETITIVE). E) EXACERBATED WITHDRAWAL (ANTAGONIST). F) ANTAGONIZES MU AND DELTA AGONISTS (NON-COMPETITIVE AT KAPPA RECEPTOR).

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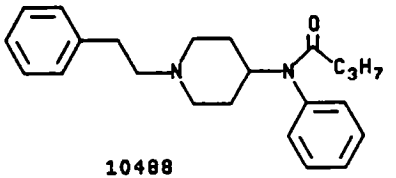
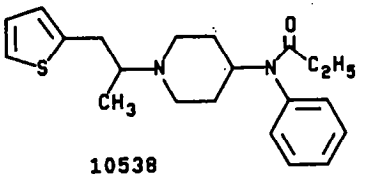
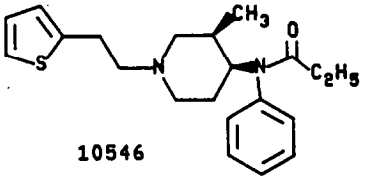
TABLE 3. FENTANYL-RELATED COMPOUNDS[a]



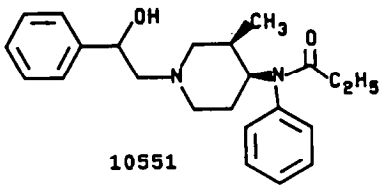
10488



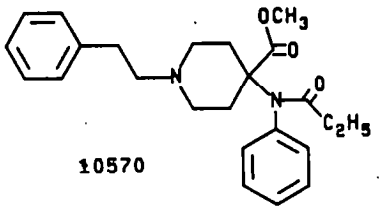
10538



10546



10551

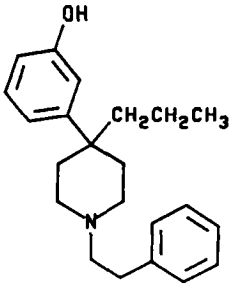


10570

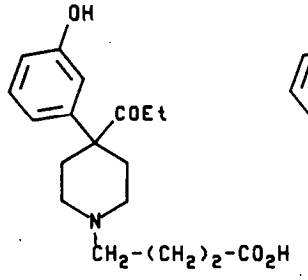
	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES
NIH #	HP	PPQ	TF	TFA	RBH	VD	SDS
10488	----PR (1987)----------				215	NE	PR (1987)
10538	----PR (1987)----------				15.7	9E-9(95)[A]	PR (1987)
10546	-	0.005	0.004	I	5.13	2E-10(100)[A][B]	CS (0.0025,0.01)[1000xM]
10551	<0.0003	0.00013	0.0002	I	4.42	4E-10(98)[A]	CS (0.002-0.005)[25000xM]
10570	<0.0004	0.00006	0.0002	I	72	1E-10(97)[A]	CS (0.005-0.00007)[25000xM

A) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS. B) EXTREMELY POTENT DELTA RECEPTOR AGONIST.

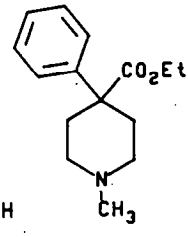
TABLE 4. 4-PHENYLPIPERIDINE-RELATED COMPOUNDS[a]



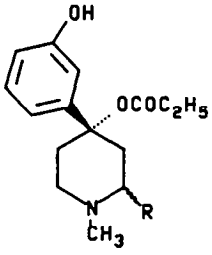
10345



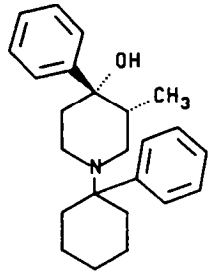
10475



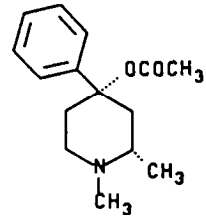
10522 (5221)
PETHIDINE



10526: R=—CH3
10527: R=...CH3



10531: RACEMATE
10553: (+)

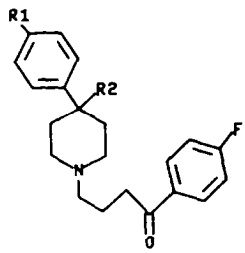


10541: RACEMATE
10543: (-)

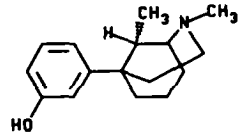
	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES
NIH #	HP	PPQ	TF	TFA	RBH	VD	SDS
10345	1.8	0.4	2.1	I	-	-	CS (1,0.4)
10475	-------PR (1987)----------				>10uM	NE	-
10522 (5221)	---PR (1987,1956,1955)----				>10uM	NE	PR (1987,1955)
10526	I	3.7	I	I	-	-	-
10527	~20	2.97	32.8	I	-	-	-
10531	I	--PR (1987)-------			680	1E-6(99)[A]	-
10541	I	2.2	9.8	I	-	-	-
10543	I	0.9	15.2	I	-	-	-
10553	I	0.7	8.7	I	-	-	-

A) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS.

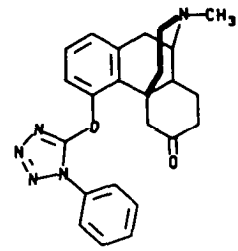
TABLE 5. COMPOUNOS RELATED TO HALOPERIDOL, THE 5-PHENYLMORPHANS AND THE MORPHINANS[a]



8032: R1=Cl, R2=OH
(HALOPERIDOL)
10494: R1=Cl, R2=OCOEt
10495: R1=H, R2=OCOEt



9971

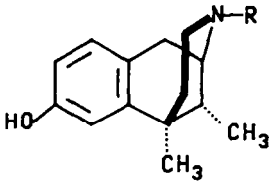


9973

	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES
NIH #	HP	PPQ	IE	IEA	RBH	VD	SDS
8032	-	0.01[B]	14.6	1	-	-	PR (1963)
9971	---PR (1983,1982)-----				780	2E-9(34)[A][C]	PR (1982)[D]
9973	2.4	-	-	-	19.2	6E-8(96)[A][E]	-
10494	30	0.4	20.6	1	279	8E-7(100)[NA][F]	PS (0.4,1.2)[G,H]
10495	0.3	0.07	0.3	1	30	5E-8(99)[A]	CS (0.1)[I]

A) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS. B) ANTAGONIZED BY NALOXONE, C) PARTIALLY REVERSED THE EFFECT OF MORPHINE (AGONIST-ANTAGONIST). D) NW STUDIES ALSO PREVIOUSLY REPORTED IN 1982. E) MOSTLY KAPPA RECEPTOR AGONIST F) NO SHIFT TO RIGHT, BUT MAXIMUM RESPONSE DECREASED TO 24%. MU RECEPTOR AGONIST WITH COMPONENT OF ACTION MEDIATED BY KAPPA RECEPTORS OR BY A NON-OPIATE MECHANISM. G) SIDE-EFFECTS RESEMBLED THOSE FROM NEUROLEPTICS. H) OTHER TESTS: PPD (RI) - NE (TOXIC); SIGMA BINDING - IC50=2.7E-8; PCP BINDING - IC50=100uM. I) OTHER TESTS: SIGMA BINDING - IC50=4.4E-8.

TABLE 6. N-SUBSTITUTED 6,7-BENZOMORPHINANS[a]

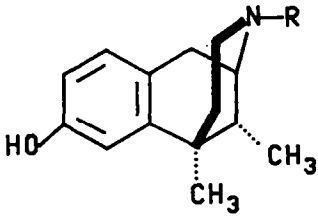


10559: R=ETHYL (+)
10560: R=ETHYL (-)
7549: R=n-PROPYL (RACEMIC)
10556: R=n-PROPYL (+)
10557: R=n-PROPYL (-)
10565: R=n-BUTYL (+)
10566: R=n-BUTYL (-)
10568: R=n-PENTYL (+)
10569: R=n-PENTYL (-)

	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES
NIH #	HP	PPQ	TF	TFA	RBH	VD	SDS
7549	I	17.2	I	0.35	-	-	-[B]
10556	I	I	I	I	>10uM	NE[C]	-
10557	I	22	I	0.4	14.5	NE[D]	NS (0,025,0.1)[E]
10559	I	33.5	I	I	>10uM	NE	NS (0.5,2.0)[F]
10560	-	1.4	I	3.7	144	NE[D]	NS (0.5,2.0)[G]
10565	-	-	-	-	>10uM	NE[H]	-
10566	I	I	I	1.5	47.2	3E-7(64)[A][I]	PS (2.0,8.0)[F,J]
10568	I	5.6	21.3	I	>6uM	SE [NA][H,K]	NS (0.75,3.0)
10569	I	-	-	-	102	7E-7(100)[A]	-

A) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS. B) OTHER TESTS: NW - PR (1972). C) WEAK ANTAGONISTS OF MU AND KAPPA AGONIST (NOT DELTA). D) ANTAGONIST OF MU AND DELTA AGONISTS (NOT SIMPLE COMPETITIVE), AND KAPPA AGONIST (NON-COMPETITIVE). E) EXACERBATES WITHDRAWAL (ANTAGONIST). F) UNUSUAL SIDE-EFFECTS. G) MAY EXACERBATE WITHDRAWAL, SEIZURES AT 20 MG/KG. H) WEAK ANTAGONIST OF MU, DELTA AND KAPPA AGONISTS. I) PARTIAL OPIATE AGONIST WITH AFFINITY FOR DELTA RECEPTORS. J) EFFECTED NORMAL, NON-DEPENDENT, MONKEY, EFFECTS NOT REVERSED BY NALOXONE. K) SOMEWHAT KAPPA-SELECTIVE.

TABLE 7. N-SUBSTITUTED 6,7-BENZOMORPHANS (CONTINUED)[a]



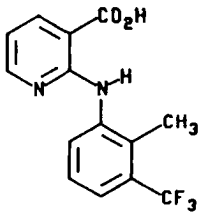
10502: R=CH_2CO_2H
10564: R=$(CH_2)_2CO_2H$
10555: R=$(CH_2)_3CO_2H$

10503: R=$CH_2CO_2C_2H_5$
10548: R=$(CH_2)_3CO_2C_2H_5$

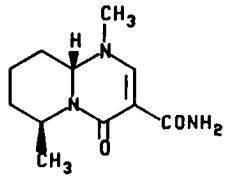
	MOUSE ED50 OR AD50				IN VITRO	
NIH #	HP	PPQ	TF	TFA	RBH	VD
10502	-----PR (1987)--------				>6uM	9E-6(78)[A]
10503	-----PR (1987)--------				2080	NE[B]
10548	I	7.1	I	I	1020	2E-5(100)[A]
10555	I	11.4	I	I	>10uM	NE
10564	I	1.4	I	I	>10uM	NE

A) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS.
B) WEAK ANTAGONIST OF MU, DELTA AND KAPPA AGONISTS.

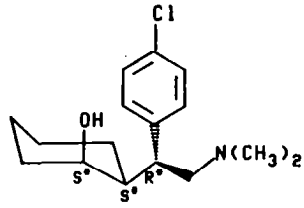
TABLE 8. MISCELLANEOUS COMPOUNDS[a]



10250 (9249)

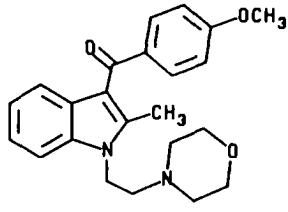


10407



10435: RACEMIC
10436: (+)
10437: (-)

ADRENAL CORTEX EXTRACT
10431

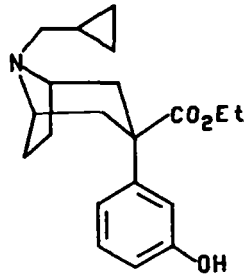


10474

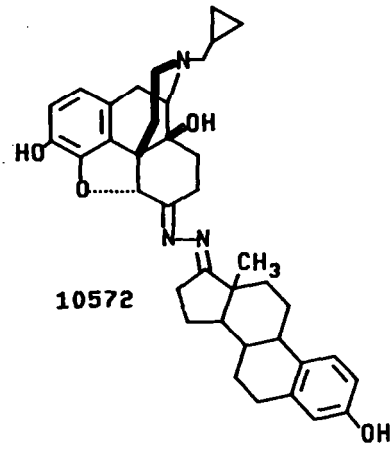
	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES
NIH #	HP	PPQ	TF	TFA	RBH	VD	SDS
10250 (9249)	----PR (1984,1977)-----				>10uM	3E-5(18)[NA][B]	PR (1984,1977)
10407	I	20	I	I	>6uM	2E-5(44)[SA]	NS (2.0-8.0)[C]
10431	-	-	-	-	-	-	NE[D]; PS[E]
10435	I	5.9	I	I	>10uM	4E-7(26)[NA]	NS (2.5,10.0)
10436	I	I	I	I	>10uM	5E-8(32)[NA]	NS (1.5-10.0)
10437	I	8	I	I	>6uM	2E-8(25)[NA][F]	PS (1.0,4.0)[G]
10474	I	5.1	I	I	>10uM	1E-6(100)[NA][H]	NS (3.0,12)[I]

A) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS. B) VERY WEAK ANTAGONIST ACTION, C) OTHER TESTS: SA - NE (0.3-5.5) [AVERSIVE EFFECT SEEN, TYPICAL OF KAPPA AGONIST]; DD (EKC MODEL) - SE [1/3 ANIMALS EFFECTED. LITTLE OR NOT KAPPA AGONIST ACTION]. D) PRETREATMENT 6x/DAY FOR 2 DAYS WITH 8.2 ML/KG. E) PRETREATMENT 6X/DAY FOR 6 DAYS WITH 1.0 ML/KG F) WEAK MORPHINE ANTAGONIST. G) NON-DOSE-RELATED MILD SUPPRESSION OF WITHDRAWAL. H) NON-OPIOID AGONIST I) OTHER TESTS: NW - NP; PPD - NE (1.0-32.0): SA - SE (0.0003-0.1); DD - SE (0.01-18).

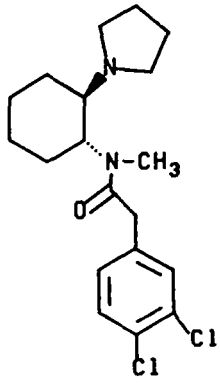
TABLE 9. MISCELLANEOUS COMPOUNDS (CONTINUED)[a]



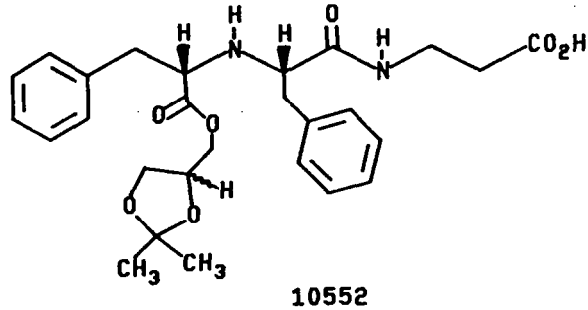
10528



10572



10532: (+)
10533: (-)



10552

	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES
NIH #	HP	PPQ	TF	TFA	RBH	VD	SDS
10528	-	I	I	I	-	-	-
10532	I	6.5	I	I	>12uM	1E-6(60)[A][B]	-
10533	8.9	0.2	2.5	I	4140	2E-8(94)[A][B]	NS (0.25,3.0)[C]
10552	I	I	I	I	-[D]	NE	NS (2.0-8.0)
10572	-	I	I	0.03	-	-	-

A) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS. B) SIGNIFICANT KAPPA RECEPTOR ACTIVITY. C) HIGH DOSE CAUSED DISORIENTATION, STUPOR. D) INSOLUBLE.

Especially Interesting Drugs

Our previous record holder for potency, etorphine, has been surpassed by two fentanyl derivatives (NIH 10551 and 10570, table 3). Both compounds are exceedingly potent. The NIH 10570, carfentanil, is a quick-acting analgesic with a short duration of action, and may be placed in Schedule II for use in capturing wild animals. It is supposedly safe for that purpose. It is said to be relatively non-toxic to deer, causing less hypothermia than etorphine, and the deer do not appear to suffer from respiratory depressant side-effects.

A third fentanyl-like compound, NIH 10546 (table 3), was noted to be an extremely potent delta opioid receptor agonist in the electrically stimulated mouse vas deferens preparation. This poses a quandary in that the compound is a potent antinociceptive agent and completely suppresses morphine withdrawal in the single dose substitution assay in monkeys, properties thought to be mu opioid-receptor mediated.

The haloperidol-like drugs, NIH 10494 and 10495, have analgesic and neuroleptic properties. NIH 10495 is considerably more tent than NIH 10494 as an analgesic. Both compounds displaced ^{3}H-(+)-SKF 10,047 from the sigma binding site with good affinity, and NIH 10494 did not interact with the phencyclidine binding site. Further work with these compounds is in progress. The submitter is preparing a number of chemically related compounds which we will test in the future.

NIH 10518 (table 2), like NIH 10494 and NIH 10495, was designed to display dual effects. It is an endoethenooripavine covalently attached through peptide links to a xanthine component and it has been found, by displacement assays, to bind to both mu opioid and adenosine receptors. Its antinociceptive potency was found to be comparable to morphine in the PPQ assay.

It is interesting to compare the effect of substitution at the C-6 position in two 4,5-epoxymorphinans, NIH 10458 and 10459 (table 1). These are N-butenyl derivatives, a rather uncommon substituent on nitrogen), with either an oxygen atom (in NIH 10458) or a methylene group (in NIH 10459) at C-6. Although both compounds appear to be agonist-antagonists in the mouse vas deferens preparation, only NIH 10458 shows narcotic antagonist activity in the TFA assay, and it is about 45 times more potent than NIH 10459 as an antinociceptive in the PPQ assay. Neither compound suppresses withdrawal in the SDS assay.

The reported data on NIH 10443 (table 1) supplement the data received on that compound last year. This is one of a series of such structural types which have been examined during the past few years. In the mouse vas deferens assay, NIH 10443 was found to be an extremely potent, albeit promiscuous, irreversible antagonist of mu, delta and kappa opioid receptors. Some of the compounds in this series have interesting research potential.

NIH 10535 (table 1), which we call cyclobroxy, was found to be a "pure" narcotic antagonist. It was equipotent with naltrexone as. a mu antagonist, and ten times more potent than naltrexone as a delta antagonist. In monkeys, it precipitated withdrawal with a potency equivalent to naloxone.

A full battery of tests were run on NIH 10474 (table 8), a compound with a structure which cannot be classified as a normal opioid-like structure. It displayed antinociceptive activity only in the PPQ assay, where it was found to be weakly potent (ca. one-twentieth as potent as morphine). It gave only slight effects in self-injection and drug discrimination assays and appeared to be a non-opioid agonist in the mouse vas deferens preparation. An SDS assay and a primary physical dependence study in monkeys showed it to be without morphine-like effects.

A number of 6,7-benzomorphans were examined (tables 6 and 7), one of which had surprising properties. In the mouse vas deferens assay, NIH 10566 (table 6) was found to be a partial opiate agonist with affinity for delta opioid receptors. In vivo, it displayed unusual side-effects in an SDS study and was inactive in all of the antinociceptive assays. It had narcotic antagonist activity in the mouse TFA assay (twice as potent as nalorphine). Some of the drug's effects were displayed in a normal, non-dependent monkey, and these effects were not reversed by naloxone.

Lastly, the adrenal cortex extract (NIH 10431, table 8) was examined because it was purported to ameliorate the opioid withdrawal syndrome. The extract was used in a pretreatment paradigm. It was given to the monkeys six times a day for 2 days. The extract was given before and during the first 12 hours of abrupt withdrawal of morphine. It did not lessen or attenuate the abrupt withdrawal syndrome in morphine-dependent monkeys. However, when the extract was concentrated, and five times the initial concentration of the material was given, a few lessened signs of withdrawal were noted. There have now been a few compounds submitted to our program which are meant to be used for amelioration or treatment of opioid physical dependence.

Statistical survey

A) Who Sends Us Drugs, And Why Do They Send Them?

Drugs have been received from three of our constituencies, industrial groups (domestic and foreign), universities (domestic and foreign), and governmental organizations. Their reasons for submitting compounds for testing under the auspices of the CPDD are as diverse as the organizations themselves.

This year, 26% of our samples came from industrial groups, 56% originated from universities, and 15% from various governmental groups (8% from NIH and 7% from NIDA). The industrial groups submit their compounds during different phases of their drug-testing procedures. Several were sent in response to the FDA's

request for further information about the physical dependence potential and abuse liability of their compound, or as part of their initial package to FDA. Others, which are not at all meant to be marketed as analgesics, as mentioned previously, wish to see the effects of their drug in our testing procedures in order to relate them to, extend, or confirm their own work.

Investigators based at universities generally do not have the facilities to do the testing which we so ably supply. Many of them (but not all) have NIDA grants for the synthetic work, and state in their grant proposal that the CPDD will provide the biological testing. I have sent several letters confirming the fact that we will test their compounds, in support of various grant applications. These scientists use the data which we provide, it is hoped with acknowledgement, in their publications in various journals. The knowledge gained from our data is used to determine structure-activity relationships for their compounds, and to generate further grant proposals.

The National Institute on Drug Abuse continues not only to support the work of the groups on whose reports we rely, but sends us compounds which they have had synthesized for their own reasons or by request of the DEA. The DEA needs our data on the physical dependence potential of drugs for scheduling purposes. Compounds which we receive from NIH are sent from the intramural research groups for purposes similar to those sent from universities.

B) Is The Supply Of Drugs Vanishing?

A comparison of the number of drugs (total of 69) sent to MCV and UM this year (5/1/87 to 4/30/88) to the mean of the number (94) sent over the past eight years (5/1/79 through 4/30/87) supplies a quick, if not quite decisive, answer to the posed question. Although we cannot, due to the standard deviation of the mean (± 26), say that the supply this year is radically different than the mean of the number sent over the past eight years, we can certainly see that we are on the low side of the standard deviation of the mean. This will, perhaps, be reflected in a lower number of reports from MCV and UM in the future. For this year, however, the number of reports by Aceto et al. (1989) and Woods et al. (1989), is about average (total of 89 vs. a mean of 94 ± 18).

The percentage of drugs received from two of our main sources, industrial and university, has remained reasonably constant. In fact we have, this year, received more drugs from domestic industry than during the previous six years. We received somewhat fewer drugs this year from foreign industrial sources (7% this year compared with a mean, from the previous eight years, of 11%), and the NIH (8% this year vs. 16% for the mean of the previous years).

C) Is There A Future For The Opioid Program?

There is, annually, a considerable fluctuation in the number of

drugs which we receive from various sources (a remarkably large standard deviation of the mean number from each source and in the total). It is difficult, then, to reach valid conclusions by comparison of a mean number over time, or to find any one time period which could be denoted as a representative year. The numbers which have been presented are the mean of data obtained over eight years. With that caveat, one possible conclusion might be that the general trend, overall, is towards diminution. The pertinent question as to whether we have, finally, reached a steady state or whether the opioid program will, some day, vanish for lack of supplied drugs can only be answered in retrospect, perhaps from the vantage point of the 21st. century.

Stimulant/Depressant Testing

The stimulant/depressant testing facilities and the assays which are used have evolved since the program was instituted (or re-instituted) in 1982. During the current year, unlike previous years (when drugs were tested mainly for evaluation of the procedures or for purposes of standardization and, during the past few years, those drugs tested at the request of the World Health Organization), we have opened our facility for admittance of samples from industrial groups. Lately, we have received a number of letters inquiring about our testing procedures, and we have received our first compound from the pharmaceutical industry.

We hope to have a 6 month turn-around time for the testing at the Medical College of Virginia with Drs. Patrick and Harris (assessment of activity in an inverted screen test and spontaneous locomotor activity in mice, assessment of physical dependence liability by substitution in pentobarbital-dependent rats using continuous intraperitoneal infusion, primary physical dependence determination in rats, by infusion), the University of Michigan with Drs. Woods and Winger (self-administration - the reinforcing properties of a drug determined by self-injection in rhesus monkeys), and at the University of Chicago with Dr. Woolverton (drug discrimination - the discriminative stimulus properties of drugs determined in rhesus monkeys trained to discriminate pentobarbital or d-amphetamine from saline).

Report to the World Health Organization on Compounds Evaluated as Opioids and as Stimulant/Depressants

The groups concerned with both the stimulant/depressant and the opioid testing facilities were involved in a report to the World Health Organization this year. The CPDD was formally asked in July, 1987, to evaluate a number of compounds, and the report which follows constitutes the work of the CPDD in response to that request.

