1978

EFFECTIVENESS OF BUTYROPHENONES AND RELATED DRUGS IN NARCOTIC WITHDRAWAL IN THE RAT

Martin Dennis Hynes III
University of Rhode Island

EFFECTIVENESS OF BUTYROPHENONES AND RELATED DRUGS IN NARCOTIC WITHDRAWAL IN THE RAT

BY

MARTIN DENNIS HYNES III

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

IN

PHARMACOLOGY AND TOXICOLOGY
(Pharmaceutical Sciences)

UNIVERSITY OF RHODE ISLAND

1978

DOCTOR OF PHILOSOPHY DISSERTATION

OF

MARTIN DENNIS HYNES III

Approved:

Dissertation Committee

Major Professor ______________________

____

____

____

____

Dean of the Graduate School

UNIVERSITY OF RHODE ISLAND

1978

BUTYROPHENONES IN NARCOTIC WITHDRAWAL

ABSTRACT

Narcotic dependence was established by continuous intravenous infusion of gradually increasing doses of morphine. Several days at the terminal concentration of morphine (100 mg/kg/day) was allowed prior to withdrawal. Morphine withdrawal body shakes were reliably observed upon the termination of continuous morphine infusion. The administration of morphine, methadone or fentanyl, potent narcotic drugs, reliably abolished the occurrence of withdrawal body shakes 8 hours after the termination of morphine infusion. The narcotic antagonist naloxone or pentazocine had no significant effect on the rate of occurrence of withdrawal body shakes; however, there was a slight trend towards an increase in the frequency of this withdrawal index. A wide variety of neuroleptics were investigated for their ability to reduce withdrawal. The order of potency for reduction of withdrawal body shakes on a mg/kg basis for those neuroleptics tested was spiperone, benperidol, +butaclamol, loxapine, oxiperomide, haloperidol, spiramide, chlorpromazine, trifluoperazine, pimozide and pipamperone. The dopaminergic agonists amphetamine, apomorphine and L-DOPA were also found to dose dependently reduce withdrawal body shakes. Azaperone, a butyrophenone possessing high alpha noradrenergic blocking potency, reduces withdrawal body shakes in a dose related manner, while the alpha adrenergic blocker phenoxybenzamine

and the beta adrenergic blocker propronalol failed to reduce withdrawal shakes to any significant extent. Dose dependent decreases in morphine withdrawal body shakes were produced by clonidine and desmethylimipramine, adrenergic agonists. Reserpine and alpha-methyl-p-tyrosine also reduced withdrawal shakes, but the effect was dose dependent for only reserpine. The serotoninergic agents fluoxetine, 5-HTP and methysergide were without effect on the occurrence of withdrawal body shakes at the doses tested. A slight but non-significant increase in the rate of withdrawal shakes was observed after the administration of the anticholinergic drugs atropine, benztropine, dexetimide and scopolamine. Conversely, the cholinergic agonists physostigmine and pilocarpine, reduced withdrawal body shakes in a dose related fashion. Modification of gabaminergic system by the administration of bicuculline, depakene and picrotoxin had no significant effect. Chlordiazepoxide, flurazepam and pentobarbital, sedative-hypnotic agents, did not produce dose dependent effects on the occurrence of withdrawal shakes. However, the highest dose of each sedative hypnotic employed did reduce withdrawal body shakes. The exact mechanism by which neuroleptics reduce withdrawal is not known. Manipulation of each of the transmitters effect by neuroleptics indicates that dopamine and acetylcholine are involved in mediating the reduction in withdrawal body shakes. The fact that the antiwithdrawal activity of certain neuroleptics is reversed by the narcotic antagonist naloxone implicates opiate receptor mechanisms.

However, not all neuroleptics were antagonized by naloxone, suggesting that neuroleptics may be working at more than one site to reduce withdrawal. Thus neuroleptics may be reducing withdrawal body shakes by several different mechanisms. The data gathered from these studies suggest a role for cholinergic, dopaminergic and narcotic mechanisms in the anti-withdrawal activity of neuroleptic drugs.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to his sister Catherine and to his aunt Miss Ann G. Lynch for their generosity, inspiration and understanding.