General Information -
Pentazocine, nalbuphine, meptazinol, butorphanol, dezocine, clonidine, and buprenorphine have been examined for their

dependence potential and abuse liability in laboratories at the Medical. College of Virginia, Virginia Commonwealth University, and the University of Michigan Medical School, under the auspices of the Committee on Problems of Drug Dependence, and the data which have been obtained are summarized in table 10. The molecular structures of the compounds are shown in figure 1. The original data from which table 10 was prepared were included for the WHO as appendices to the report and are available upon request.

The test procedures which were utilized were opioid receptor binding (displacement of specific [^{3}H]-etorphine binding from membrane preparations of rat cerebrum), inhibition of twitch in electrically-driven mouse vas deferens or guinea pig ileum preparations, antinociceptive tests in mice (tail flick, PPQ, hot plate), test for narcotic antagonism in mice (tail flick vs. morphine [TFA]), single-dose suppression in morphine-dependent rhesus monkeys (after a 15 hour withdrawal period), precipitated withdrawal (test drug injected 2 1/2 hours after morphine injection), substitution for morphine in morphine-dependent rats, primary physical dependence in naive rats (test drug administered by infusion for 6 days and the animal placed in abrupt withdrawal), primary physical dependence in naive rhesus monkeys, self-administration of test drug in rhesus monkeys trained to self-inject codeine (saline used as negative control), and drug-discrimination assays in rhesus monkeys (trained to discriminate etorphine or ethylketazocine from saline).

All of the compounds examined were tested in some, or many, of these assays. All compounds were initially tested under NIH code number and the laboratories were not aware of the name and structure of the substance until their reports were completed. The studies noted in the papers by Young et al. (1984), and Woods and Gmerek (1985), which have been included in the appendix, were done on a non-blind basis.

Summary of Data on Specific Compounds Tested as Opioids -

Pentazocine (NIH 7958)

Pentazocine (see table 10 and figure 1) showed weak antinociceptive and narcotic antagonist activity. It was 1/8 to 1/30 as potent as morphine as an antinociceptive in the PPQ and hot plate assays, respectively, and it was inactive in the tail flick assay. Pentazocine was 1/3 as potent as nalorphine as an antagonist in the TFA assay. Partial substitution was observed in single dose substitution, and atypical withdrawal signs were noted in precipitated withdrawal. Pentazocine was noted to be an atypical or partial narcotic antagonist. It did not produce drug-appropriate responding in monkeys trained on etorphine or ethylketazocine. The self-administration of pentazocine is somewhat variable; it maintained rates that were either 34 or 86% of rates maintained by codeine in different studies (Young et al. (1984), and Woods and Gmerek (1985)). Pentazocine, thus, is considerably different from morphine. It cannot be considered as a typical morphine-like agonist.

TABLE 10. SUMMARY OF RESULTS FROM THE REPORTS OF THE OPIOID TESTING GROUP OF THE COMMITTEE ON PROBLEMS OF DRUG DEPENDENCE

TESTS	PENTAZOCINE (NIH 7958)	NALBUPHINE (NIH 8359, 10274)	MEPTAZINOL (NIH 8683)	BUTORPHANOL (NIH 8791, 10275)	DEZOCINE (NIH 8834)	CLONIDINE (NIH 9549, 9571)	BUPRENORPHINE (NIH 8805, 10276)	MORPHINE (NIH 0001)
RECEPTOR BINDING[1]	-	-	1600 nM	-	54 nM	>2 uM	-	28 nM
MOUSE VAS DEF.[2]	-	-	NO EFFECT	-	-	NOTE[3]	-	4×10^{-7}
GUINEA PIG ILEUM[4]	-	-	-	-	-	NOTE[5]	-	4×10^{-7}
TAIL FLICK[6]	INACTIVE	NOTE[7]	12.8	INACTIVE	2.6	1.2	0.14	5.8
TAIL FLICK vs M[8]	8.5	NOTE[9]	INACTIVE	INACTIVE	NOTE[10]	INACTIVE	1.0	INACTIVE
PPQ[11]	7.6	0.26	1.6	15.0	0.3	0.005	0.016	0.23
HOT PLATE[12]	9.3[13]	13.	5.3	0.8[14]	0.7[15]	1.0	0.035	1.2
SDS (MONKEY)[16]	PARTIAL	PARTIAL	NO	NO	NO[17]	PARTIAL	PARTIAL[18]	YES
NW (MONKEY)[19]	PARTIAL[20]	YES[21]	YES[21]	PARTIAL[20]	-	NO	YES[22]	NO
SUBST. (RAT)[23]	-	-	NO	-	-	-	-	YES
PPD (RAT)[24]	-	-	YES	-	YES[25]	-	SLIGHT[26]	YES
PPD (MONKEY)[27]	-	-	MILD[28]	YES[29]	-	NOTE[30]	-	YES
S.I. (MONKEY)[31]	SLIGHT	INTERMEDIATE	SLIGHT[32]	INTERMEDIATE	SLIGHT[33]	SLIGHT	INTERMEDIATE	POSITIVE
DD (ETORPHINE)[34]	NEGATIVE	POSITIVE	POSITIVE[35]	POSITIVE	POSITIVE	NEGATIVE	POSITIVE	POSITIVE
DD (EKC)[36]	NEGATIVE	NEGATIVE	POSITIVE[37]	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	-

FOOTNOTES TO TABLE 10:

(1) Displacement of specific [^{3}H]-etorphine binding from membrane preparation of rat cerebrum.

(2) Inhibition of twitch in electrically-driven mouse vas deferens preparations.

(3) Inhibited twitch; inhibition blocked by naltrexone. A small degree of cross tolerance to morphine.

(4) Inhibition of twitch in electrically-driven guinea pig ileum preparations.

(5) Potent agonist; not blocked by naltrexone or UM 979.

(6) Tail-flick agonist test, in mice. The ED50, in mg/kg, is listed; see enclosed data for 95% confidence limits (morphine = 5.8 (5.7-5.9).

(7) Insufficient activity for statistical analysis (22% of the mice were effected at 10 mg/kg).

(8) Morphine antagonist test, in mice. The AD50, in mg/kg, is listed; see enclosed data for 95% confidence limits (naloxone = 0.04 (0.01-0.09).

(9) Insufficient activity for statistical analysis (69% of the mice were effected at 3 mg/kg).

(10) Insufficient activity for statistical analysis (30% of the mice were effected at 30 mg/kg).

(11) Phenylquinone test for agonist activity (mouse). The ED50, in mg/kg, is listed; see enclosed data for 95% confidence limits (morphine = 0.23 (0.20-0.25).

(12) Hot plate assay for agonist activity. The ED50, in mg/kg, is listed; see enclosed data for 95% confidence limits (morphine = 1.0 (0.83 - 1.1).

(13) In Nilsen assay for agonist activity: ED50 = 6.5 (morphine = 1.3 (1.0-1.7).

(14) In Nilsen assay for agonist activity: ED50 = 0.09 (morphine = 1.3 (1.0-1.7).

(15) In Nilsen assay for agonist activity: ED50 = 0.9 (morphine = 1.3 (1.0-1.7).

(16) Single dose suppression test, in morphine-dependent. monkeys after a 15 hour withdrawal period (“Partial” = suppression of some, but not all, of the withdrawal signs).

(17) Exacerbates withdrawal syndrome.

Footnotes to Table 10 - continued -

(18) No suppression observed in special, 9 hour, withdrawal; partial in special, 19 hour, withdrawal.

(19) Precipitated withdrawal test initiated by test drug injection 2 1/2 hr after an injection of morphine.

(20) Atypical withdrawal observed.

(21) Withdrawal symptoms precipitated by test drug.

(22) Very long duration of action.

(23) Substitution of test drug for morphine in morphine-dependent rats.

(24) Primary physical dependence study in naive rats. Test compound administered by infusion for 6 days and then placed in abrupt withdrawal.

(25) Withdrawal symptoms less intense than those obtained with morphine.

(26) Slight withdrawal symptoms observed at low dose (0.025 mg/kg); none observed at high dose (4.0 mg/kg). In combination with morphine, observed increased physical dependence at low dose and antagonism at high dose (at very low dose, 0.006 mg/kg, no attenuation of morphine was observed).

(27) Primary physical dependence study in naive rhesus monkeys.

(28) Only mild physiological dependence obtained with test drug.

(29) Both tolerance and physical dependence with test drug.

(30) High tolerance obtained, but no significant physical dependence with test drug.

(31) Self-administration of test drug in rhesus monkeys trained to self-inject codeine. Saline is used as negative control.

(32) Self-administration rate of administration only slightly above saline, at 0.03-1.0 mg/kg.

(33) Self-administration rate of administration higher than saline, but less than with codeine.

(34) Drug discrimination assay in rhesus monkeys trained to discriminate the administration of etorphine, a mu receptor opioid agonist, from saline.

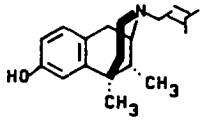
(35) Cumulative dose regimen at 0.1-5.6 mg/kg. Responses blocked by prior administration of quadazocine, a mu and kappa receptor opioid antagonist.

Footnotes to Table 10 - continued -

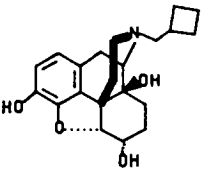
(36) Drug discrimination assay in rhesus monkeys trained to discriminate the administration of ethylketocyclazocine, the prototypic kappa receptor opioid compound, from saline.

(37) Experiment, at 0.1-10 mg/kg, replicated in two of four animals.

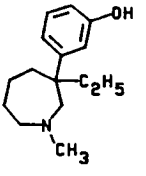
FIGURE 1. COMPOUNDS EVALUATED AS OPIOIDS UNDER CPDD AUSPICES



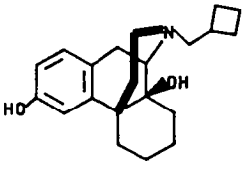
PENTAZOCINE
(NIH 7958)



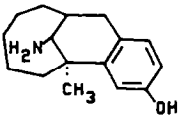
NALBUPHINE
(NIH 8359, 10274)



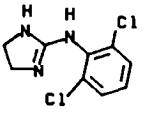
MEPTAZINOL
(NIH 8683)



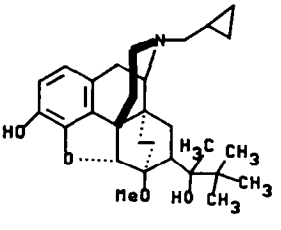
BUTORPHANOL
(NIH 8791, 10275)



DEZOCINE
(NIH 8834)



CLONIDINE
(NIH 9549, 9571)



BUPRENORPHINE (NIH 8805, 10276)

Nalbuphine (NIH 8359. 10274)

Nalbuphine displayed morphine-like antinociceptive action in the PPQ assay, and was 1/10 as potent as morphine in the hot plate. It was inactive in the tail flick assay, and inactive as a narcotic antagonist in the TFA assay in mice. In single dose suppression studies it partially suppressed withdrawal at 10 and 20 mg/kg. The studies indicated that the drug was weaker than morphine as an opioid agonist. It does, however, precipitate abstinence in the non-withdrawn dependent monkey (1/80 the potency of nalorphine). Nalbuphine, thus, is distinctly different from morphine. However, nalbuphine produced drug-appropriate responding in monkeys trained to discriminate etorphine but not in monkeys trained to discriminate ethylketazocine. It maintained self-injection rates intermediate between those maintained by codeine and by saline. Thus, nalbuphine might be considered as having somewhat less abuse liability than morphine.

Meptazinol (NIH 8683)

Meptazinol displaced etorphine from opioid receptors only at very high concentrations. It was neither an opioid agonist or antagonist in the mouse vas deferens assay. It was weaker than morphine as an antinociceptive in mice. In the morphine-dependent monkey it was a weak morphine antagonist. It did not substitute for morphine in the rat, but produced physical dependence indistinguishable from morphine in a primary physical dependence study in rats. It produced generalization to etorphine in monkeys, a mild degree of opioid-like physiological dependence, and had a weak reinforcing effect. It also generalized to ethylketo-cyclazocine in monkeys *on* initial evaluation, but not on repeat evaluation on two of the four animals. Meptazinol clearly acted as an opioid antagonist in monkeys physiologically dependent on morphine, with about 1/40 the potency of nalorphine. Thus, it showed an unusual profile of activity. From the rat primary physical dependence study, its dependence potential is comparable to morphine, but it showed a lesser effect than morphine in that assay in the monkey. Meptazinol's dependence potential may be comparable to morphine in naive animals but, because of its weak reinforcing effect, its abuse liability would probably be somewhat less than that observed with morphine.

Butorphanol (NIH 8791, 10775)

Butorphanol was inactive, or much less potent than morphine as an antinociceptive in some assays (tail flick, PPQ), and was as potent as morphine in other assays (hot plate, Nilsen). It was not active as a narcotic antagonist in mice. It did not substitute for morphine in single dose substitution, and acted as an atypical antagonist in precipitated withdrawal. Butorphanol behaves like an effective narcotic agonist, a weak narcotic antagonist or a convulsant, depending upon the circumstances of its administration. In the normal, non-dependent monkey, butorphanol produces narcotic-like CNS depression, and tolerance and physical dependence develop upon repeated administration. Butorphanol produced drug-appropriate responding in monkeys trained to discriminate etorphine but not in monkeys trained to discriminate ethylketazocine. It maintained rates of responding intermediate between those of

codeine and those of saline. Butorphanol, to some extent, has dependence potential and abuse liability of the morphine-type.

Dezocine (NIH 8814)

Dezocine was morphine-like in its ability to displace etorphine from opioid receptors. It was morphine-like in potency as an antinociceptive and did not have narcotic antagonist activity in mice. Dezocine does not substitute for morphine in single dose substitution. It exacerbated withdrawal in that assay. It produced physical dependence in rats, although the degree of dependence was not as intense as that for morphine. Dezocine produced drug-appropriate responding in monkeys trained to discriminate etorphine but not in monkeys trained to discriminate ethylketazocine. It also maintained responding, in self-injection in monkeys, at rates intermediate to those of codeine and saline. Dezocine is likely to have dependence potential and abuse liability of the opioid type, but would probably be less effective than morphine in those actions.

Clonidine (NIH 9549, 9571)

Displacement studies indicated that clonidine did not interact at opioid receptors. On the guinea pig ileum clonidine did not exert effects through a morphine-like mechanism. In the mouse vas deferens, however, there was indication of interaction between clonidine sites and opioid receptors. It substituted only partially for morphine in monkeys in single dose substitution, and did not produce a significant degree of morphine-like physical dependence in a primary physical dependence study in monkeys. The actions of clonidine in the behavioral preparations point to non-morphine-like sedative properties which are not reversed by naloxone. However, the drug was very potent and had strong behavioral effects. The data indicate that clonidine would be unlikely to have dependence potential or abuse liability comparable to morphine. Its actions clearly cannot be classified as morphine-like.

Buprenorphine (NIH 8805, 10276)

Buprenorphine was a potent antinociceptive (14 to 40 times as potent as morphine in the mouse), and was two to three times as potent as nalorphine as a narcotic antagonist. It significantly alleviated many withdrawal signs in single dose substitution, but did not completely substitute for morphine. Buprenorphine precipitated withdrawal in non-withdrawn animals. It had a very long duration of action as an antagonist. Indeed, it appeared to be an irreversible antagonist in monkeys. In a primary physical dependence study in rats, buprenorphine produced physical dependence at a low, but not at a higher, dose level. In that assay, buprenorphine when introduced in combination with morphine appeared to act as an opioid antagonist, interfering with the development of morphine physical dependence in the rat. Thus, buprenorphine might have some degree of dependence potential at some dose level in naive animals, but clearly does not have the dependence potential of morphine. Buprenorphine precipitates withdrawal in opioid-dependent animals, whether administered after or in combination with an opioid. Buprenorphine produced drug-

appropriate responding in monkeys trained to discriminate etorphine but not in monkeys trained to discriminate ethylketazocine. It maintained rates of responding intermediate between those of codeine and those of saline. Buprenorphine might be considered, thus, as having lesser potential for abuse liability than morphine.

Compounds Evaluated as Stimulants or Depressants

General Information -

Propylhexedrine, methaqualone, and carbromal were assigned for evaluation to the CPDD Stimulant/Depressant Testing Group. Dr. A. E. Jacobson (Chairman, Drug Testing Committee, CPDD) served as the coordinator at NIH, NIDDK, and the laboratories at the University of Chicago, the University of Michigan, Johns Hopkins University and Virginia Commonwealth University served as the biological testing sites. All compounds were tested by code number and the laboratories were blind as to the structures until the report was prepared. The data on carbromal have been added to this report because of its structural similarity to two compounds (bromisoval and carbromide) which are to be considered for scheduling by the World Health Organization. However, neither bromisoval nor carbromide have been evaluated under the auspices of the Committee on Problems of Drug Dependence. The data on methaqualone have not been included with this report; these data were submitted as part of the report to the World Health Organization in 1987 by the Committee on Problems of Drug Dependence. However, a summary of those data has been included in this report. Summarization of the data obtained for propylhexedrine, methaqualone, and carbromal can be found in table 11. The molecular structures of propylhexedrine and methaqualone are shown in figure 2.

The test procedures were designed to give some information concerning the pharmacological resemblance to known drugs, their physical dependence potential and the reinforcing effects of the compounds. The results will be presented in this order for all of the drugs tested.

Summary of Data on Specific Compound Tested as Stimulants/ Depressants - (The origina1 data have been included in an Appendix, available on request).

N,α–Dimethylcyclohexaneethanamine (Propylhexedrine,CPDD 0019)
Propylhexedrine was inactive in the inverted screen test at doses less than near-lethal. It produced stimulant-like, dose-related increases in spontaneous locomotor activity, with maximal excitation occurring as lethality began to occur. Physical-dependence studies to determine dependence liability of the depressant-drug type were assessed by substitution studies in pentobarbital-dependent rats. The data indicated that propylhexedrine did not substitute for pentobarbital in preventing signs of withdrawal, but rather worsens those signs. A primary physical dependence study in rats infused with propylhexedrine gave data which were consistent with its stimulant nature. The pattern observed with initial loss of body weight, followed by lesser weight gain than vehicle-treated rats,

was typical of stimulant drugs. The effects,however, were not as great as those previously observed with amphetamine or MDMA. Drug discrimination studies were conducted in the rhesus monkey (i.g. administration) trained to discriminate d-amphetamine (0.56 or 1.0 mg/kg) or pentobarbital from saline. The discriminative stimulus effects of propylhexedrine were similar (at 3.0 - 10.0 mg/kg i.g.) to those of d-amphetamine, but not similar to pentobarbital. Thus, primary physical dependence studies indicate that propylhexedrine acts as a stimulant, and drug discrimination data demonstrate that propylhexedrine has amphetamine-like subjective effects. It is, however, considerably less potent (ca. 1/10th.) than d-amphetamine in drug discrimination. Propylhexedrine was self-injected by rhesus monkeys trained to self-inject cocaine. It was somewhat less efficacious but active over the same dose range as cocaine. Thus, propylhexedrine would appear to have stimulant-type abuse liability.

Methaqualone (CPDD 0007)

The data for methaqualone were included in the 1987 report from the Committee on Problems of Drug Dependence to the World Health Organization. These data have not been added to the appendices. However, the summary of that work is noted herein.

Methaqualone is active in the inverted screen test and in depression of locomotor activity. Drug discrimination studies reveal that it substitutes for pentobarbital in the rhesus monkey and diazepam in the rat, but not for pentobarbital in the pigeon or baboon nor for lorazepam in the baboon. In physical dependence studies, it substituted for pentobarbital in dependent rats, and signs of abstinence occurred upon withdrawal. Methaqualone was self-administered by rhesus monkeys. The potency of methaqualone was similar to that of pentobarbital in most studies. Methaqualone, thus, appears to have abuse liability.

N-(Aminocarbonyl)-2-ethylbutanamide (Carbromal, CPDD 0013)

The carbromal data in this report were obtained last year for the World Health Organization and are included because carbromal is structurally similar to bromisoval and to carbromide (see figure 3). The structural similarity of the three compounds may reflect, or have bearing upon, their pharmacological actions. We did not evaluate either bromisoval or carbromide for this report.

Carbromal may be metabolized to a barbiturate-like compound or to a bromide carrier. It is active in the inverted screen test, and produced mild stimulant effects on spontaneous activity in the same dose range. In drug discrimination studies it was identified as pentobarbital-like by the pigeon, and there was partial generalization in the pentobarbital-trained rhesus monkey. It has not been tested in diazepam-trained rats or baboons. Carbromal showed little evidence of barbiturate-like dependence properties in the barbiturate dependent rat. However, the doses employed were quite low relative to the dose of pentobarbital that the rats had been receiving, due to difficulty in solubilizing the compound for intraperitoneal infusion. The compound has not been tested in drug self-administration. The potency of carbromal appears to be in the range of 1/3 to 1/5 that of pentobarbital.

TABLE 11. SUMMARY OF RESULTS FROM THE REPORTS OF THE STIMULANT/ DEPRESSANT TESTING GROUP OF THE COMMITTEE ON PROBLEMS OF DRUG DEPENDENCE

TESTS	PROPYLHEXEDRINE (CPDD 0019)	METHAQUALONE (CPDD 0007)	CARBROMAL (CPDD 0013)
INVERTED SCREEN (IP)	NO EFFECT	POSITIVE	POSITIVE
ACTIVITY CAGE (IP)	NEGATIVE[1]	POSITIVE	POSITIVE[2]
PHYSICAL DEPENDENCE			
SUBSTITUTION (IP)	NO EFFECT[3]	POSITIVE	NO EFFECT
PRIMARY (IP)	POSITIVE[4]	POSITIVE	NOT TESTED
DRUG DISCRIMINATION			
PB PIGEON (IM)	NOT TESTED	NEGATIVE[5]	POSITIVE
PB RHESUS (PO)	NEGATIVE[6]	POSITIVE	PARTIAL
DA RHESUS (IG)[7]	POSITIVE		
DZ RAT (IP)	NOT TESTED	POSITIVE	NOT TESTED
SELF-ADMINISTRATION			
BABOON (PO)	NOT TESTED	NEGATIVE-LZ NEGATIVE-PB	NOT TESTED
RHESUS (IV)	POSITIVE	POSITIVE	

FOOTNOTES TO TABLE 11:

(1) Increased spontaneous motor activity.

(2) Effect noted at 125 mg/kg, at 5-15 min time interval.

(3) No dependence of barbiturate type.

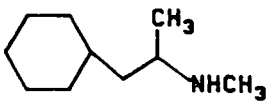
(4) Dependence of stimulant type.

(5) Only tested in midazolam trained pigeons.

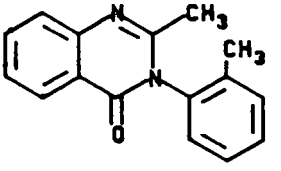
(6) Intragastric (ig) route of administration.

(7) D-Amphetamine trained animals.

FIGURE 2. STRUCTURE OF PROPYLHEXEDRINE AND METHAQUALONE

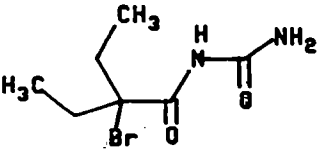


PROPYLHEXEDRINE
(CPDD 0019)

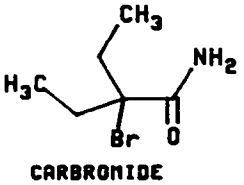


METHAQUALONE
(CPDD 0007)

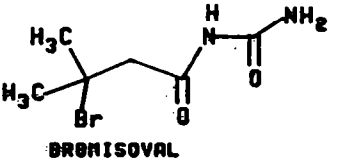
FIGURE 3. STRUCTURAL RESEMBLANCE OF CARBROMAL TO CARBROMIDE AND BROMISOVAL



CARBROMAL (CPDD 0013)



CARBROMIDE



BROMISOVAL

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AUTHOR

A. E. Jacobson, Ph.D., Drug Design and Synthesis Section, Laboratory of Neuroscience, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892.

Evaluation of New Compounds for Opioid Activity

J. Woods, F. Medzihradsky, C. Smith, G. Winger and C. France

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, Drug Design and Synthesis, NIDDK, National Institutes of Health, Bethesda, MD. The drugs, which come originally from pharmaceutical companies, universites, government laboratories, and international organizations 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 is trained to discriminate the administration of the kappa agonist ethylketazocine (EKC). The other group is trained to discriminate the mu agonist, codeine.

The procedures used with the EKC-trained monkeys have been described by Bertalmio et al. (1982). The monkeys are removed from their home cages each day and seated in primate restraining chairs. These chairs are placed in isolation chambers 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 monkeys is similar, but not identical. 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 required to make 20 rather than 100 responses, 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 is illuminated, the 30th response produce a five-sec intravenous drug injection accompanied by another light that is illuminated during drug delivery. After each injection, a ten-min timeout condition is in effect, during which responses have no scheduled consequence and neither light is illuminated. Each of the two daily sessions consist of 13 injections or 130 min. whichever occurs first. Other details of the procedure and' initial findings with a variety of narcotics are given in previous reports (Woods, 1977; 1980).

Doses of the drugs are typically described in terms of mg/kg/injection (inj). Duplicate observations of codeine (0.32 mg/kg/inj) and of saline are obtained for each monkey. A saline substitution is conducted before and after the series of observations on a test drug; the control rates of codeine-reinforced responding are obtained by a random sampling of two sessions interpolated between the drug-substitution sessions. These data are represented in the following graphs with individual symbols for each of the monkeys; each symbol is the mean of duplicate observations for a given dose in each monkey. The closed circles indicate the averaged data for observations on the subset of monkeys used to study each drug under each of the

experimental conditions. In all cases, the rates of responding given are those calculated during only the fixed-ratio portion of each session.

DISPLACEMENT OF RADIOLABELED LIGAND BINDING

Details of the binding assay have been described previously (Medzihradsky et al., 1984: Clark et al., 1988). Briefly, aliquots of a membrane preparation from rat cerebrum are incubated with ^{3}H-etorphine in the presence of 150 mM NaCl, and in the presence of different concentrations of the drug under investigation. Specific, i.e., opioid-receptor-related interaction of ^{3}H-etorphine is determined as the difference in binding obtained in the absence and presence of an appropriate excess of unlabeled etorphine. The potency of the drugs in displacing the specific binding of ^{3}H-etorphine is determined from log-probit plots of the data. It should be noted that since April 1982 the concentration of 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. of a given drug. However, due to the different concentration 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 and 3.0 nM ^{3}H-etorphine.

As part of our goal to develop advanced procedures to assess the interaction of newly synthesized compounds with opioid receptors (Medzihradsky, 1987), this laboratory is now in the position to determine the selectivity of ligands in binding to the mu, delta, and kappa opioid receptors. Thus, we can now provide EC50 values of tested compounds in displacing the following radiolabeled opioid ligands in rat and monkey brain membranes:

etorphine (nonselective)
sufentanil (mu selective)
[D-Pen^{2}-D-Pen^{5}]enkephalin (delta selective)
U-69,593 (kappa selective)

Using these binding assays, we have described the selectivity of various established opiates in membranes from rat, guinea-pig, and monkey brain (Clark et al., 1988).

INHIBITION OF TWITCH IN ELECTRICALLY-DRIVEN MOUSE VAS DEFERENS PREPARATIONS.

The development of new, highly selective antagonists such as the irreversible mu receptor antagonist beta-funaltrexamine (beta-FNA) and the reversible delta receptor antagonist ICI-174864 have made possible the evaluation of selectivity of opioid agonists and antagonists by use of the mouse vas deferens preparation.

Male, albino ICR mice, weighing between 25 and 30 g, are used. The mice are decapitated, the vasa deferentia removed, and 1.5 cm segments are suspended in organ baths which contain 30 ml of a modified Kreb's physiological buffer. The buffer contains the following (mM): NaCl, 118; KCl, 4.75; $CaCl_2$, 2.54; $MgSO_4$, 1.19; KH_2PO_4, 1.19; glucose, 11; $NaHCO_3$, 25; pargyline HCl, 0.1; tyrosine, 0.2; ascorbic acid, 0.1 and disodium edetate, 0.03. HCl, 0.3, The buffer is saturated with 95% O_2 - 5% CO_2 and kept at 37° C. The segments are attached to strain gauge transducers and suspended between two platinum electrodes. After a 30-min equilibration period, the segments are stimulated once every 10 sec with pairs of pulses of 2 msec duration, 1 msec apart and at supramaximal voltage.

The following antagonists are studied: naltrexone HCl, ICI-174864 [N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH] and beta-FNA. Naltrexone and ICI-174864 are added to the organ baths 15 minutes before the determination of cumulative concentration-effect relationships for the various agonists. Beta-FNA is added to the organ baths after the initial equilibration period. Thirty min later, the beta-FNA is removed from the organ baths by repeated washings with fresh buffer. The tissues are washed three times every 5 min for 30 min. Cumulative concentration-effect relationships for the various agonists are then determined 10 min after the last wash (i.e., 30 min after the beta-FNA was removed from the organ baths). EC50's are calculated by probit analysis, and pA_2 values are determined to assess relative potencies of antagonists. All drugs which are submitted for evaluation are studied in the following manner: 1) the submitted drug is tested on the vas deferens preparation in the absence and in the presence of 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. For further details of the procedure see Smith (1986). Drugs studied in the preparation prior to 1987 were evaluated with the protocol reported in the 1985 Annual Report.

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[a-c] and Nilsen[d] 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	0.98 (0.83-1.1)	6.3 (4.7-8.3)	1.3 (1.0-1.7)	8.3 (6.0-11.4)
NIH 0001, 9929	2.9 (2.5-3.3)	18.9 (14.1-24.9)	3.9 (3.0-5.1)	24.9 (18.0-34.1)
Codeine phosphate	6.8 (4.5-10.2)	13.5 (9.7-18.7)	7.4 (4.9-11.0)	14.7 (9.2-23.3)
NIH 0002	17.1 (11.3-25.7)	34.0 (24.4-47.1)	18.6 (12.3-27.7)	37.0 (23.2-58.7)
Levorphanol tartrate	0.2 (0.1-0.3)	-	0.2 (0.16-0.3)	2.5 (1.7-3.7)
NIH 4590	0.5 (0.2-0.7)	-	0.5 (0.4-0.7)	6.2 (4.2-9.1)
Meperidine.HCl	5.3 (4.0-7.1)	-	-	-
NIH 5221	18.7 (14.1-25.0)	-	-	-
(-)-Metazocine.HBr	0.6 (0.5-0.9)	10.6 (8.0-14.1)	0.5 (0.3-0.7)	26.0 (21.0-33.0)
NIH 7569	1.9 (1.4-2.8)	34.1 (25.7-45.3)	1.6 (1.0-2.3)	83.6 (67.5-106.1)

TABLE 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 8503				
Naloxone.HCl			No dose response	
NIH 7890				

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's of representative opioids for displacement of ^{3}H-etorphine from rat brain membrane, and inhibition of the twitch of the mouse vas deferens preparation.

	BINDING (+ 150 mM NaCl) EC50		MVD
Compound	^{3}H-etorphine (0.5 nM)	^{3}H-etorphine (3.0 nM)	EC50 (nM)
DPDPE	---	---	5.52
U50,488	---	---	6.29
Fentanyl	---	---	37.1
DAGO	---	---	81.3
Etorphine	0.37	4.2	0.0068
(-)Cyclazocine	0.53	3.40	11.9
Naltrexone	0.63	2.34	---
Bremazocine	1.42	---	---
UM 1071R*	1.55	4.71	---
Sufentanil	1.60	---	4.43
(-)SKF 10047	3.93	20.5	---
Ethylketazocine	6.60	19.3	11.6
Ketazocine	14.1	63.1	1.18
Morphine	23.6	140	395
DSLET	43.0	---	1.71
Dextrorphan	9820	18000	1010

* 1R-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

NIH	CHEMICAL CLASS AND/OR GENERIC NAME	SA	MVD	BIND	DD	REPORT*
9971	phenylmorphan	-	+	+	-	04/04/88
9973	morphinan-6-one	-	+	+	+	02/12/88
9992	quaternary morphine	-	+	+	-	12/04/88
10250	flunixin meglumine	-	+	+	-	02/08/88
10407	pyrimidine	+	+	+	+	01/27/86
10435	morphine	-	+	+	-	11/19/85
10436	arylcyclohexylamine	-	+	+	-	11/22/85
10437	arylcyclohexylamine	-	+	+	-	11/22/85
10443	14-substituted morphine	-	+	+	+	01/13/88
10458	oxymorphone	-	+	+	-	05/02/86
10459	6-methylenenoroxymorphone	-	+	+	-	05/02/86
10474	morpholinyoethylindole	+	+	+	+	04/24/87
10475	ketobemidone	-	+	+	-	05/12/87
10488	phenylvaleramide	-	+	+	-	10/12/86
10494	butyrophenone	-	+	+	-	02/03/87
10495	butyrophenone	-	+	+	-	02/03/87
10502	benzomorphan	-	+	+	-	04/04/88
10503	benzomorphan	-	+	+	-	05/12/87
10504	morphinone	-	+	+	-	05/12/87
10522	meperidine	-	+	+	-	10/30/87
10531	phenylpiperidine	-	+	+	-	12/05/87
10532	benzacetamide	-	+	+	-	05/12/87
10533	benzacetamide	-	+	+	-	05/12/87
10535	morphinan	-	+	+	-	12/01/87
10538	phenylpropamide	-	+	+	-	05/12/87
10539	morphine	-	+	+	-	05/12/87
10546	phenylpropamide	-	+	+	-	10/30/87
10548	benzomorphan	-	+	+	-	10/30/07
10550	oxymorphone	-	+	+	-	12/17/87
10551	phenylpropanamide	-	+	+	-	10/30/87
10552	phenylanylalanine	-	+	+	-	12/17/87
10555	benzomorphan	-	+	+	-	10/30/87
10556	benzomorphan	-	+	+	-	12/02/87
10557	benzomorphan	-	+	+	-	10/30/87
10558	oxymorphone	-	+	+	-	12/23/87
10559	benzomorphan	-	+	+	-	10/30/87
10560	benzomorphan	-	+	+	-	12/02/87
10561	oxymorphone	-	+	+	-	12/17/87
10562	naloxone	-	+	+	-	12/02/87
10564	benzomorphan	-	+	+	-	10/30/87
10565	benzomorphan	-	+	+	-	11/21/87
10566	benzomorphan	-	+	+	-	12/02/87
10568	benzomorphan	-	+	+	-	01/05/88
10569	benzomorphan	-	+	+	-	01/13/88
10570	carfentanil	-	+	+	-	02/16/88

* Date report was submitted to CPDD Biological Coordinator.

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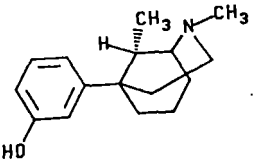
NIH 9971 (-)-2,9α-Dimethyl-5-(m-hydroxyphenyl)morphan hydrochloride



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 10.0 (7.2 - 13.9)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 780 nM in the presence of 150 mM NaCl.

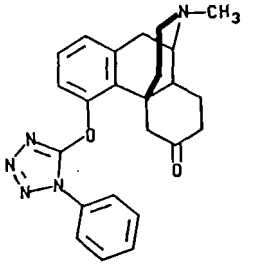
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.19×10^{-9}	33.6%
After naltrexone	6.22×10^{-8}	39.1%
With equimolar concentration of naltrexone	Slight reversal	
Equimolar concentration with morphine	Partial-reversal	

SUMMARY

NIH 9971 has agonist-antagonist properties in the vas deferens preparation; it also appears to have opioid activity in the binding assay.

NIH 9973 (-)-N-Methyl-4-(1-phenyl-1H-5-tetrazolyloxy)-morphinan-6-one



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 2.4 (1.9 - 2.9)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 19.2 nM in the presence of 150 mM NaCl.

NIH 9973 (-)-N-Methyl-4-(1-phenyl-1H-5-tetrazolyloxy)-morphinan-6-one

...(continued)

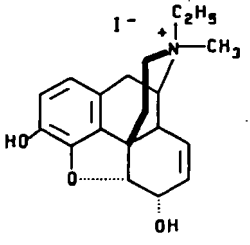
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	5.94×10^{-8}	95.9%
After naltrexone	2.65×10^{-6}	98.4%
After ICI-174864	1.08×10^{-7}	97.9%
After beta-funaltrexamine	1.28×10^{-7}	96.9%
With equimolar concentration of naltrexone	Marked reversal	

SUMMARY

NIH 9973 is a potent displacer of etorphine, and appears to be a kappa-receptor agonist in the mouse vas deferens preparation, although actions at other receptors cannot be excluded.

NIH 9992 N-ethylmorphinium iodide



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 50

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 510 nM in the presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	3.32×10^{-6}	35.1%
After UM 979	1.03×10^{-5}	29.9%
After naltrexone	4.83×10^{-6}	18.9%
With equimolar concentration of naltrexone	No reversal	
Equimolar concentration with UM 979	Reversal	

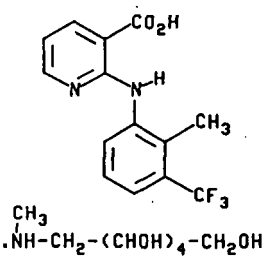
NIH 9992 N-ethylmorphinium iodide

...(continued)

SUMMARY

On both preparations NIH 9992 seems to have opiate activity. This drug is of low potency and efficacy. The observations upon the mouse vas deferens. suggest that this drug might be more selective for the kappa receptor, but it is difficult to draw conclusions from a drug of such limited efficacy.

NIH 10250, 9249 Flunixin meglumine



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 10% at 100

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (17.7% at 6000 nM) in the presence of 150 mM NaCl.

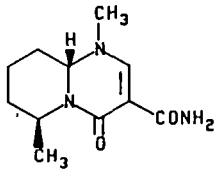
MOUSE VAS DEFERENS PREPARATION

Only a concentration of 3 x 10^{-5} M caused an inhibition of the twitch. This was a 17.8% inhibition that was not blocked by naltrexone. At a concentration of 10 M, NIH 10250 caused a 4.1-fold shift to the right of the sufentanil concentration-effect curve, Because of its lack of potency, pA_2 values could not be determined.

SUMMARY

NIH 10250 has opioid activity in only one of the two assay systems. Its opioid antagonistic activity in the vas deferens preparation was obtained only at very high concentrations.

NIH 10407 1,6β-Dimethyl-4-oxo-1,6,7,8,5,10 β-hexahydro-4H-pyrido[1,2a]pyrimidine-3-carboxamide



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6000 nM (12.5% at 6000 nM) in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

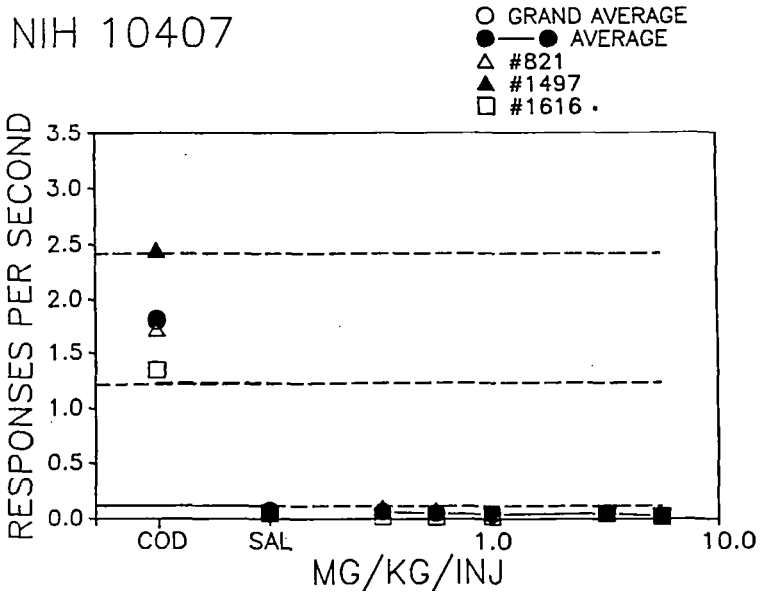
	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.37×10^{-5}	43.7%
After naltrexone	8.50×10^{-6}	38.3%
With equimolar concentration of naltrexone	No Reversal	
Equimolar concentration with morphine	Slight reversal	

SELF ADMINISTRATION IN RHESUS MONKEYS

The reinforcing effects of NIH 10407 were evaluated in three rhesus monkeys trained to respond for 0.32 mg/kg injection codeine (Woods, Drug and Alcohol Dependence, 1980, 8:223-230). Doses of NIH 10407 were substituted for codeine in single, 130 min test sessions. Each dose was tested twice in each of the three monkeys. Rates maintained by NIH 10407 were equal to or below those maintained by saline at all tested doses (0.32 to 5.6 mg/kg/injection). The attached figure gives details of the results of this study: 821, 1497 and 1616 are identification numbers of the three individual monkeys. Average refers to the mean of data from these animals, while Grand Average refers to-a historical control value for 20 monkeys under the codeine and saline conditions. The two topmost slashed horizontal lines indicate ± 3 SEM for the codeine grand average. The bottommost slashed horizontal line is + 3 SEM for the-saline grand average.

NIH 10407 1,6β-Dimethyl-4-oxo-1,6,7,8,9,10 β-hexahydro-4H-pyrido[1,2a]pyrimidine-3-carboxamide

...(continued)



DRUG DISCRIMINATION STUDIES IN RHESUS MONKEYS

Cumulative doses of from 3.2 to 32 mg/kg of NIH 10407 were evaluated in monkeys trained to discriminate 0.0032 or 0.0056 mg/kg ethylketocyclazocine (EKC) from sham injections in a drug discrimination paradigm (Bertalmio et al., J. Pharmacol. Methods, 1982, 7:289-299). Only one of the three monkeys responded on the EKC-appropriate lever following administration of 10 mg/kg NIH 10407. A second monkey showed complete suppression of responding at 32 mg/kg. Because of the variability among monkeys, this dose response curve was repeated. The effects on rate were identical upon replication; however, none of the monkeys responded on the EKC-appropriate lever.

SUMMARY

NIH 10407 did not maintain rates of responding above those maintained by saline at any tested dose, and in fact maintained rates lower than those maintained by saline at each tested dose. This pattern of self-administration is typical for kappa

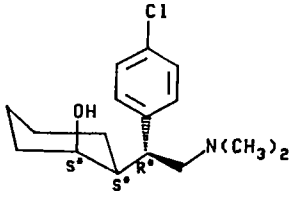
NIH 10407 1,6β-Dimethyl-4-oxo-1,.6,7,8,9,10β-hexahydro-4H-pyrido[1,2a]pyrimidine-3-carboxamide

...(continued)

agonists, but NIH 10407 produced EKC-appropriate responding in only one of three monkeys, and then on only one of the two occasions on which it was administered. NIH 10407 may have an unusual spectrum of action in that it may be aversive, but have no kappa-agonist effects.

None of the assays of NIH 10407 suggest that it has significant opioid agonist activity.

NIH 10435 (1S*)-cis-2-[(R*)-p-Chloro-α-[(dimethylamino)methyl] benzyl]cyclohexanohydrochloride



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20

DISPLACFMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (10.3% at 6.000 nM) in the presence of NaCl.

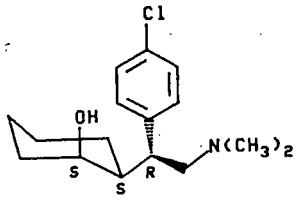
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	3.62×10^{-7}	26.3 ± 8.7%
After naltrexone	2.69×10^{-7}	17.9 ± 3.1%
With equimolar concentration of naltrexone	No Reversal	
Equimolar concentration with morphine	No reversal	

SUMMARY

NIH 10435 did not have opioid activity upon the mouse vas deferens. It failed to have significant affinity for the etorphine binding site, as well.

NIH 10436 (+)-(1S)-cis-2-[(R)-p-Chloro-α-[(dimethylamino)methyl]benzyl]cyclohexanol hydrochloride



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6.000 nM (14% inhibition at 6000 nM) in presence of 150 mM NaCl.

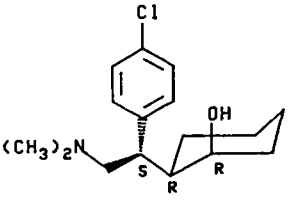
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	4.66×10^{-4}	32.4 ± 6.9%
After naltrexone	6.24×10^{-8}	23.7 ± 3.0%
With equimolar concentration of naltrexone	Slight reversal	
Equimolar concentration with morphine	Slight reversal	

SUMMARY

NIH 10436 has very slight mixed agonist-antagonist opioid activity on the mouse vas deferens preparation. It failed to have significant affinity for the etorphine binding site.

NIH 10437 (-)-(1R)-cis-2-[(S)-p-Chloro-α-[(dimethylamino)methyl] benzyl]cyclohexanol hydrochloride



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20

NIH 10437 (-)-(1R)-cis-2-[(S)-p-Chloro-α-[(dimethylamino)methyl] benzyl]cyclohexanol hydrochloride

... (continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (20% inhibition at 6000 nM) in presence of 150 mM NaCl.

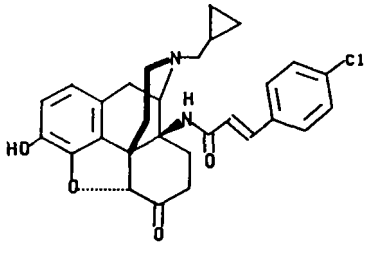
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	22.7×10^{-7}	24.5 ± 10.4%
After naltrexone	1.42×10^{-7}	14.3 ± 3.3%
With equimolar concentration of naltrexone	No reversal	
Equimolar concentration with morphine	Slight reversal	

SUMMARY

NIH 10437 appears to be a weak morphine antagonist upon the mouse vas deferens preparation. It has a very low affinity for the etorphine binding site.

NIH 10443 14β-(p-Chlorocinnamoylamino)7,8-dihydro-N-cyclopropylmethylnormorphine mesylate



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 0.66 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10443 was studied upon the isolated, electrically stimulated mouse vas deferent preparation in concentrations which ranged from 10^{-9} M to 10^{-4} M. At no concentration did this drug cause

...(continued)

an inhibition of the twitch. NIH 10443 was studied as an antagonist at concentrations of 10^{-8}, 10^{-7} and 10^{-6} M. All three concentrations completely abolished all responses to alfentanil and morphine. Its ability to block the actions of mu, kappa and delta receptor agonists were evaluated further. In these experiments, the tissues were exposed to NIH 10443 in a concentration of 10^{-8} M for 30 min and then washed with fresh buffer three times every five minutes for an additional 30 min period. At this time complete concentration-effect relationships were determined for sufentanil (mu), DSLET (delta) and U50,488 (kappa). Under these experimental conditions. NIH 10443 antagonized markedly the inhibitory actions of these opioid agonists upon the mouse vas deferens. The DSLET concentration-effect curve was shifted 100-fold to the right. The sufentanil curve was shifted to an equivalent degree to the right, and the maximum response was diminished. The U50,488 curve was shifted downward. and the maximum response was approximately 25% of that produced in the absence of the antagonist.

DRUG DISCRIMINATION IN RHESUS MONKEYS

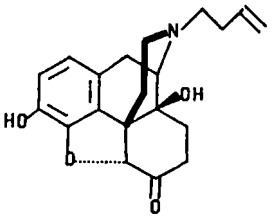
The discriminative effects of NIH 10443 were evaluated in rhesus monkeys trained to discriminate the stimulus effects of 1.0 or 1.8 mg/kg codeine. In doses as high as 10 mg/kg, NIH 10443 did not produce codeine-appropriate responding by these monkeys. On the following day, the effects of codeine, in doses as high as 18 mg/kg, were antagonized.

Due to the opioid antagonist properties of NIH 10443, it was not evaluated in self-administration experiments.

S U M M A R Y

NIH 10443 has a sub nanomolar EC50 in the binding assay, and is an extremely potent, irreversible opioid antagonist on the mouse vas deferens preparation. In addition, the compound is potent and long-lasting when administered systemically. This drug might be a valuable substitute for beta-chlornaltrexamine in the study of opioid receptors.

NIH 10458 N-3-Butenyl-3,14-dihydroxy-4,5-α-epoxy-6-methylene-morphinan hydrochloride



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 7.8 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

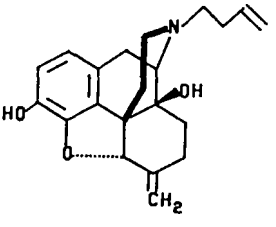
	Inhibitory EC50 (M)	Maximum Response
Drug alone	6.28×10^{-8}	48.1 ± 8.3%
After naltrexone	2.84×10^{-7}	36.6 ± 6.2%
With equimolar concentration of naltrexone	Slight reversal	
With equimolar concentration of sufentanil	Complete reversal	

NIH 10458, 10^{-7} M, shifted the sufentanil concentration-effect curve to the right (a 2.7-fold shift).