The guidance provided by Dr. Harbans Lal during the course of these studies is sincerely appreciated. The author wishes to convey his appreciation to the members of his committee and the faculty of the Department of Pharmacology and Toxicology of the University of Rhode Island. The author is also grateful for the help and understanding provided by his fellow students in the Department of Pharmacology and Toxicology.

The invaluable help of Mr. Michael D. McCarten, Mr. Frank D. Sistare, Mr. E. Francis Cook and Mrs. Nadylis Wood is gratefully acknowledged. The author wishes to thank Mrs. Edith Williams for typing this manuscript and many others over the past five years.

In addition, the author acknowledges the financial support provided by a Teaching Assistantship from the Department of Pharmacology and Toxicology and a Fellowship from the National Institute of Mental Health (1 F31 MH05846-01) without which these studies would have never been possible.

To my Mother and

the Memory of my Father

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I

INTRODUCTION

Treatment of narcotic dependence invariably requires the discontinuation of the abused narcotic. Discontinuation of narcotic drugs results in an abstinence syndrome which is so aversive that patients hesitate undergoing treatment and continue using illicit narcotics. Presently, methadone is employed to prevent the narcotic abstinence syndrome. Although methadone is an effective agent in blocking withdrawal, it is a narcotic drug (Jaffe and Martin, 1975) and is therefore likely to perpetuate the underlying cause of narcotic dependence. Thus chemotherapeutic approaches to the treatment of narcotic addiction require the development of non-addicting drugs which are able to successfully relieve the withdrawal syndrome. This research was undertaken to investigate the effectiveness of butyrophenone neuroleptics and related compounds in relieving morphine withdrawal body shakes in the rat. The mechanism by which these drugs inhibit the narcotic withdrawal syndrome was also explored.

Interest in the use of butyrophenones in narcotic withdrawal was stimulated by their known effectiveness in blocking brain dopamine receptors. Several lines of evidence have suggested that supersensitivity of dopamine receptors is intimately involved in the development of

narcotic dependence. This evidence has been in part generated by studies of morphine withdrawal aggression. Withdrawal from morphine elicits intense aggression upon grouping of dependent rats. Pretreatment with L-dihydroxyphenylalanine (L-DOPA), amphetamine or apomorphine enhanced that aggression severalfold. These agents are known to stimulate brain dopamine receptors. Haloperidol, a potent dopaminergic blocking agent, inhibits this aggression (Puri and Lal, 1973). Several other studies have suggested a role for brain dopamine in narcotic dependence. Lesions placed stereotaxically in monkeys at the origin of the nigrostriatal system and the tegmenti ventralus, known to be mainly dopaminergic pathways (Anden et al., 1964) abolish craving for morphine and the signs of withdrawal (Pozuelo and Kerr, 1972). Spontaneous aggression is reliably seen when morphine dependent rats are grouped after 30 days of abstinence. Electrolytic lesions of the nigrostriatal nerve tract abolished this morphine withdrawal aggression. A low dose of the dopaminergic stimulant apomorphine was effective in reinstating the withdrawal aggression in these lesioned animals (Gianutsos et al., 1973; 1974). For a detailed review of narcotic dependence and brain dopamine see Lal (1975).

More recently, another line of evidence became available to suggest that butyrophenones may serve as nonaddicting substitutes for narcotic drugs in the treatment of withdrawal. Clay and Brougham (1975) reported a competition for binding at selective brain sites between the butyrophenone, haloperidol

and the narcotic antagonist, naloxone, suggesting that haloperidol binds with narcotic-specific receptors. Creese et al. (1976) and Leysen et al. (1976) have shown marked affinity between several neuroleptics and opiate binding sites to suggest that the binding was due to a structural feature common to both narcotics and butyrophenone type neuroleptics. Their conclusions are supported by a number of pharmacological similarities between neuroleptics and narcotics (Lal et al., 1975; Lal et al., 1976). It therefore became apparent that butyrophenones and related drugs are likely to block withdrawal signs. Results from preliminary experiments with haloperidol in laboratory animals (Lal et al., 1971; Lal and Numan, 1976; Martin et al., 1974) and human patients (Karkalas and Lal, 1973) were encouraging.