SUMMARY

NIH 10458 has opioid activity in both preparations. It was slightly more potent in the binding assay and had agonist-antagonist activity in the vas deferens.

NIH 10459 N-3-Butenyl-3.14-dihydroxy-4,5-α-6-oxomorphinan hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 8.1 nM in presence of 150 mM NaCl.

NIH 10459 N-3-Butenyl-3,14-dihydroxy-4,5-α-6-oxomorphinan hydrochloride

. . . (continued)

MOUSE VAS DEFERENS PREPARATION

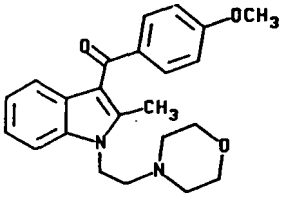
	Inhibitory EC50 (M)	Maximum Response
Drug alone	8.01×10^{-8}	75.7 ± 4.1%
After naltrexone	7.88×10^{-7}	63.1 ± 8.1%
With equimolar concentration of naltrexone	Slight reversal	

At a concentration of 10^{-7} M, NIH 10459 caused a 7.1-fold shift to the right in the sufentanil concentration-effect curve.

SUMMARY

NIH 10459 is a potent opioid. In the mouse vas deferens it has an agonist-antagonist profile of activity.

NIH 10474 (4-Methoxyphenyl)[2-methyl-1-[2-(4-morpholinyl)ethyl]1H-indol-3-yl]methanone (Z)-2-butenedioate (1:1)



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (9.4% inhibition at 6,000 nM) in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	EC50 (M)	Maximum Response
Drug alone	1.01×10^{-6}	100%
After naltrexone	1.06×10^{-6}	100%

NIH 10474 (4-Methoxyphenyl)[2-methyl-1-[2-(4-morpholinyl)ethyl]1H-indol-3-yl]methanone (Z)-2-butenedioate (1:1)

...(continued)

SELF-ADMINISTRATION IN RHESUS MONKEYS

The reinforcing effects of NIH 10474 were evaluated in three rhesus monkeys trained to respond for 0.32 mg/kg/injection codeine (Woods, J.H., Drug and Alcohol Dependence, 1980, 8:223-230). Doses of NIH 10474 were substituted for codeine in single, 130 min test sessions; each test session was separated by at least three sessions with the baseline codeine dose. Doses of 0.003, 0.01, 0.03, and 0.10 mg/kg/injection of NIH 10474 were evaluated. Each dose was tested twice in each of the three monkeys. Rates maintained by NIH 10474 were quite low in each of the three monkeys. The highest rate of responding, which was slighlty above rates maintained by saline in two of the three monkeys, occurred at a dose of 0.01 mg/kg/inj.

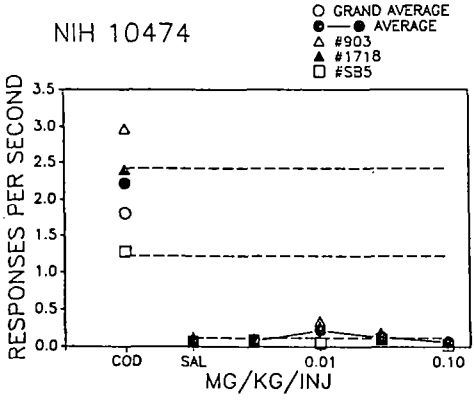


Figure 1 gives details of the results of this study. The data from the individual monkeys are indicated by each animal's identification numbers (903, 1718, SB5). Average refers to the mean of data from these three monkeys, while Grand Average refers to a historical control value for 20 monkeys under the codeine and saline conditions. The two topmost horizontal lines indicate ± 3 SEM for codeine grand average. The bottommost slashed horizontal line, is + 3 SEM for the saline grand average.

DRUG DISCRIMINATION IN RHESUS MONKEYS

Cumulative doses of from 0.01 to 18 mg/kg were evaluated in monkeys trained to discriminate 1.0 or 1.8 mg/kg codeine from sham injections in a drug discrimination paradigm (Bertalmio, et al., J. Pharmacol. Methods, 1982, 7:289-299). One monkey generalized completely to codeine following administration of 3.2 mg/kg on one of two occasions, and generalized completely to

NIH 10474 (4-Methoxyphenyl)[Z-methyl-1-[2-(4-morpholinyl)ethyl]1H -indol-3-yl]methanone (Z)-2-butenedioate (1:1)

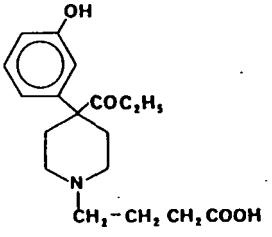
...(continued)

codeine following administration of 10 mg/kg on one of two occasions. No other monkey made any responses on the codeine-appropriate lever following administration of any dose of NIH 10474. The doses of NIH 10474 did not produce any suppression of response rates. Rates were never below 2.0 responses per second for any monkey at any tested dose of NIH 10474.

SUMMARY

NIH 10474, at the doses tested, did not have opioid activity in any of the preparations. It would appear to have insignificant opioid abuse liability, based on the results in these assay systems.

NIH 10475 N-3-Carboxypropyl-N-norketobemidone



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (30.7% inhibition at 6,000 nM) in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

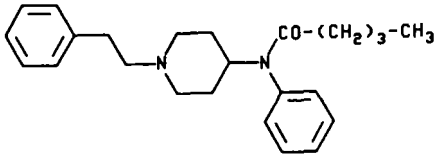
NIH 10475 was inactive upon the isolated, electrically-stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-8} M to 3 x 10^{-5} M. It neither altered responses to nor reversed the inhibitory effects of sufentanil.

SUMMARY

NIH 10475 appears to be devoid of activity upon opioid receptors in either preparation.

NIH 10488 N-[-(2-Phenylethyl)4-piperidyl]-N-phenylvaleramide hydrochloride

MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 7.5 (5.4 - 10.3)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 215 nM in the presence of 150 mM NaCl.

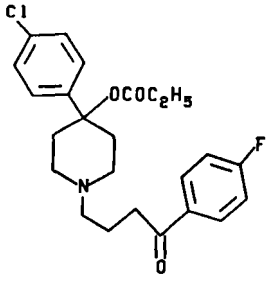
MOUSE VAS DEFERENS PREPARATION

NIH 10488 was inactive upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-8} M to 3 x 10^{-9} M. NIH 10488 neither altered responses to sufentanil nor reversed the inhibitory effects of sufentanil.

SUMMARY

NIH 10488 does not have opioid activity upon the mouse vas deferens. However, it does show displacement at the etorphine specific binding site.

NIH 10494 Haloperidol Propionate



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 30% at 100

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 279 nM in presence of 150 mM NaCl.

NIH 10494 Haloperidol Propionate

...(continued)

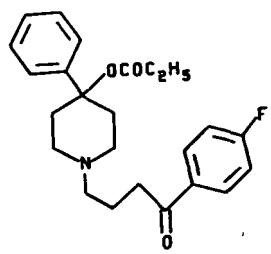
MOUSE'VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	7.52 x 10 -7	100%
After naltrexone	3.86 x 10^{-8}	24.5%
After ICI 174864	8.84 x 10^{-7}	100%
After beta-funaltrexamine	1.62 x 10^{-5}	100%

SUMMARY

NIH 10494 appears to be an opioid receptor agonist upon the mouse vas deferens preparation. The opioid antagonists produced an unusual set of responses to NIH 10494.

NIH 10495 N-3-(E-Fluorobenzoyl)propyl-4-phenyl-4-propionyloxy-piperidine



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 0.32 (0.25 - 0.42)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 30.3 nM in presence of 150 mM NaCl.

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	Inhibitory EC50 (M)	Maximum Response
Drug alone	4.95 x 10^{-8}	99.3%
After naltrexone	7.52 x 10^{-7}	54.1%
After ICI 174864	5.69 x 10^{-8}	85.4%
After beta-funaltrexamine.	1.05 x 10^{-7}	47.7%

NIH 10495 N-3-(p-Fluorobenzoyl)propyl-4-phenyl-4-propionyloxy-piperidine

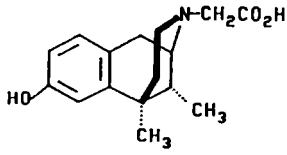
...(continued)

SUMMARY

NIH 10495 is an unusual opioid agonist on the mouse vas deferens preparation in that its actions are antagonized by naltrexone in a non-competitive manner. The binding assay shows a receptor affinity similar to that of morphine for opioid binding sites.

NIH 10502 2-Carboxymethyl-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride

MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (7.0% inhibition at 6000 nM) in presence of 150 mM NaCl.

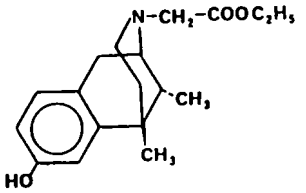
INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	Inhibitory EC50 (M)	Maximum Response
Drug alone	9.79×10^{-6}	78.3%
After naltrexone	1.93×10^{-5}	25.2%
After ICI 174864	9.91×10^{-6}	74.9%
After beta-funaltrexamine	1.51×10^{-5}	57.3%

SUMMARY

NIH 10502 has a low potency in both preparations. In the vas deferens, it has mu-opioid activity.

NIH 10503 2-Carbethoxymethyl-5,9 α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 50

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 2080 nM in presence of 150 mM NaCl.

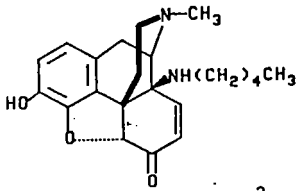
INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

NIH 10503 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-5} M. At no concentration did this drug inhibit the twitch of this preparation. NIH 10503 was a weak antagonist of sufentanil, U50,488H and DSLET.

SUMMARY

NIH 10503 had opioid activity only at high concentrations in both assays. It had an antagonist profile in mouse vas deferens like that of naltrexone.

NIH 10504 14-β-n-Pentylaminomorphinone.



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 2.86 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.20 x 10^{-9}	100%
After naltrexone	1.06 x 10^{-7}	100%
After ICI 174864	3.52 x 10^{-9}	100%
After beta-funaltrexamine	2.02 x 10^{-8}	98.0%

NIH 10504 14-β-n-Pentylaminomorphinone.

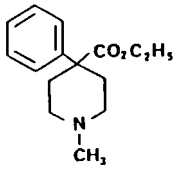
...(continued)

NIH 10504 neither altered responses to sufentanil nor reversed the inhibitory effects of sufentanil.

SUMMARY

NIH 10504 was a highly potent opioid in both preparations. In the mouse vas deferens preparation, it appears to be a selective mu-receptor agonist.

NIH 10522, 5221 Pethidine hydrochloride (4-Carbethoxy-1-methyl-4-phenylpiperidine hydrochloride)



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 4.1 (2.8 - 6.1)

DISPLACEMENT OF SPECIFIC ^{3}H-ETCRPHINE BINDING

EC50 of > 6,000 nM in the presence of NaCl (15.2% inhibition at 6000 nM).

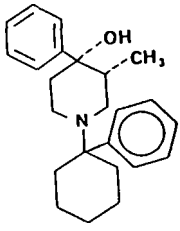
MOUSE VAS DEFERENS PREPARATION

NIH 10522 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. This drug did not inhibit the twitch at any concentration. NIH 10522, 10^{-5} M, neither blocked nor reversed the actions of sufentanil, DSLET or U50,488H. Thus, NIH 10522 is devoid of opioid activity on the mouse vas deferens preparation.

SUMMARY

NIH 10522 failed to display significant opioid activity in these in vitro preparations.

NIH 10531 4-Hydroxy-3-methyl-4-phenyl-1-(1-phenylcyclohexyl)-piperidine (trans 3-methyl, 4-phenyl) hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 680 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

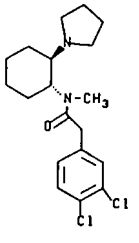
	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.42×10^{-6}	99.2%
After ICI 174864	7.47×10^{-7}	100%

Naltrexone abolished all responses to this drug. Pretreatment of NIH 10531 with beta-funaltrexamine, 10^{-7} M, abolished all responses to the compound except at the highest concentration studied.

SUMMARY

NIH 10531 has opioid activity in both preparations, although its potency was low. It was a mu receptor agonist on the mouse vas deferens preparation.

NIH 10532 (+)-trans-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl-1]benzeneacetamide d-tartrate ((+)-U50,488 .d-tartrate)



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (32.6% inhibition at 60000 nM) in the presence
of NaCl.

NIH 10532 (+)-trans-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl-1]benzeneacetamide d-tartrate ((+)-U50,488.d-tartrate)

...(continued)

MOUSE VAS DEFERENS PREPARATION

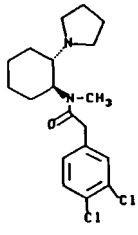
	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.40×10^{-6}	59.6%
After ICI 174864	2.03×10^{-6}	73.1%
After beta-funaltrexamine	2.01×10^{-6}	35.7%

Naltrexone, 10^{-7} M, completely blocked all responses to this drug.

SUMMARY

NIH 10532 appears to have very low affinity for opioid recognition sites, but to have some kappa receptor activity in the mouse vas deferens.

NIH 10533 (-)-trans-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl-1]benzeneacetamide l-tartrate ((-)-U50,488.l-tartrate)



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: ca. 4.5

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 4140 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.62×10^{-8}	93.6%
After naltrexone	1.36×10^{-7}	83.3%
After ICI 174864	3.01×10^{-8}	98.6%
After beta-funaltrexamine	1.85×10^{-8}	100%

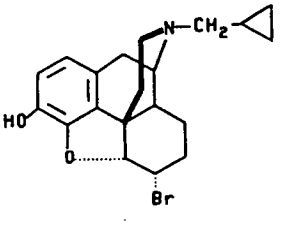
NIH 10533 (-)-trans-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl-1]benzeneacetamide l-tartrate ((-)-U50,488 .l-tartrate)

...(continued)

SUMMARY

NIH 10533 had opioid activity in both preparations. It was considerably less active in the binding assay than in the mouse vas deferens. It has kappa opioid activity in the mouse vas deferens.

NIH 10535 6α-Bromo-N-cyclopropylmethyl-4,5 α-epoxymorphinan hydrochloride (Cyclobroxy)



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1.92 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

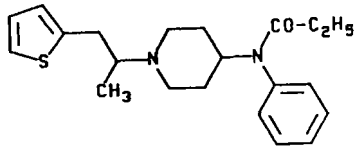
NIH 10535 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 10^{-4} M. No concentration of this drug significantly inhibited the contractions of the vas deferens. pA_2 values against the following agonists were:

Agonist	pA_2 values
Sufentanil	8.53 ± 0.13
DSLET	8.46 ± 0.15
U50,488	non-competitive (10^{-6} M)

SUMMARY

NIH 10535 is an antagonist equivalent in potency to naltrexone at mu and delta receptors, but is much less potent at kappa receptor sites.

NIH 10538 N-[1-Methyl-2-(2-thienyl)ethyl]-4-piperidyl]-N-phenylpropanamide hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 15.7 nM in presence of 150 mM NaCl.

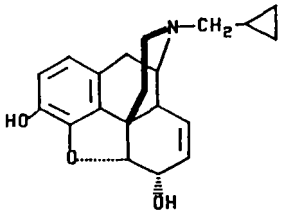
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	9.37×10^{-9}	94.9%
After naltrexone	2.08×10^{-7}	92.0%
After ICI-174864	8.63×10^{-9}	94.0%
After beta-funaltrexamine	8.90×10^{-8}	69.4%

SUMMARY

NIH 10538 was a potent opioid in both preparations. On the basis of selective antagonism, the compound appears to exert mu-receptor opioid agonist action on the mouse vas deferens.

NIH 10539 N-Cyclopropylmethylnormorphine hydrochloride



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 33% at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1.45 nM in the presence of NaCl.

NIH 10539 N-Cyclopropylmethylnormorphine hydrochloride

...(continued)

MOUSE VAS DEFERENS PREPARATION

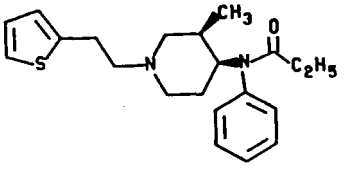
NIH 10539 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 10^{-5} M. No concentration of this drug significantly inhibited the contractions of the vas deferens. pA_2 values against the following agonists were:

Agonist	pA_2 values
Sufentanil	8.10 ± 0.13
DSLET	6.57 ± 0.14
U50,488	6.58 ± 0.32

SUMMARY

NIH 10539 is a highly potent opioid compound in both preparations. The pA_2 analysis of its selective affinity for agonists in the mouse vas deferens suggests that it could be a useful mu-selective antagonist.

NIH 10546 cis-N-[1-[2-(2-Thienyl)ethyl]-3-methyl-4-piperidyl]-N-phenylpropanamide hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 5.13 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.92×10^{-10}	100%
After naltrexone	2.79×10^{-8}	100%
After ICI-174864	1.34×10^{-8}	100%
After beta-funaltrexamine	1.16×10^{-8}	100%

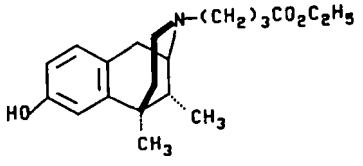
NIH 10546 cis-N-[1-[2-(2-Thienyl)ethyl]-3-methyl-4-piperidyl]-N-phenylpropanamide hydrochloride

...(continued)

SUMMARY

NIH 10546 was a potent delta receptor agonist upon both preparations, especially the mouse vas deferens preparation. The possiblity that this drug also stimulates mu and/or kappa receptors cannot be ruled out.

NIH 10548 2-(3-Carbethoxypropyl)5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1020 nM in presence of NaCl.

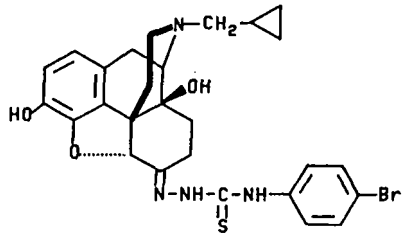
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.79×10^{-5}	100%
After naltrexone	1.74×10^{-4}	100%
After ICI-174864	2.86×10^{-5}	100%
After beta-funaltrexamine	3.60×10^{-5}	100%

SUMMARY

NIH 10548 has a low potency in both preparations. It appears to selectively activate mu receptors in the mouse vas deferens, but also has a non-opioid component action on this preparation at quite high concentrations.

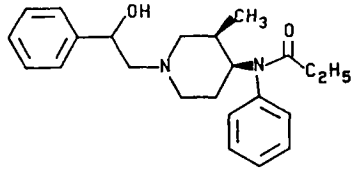
NIH 10550 1-(N)-p-Bromophenylnaltrexone thiosemicarbazone (80% anti: 20% syn)



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 20

NIH 10550 was insoluble in both in vitro preparations.

NIH 10551 cis-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]- N-phenylpropanamide hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: < 0.003

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 4.42 nM in the presence of NaCl.

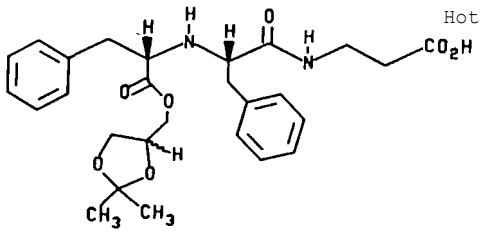
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	3.97×10^{-10}	98.0%
After naltrexone	2.80×10^{-8}	100.0%
ICI-174864	6.96×10^{-10}	96.7%
After beta-funaltrexamine	3.66×10^{-9}	91.3

SUMMARY

NIH 10551 was a very potent compound in both, in vitro preparations. It appears to act as a mu-receptor agonist in the mouse vas deferens preparation.

NIH 10552 N-[N-[L-[1-[D,L-(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]carbonyl]-2-phenylethyl]-L-phenylalanyl]-β-alanine



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

NIH 10552 was insoluble under standardized conditions.

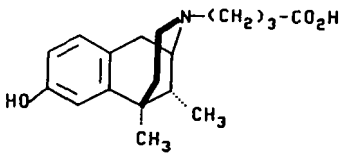
MOUSE VAS DEFERENS PREPARATION

In a concentration of 10^{-5} M, NIH 10552 failed to antagonize the actions of sufentanil, DSLET, or U50,488 upon the vas deferens.

SUMMARY

It would appear that NIH 10552 fails to have significant opioid activity in the mouse vas deferens preparation.

NIH 10555 2-(3-Carboxypropyl)-5-9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (26% inhibition at 6000 nM) in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10555 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. This drug did not inhibit the twitch

NIH 10555 2-(3-Carboxypropyl)-5-9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride

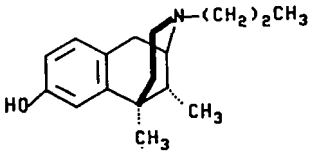
...(continued)

at any concentration. NIH 10555, 10^{-5} M, neither blocked nor reversed the actions of sufentanil, DSLET or U50,488H. Thus, NIH 10555 is devoid of opioid activity on the mouse vas deferens preparation.

SUMMARY

NIH 10555 failed to display significant opioid activity in the in vitro preparations.

NIH 10556 (+)-5,9α-Dimethyl-2'-hydroxy-2-n-propyl-6,7-benzomorphan hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (37% inhibition at 6000 nM) in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10556 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-5} M. This drug did not inhibit the twitch at any concentration. NIH 10556 acted as an opioid antagonist of very low potency. This drug caused shifts to the right in the concentration-effect curves for sufentanil (a mu agonist) and U50,488 (a kappa agonist), but not for DSLET (a delta agonist). pA_2 values were not determined because of the low potency of this drug. At a concentration of 10^{-5} M, NIH 10556 caused a 4.2-fold shift to the right in the sufentanil concentration-effect curve and a 4.3-fold shift to the right in the U50,488 concentration-effect curve. This concentration of NIH 10556 did not block the actions of DSLET. NIH 10556 is a very weak opioid receptor antagonist which blocks mu and kappa, but not delta, receptors in the mouse vas deferens preparation.

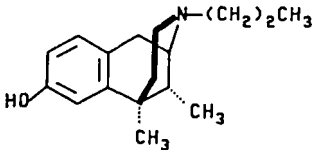
NIH 10556 (+)-5,9α-Dimethyl-2'-hydroxy-2- n-propyl-6,7-benzomorphan hydrochloride

...(continued)

SUMMARY

NIH 10556 is an opioid antagonist on the vas deferens, but it is of very low potency in this preparation. The potency of this compound in the binding assay was too low to be detected with the current protocols.

NIH 10557 (-)-5,9α-Dimethyl-2'-hydroxy-2-1-propyl-6,7-benzomorphan hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 14.5 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

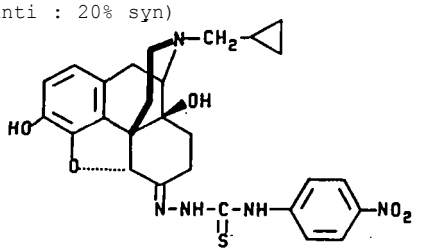
NIH 10557 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. This drug did not inhibit the twitch at any concentration. pA_2 values against the following agonists were:

Agonist	pA_2 values
Sufentanil	7.17 ± 0.05
DSLET	6.50 ± 0.25
U50,488	Non-competitive

SUMMARY

NIH 10557 is an opioid antagonist on the mouse vas deferens preparation and very potent in the binding assay.

NIH 10558 1-(N)-p-Nitrophenylnaltrexone thiosemicarbazone (80% anti : 20% syn)



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

NIH 10558 was insoluble under standardized conditions.

MOUSE VAS DEFERENS PREPARATION

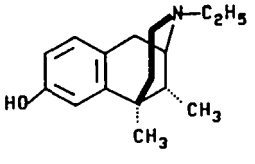
NIH 10558 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged FROM 10^{-9} M to 3 x 10^{-4} M. This drug did not inhibit the twitch at any concentration. pA_2 values against the following agonists were:

Agonist	pA_2 values
Sufentanil	8.15 ± 0.69
DSLET	8.10 ± 0.62
U50,488	7.53 ± 0.63

SUMMARY

NIH 10558 is a non-selective opioid antagonist in the mouse vas deferens preparation. Because of its limited solubility, it could not be studied in the binding assay.

NIH 10559 (+)-5,9α-Dimethyl-2-ethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (8.4% inhibition at 6000 nM) in the presence of NaCl.

NIH 10559 (+)-5,9α-Dimethyl-2-ethyl-2'-hydroxy-6,7-benzomorphan hydrochloride

...(continued)

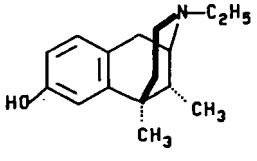
MOUSE VAS DEFERENS PREPARATION

NIH 10559 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-5} M. No concentration of this drug inhibited the twitch of the vas deferens. Furthermore, NIH 10559, in a concentration of 10^{-5} M, did not alter the inhibitory actions of sufentanil (mu), DSLET (delta) or U50,488H (kappa) upon the vas deferens. NIH 10559 is devoid of opioid agonistic and antagonistic activity upon the mouse vas deferens preparation.

SUMMARY

NIH 10559 failed to display significant opioid activity in either in vitro preparation.

NIH 10560 (-)-5,9α-Dimethyl-2-ethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 144 nM in the presence of NaCl.

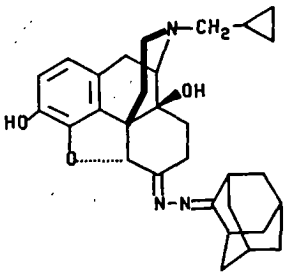
MOUSE VAS DEFERENS PREPARATION

NIH 10560 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. This drug did not inhibit the twitch at any concentration. NIH 10560 was a non-competitive antagonist of sufentanil, DSLET, and U50,488.

SUMMARY

NIH 10560 is an unusual narcotic antagonist; as suggested by the mouse vas deferens, NIH 10560 may be noncompetitive at mu and delta types of receptors in this assay, and noncompetitive at the kappa receptor at very high concentrations.

NIH 10561 6-(2-Adamantyl)naltrexonazine (80% anti : 20% syn)



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 2.22 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.64×10^{-6}	49.4%
After naltrexone	3.21×10^{-6}	54.7%

NIH 10561 acted as an antagonist. pA_2 values against the following agonists were:

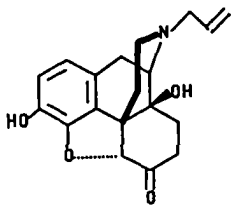
Agonist	pA_2
sufentanil	7.55 ± 0.38
DSLET	7.35 ± 0.05

Because of low potency, a pA_2 value could not be determined against U50,488.

SUMMARY

NIH 10561 was a mixed agonist-antagonist on the mouse vas deferens. It was extremely potent in displacing etorphine.

NIH 10562, 7890 Naloxone hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: 20% at 50
Nilsen Assay: 12% at 100

NIH 10562, 7890 Naloxone hydrochloride

...(continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 6.26 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

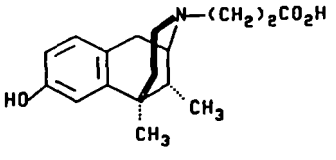
NIH 10562 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. This drug did not inhibit the twitch at any concentration. pA_2 values against the following agonists were:

Agonist	pA_2 values
Sufentanil	7.99 ± 0.34
DSLET	7.35 ± 0.10
U50,488	6.90 ± 0.12

SUMMARY

NIH 10562 is an antagonist similar to, but less potent than, naltrexone in both in vitro preparations.

NIH 10564 2-(2-Carboxyethyl)-5,9 α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 50

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (27% inhibition at 6000 nM) in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10564 was studied upon the isolated, electrically stimulated, mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. This drug did not inhibit the twitch at any concentration. NIH 10564, 10^{-5} M, neither. blocked nor reversed the actions of sufentanil, DSLET or U50,488H.

229-045 0 - 89 - 16 QL3

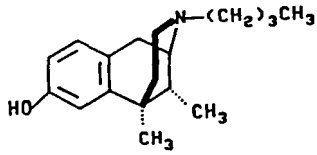
NIH 10564 2-(2-Carboxyethyl)-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride

...(continued)

SUMMARY

NIH 10564 failed to display significant opioid activity in these in vitro preparations.

NIH 10565 (+)-2-n-Butyl-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (21% inhibition at 6000 nM) in the presence of NaCl.

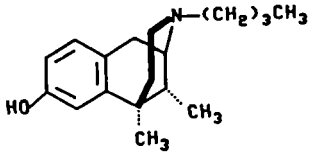
MOUSE VAS DEFERENS PREPARATION

NIH 10565 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. This drug did not inhibit the twitch at any concentration. NIH 10565 acted as an opioid antagonist. This drug caused shifts to the right in the concentration-effect curves for sufentanil (a mu agonist), U50,488H (a kappa agonist) and DSLET (a delta agonist). Because of the low potency of this antagonist, pA_2 values could not be determined, but would have been less than 5.0.

SUMMARY

NIH 10565 fails to have significant activity in the binding assay, but does have partial agonist activity at the delta receptors in the vas deferens preparation.

NIH 10566 (-)-2-n-Butyl-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 47.2 nM in the presence of NaCl.

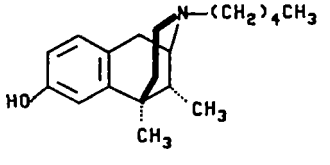
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	3.41×10^{-7}	63.7%
After naltrexone	1.62×10^{-6}	54.0%
After ICI 174,864	1.38×10^{-6}	54.4%
After beta-funaltrexamine	7.62×10^{-7}	34.2%

SUMMARY

NIH 10566 is a partial agonist on the mouse vas deferens with activity at delta receptors. It would be of interest to know its binding profile in greater detail, and its in vivo activities.

NIH 10568 (+)-5,9α-Dimethyl-2'-hydroxy-2-n-pentyl-6,7-benzomorphan hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (40.3% inhibition at 6000 nM) in the presence of NaCl.

NIH 10568 (+)-5,9α-Dimethyl-2'-hydroxy-2-1-pentyl-6,7-benzomorphan hydrochloride

...(continued)

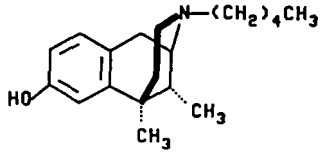
MOUSE VAS DEFERENS PREPARATION

NIH 10568 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. Concentrations between 10^{-8} and 10^{-5} M caused a very slight inhibition of the twitch. The maximum response was less than a 20% inhibition of the twitch, and an EC50 could not be determined reliably (n=3). Naltrexone did not appear to antagonize the inhibitory actions of NIH 10568. NIH 10568 is a very weak antagonist at mu, kappa, and delta receptors. In a concentration of 10^{-5} M, it caused a 4.5-fold shift to the right in the DSLET concentration-effect curve, a 24-fold shift to the right in the U50,488 concentration-effect curve and a 2.1-fold shift to the right in the sufentanil concentration-effect curve. Because of the low potency of NIH 10568 as an antagonist, pA_2 values were not determined.

SUMMARY

NIH 10658 was only marginally active in both preparations at high concentrations. In the mouse vas deferens, it displayed antagonist activity that appeared somewhat kappa-selective.

NIH 10569 (-)-5,9α-Dimethyl-2'-hydroxy-2-1-pentyl-6,7-benzomorphan hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: 33% at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 102 nM in the presence of NaCl.

NIH 10569 (-)-5,9α-Dimethyl-2'-hydroxy-2-n-pentyl-6,7-benzomorphan hydrochloride

...(continued)

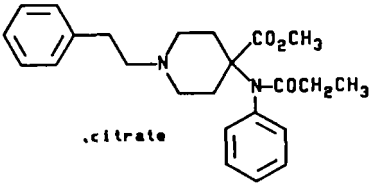
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (A)	Maximum Response
Drug alone	7.16×10^{-7}	100%
After naltrexone	1.29×10^{-5}	91.7%
After ICI 174864	1.91×10^{-6}	100%
After beta-funaltrexamine	6.07×10^{-6}	100%

SUMMARY

NIH 10569 appears to have opioid activity in both preparations; in the mouse vas deferens preparation it had activity at delta receptors.

NIH 10570 Carfentanil citrate



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: < 0.00004

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 72.0 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.05×10^{-11}	97.0%
After naltrexone	1.35×10^{-10}	98.7%
After ICI 174864	3.01×10^{-11}	100%
After beta-funaltrexamine	3.76×10^{-11}	93.5%

SUMMARY

NIH 10570 is an extremely potent agonist in the mouse vas deferens. with activity at more than one receptor type. The potency of the compound in the binding assay doesn't bear out the extremely high potency in the vas deferens.

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Dependence Studies of New Compounds in the Rhesus Monkey, Rat and Mouse (1988)

M. Aceto, E. Bowman, L. Harris and E. May

All the drugs except haloperidol were supplied by Dr. Arthur Jacobson, Chairman, Drug Testing Committee (NIH, NIDDK) 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 3.0 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 l978). The PPt-W test was initiated by the injection of a test drug 2 1/2 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 drugs were noted. In both tests, a vehicle control and an appropriate positive control (naloxone hydrochloride, 0.05 mg/kg or morphine sulfate, 3.0 mg/kg) along with 2 or 3 different treatments (doses) of a test compound were randomly allocated to the 4 or 5 monkeys of a group. Usually, 3 or 4 groups per compound. were used. All drugs were given subcutaneously (1 ml/kg) and the vehicle was water except where indicated. 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) test, 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 subcutaneously from the nape of the neck to the lateral side of the lower abdomen and then inserted into the peritoneal cavity. The cannula was anchored at both ends with silk sutures and attached to a flow-through, swivel mechanism which allowed the animal to move about in the cage and eat and drink normally. The swivel was connected to a syringe which was attached to a syringe pump. The animals received 7-10 ml of solution every 24 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 1/2 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 with morphine.

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 (TV 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 sometimes 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 1

Comparative Data-ED50 or AD50, mg/kg s.c. (95% C.L.) of Selected Standards in 3 Mouse Agonist-Antagonist Tests

Drug	Tail-Flick	Tail-Flick vs Morphine	Phenylquinone
Pentazocine	15% at 10.0	18 (12-26)	1.7 (1.0-2.5)
Cydazocine	17% at 1.0[a]	0.03 (0.020-0.78)	0.01(0.005-0.03)
Nalorphine•HCl	None at 10.0	2.6 (0.7-10.0)	0.6 (0.03-1.44)
Naloxone•HCl	None at 10.0	0.04 (0.01-0.09)	No Activity
Naltrexone•HCl	None at 10.0	0.007 (.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 there was no further increase in reaction time.

Table 2

Comparative Data (ED50 mg/kg) [95% C.L.] from the Hot Plate and Nilsen Assays

	Hot Plate s.c./p.o.	Nilsen s.c./p.o.
Morphine Sulfate	0.98 (0.83-1.1) / 6.3 (4.7-8.3)	1.3 (1.0-1.7) / 8.3 (6.0-11.4)
Codeine Phosphate	6.8 (4.5-10.2) / 13.5 (9.7-18.7)	7.4 (4.9-11.0) / 14.7 (9.2-23.3)
Levorphanol Tartrate	0.2 (0.1-0.3) / -	0.2 (0.16-0.3) / 2.5 (1.7-3.7)
Meperidine•HCl	5.3 (4.0-7.1) / -	- / -
(-)-Metazocine•HBr	6 (0.5-0.9) / 10.6 (8.0-14.1)	0.5 (0.3-0.7) / 26.0 (21.0-33.0)
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)
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) / -	- / -

No dose response for naloxone and naltrexone.
Phenobarbital, amobarbital, oxazepam, flurazepam, meprobamate and mescaline are inactive on the hot plate test.

SUMMARY OF TESTING

Compound NIH	Chemical Name or Generic Class	MOUSE					RAT		MONKEY		
		TF	TF vs M	PPQ	HP	N	SM	PPD	SDS	PPt-W	PPD
7549	6,7-Benzomorphan	+	+	+	+	+	-	-	-	-	-
8032	Halopelidol[a]	+	+	+	-	-	-	-	-	-	-
10345	4-Phenylpiperidine	+	+	+	+	-	-	-	+	-	-
10407	4-H-Pyridopyrimidine	+	+	+	+	+	-	-	+	-	-
10431	Adrenal Cortex extract	-	-	-	-	-	-	-	+[b]	-	-
10435	Benzyl-Cyclohexanol	+	+	+	+	-	-	-	+	-	-
10436	Benzyl-Cyclohexanol	+	+	+	+	-	-	-	+	-	-
10437	Benzyl-Cyclohexanol	+	+	+	+	-	-	-	+	-	-
10458	Oxymorphone	+	+	+	+	-	-	-	+	-	-
10459	Oxymorphone	+	+	+	+	-	-	-	+	-	-
10474	14-Indolyl-methanone	+	+	+	+	-	-	-	+	-	+
10494	Haloperidol propionate[c]	+	+	+	+	-	+	+	+	-	-
10495	4-Phenylpiperidine[c]	+	+	+	+	-	+	+	+	-	-
10501	6,14-Ethenoisomorphinan[c]	+	+	+	-	-	-	-	-	-	-
10504	14-Aminomorphinone	+	+	+	-	-	-	-	+	-	-
10518	Endoethethancoripavine	+	+	+	-	-	-	-	-	-	-
10526	4-Phenylpiperidine	+	+	+	+	-	-	-	-	-	-
10527	4-Phenylpiperidine	+	+	+	+	-	-	-	-	-	-
10528	Azabicyclooctane	+	+	+	-	-	-	-	-	-	-
10532	Cyxclohexylbenzeacetamide	+	+	+	+	-	-	-	+	-	-
10533	Cyclohexylbenzeacetamide	+	+	+	+	-	-	-	+	-	-
10535	4-5α-Epoxymorphinan	+	+	+	-	-	-	-	+	+	-
10539	Normorphine	+	+	+	+	-	-	-	+	+	-
10541	4-Phenylpiperidine	+	+	+	+	-	-	-	-	-	-
10543	4-Phenylpiperidine	+	+	+	-	-	-	-	-	-	-
10546	4-Piperidyl-N-phenylpropanamide	+	+	+	-	-	-	-	+	-	-

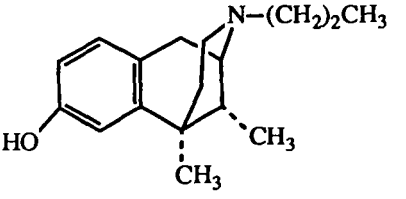
(continued)

SUMMARY OF TESTING

Compound NIH	Chemical Name or Generic Class	MOUSE TF	MOUSE TF vs M	MOUSE PPQ	MOUSE HP	MOUSE N	RAT SM	RAT PPD	MONKEY SDS	MONKEY PPT-W	MONKEY PPD
10548	6,7-Benzomorphan	+	+	+	+	-	-	-	-	-	-
10550	Naltrexone	+	+	+	+	-	-	-	+	+	-
10551	4-Piperidyl-N-phenylpropanamide	+	+	+	+	-	-	-	+	-	-
10552	Dioxalanyl-β-alanine	+	+	+	+	-	-	-	+	-	-
10553	4-Phenylpiperidine	+	+	+	+	-	-	-	-	-	-
10555	6,7-Benzomorphan	+	+	+	+	-	-	-	-	-	-
10556	6,7-Benzomorphan	+	+	+	+	-	-	-	-	-	-
10557	6,7-Benzomorphan	+	+	+	+	-	-	-	+	-	-
10558	Naltrexone	+	+	+	+	-	-	-	+	-	-
10559	6,7-Benzomorphan	+	+	+	+	-	-	-	+	-	-
10560	6,7-Benzomorphan	+	+	+	-	-	-	-	+	-	-
10561	Naltrexonazine	+	+	+	+	-	-	-	+	-	-
10562	Naloxone	+	+	+	+	+	-	-	+	-	-
10564	6,7-Benzomorphan	+	+	+	+	-	-	-	-	-	-
10566	6,7-Benzomorphan[d]	+	+	+	-	-	-	-	+	-	-
10568	6,7-Benzomorphan	+	+	+	+	-	-	-	+	-	-
10570	Carfentanil	+	+[e]	+	+	-	-	-	+	-	-
10572	Naltrexone-estrone (mixed azine)	+	+	+	-	-	-	-	-	-	-

[a]Special Naloxone Antagonism Studies
[b]Special Pretreatment Study
[c]Special Naloxone Antagonism and Special Binding Study
[d]Special Niave Monkey Experiment
[e]Special Naloxone Antagonism of TF ED_{80}

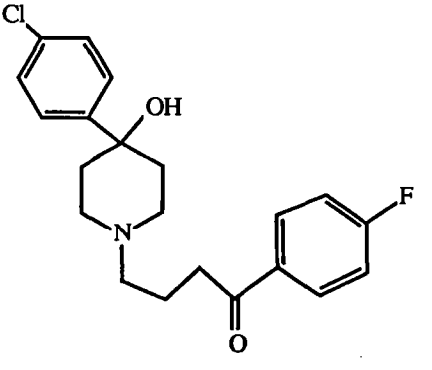
NIH 7549 (±)-5,9α-Dimethyl-2'-hydroxy-2-*n*-propyl-6,7-benzomorphan hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - Inactive at 1.0 and 10.0 and 30.0
2) TF vs. M. - 0.35 (0.11 - 1.11)
3) PPQ -17.2 (11.1 - 26.7)
4) HP- Inactive at 20.0
5) N - Inactive at 40.0

NIH 8032 1-(3-*p*-Fluorobenzoylpropyl)-4-*p*-chlorophenyl-4-hydroxypiperidine (Haloperidol)



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - 14.6 (10.9 - 19.5)[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.011 (0.002 - 0.048)
4) HP -

[a]Very erratic results

A) Special: Naloxone antagonism of Haloperidol ED60[a] in Tail-Flick

Naloxone mg/kg s.c.	%Antagonism
5	82
1	65
0.5	82
0.1	12

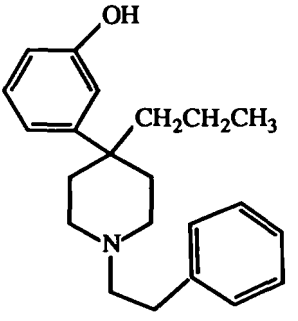
[a]Highest efficacy

B) Special: Naloxone antagonism of Haloperidol ED80 in PPQ

Naloxone mg/kg s.c.	%Anatagonism
10	0.0
1	2.0
0.1	12.0

All the data given above for NIH 8032 should be compared with those of NIH 10494 and NIH 10495.

NIH 10345 4-(*m*-Hydroxyphenyl)-4- *n*-propyl-1-phenethylpiperidine hydrochloride

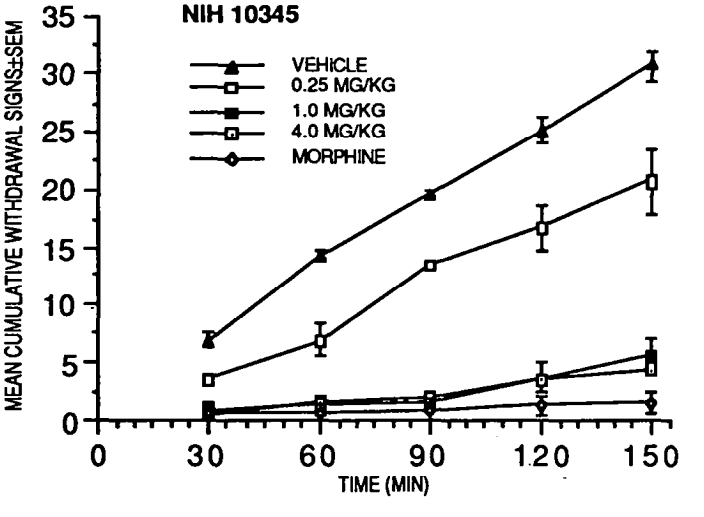


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - 2.1 (1.3 - 3.5)
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.4 (0.3 - 0.7)
4) HP- 1.8 (1.3 - 2.6)

MONKEY DATA (SDS)

At the 2 higher doses, NIH 10345 substituted completely for morphine. Onset of action was prompt and duration was at least 2 1/2 hr. This drug and morphine are approximately equal in potency.

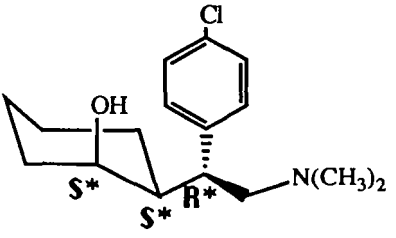


NIH 10431 Adrenal Cortex Extract

MONKEY DATA SDS (Special)

The extract was concentrated 10 fold, 5 times the concentration of the adrenal cortex extract used previously. Two morphine-addicted monkeys were pretreated for 6 days (6 x day at 6 a.m., 10 a.m., noon, 3 p.m., 6 p.m. and midnight with 0.2 ml/kg) before the abrupt withdrawal of morphine and at 10:30 a.m. during withdrawal. The monkeys showed the signs designated: lying on side or abdomen, restlesness. wet-dog shakes, retching and masturbation. However, they only vocalized 3 of 16 times when palpated and had relaxed abdomen during 14 of 16 palpations. During abrupt withdrawal, monkeys will normally vocalize and have rigid abdomens when palpated. Higher doses might alleviate all the symptoms.

NIH 10435 (1S*)-cis-2-[(R*)-*p* -Chloro-α-[(dimethylamino)methyl]-benzyl]cyclohexanol hydrochloride

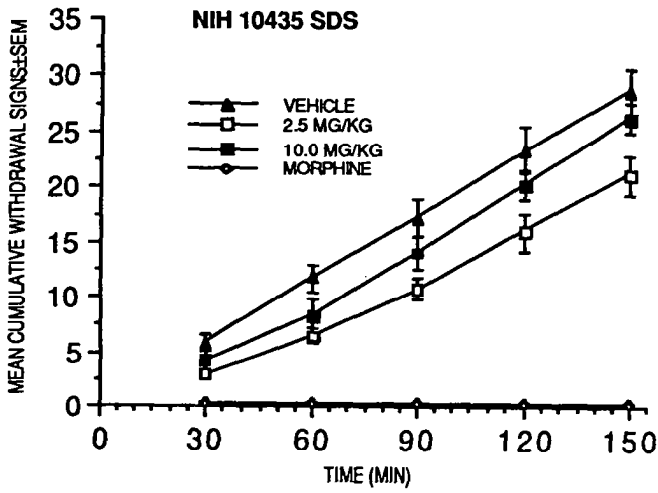


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

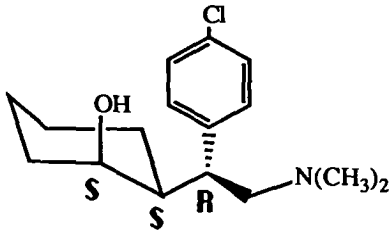
1) TF - 1% at 1.0, 12% at 10.0 and 19% at 30.0
2) TF vs. M. - 3% at 1.0, 16% at 10.0 and 55% at 30.0
3) PPQ - 5.9 (2.0 - 17.3)
4) HP - Inactive at 5.0 and 20.0

MONKEY DATA (SDS)

NIH 10435 did not substitute. completely for morphine in the dose range of 2.5 - 10.0 mg/kg. Some abdominal relaxation probably accounted for the slight suppression (see fig.) of the total number of withdrawal signs.



NIH 10436 (+)-(1S)-cis-2-[(R)- *p* -Chloro-α-[(dimethylamino)methyl] benzyl]cyclohexanol hydrochloride

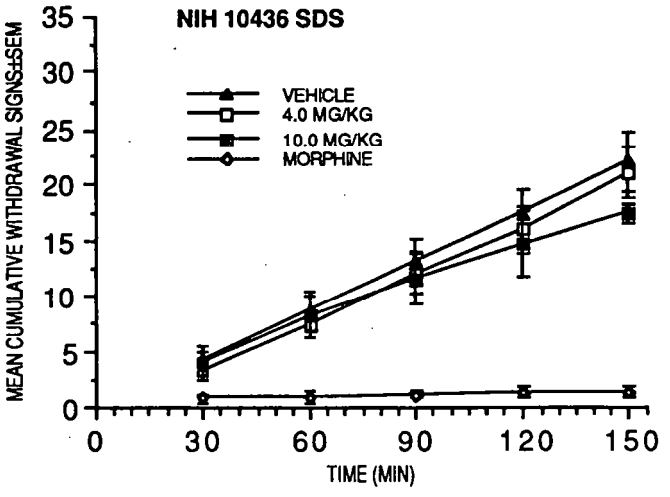


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

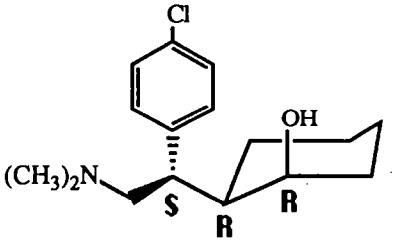
1) TF - 3% at 1.0, 14% at 10.0 and 11% *at 30.0*
2) TFvs.M. - 4% at 1.0, 9% at 10.0, 24% at 30.0 and 72% at 30.0
3) PPQ - 4% at 1.0, 14% at 10.0 and 20% at 30.0
4) HP - Inactive at 20.0

MONKEY DATA (SDS)

NIH 10436 did not substitute for morphine (1.5-10.0 mg/kg). The weak suppression seen at 10.0 mg/kg could be attributed to the relaxed abdominal muscles in all the monkeys (see fig.).