The present study was undertaken to investigate butyrophenones and related drugs in reducing morphine withdrawal body shakes, an important sign of narcotic withdrawal in rats. Morphine withdrawal body shakes are employed as an index of withdrawal since they are a consistent and objective sign of narcotic withdrawal (Gianutsos et al., 1975, Martin et al., 1963). The anti-withdrawal mechanism of butyrophenones were explored in detail. The specific hypothesis to be tested with respect to the anti-withdrawal mechanisms of neuroleptic drugs are that these drugs interfere with transmitter substances in the brain thereby reducing withdrawal intensity or alternatively these drugs bind to narcotic receptors to suppress withdrawal.

Neuroleptics have many important and well demonstrated effects on neurotransmitters such as dopamine, norepinephrine, serotonin, γ-aminobutyric acid, and acetylcholine. It is conceivable that the anti-withdrawal actions of neuroleptic agents are mediated by their effects on the aforementioned neurotransmitters. Alternatively, in view of the fact that neuroleptic drugs have been shown to bind to opiate receptor sites within the central nervous system it is possible that this binding is responsible for the anti-withdrawal activity of neuroleptics.

In order to test the hypothesis that the anti-withdrawal mechanism of neuroleptics are related to effects on neurotransmitters it was necessary to modify the activity of each transmitter individually. This was accomplished by studying the effects of specific neurotransmitter agonist and antagonist on morphine withdrawal body shakes. To determine if anti-withdrawal activity is due to antagonism of dopamine receptor, a number of anti-dopamine drugs were adminstered. The dopaminergic antagonists employed were haloperidol, benperidol, spiroimide, oxiperimide, spiperone, loxapine, chlorpromazine, trifluoperazine, pimozide, butaclamol and pipamperone. Stimulation of dopamine receptors was accomplished by the administration of apomorphine, L-DOPA and amphetamine. Norepinephrine receptors were antagonized by the use of propranolol, phenoxybenzamine and azaperone. Clonidine, imipramine, tranylcypromine and amitriptyline were the noradrenergic agaonists employed. Synthesis and storage of the

catecholamines was inhibited by the administration of alpha-methy-p-tyrosine and reserpine, respectively. Serotonin activity was mimicked by 5-hydroxytryptophan (5-HTP) and fluoxetine. Antagonism of serotonergic receptors was achieved by administration of methysergide. Acetylcholine was also systematically explored. The following anticholinergic drugs were investigated - scopolamine, atropine, benztropine and dexetimide while the cholinomimetics employed were pilocarpine, deanol, choline chloride, oxotremorine and physostigmine. Additionally, the effect of the gabaminergic agents bicuculline, depakene and picrotoxin were investigated for their effects on morphine withdrawal body shakes. Chlordiazepoxide, flurazepam and pentobarbital, sedative-hypnotic agents, were administered to investigate the possibility that neuroleptics induce these anti-withdrawal effects by their ability to produce sedation. A dose response curve was established for all drugs employed in modifying neurotransmitter activity. These experiments will determine if reduction of withdrawal intensity is due to modification of dopamine, norepinephrine, serotonin, acetylcholine, or γ-aminobutyric acid activity. A series of saline injections were interspersed with drug trials to control for a spontaneous decrease in withdrawal intensity, the effect of handling and the injection procedure.

The hypothesis that the anti-withdrawal activity of neuroleptics is mediated by an interaction with narcotic binding sites or release of endorphins was tested with the

aid of naloxone a specific narcotic antagonist. Neuroleptics which were found to reduce morphine withdrawal body shakes were interacted with naloxone. Theoretically, if neuroleptic binding to opiate receptors mediates the anti-withdrawal activity then this effect should be reversed by the narcotic antagonist, naloxone. The maximum effective dose of each neuroleptic was administered in conjunction with naloxone to determine if the effect was reversible by this narcotic antagonist.

This research has significance in that evidence for the role of various neurotransmitters in the expression of morphine withdrawal body shakes will be gathered. It will give expanded insight into the role of dopamine systems in narcotic dependence and withdrawal. Drugs which have potential for use in reducing the narcotic withdrawal syndrome in humans will hopefully be found. Additionally, further information on the pharmacological mode of action of narcotics and neuroleptics will be achieved.