NIH 10437 (-)-(1R)-cis-2-[(S)-*p*-Chloroα-[(dimethyl,amino)methyl]-benzyl]cyclohexanol hydrochloride

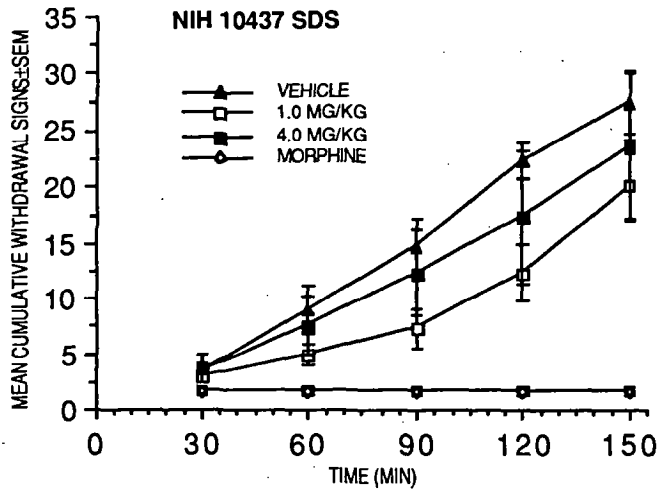


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

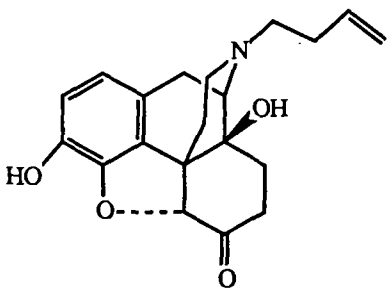
1) TF - Inactive at 1.0, 10.0 and 30.0
2) TFvs.M. - 6% at 1.0, 27% at 10.0, and 47% at 30.0
3) PPQ - 8.0 (2.1 - 30.6)
4) HP - Inactive at 20.0

MONKEY DATA (SDS)

NIH 10437 produced a non-dose-related, mild suppression of withdrawal. Most of the suppression was due to abdominal muscle relaxation (see fig.).



NIH 10458 N-3-Butenyl-3,14-dihydroxy-4,5α-epoxy-6-oxomorphinan hydrochloride

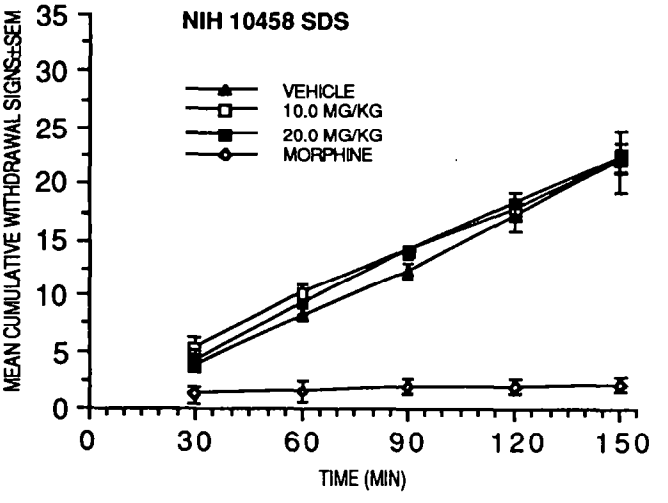


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

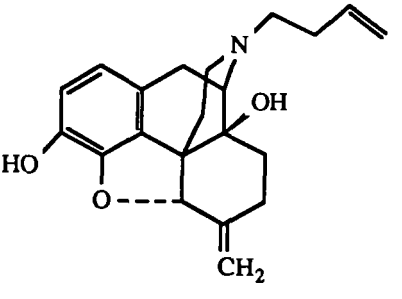
1) TF - 10% at 1.0, 15% at 10.0 and 16% at 30.0
2) TF vs. M. - 3.8 (1.2 - 11.3)
3) PPQ - 0.13 (0.05 - 0.38)
4) HP - Inactive at 5.0 and 20.0

MONKEY DATA (SDS)

NIH 10458 did not substitute for morphine or exacerbate withdrawal at 10.0 and 20.0 mg/kg. Jaw sag was noted in one monkey receiving 10.0 mg/kg and tremors were noted in one animal receiving the highest dose.



NIH 10459 N-3-Butenyl-3,14-dihydroxy-4,5 α-epoxy-6-methylenemorphinan hydrochloride

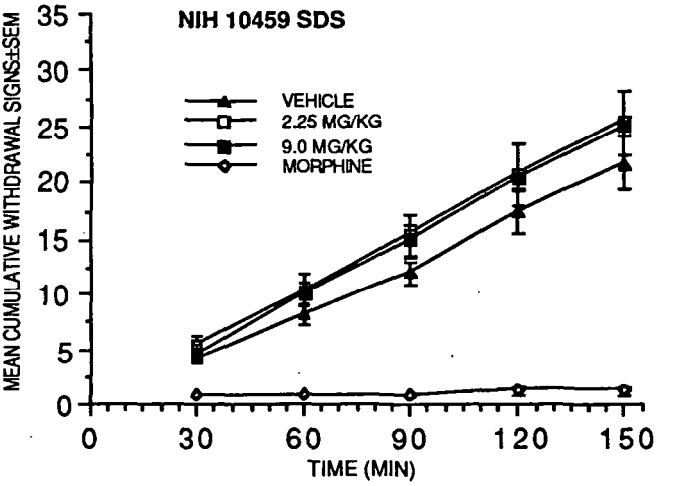


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

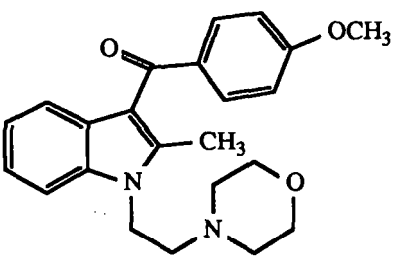
1) TF - 11% at 1.0, 37% at 10.0 and 38% at 30.0
2) TF vs. M. - 17% at 0.5, 40% at 1.0, 55% at 10.0 and 65% at 30.0
3) PPQ - 5.8 (1.8 - 18.6)
4) HP - Inactive at 5.0 and 20.0

MONKEY DATA (SDS)

In the dose range studied (2.25 and 9.0 mg/kg), NIH 10459 did not substitute for morphine (see fig.). The drug also produced ataxia, slowing and jaw sag at both doses.



NIH 10474 (4-Methoxyphenyl)[2-methyl-1[2-(4-morpholinyl)etyl]-1 H-indol-3-yl]methanone (Z)-2-butenedioate (1:1)



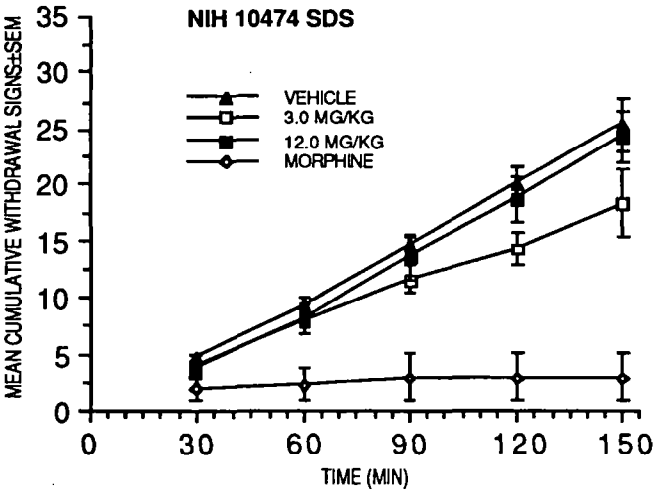
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 5.1 (1.9 - 14.0)[a]
4) HP - Inactive at 5.0 and 20.0

[a]Vehicle - Tween 80 + H_2O

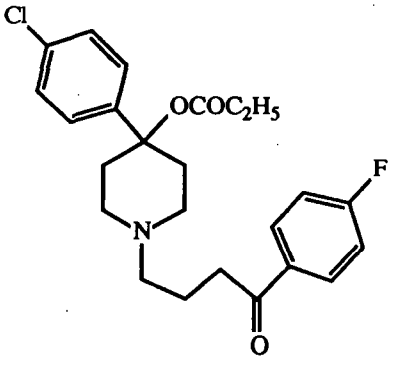
MONKEY DATA (SDS)

As shown in the fig., NIH 10474 did not substitute for morphine or exacerbate withdrawal. The reduction in withdrawal signs can be accounted for by the low incidence of the signs designated wet-dog shakes and retching; it is probably a spurious result. The drug was very insoluble and precipitated after 1/2 hr when suspended in Tween 80. DMSO helped prevent this problem and was used to solubilize the drug in one assay.



MONKEY DATA (PPD)

Three rhesus monkeys were medicated as indicated in the accompanying table (next page). As can be seen, neither abrupt withdrawal of drug nor challenge with naloxone produced a withdrawal syndrome. Few signs of withdrawal were noted. NIH 10474 appears to have a low physical dependence liability in the dose range studied.



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - 20.6 (6.2 - 69.2)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.4 (0.1 - 2.7)
4) HP - 30% at 100

Summary of a Primary Physical Dependence Study with NM 10474

Day	Dose (mg/kg/s.c.) 4-6 time/day	Comments
1	1.0	Throughout the entire experiment few overt behavioral signs were seen. Occasionally, wet-dog shakes, restlessness and drowsiness were noted. Body weight remained unchanged at 6.6 kg in one monkey, changed from 4.5 to 3.5 kg in another and rose from 4.1 to 4.9 in the third monkey.
2	2.0	
3-5	4.0	
6-7	6.0	
8	8.0	
9	10.0	PPt-W
10-12	12.0	Two of the three animals were injected with 1.0 mg/kg s.c. of naloxone hydrochloride, the third animal received vehicle. One animal receiving naloxone showed wet-dog shakes during the first 30 minutes after challenge. However, the vehicle-treated animal also showed wet-dog shakes and restlessness. In essence, no withdrawal syndrome developed.
13	14.0	
14-15	16.0	
16-PPt-W Test		
17-19	22.0	
20	24.0	
21	26.0[a,b]	
22	22.0[a,b]	
23	24.0[a,c]	
24	26.0[a,c]	
25-26	26.0[a]	In the abrupt withdrawal test on day 33, although a few signs designated, wet-dog shakes, restlessness and drowsiness were noted, no withdrawal syndrome developed. In the precipitated withdrawal test, (1.0 mg/kg of naloxone s.c. to 2 of 3 monkeys and vehicle to the third monkey), the same signs indicated above were seen, but again, no syndrome developed.
27	28.0[c]	
28	30.0[c,d]	
29-32	32.0[d,e]	
33 Abrupt Withdrawal at 6:00 a.m.; PPt-W at 1:00 p.m.		

Conclusion: NM 10474 does not produce opioid-type physical dependence in the dose range of 1.0 - 32.0 mg/kg given 4-6 times a day as evidenced by a lack of a withdrawal syndrome during either abrupt or precipitated withdrawal.

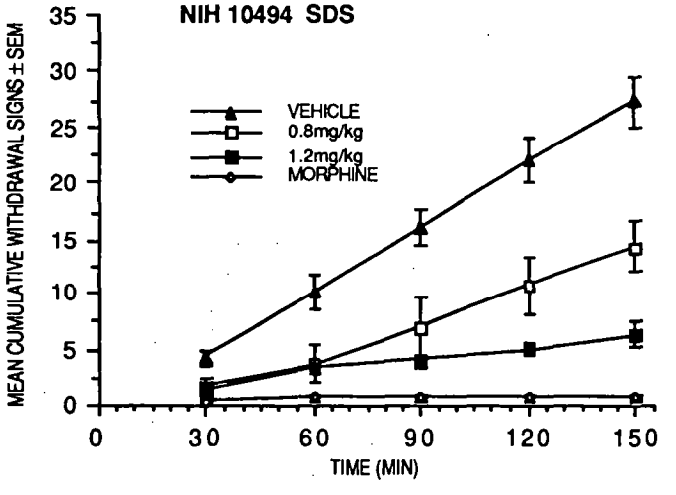
[a] Six injections/day; [b] One monkey developed an ulcer on its skin on its left hip. A veterinarian cleaned and treated ulcer, [c] Volume of solution was changed from 1/4 to 1 ml/kg to dilute solution and minimize possible irritating effects; [d] Drug solution crystallized; [e] Drug solution was cloudy.

Special A. Naloxone Antagonism of TF ED80 of NIH 10494:
AD50 = 0.2 (0.0-0.5)

Special B. Displacement of (+)-(3H)-NANM in Mouse Brain:
IC50=2.7 x 10^{-8}M

MONKEY DATA (SDS)

NIH 10494 reduced the incidence of most of the withdrawal signs including those designated relaxed abdominal muscles and vocalization when abdomen palpated. However, the drug had other actions. It produced drowsiness and restlessness per se. These actions interfered with the interpretation of the results because they are normally counted as withdrawal signs. Furthermore, the period of restlessness/agitation/vocalization was interspersed between periods of drowsiness. The drug also produced other signs designated slowing, jaw sag, and catalepsy. Other rarely seen oven signs included peculiar head and body tremors, arched back, as if a cat were stretching, and hyperextension of the neck. The syndrome, restlessness and agitation sometimes accompanied by vocalization, brought to mind a side effect of neuroleptic drugs termed akathisia. This drug should be studied further. It seemed to incorporate opioid and neuroleptic properties.



RAT INFUSION STUDIES

A. P P D When NIH 10494 was given continuously for 6 days and then withdrawn abruptly, little evidence for the development of primary physical dependence was noted. See the figs. and table.

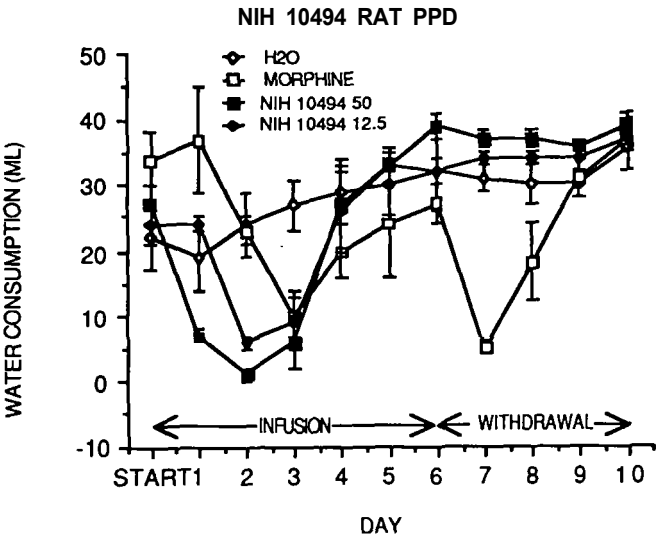
Table; Primary Physical Dependence (PPD) and Substitution for Morphine Studies (SM) with NIH 10494 in Continuously-Infused Rats

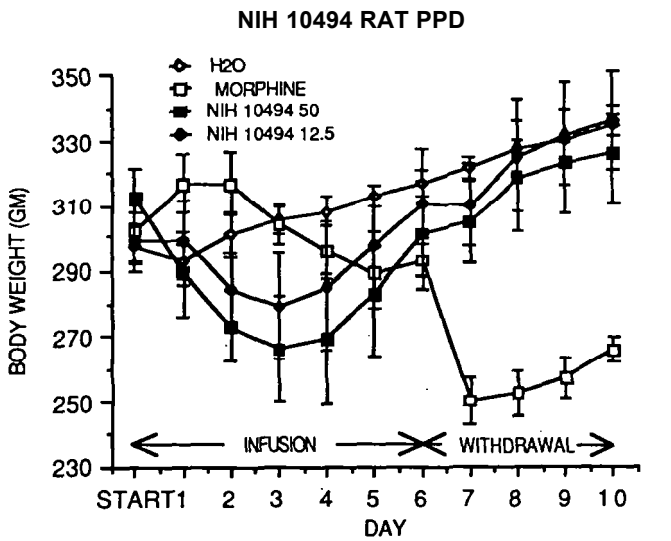
Treatment	Hr in Withdrawal			
	24	48	72	96
	Mean Number of Withdrawal Signs [a,b]			
1. H_2O Controls[c]	0	0.8	1.5	1.5
2. Morphine Controls[d]	9.3[b]	7.8[b]	4.8[b]	4.8[b]
3. NIH 10494-PPD[e] (high dose)	0.3	0	0	2.3
4. NIH 10494-PPD[f] (low dose)	2.5[b]	0.5	1.8	0.5
5. NIH 10494-SDS[g] (high dose)	2.5[b]	7.0[b]	5.3[b]	4.5
6. NIH 10494-SDS[h] (low dose)	5.9[b]	6.8[b]	10.0[b]	3.5

[a]Hypersensitivity, squeaking, aggression, wet-dog shakes, rubbing and chewing;
[b]One-tailed test Mann-Whitney U test, $p < 0.05$, probability value vs. water controls;
[c]8 ml/24 hr. N= 4;
[d]Dose regimen of morpine SO_4, 50 mg/kg on day 1, 100 mg/kg on day 2, 200 mg/kg on days 3-6. N= 4;
[e]Dose regimen of NIH 10494, 50 mg/kg on day 1, 25 mg/kg on day 2, 6.25 mg/kg on day 3 and 1.56 mg/kg on days 4-6, then, H_2O as above during withdrawal. N=4;
[f]Dose regimen of NIH 10494 12.5 mg/kg on day 1.25 mg/kg on day 2, 6.25 mg/kg on day 3, 1.56 mg/kg on days 4 and 5 and 3.12 mg/kg on day 6. Then, H_2O as above during withdrawal. N=4;
[g]Morphine SO_4 Infusion, days 1-6 as above then, NIH 10494 on days 7 and 8, 25 mg/kg, and H_2O as above on days 9 and 10. N=4 on days 7 and 8, 3 on day 9 and 2 on day 10;
[h]Morphine SO_4 Infusion days 1-6 as above. Then, NIH 10494 12.5 mg/kg on days 7 and 8, and H_2O on days 9 and 10. N=4 on days 1-8 and 2 on days 9 and 10.

RAT PPD (continued)

It should be noted that the starting dose produced drastic changes in body weight and drinking and had to be lowered instead of raised during the infusion period to prevent the rats from becoming moribund. The doses initially selected were based on the relative potency of NIH 10494 in the tail-flick experiment.


NIH 10494 RAT PPD
H2O
MORPHINE
NIH 10494 50
NIH 10494 12.5
WATER CONSUMPTION (ML)
50
40
30
20
10
0
-10
INFUSION
WITHDRAWAL
START1 2 3 4 5 6 7 8 9 10
DAY

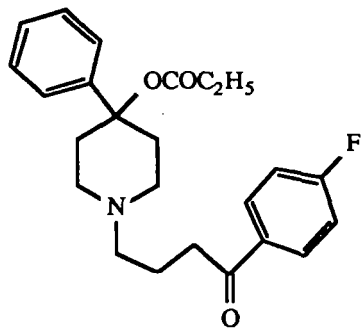

NIH 10494 RAT PPD
H2O
MORPHINE
NIH 10494 50
NIH 10494 12.5
BODY WEIGHT (GM)
350
330
310
290
270
250
230
INFUSION
WITHDRAWAL
START1 2 3 4 5 6 7 8 9 10
DAY

B. RAT SM

When NIH 10494 was substituted for morphine in morphine-addicted rats the drug did not attenuate the withdrawal syndrome with regard to loss of body weight, dipsia, or overt behavioral signs. Unfortunately, the doses were in the toxic range, and 4 animals died on the last 2 days of the experiment.

In the rat-infusion studies, the dose regimens were in the toxic range. In any case, it appears that NIH 10494 neither substitutes for morphine nor produces physical dependence in the rat. However, lower doses should also be tested.

NIH 10495 N - 3 - (*p*-Eluorobenzoyl)propyl-4-phenyl-4-propionyloxypiperidine



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

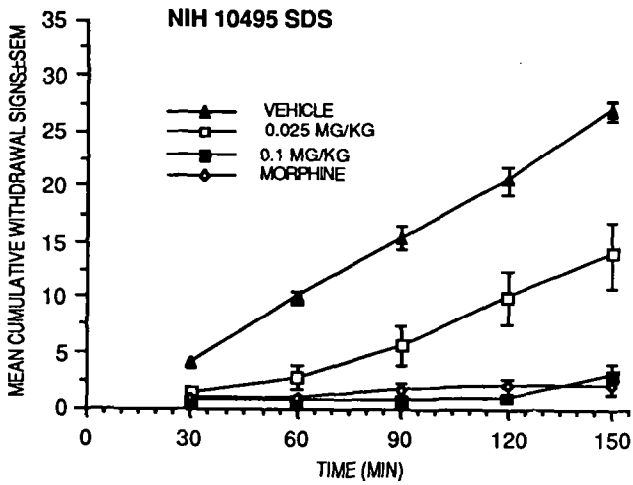
1) TF - 0.3 (0.1 - 1.1)
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.07 (0.02 - 0.18)
4) HP - 0.32 (0.25 - 0.42)

Special Naloxone Antagonism of NIH 10495 TF ED80; AD50 = 0.2 (0.5)

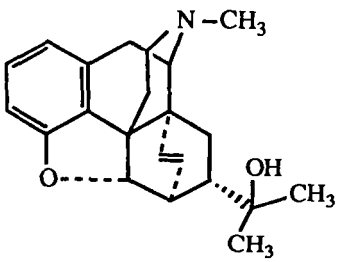
Special B, Displacement of (+)-(^{3}H)-NANM in Mouse Brain:
IC50 = 4.4 x 10^{-8}M

MONKEY DATA (SDS)

As shown in the fig., dose-related suppression of withdrawal signs was noted after the administration of NIH 10495. The drug substituted completely for morphine at 0.1 mg/kg. Onset and duration of action are similar to morphine. The drug is approximately 30 times more potent than morphine at peak effect. At the highest dose scratching, ataxia, slowing, and body sag were noted.



NIH 10501 4-5α-Epoxy-α,α,]N-trimethyl-6,14-ethenoisomorphinan-7 α-methanol

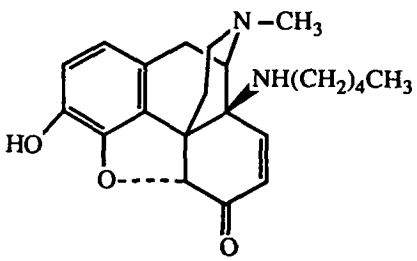


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - 0.05 (0.02 - 0.12)[a]
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.2 (0.08 - 0.48)[a]
4) HP-

[a]Vehicle - lactic acid + H_2O

NIH 10504 14ß-*n*-Pentylaminomorphinone



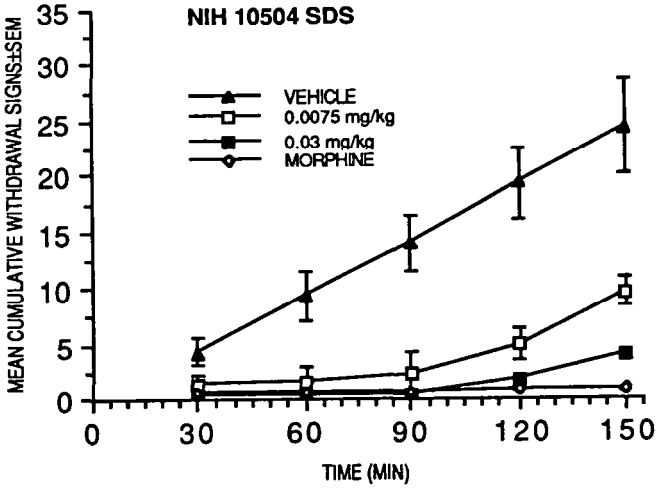
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - 0.008 (0.003 - 0.021)[a]
2) TF vs. M. - Inactive at 1.0 10.0 and 30.0
3) PPQ - 0.0005 (0.0002 - 0.001)
4) HP-

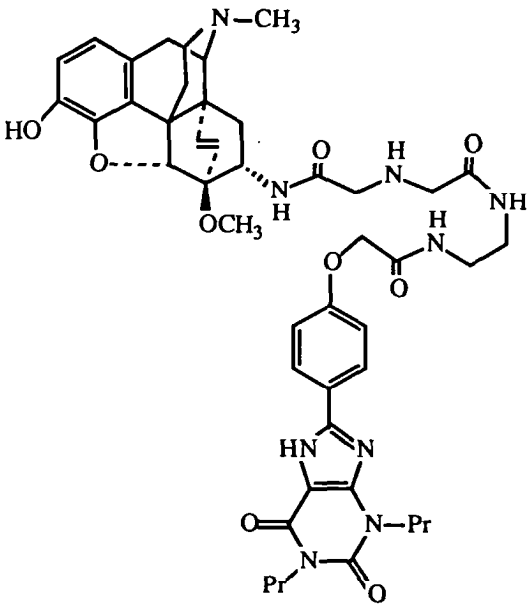
[a]Vehicle: lactic acid + H_2O

MONKEY DATA (SDS)

As can be seen in the fig., NIH 10504 substituted completely for morphine in a dose-related manner. Onset of action was prompt and duration of action was about 2 hr. This drug is estimated to be 100x more active than morphine at peak effect. The signs designated, jaw and body sag, scratching, and slowing were also seen.