II

LITERATURE SURVEY

A. The Narcotic Withdrawal Syndrome in the Rat

The study of the pathophysiology of narcotic dependence requires an appropriate animal model . The rat has proven to be a useful subject for the study of the mechanism of narcotic addiction since it is easily made dependent on narcotics. Dependence in the rat has been produced by chronic intraperitoneal injections of morphine sulfate (Martin et al., 1963), intravenous injections (Numan et al., 1975), subcutaneous implantation of morphine alkaloid pellets (Collier et al., 1972; Wei and Way, 1975), administration of narcotics in the drinking water (Gianutsos et al., 1975a) or continuous intraperitoneal infusion (Teiger, 1974; Harris, 1976).

The withdrawal syndrome exhibited by the rat is consistent, reliable and can be objectively measured (for review see Gianutsos et al., 1975a). This syndrome may be brought about either by administration of a narcotic antagonist or by the abrupt termination of chronic morphine administration. The withdrawal precipitated by an antagonist is immediate, shortlived and quantifiable. However, except for special circumstances, it is never clinically encountered in man. In contrast, the spontaneous withdrawal caused by cessation of narcotic drug administration has wider application to research and the screening of antiabstinence drugs (Gianutsos et al., 1975a).

1. Withdrawal Signs, Acute and Protracted

The withdrawal syndrome can be divided into two phases, primary abstinence and protracted abstinence (Martin and Sloan, 1971; Martin, 1972). The primary abstinence syndrome begins to appear 12-24 hours after discontinuing chronic morphine. It consists of the following symptoms: "wet shakes," loss of body weight, decreased metabolic rate, increased urination and defecation, decreased fluid consumption, hypothermia, sleeplessness, irritability on handling, enhanced excretion of norepinephrine and epinephrine. In addition, there is a decline in the voltage output of the EEG (Khazan et al., 1967), writhing (Buckett, 1964), social aggression (Lal et al., 1971a), ptosis and piloerection (Wikler et al., 1960). This syndrome lasts for approximately 72 hours after which the syndrome begins to change (Gianutsos et al., 1975a). During the protracted phase, food and water intake, body weight, temperature and locomotor activity increase above normal levels and may persist for several months (Martin et al., 1963). Additionally, there is a continuation of wet shakes (Martin et al., 1974) and EEG abnormalities (Khazan and Colasanti, 1971) during the protracted phase. Many of the other symptoms begin to return to normal levels during this time (Gianutsos et al., 1975a).

2. Morphine Withdrawal Body Shakes

Body shakes constitute a widely used and reliable criterion of narcotic withdrawal (Martin et al., 1963; Gianutsos et al., 1975a). They are defined as violent shaking

movements of the head and/or body of the rat which resemble the action of an animal that has been drenched with water and are readily distinguishable from tremors or jerky movements (Gianutsos et al., 1975a). The frequency at which wet shakes occur is greatest shortly after handling the rat or after changing its place of residence and decreases with time (Gianutsos et al., 1975a). Withdrawal body shakes are most intense 24 hours after the last morphine injection and begin to decrease by 72 hours of withdrawal (Gianutsos et al., 1975a).

Withdrawal body shakes have been shown to occur upon abrupt termination of morphine treatment (Martin et al., 1963; Gianutsos et al., 1975a), the administration of narcotic antagonist (Wei et al., 1973c) and to a lesser extent in drug naive rats (Martin et al., 1963; Gianutsos et al., 1975a). Rats rendered chronically epileptic by the implantation of cobalt in the right parietal cortex exhibited a significant number of wet dog shakes (Colasanti et al., 1975). Wet dog shakes have also been elicited in non-addicted rats without concomitant symptoms by the administration of [(4-chlorobenzylidene-amino)-oxy)] isobutyric acid (Jahn and Mixich, 1976). Additionally, repetitive shaking movements can be elicited in pentobarbital anesthetized rats after immersion in water. The frequency of shakes is inversely related to the temperature of the water and is a function of the drop in body temperature (Wei et al., 1973b). However, hypothermia per se was not found to be a necessary condition for

eliciting shaking behavior (Wei et al., 1973b). This wet shake response was facilitated by stimuli to the ear (Wei et al., 1973b). The endogenous morphinomemetic brain peptides metenkephalin and α, B and γ endorphins have been found to produce acute episodes of wet-dog-like shaking behavior in animals not previously treated with drugs. This response began within 90 seconds after lateral ventricular injection (Bloom et al., 1976). These shaking episodes were found to be more intense and prolonged with the α and γ -endorphins. The administration of naloxone completely abolished these wet-dog-shaking episodes from lateral ventricular peptide injections (Bloom et al., 1976). Intraperitoneal administration of n-dipropylacetate (DPA) to naive rats produced abstinence behavior which included shaking (de Boer et al., 1977). This behavioral effect of DPA, which increases GABA concentrations in the brain, is suppressed by bicuculline and morphine (de Boer et al., 1977).

Several investigations have been undertaken to study the neuroanatomical pathways related to morphine abstinence, in particular the effects of brain lesions on the wet shake behavior of morphine abstinence. Wei et al. (1973a) have explored the brains of morphine-dependent rats for areas sensitive to naloxone-precipitated withdrawal. Wet shakes were most frequently elicited after application of naloxone to the medial thalamus and in medial areas of the diencephalic-mesencepholic junctures. Neocortical, hippocampal, hypothalamoic, tegmental, lateral thalamic and striatal

areas of the brain were found less sensitive to naloxone-precipitated withdrawal body shakes (Wei et al., 1972; 1973a). Transverse brain lesions made at the mid-collicular level completely inhibit the wet shake response to ice water in non-tolerant rats, while lesions at the mid-thalamic level did not significantly affect the wet shake response (Wei et al., 1973a). The areas in the rat brain where knife cuts alternate the wet shake response to ice water and where naloxone precipitates the shaking behavior of the morphine abstinence syndrome (Wei et al., 1973a) bears considerable resemblance to the primary motor center for shivering in the cat and brain areas thought to be intimately related to subcortical mechanism for arousal and emotional behavior (Wei et al., 1973a).

In addition to those studies employing lesions of various brain regions to study the neuroanatomical pathways related to morphine abstinence, there are studies which have employed intraventricular injections of morphine antagonist into dependent animals. Withdrawal signs have been precipitated by injection of morphine antagonist into the ventricular system of dependent rats (Watanabe, 1971), rabbits (Herz et al., 1972) and monkeys (Eidelberg and Barstow, 1971). The withdrawal pattern precipitated by intraventricular injection generally resembles that observed after systemic administration of morphine antagonist (Laschka et al., 1976a). When antagonists were applied into restricted parts of the ventricular system of rabbits, severe withdrawal signs were elicited after injection of antagonists into the 4th ventricle, whereas

only weak signs were observed when antagonists were administered into restricted parts of the anterior ventricular system, consisting of the lateral ventricles and third ventricle (Herz et al., 1972). Similar studies in the rat show a weak withdrawal syndrome when the antagonists could spread only within the lateral ventricles and the 3rd ventricle; however, when antagonist administration was restricted to the 4th ventricle, strong withdrawal signs were elicited by small doses (Laschka et al., 1976b). Following intraventricular injection of antagonist wet dog shakes were readily obtained; this was most clearly demonstrated by the fact that 5H 254, a partial agonist, induces wet dog shakes at low doses following intraventricular administration but which after systemic administration induces almost no wet dog shakes (Laschka et al., 1976). Similarly, naloxone which induced only a few wet dog shakes in rats made dependent by pellet implantation (Blasig et al., 1976), elicits this symptom reliably after intraventricular injection (Laschka et al., 1976a). These studies lead to the conclusion that brain structures located in the anterior parts of the floor of the 4th ventricle and caudal parts of the periaqueductal gray matter are important sites in the development of morphine physical dependence.