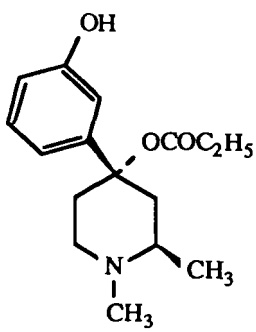
NIH 10518 Endoethenotetrahydrooripavine-xanthine conjugate trifluoro-acetate



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - Inactive at 1.0 and 10.0 and 13% at 30.0
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.22 (0.05 - 1.02)
4) HP - Inactive at 20.0

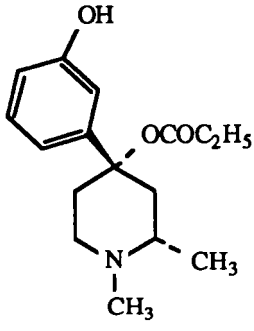
NIH 10526 1,2ß-Dimethyl-4-(3-hydroxyphenyl)-4-propionyloxypiperidine hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - 4% at 1.0, 28% at 10.0 and 31% at 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 3.7 (1.6 - 8.1)
4) HP - Inactive at 5.0 and 20.0

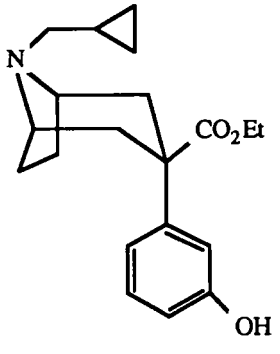
NIH 10527 1.2α-Dimethyl-4-(3-hydroxyphenyl)-4-propionyloxypiperidine hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - 32.8 (16.2 - 66.5)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 2.97 (1.22 - 7.23)
4) HP - 50% at 20.0

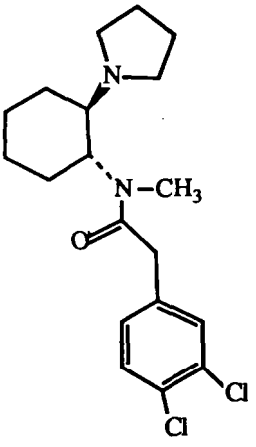
NIH 10528 Ethyl N-(cyclopropylmethyl)-3α-(3-hydroxyphenyl)-8-aza-bicyclo[3.2.1]octane carboxylate hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M- 16% at 1.0, 10% at 10.0 and 4% at 30.0)
3) PPQ - 20% at 1.0, 17% at 10.0 and 31% at 30.0
4) HP-

NIH 10532 (+)-trans-3,4Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclo-hexyl-1]benzeneacetamide *d*-tartrate [(+)-U50,488 *d*-tartrate)]

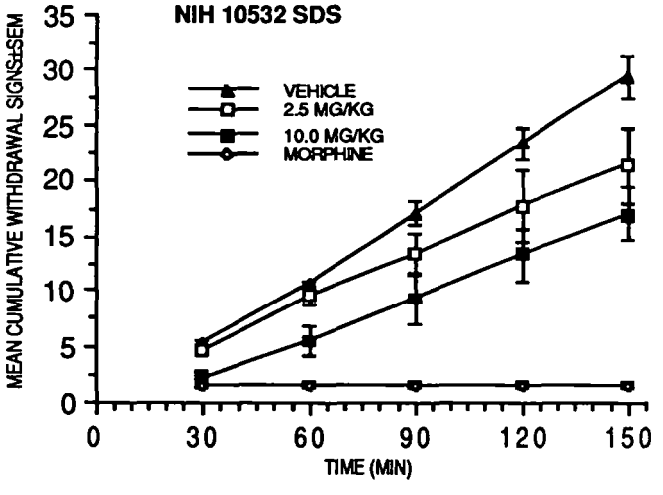


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

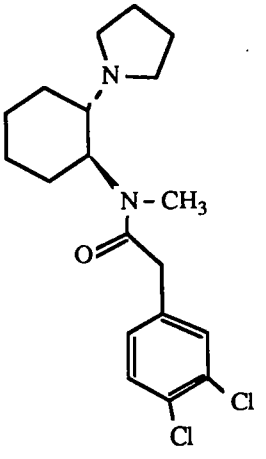
1) TF-Inactive at 1.0 and 10.0 and 30.0
2) TF vs. M - 0% at 1.0, 2% at 10.0 and 21% at 30.0
3) PPQ - 6.5 (2.0 - 20.9)
4) HP - Inactive at 20.0

MONKEY DATA (SDS)

Although NIH 10532 reduced the number of withdrawal signs. it did not substitute completely for morphine (see fig.). At the highest dose, drowsiness, tremors, scratching and jaw sag were noted.



NIH 10533 (-)-trans-3,4-Dichloro-N-methyl-N-[2-(1-pyrmlidinyl)cyclo-hexyl-1]benzeneacetamide tartrate [(-)-U50,488 *l*-tartrate)]

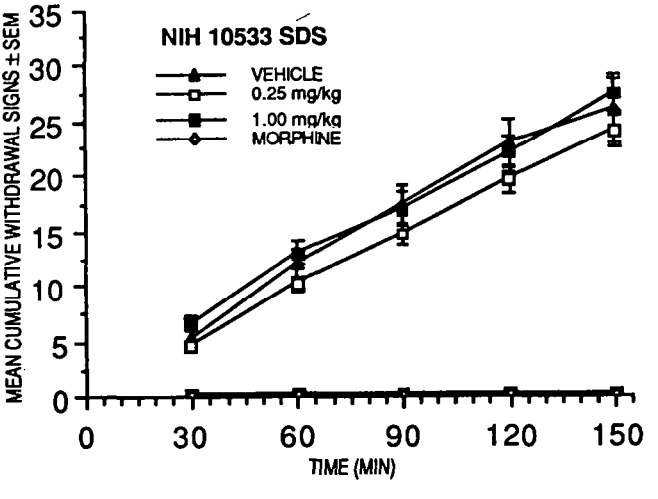


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

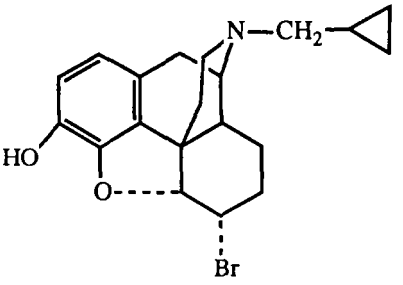
1) TF - 2.5 (1.0 - 6.0)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.2 (0.08 - 0.54)
4) HP - 8.9 (6.0 - 13.2)

MONKEY DATA (SDS)

When tested at 0.25 and 1.0 mg/kg s.c., NIH 10533 neither substituted for morphine nor exacerbated withdrawal (see fig.). Two of three monkeys receiving the high dose seemed to be disoriented or in a stupor and had eyelid ptosis. One monkey also salivated, moved about slowly, and had severe jaw sag.



NIH 10535 6α-Bromo-N-cyclopropylmethyl-4,5 α-epoxymorphinan hydrochloride (Cyclobroxy)



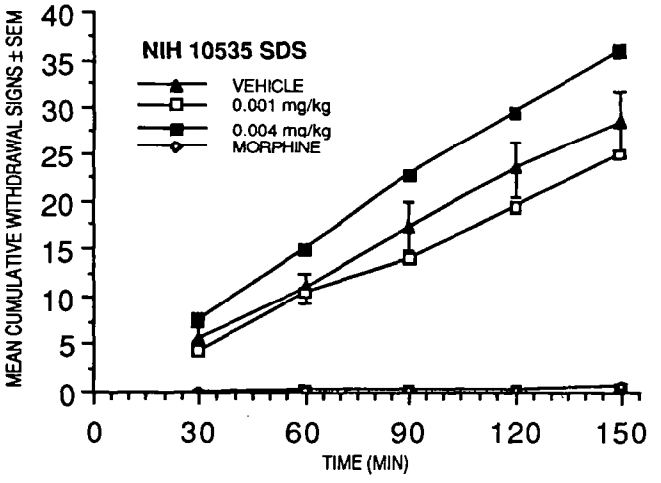
MOUSE DATA- ED or AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M -0.008 (0.002 - 0.025)
3) PPQ - Inactive at 1.0, 10.0 and 30.0
4) HP - Inactive at 20.0

MONKEY DATA

A. (SDS)

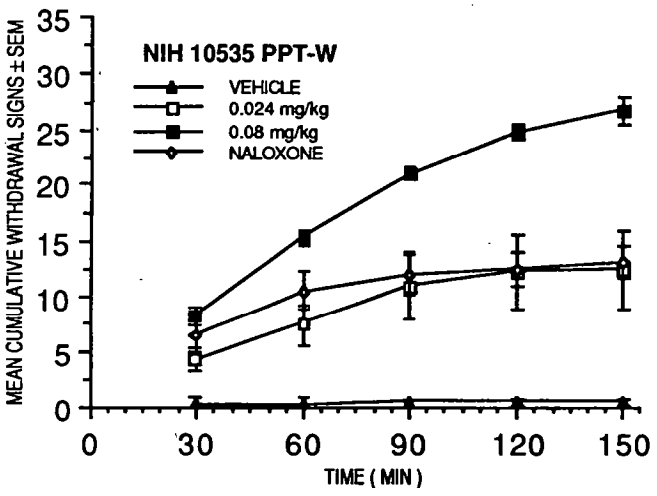
NIH 10535 did not substitute for morphine in the dose range tested (0.001 - 0.004). The drug exacerbated withdrawal (see accompanying fig.). One monkey receiving the highest dose was found dead 4 days later.



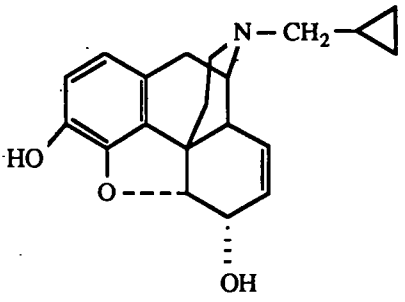
B. (PPt-W)

Dose-related withdrawal signs were noted after NIH 10535 was given (see fig.) The drug behaved as did naloxone regarding onset and duration of action and is considered equipotent with it. Frequent vomiting and tremors were seen at the highest dose.

229-045 0 - 89 - 17 QL3



NIH 10539 N-Cyclopmpylmethylnormoxphine hydrochloride



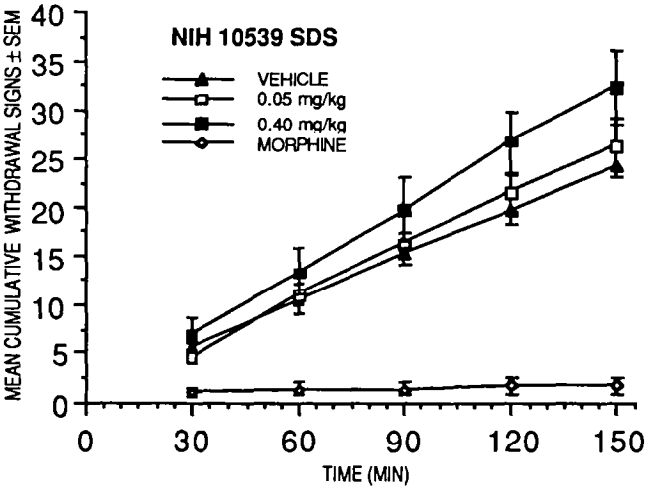
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - 34% at 0.1, 40% at 1.0, 45% at 10.0 and 40% at 30.0[a]
2) TF vs. M -
   1) 11% at 1.0, 20% at 10.0 and 13% at 30.0
   2) 21% at 1.0, 36% at 10.0 and 11% at 30
3) PPQ - 21% at 1.0, 36% at 10.0 and 18% at 30.0[b]
4) HP - 0% at 5.0 and 33% at 20.0

MONKEY DATA

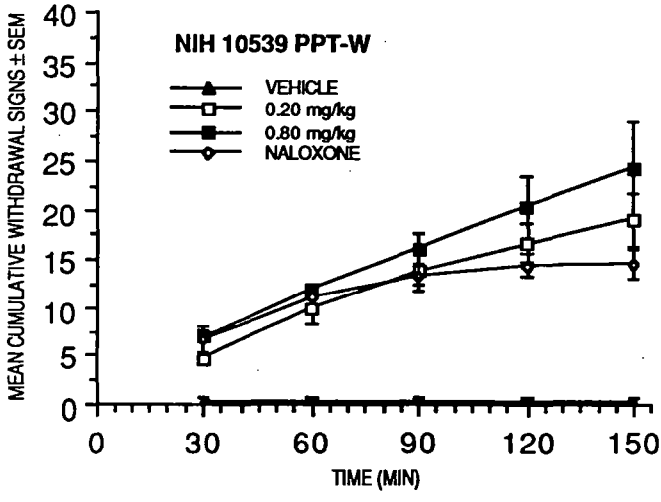
A. (SDS)

NIH 10539 did not substitute for morphine at doses of 0.05 to 0.40 mg/kg; however, the drug may have exacerbated withdrawal (see fig.)

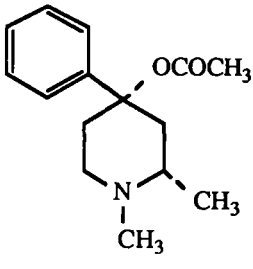


B. (PPt-w)

As shown in the graph labeled NIH 10539, the drug precipitated withdrawal in a dose-related manner. Onset of action can be described as prompt and duration of action was longer than with naloxone. When compared with peak effects with the reference compound, naloxone, the drug was approximately 1/4 as potent.



NIH 10541 (±)-4-α-Acetoxy- 1,2α--dimethyl-4-phenylpiperidine hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - 9.8 (3.3 - 29.0)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 2.2 (0.9 - 5.7)
4) HP - Inactive at 50

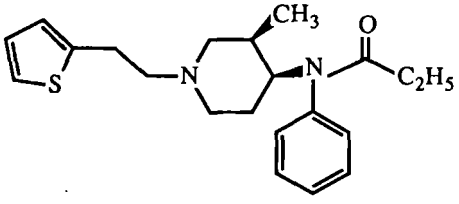
NIH 10543 (-)4α-Acetoxy-1,2-dimethyl-4phenylpiperidine hydrochloride

SEE NIH 10541

MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - 15.2 (6.3 - 36.5)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.9 (0.2 - 3.3)
4) HP - 0% at 5.0 and 33% at 50.0

NIH 10546 cis-N-[1-[2-(2-Thienyl)ethyl]-3-methyl-4-piperidyl]-N-phenyl-propanamide hydrochloride

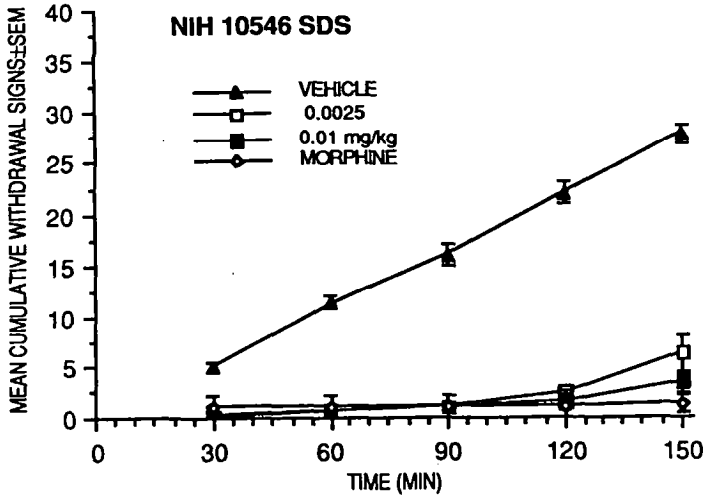


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

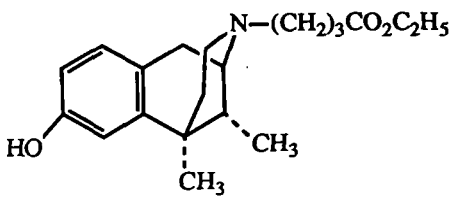
1) TF - 0.004 (0.002 - 0.006)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.0005 (0.0003 - 9.4)

MONKEY DATA (SDS)

NIH 10546 substituted completely for morphine (see fig.). The drug acts promptly but its duration of action (2 hr) is shorter than that of morphine (4 hr). At the highest dose, body and jaw sag, ataxia, slowing and scratching were noted. At peak effect the drug is about 1000 x more potent than morphine.



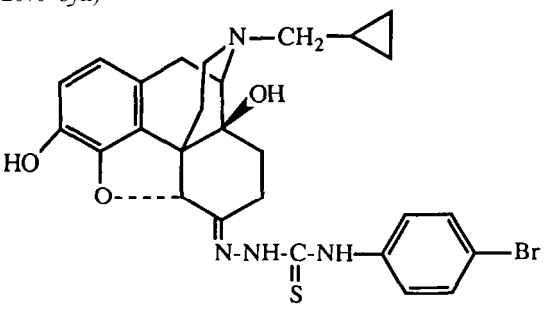
NIH 10548 (±)-2-(3-Carbethoxyptvpyl)-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/sc or % change)

1) TF - 0% at 1.0, 4% at 10.0 and 20% at 30.0
2) TF vs. M - Inactive at 1.0, 3.0, 10.0 and 30.0
3) PPQ - 7.1 (2.4 - 21.4)
4) HP - Inactive at 20

NIH 10550 1-(N)-*p* -Bromophenylnaltrexone thiosemicarbazone (80% anti: 20% syn)



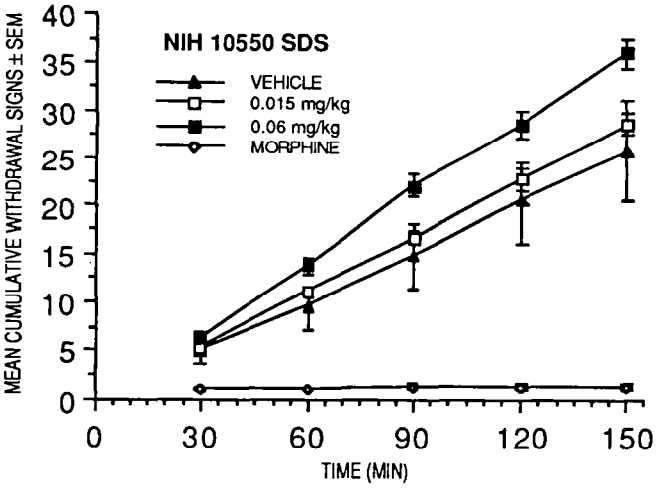
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/sc or % change)

1) TF -Inactive at 1.0, 3.0, 10.0 and 30.0
2) TF vs. M - 0.05 (0.03 - 0.09)
3) PPQ -Inactive at 1.0, 10.0 and 30.0
4) HP - Inactive at 20.0

MONKEY DATA

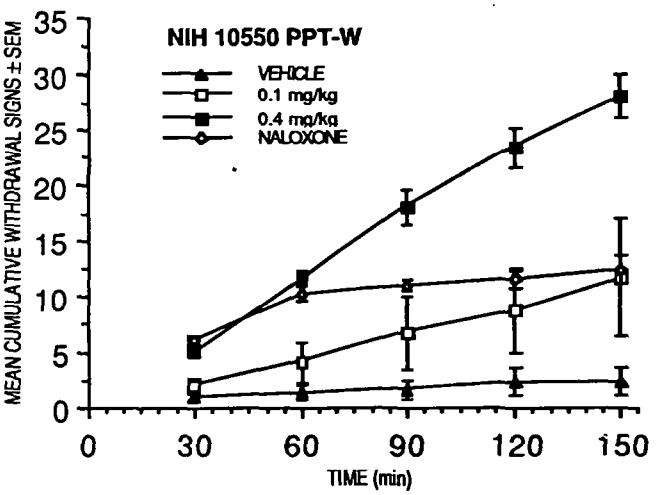
A. (SDS)

This compound did not substitute for morphine in the dose range 0.015 - 0.06 mg/kg. The drug exacerbated withdrawal at the highest dose (see fig.). In addition, at the highest dose, head and body jerks, restlessness and frequent episodes of retching were noted.

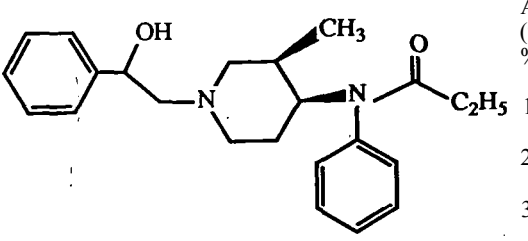


B. (PPt-W)

As can be seen in the accompanying fig., NIH 10550 precipitated withdrawal in a dose-related manner. Onset of action was slow but duration of action was longer than that of naloxone. At peak effect, the drug appears to be equal in potency to naloxone. Frequent retching, vomiting, and body jerks were noted at the high dose. In addition, one monkey stretched in a "cat-like" manner and another bit itself.



NIH 10551 cis-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl4piperidyl]-N-phenylpropanamide hydrochloride

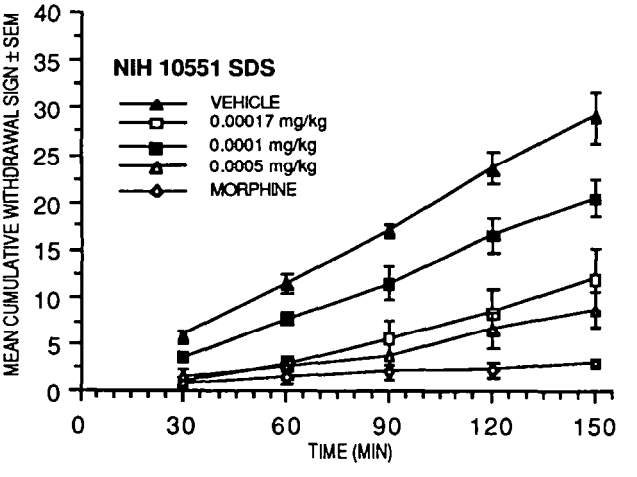


MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg sc or % change)

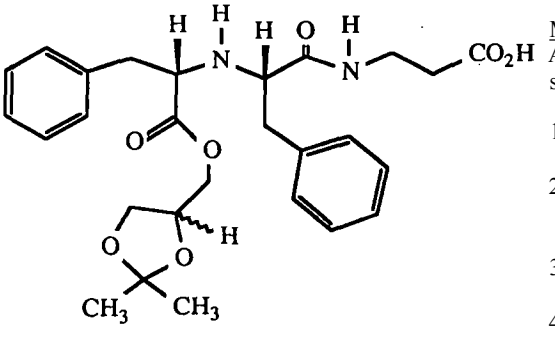
1) TF - 0.0002 (0.0001 - 0.0005)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.00013 (0.00006 - 0.0003)
4) HP - <0.003

MONKEY DATA (SDS)

In a previous study, we noted that NIH 10551 substituted completely for morphine and at peak effect the drug was at least 6000 x morphine. On the basis of this study we and now estimate that this drug is 25,000 x more potent than morphine sulfate. As shown in the fig., the drug acted promptly and duration of action was abut 90 min.