B. The Effect of Drugs on the Morphine Withdrawal Syndrome

1. Narcotic Drugs

During abstinence from morphine, the rat exhibits a large variety of measurable signs (Martin et al., 1963;

Gianutsos et al., 1975a) which may be prevented by both morphine and methadone (Gianutsos et al., 1975a). Withdrawal wet shakes, writhing, piloerection, ptosis and aggression are blocked by the administration of morphine and methadone. Weight loss during withdrawal is not immediately reversed by narcotic administration; however, if morphine is continually administered, the occurrence of weight loss is prevented. Methadone initially prevents weight loss in morphine withdrawn rats, but a tolerance rapidly develops to this effect (Gianutsos et al., 1975a). Withdrawal hypothermia is reversed by morphine administration; however, methadone is only partially able to reverse this withdrawal sign (Gianutsos et al., 1975a). It should be noted that the effectiveness of morphine and methadone is short-lived, lasting less than 24 hours, after which the syndrome again emerges. The effectiveness of methadone on certain parameters, body weight loss and wet shakes, disappears during repeated treatment, indicating the rapid development of tolerance (Gianutsos et al., 1975a). In contrast to the spontaneous withdrawal syndrome, the withdrawal precipitated by an antagonist is very difficult, if not impossible, to suppress by drugs, including opiates (Gianutsos et al., 1975a). Morphine has been found to suppress the withdrawal abstinence syndrome in the dog in a dose-related manner. However, morphine was less effective in suppressing abstinence in dogs with a high level of dependence (Martin et al., 1974). A wide variety of compounds with diverse chemical structures which possess very

potent morphine-like activity have been tested for their ability to suppress abstinence in the morphine-dependent monkey. Some of the chemical families test include the phenylpiperidines, benzimidazoles, benzomorphans, morphinans, methadones and diazabicyclo-octanes. These compounds have been found to suppress abstinence in the morphine-dependent monkey with varying degrees of potency (Villarreal, 1973). Studies done at the Addiction Research Center at Lexington, Kentucky also show these compounds to suppress morphine abstinence in man (Villarreal, 1973).

2. Non-Narcotics Drugs

a. Dopaminergic Agonist and Antagonist

Narcotic dependent rats undergoing withdrawal are hyper-irritable and when placed together show vigorous fighting (Boshka et al., 1966; Lal, et al., 197la). This aggression is blocked by continued treatment with morphine, but emerges when drug administration is discontinued. Aggression in the rat consists of vocalizations, aggressive posture, biting and other forms of attack which often leads to severe injury or death (Lal, 1975a). Haloperidol, a potent antipsychotic drug (Janssen, 1966), is very effective in blocking morphine withdrawal aggression (Puri et al., 1973; Lal, 1975b). This aggression is also reduced by chlorpromazine, a pentothiazine neuroleptic (Lal, 1975a).

Wet-dog-like body shakes are a characteristic sign of morphine withdrawal in rats (Martin et al., 1963; Gianutsos et al., 1975a). Haloperidol has been found to produce a dose dependent decrease in withdrawal shakes (Lal and Numan, 1976).

During morphine-withdrawal, animals learn to self-administer narcotic drugs directly into the bloodstream at high rates (Weeks and Collins, 1968; Schuster, 1970; Numan et al., 1975). Intraperitoneal administration of haloperidol given in small doses was shown to significantly decrease morphine self-administration in addicted rats (Cimini-Venoma and Hanson, 1972). This observation was later replicated in the monkey by Pouzolo and Kerr (1972). Spiramide, a butyrophenone neuroleptic, reduces the rate of self-administration of fentanyl, a potent narcotic (Lal and Hynes, 1977). Haloperidol has also been found to decrease morphine reinforcement and inhibit relapse in post-addict rats (Schwartz and Marchok, 1974).

Haloperidol (Lal et al., 1971b) and pimozide (Cox et al., 1975) prevent as well as reverse withdrawal hypothermia in the morphine dependent rat. Recently, Martin et al. (1974) reported that haloperidol depressed certain aspects of the morphine abstinence syndrome in the dog but not others. Haloperidol proved to be more effective than morphine in suppressing mydriasis and panting while it was relatively less effective in reducing whining, gnawing and hindlimb stepping movement (Martin et al., 1974). Morphine withdrawal jumping in the mouse is blocked by the administration of haloperidol (Takemori et al., 1976).