NIH 10552 N-[N-[L-[1-[D.L-(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]-carbonyl]-2-phenylethyl]-L-phenylalanyl]-ß-alanine



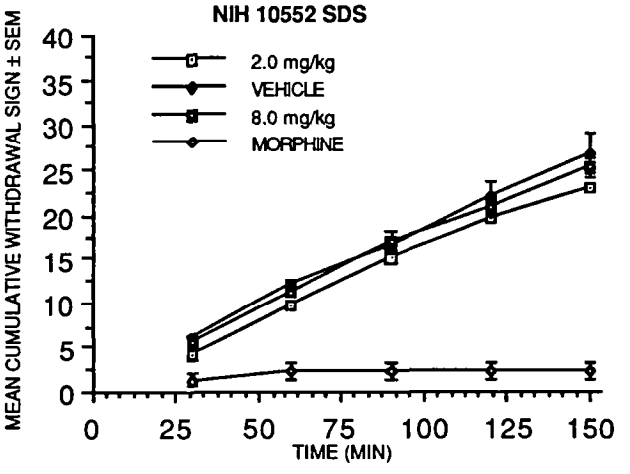
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

1) TF - Inactive at 0.1, 1.0 10.0 and 30.0[a]
2) TF vs. M - 4% at 1.0, 0% at 10.0, 12% at 30.0 and 20% at 60.0[a]
3) PPQ - Inactive at 1.0, 10.0 and 30.0[a]
4) HP - Inactive at 20.0

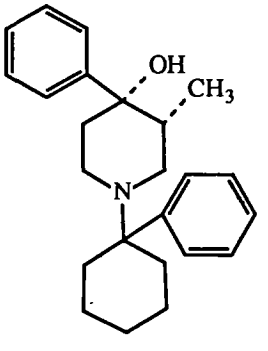
Vehicle - Tween 80 and H_2O

MONKEY DATA (SDS)

In the dose range 2.0 - 8.0 mg/kg, NIH 10552 did not substitute for morphine and behaved essentially as did the vehicle control (Tween 80 + H_2O). See accompanying fig.



NIH 10553 (+)-4-Hydroxy-3-methyl-4-phenyl-1-(1-phenylcyclohexyl) piperidine (trans 3-methyl, 4-phenyl) hydrochloride

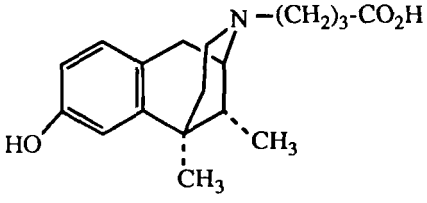


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

1) TF - 8.7 (4.1 - 18.3)[a]
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.7 (0.3 - 2.1)[a]
4) HP - 16% at 20.0

[a]Vehicle - 5% Tween80 in H_2O

NIH 10555 (±)-2-(3-Carboxypropyl)-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 11.4 (2.9 - 44.6)
4) HP - Inactive at 20.0

NIH 10556 (+)-5.9α-Dimethyl-2'-hydroxy-2- *n* -propyl-6,7-benzomorphan hydrochloride

SEE NIH 7549

MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

1) TF - Inactive at 1.0, 3.0, 10.0 and 30.0[a]
2) TF vs. M - 0% at 1.0, 6% at 10.0 and 20% at 30.0
3) PPQ -Inactive at 1.0, 10.0 and 30.0
4) HP - Inactive at 20.0

[a]slight ataxia

NIH 10557 (-)-5,9α-Dimethyl-2'-hydroxy-2- *n* -propyl-6,7-benzomorphan hydrochloride

See NIH 7549

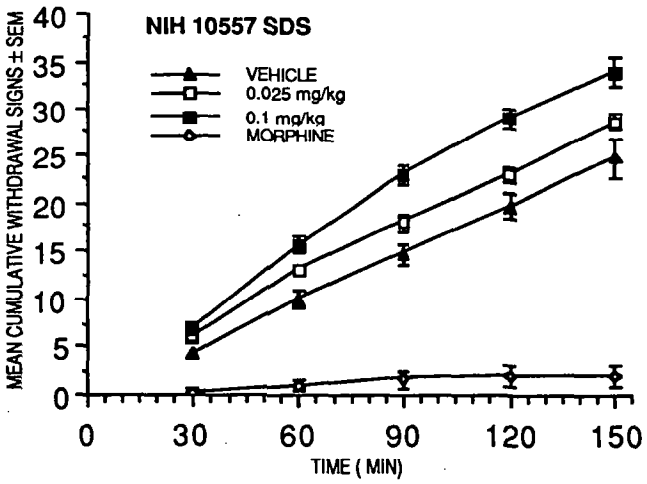
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - 0.4 (0.2 - 1.0)
3) PPQ - 22.0 (16.0 - 30.1)
4) HP - Inactive at 20.0

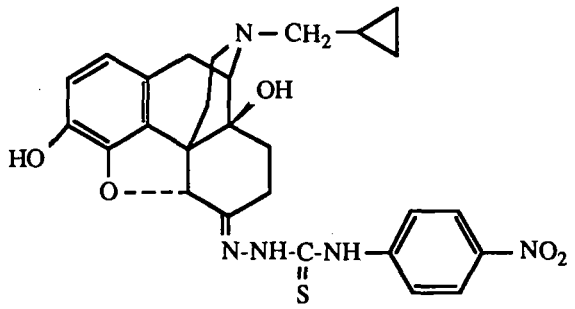
[a]Ataxia at 10.0 and 30.0

MONKEY DATA (SDS)

As illustrated in the fig., NIH 10557 did not substitute for morphine. Instead, it appeared to exacerbate withdrawal at both doses (0.025 and 0.1 mg/kg).



NIH 10558 1-(N)-*p*-Nitrophenylnaltrexone thiosemicarbazone (80% anti: 20% syn)

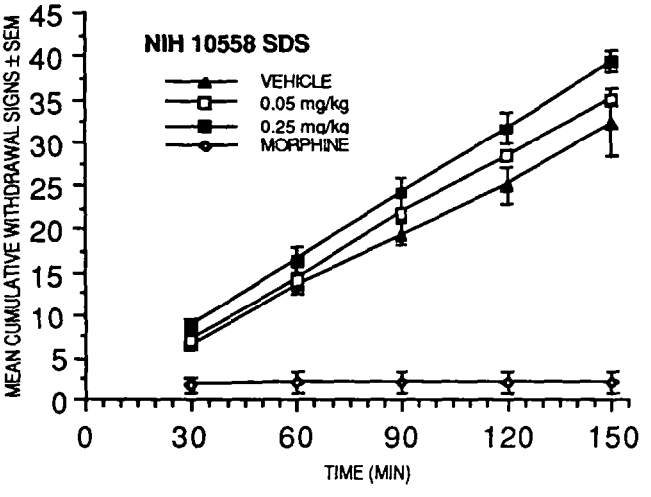


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

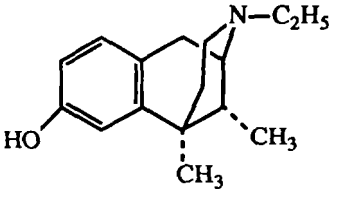
1) TF - Inactive at 1.0, 10.0 and 15% at 30.0
2) TF vs. M - 0.05 (0.03 - 0.08)
3) PPQ - 0.003 (0.001 - 0.007)
4) HP - Inactive at 20.0

MONKEY DATA (SDS)

As shown in the fig., at doses of 0.05 and 0.25 mg/kg. NIH 10558 did not substitute for morphine. Instead, the drug exacerbated withdrawal. Onset of action was prompt and duration of action was > 150 min. At the highest dose, vomiting was observed. This sign is not normally seen in vehicle controls in the SDS test. In addition, ataxia, slowing, tremors and "cat-like" stretching were noted.



NIH 10559 (+)-5,9α-Dimethyl-2-ethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



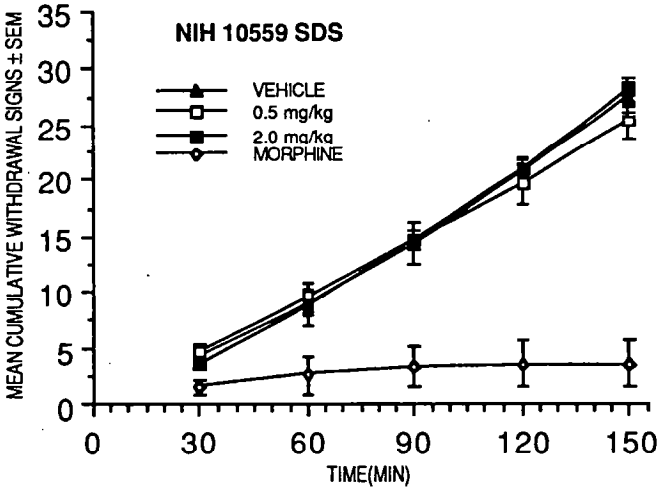
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

1) TF - Inactive at 0.1, 1.0 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 33.5 (15.5 - 72.1)
4) HP - Inactive at 20.0

[a]Straub Tail at 10.0 and 30.0. Tail-flick latencies were shorter than those of control mice. Also ataxia and clonic convulsions were noted at 10.0 and 30.0.

MONKEY DATA (SDS)

As illustrated in the fig., NIH 10559 did not substitute for morphine at 0.5 and 2.0 mg/kg/s.c. However, ataxia, sagging, slowing, biting (hands and feet), restlessness, frequent retching, rapid respiration, body spasms and loud vocalizations were noted. This is an unusual drug.



NIH 10560 (-)-5,9α-Dimethyl-2-ethyl-2'-hydroxy-6,7-benzomorphan hydrochloride

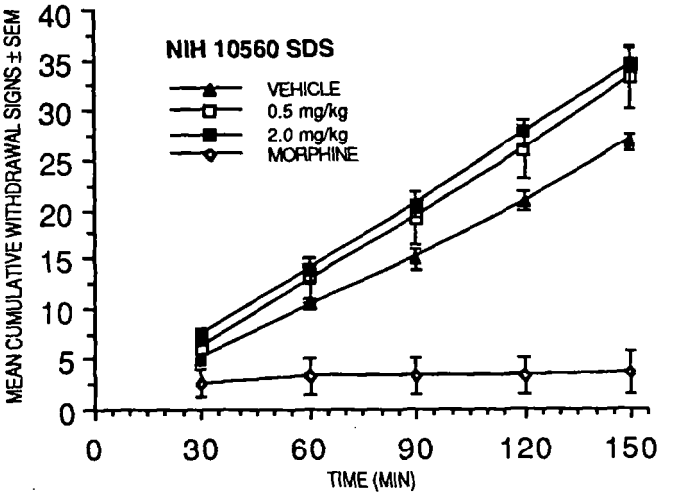
SEE NIH 10559

MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

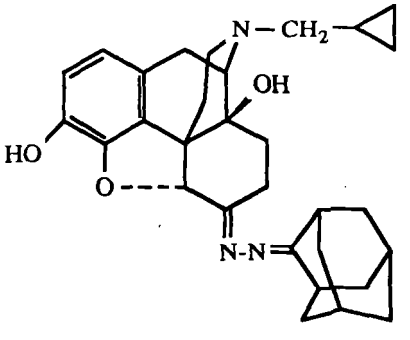
1) TF - Inactive at 1.0 10.0 and 30.0
2) TF vs. M - 3.7 (1.8 - 7.8)
3) PPQ - 1.4 (0.5 - 4.1)
4) HP-

MONKEY DATA (SDS)

At 0.5 and 2.0 mg/kg s.c., NIH 10560 did not substitute for morphine and may have exacerbated withdrawal. Frequent retching and head tremors were observed. One monkey receiving the highest dose had seizures 9 hrs after receiving this compound.



NIH 10561 6-(2-Adamantyl)naltrexonazide (80% anti : 20% syn)

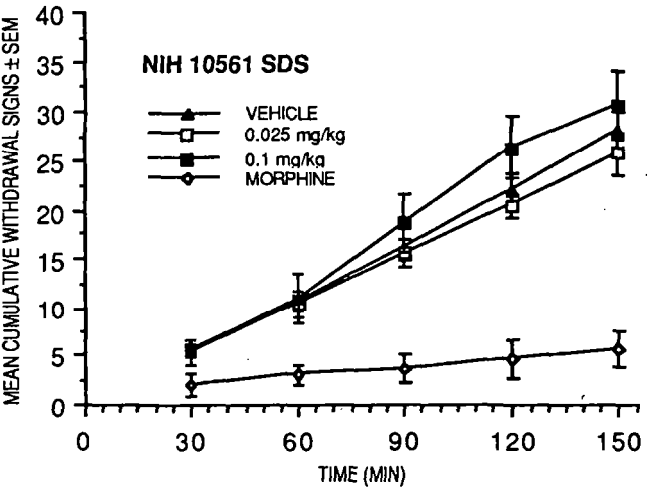


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

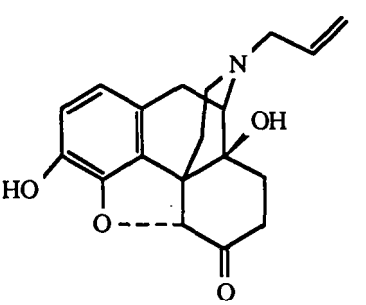
1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. - 0.03 (0.01 - 0.07)
3) PPQ - 29% at 0.1, 31% at 0.3. 17% at 1.0, 37% at 10.0 and 37% at 30.0
4) HP - Inactive at 20.0

MONKEY DATA (SDS)

NIH 10561 did not substitute for morphine. Instead, at the highest dose (0.1 mg/kg) the drug exacerbated withdrawal. The data are illustrated in the accompanying fig.



NIH 10562, 7890 Naloxone hydrochloride

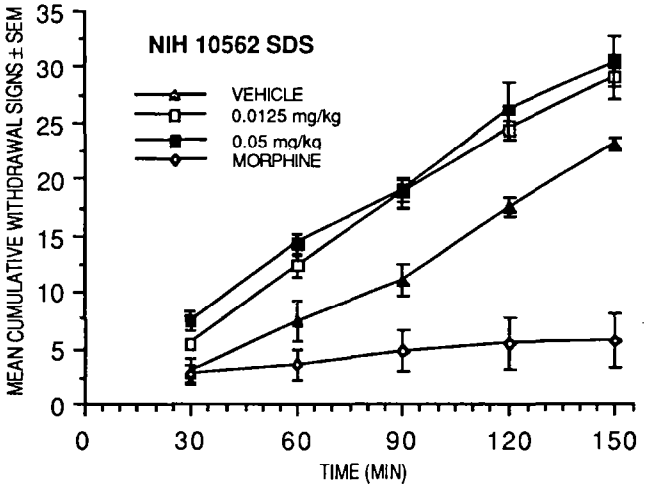


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

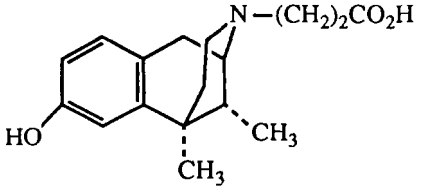
1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. - 0.03 (0.01 - 0.1)
3) PPQ - 17% at 1.0, 34% at 3.0, 45% at 10.0 and 60% at 30.0
4) HP - 20% at 50.0
5) N - 12% at 100.0

MONKEY DATA (SDS)

As shown in the fig. both doses of NIH 10562, namely 0.05 and 0.0125 mg/kg, exacerbated withdrawal. Onset of action was prompt and duration of action was longer than 150 min. In the SDS Test, duration of action of antagonists is much longer than in PPt-W Tests. The preceeding statement was added after the structure was revealed to us.



NIH 10564 (±)-2-(2-Carboxyethyl)-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. - Inactive at 0.1, 1.0, 3.0, 10.0 and 30.0
3) PPQ - 1. 1.4 (0.3 - 6.4)
   2. 16% at 0.3, 34% at 1.0, 61% at 5.0, 50% at 10.0 and 47% at 30.0
4) HP - Inactive at 50

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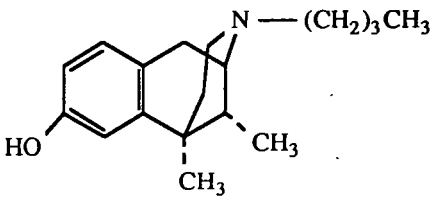
NIH 10566 (-)-2-*n*-Butyl-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. - 1.5 (0.5 - 4.2)
3) PPQ- 1. 26% at 0.1, 40% at 1.0, 37% at 10.0 and 40% at 30.0
   2. 0% at 0.3, 13% at 1.0, 62% at 3.0 and 90% at 10.0
4) HP-

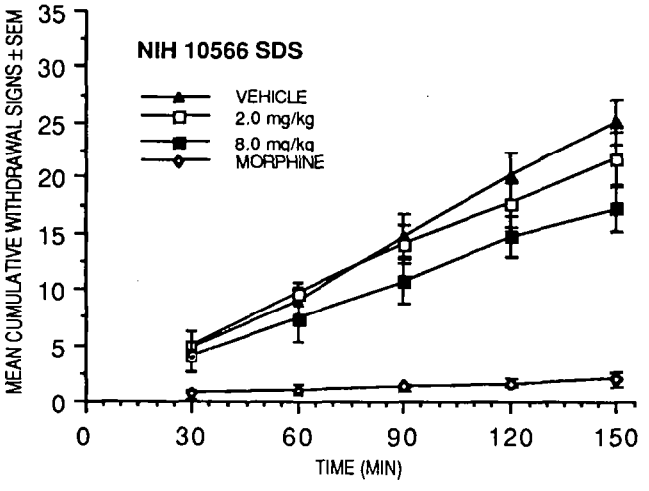
MONKEY DATA

A. (SDS)

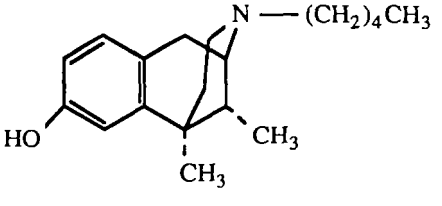
NIH 10566 reduced slightly the total number of withdrawal signs (see fig.) including vocalization to abdominal palpation. Abdominal relaxation was also observed. However, the drug produced severe ataxia, slowing, eyelid ptosis and, paradoxically, increased the incidence of retching and vomiting, wet dogs and tremors. Apparently, this compound has a complex pharmacology.

B. NON-DEPENDENT MONKEY

In a normal non-dependent monkey NIH 10566, after a total dose of 6 mg/kg s.c., the signs slowing, ptosis, ataxia, stupor, profuse salivation, jaw and body sag were noted. Naloxone (0.05 mg/kg at 30 min, 0.2 mg/kg at 45 min and 0.2 mg/kg at 60 min) did not reverse these effects.



NIH 10568 (+)-5,9α-Dimethyl-2'-hydroxy-2- *n* -pentyl-6,7-benzomorphan hydrochloride

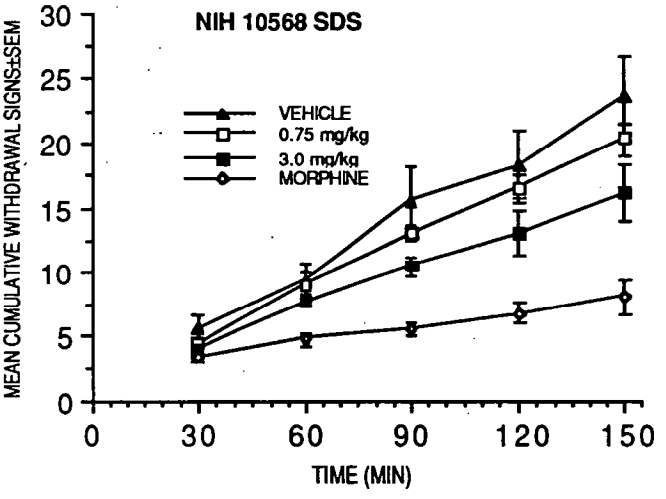


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

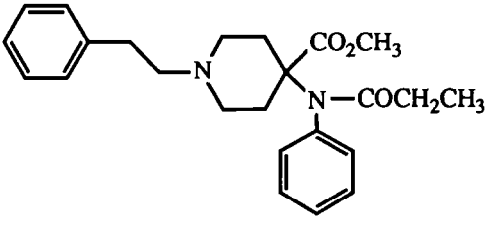
1) TF - 21.3 (15.0 - 30.2)
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 5.6 (1.8 - 1.6)
4) HP - Inactive at 20.0

MONKEY DATA (SDS)

This compound reduced the total number of withdrawal signs but did not substitute for morphine (see fig.). At the highest dose, some abdominal relaxation was noted along with failure to vocalize when palpated; otherwise the monkeys displayed the same incidence of the other withdrawal signs.



NIH 10570 Carfentanil citrate



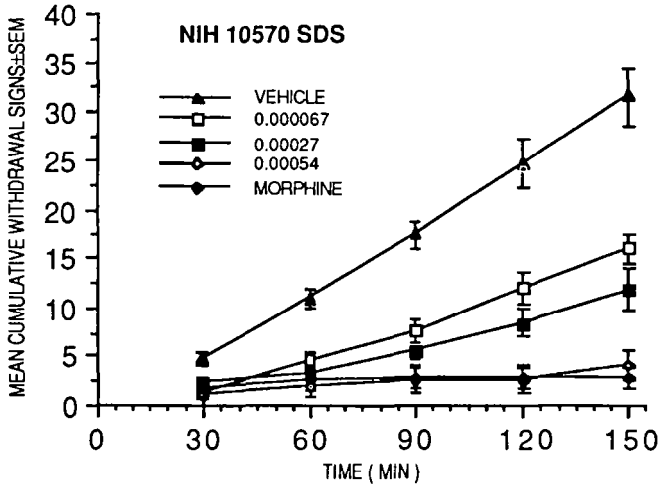
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

1) TF - 0.00021 (0.00008 - 0.005)
2) TF vs. M. - Inactive at 0.0001 and 0.003
3) PPQ - 0.000058 (0.000029 - 0.00011)
4) HP - < 0.0004

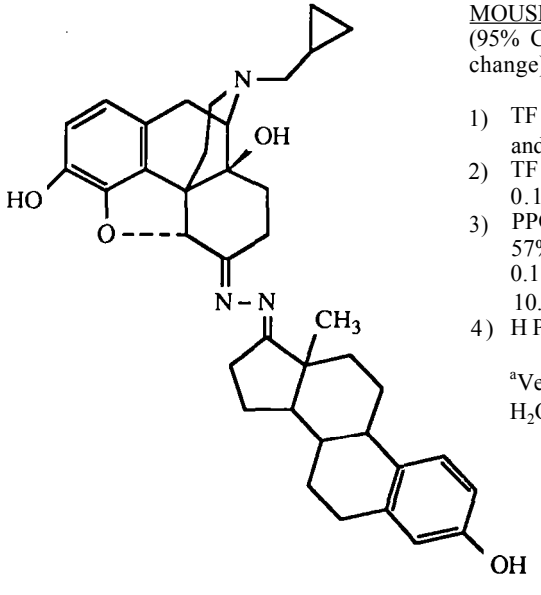
Special Naloxone Antagonism of TF ED80 of 10570: AD50 = 0.035 (0.017 - 0.074)

MONKEY DATA (SDS)

As shown in the accompanying fig.. NIH 10570 substituted completely for morphine in withdrawn morphine-dependent monkeys in a dose-related manner. Onset of action was as rapid as with morphine but duration was shorter. The drug is considered to be at least 25,000 x more potent than morphine. The potency estimate is predicated, in part, on the stated concentration of the stock solution submitted to us namely, 2.7 mg/9.0 ml.



NIH 10572 Mixed azine of naltrexone and estrone



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M. - 0.03 (0.01 - 0.1)[a]
3) PPQ - 23% at 0.001, 57% at 0.01, 49% at 0.1, 23% at 1.0. 9% at 10.0 and 14% at 30.0[a]
4) HP -

[a]Vehicle 5% Tween80 in H_2O

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AFFILIATION

Department of Pharmacology and Toxicology Medical College of Virginia, Virginia Commonwealth University, Box 613, Richmond, VA 23298

SUBJECT INDEX

In order to simplify the Index, the subject subheadings along with page numbers can be found under both the chemical name and the NIH number.

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AUTHOR INDEX

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