Several years ago it was reported (Lal et al., 1971b) that haloperidol reduced several withdrawal symptoms in heroin addicts. This initial study was replicated by Karkalas

and Lal (1973) who compared haloperidol with methadone in 18 patients. Haloperidol was found effective in completely blocking the withdrawal symptoms in half of the patients and compared well with methadone in this respect. The failure of several patients to respond to therapy was ascribed to the use of too low a dose of haloperidol. Those patients responding to haloperidol reported a reduction in their craving for heroin (Karkalas and Lal, 1973). These findings have been replicated by a number of physicians (Lal and Hynes, 1977). Haloperidol in conjunction with psychological and social counseling, has been found useful in getting patients to give up heroin for a six-month period or longer (Le Compte and Friedman, 1974).

Chlorpromazine, a phenothiazine neuroleptic, has been reported to reduce wet-dog shakes characteristic of precipitated withdrawal in the rat (Cicero et al., 1974). Narcotic withdrawal symptoms in newborn infants of drug dependent mothers have been successfully controlled with chlorpromazine (Glass, 1974; Kandall, 1976). However, adult patients who were being treated with both methadone and chlorpromazine requested the discontinuation of chlorpromazine because of adverse effects (Dufficy, 1973).

Morphine-withdrawal aggression is seen after the withdrawal of narcotic drugs (Lal, 1975; Puri and Lal, 1973). This aggression is enhanced by directly and indirectly acting dopamine-receptor stimulants such as amphetamine, L-DOPA or apomorphine (Puri and Lal, 1973). DOPA has also been found

to exacerbate withdrawal jumping, diarrhea, tremors, writhing and convulsions (Gunne, 1965). Herz (1975) found L-DOPA administered shortly before precipitating withdrawal induced a dose-dependent increase in withdrawal jumping and a decrease in wet-dog shakes. Other signs such as diarrhea and ptosis decreased, whereas rhinorrhea, lacrimation and salivation increased (Herz, 1975). Apomorphine, administered at doses between 1 and 2.5 mg/kg, increased jumping, teeth chattering and eye twitches but only the increase in jumping was statistically significant (Herz, 1975). Ptosis and diarrhea were both significantly decreased, with wet-dog shakes, writhing and rhinorrhea yielding only equivocal results (Herz, 1975). Apomorphine has been found to reduce morphine withdrawal body shakes in rats after the termination of morphine injections (Hynes, 1975).

b. Adrenergic Agonist and Antagonist

The alpha adrenergic stimulant clonidine has been found effective in reducing body shakes, weight loss, ptosis, urination, diarrhea, teeth chattering, salivation and ejaculation in dependent animals injected with naloxone (Meyer and Sparber, 1976; Tseng et al., 1975). Clonidine has been found to potentiate escape attempts induced by naloxone in dependent rats (Tseng et al., 1975).

Inhibition of brain monoamine oxidase by pargyline results in a potentiation of jumping behavior during naloxone-precipitated withdrawal in mice (Iwamoto et al., 1971). The potentiation of jumping has been attributed to the elevation

of biogenic amines in the central nervous system resulting from monoamine oxidase inhibition. Similarly, it has been reported that imipramine, a drug which inhibits the reuptake of norepinephrine potentiates morphine withdrawal activity in mice (Chiosa et al., 1968) and rats (Herz, 1975). Low doses of d-amphetamine and cocaine given shortly before precipitating withdrawal induced a dose-dependent increase in jumping and a decrease in wet-dog shakes (Herz, 1975). Signs such as diarrhea and ptosis decreased, whereas rhinorrhea, lacrimation and salivation increased after amphetamine or cocaine treatment (Herz, 1975).

Alpha adrenergic blockers such as phenoxybenzamine have been found to produce a dose-related suppression of diarrhea and wet-dog shakes in precipitated narcotic withdrawal (Cicero et al., 1974). Propranolol, the beta adrenergic blocker, has been the subject of numerous and conflicting investigations. Propranolol has been reported to prevent heroin induced euphoria, cause the use of heroin to precipitate protracted withdrawal symptoms and reduce the craving for narcotics in ex-addicts (Grosz, 1972; Black and Grosz, 1974). Other investigators have failed to find that propranolol aggravated the opiate withdrawal syndrome and on the contrary reported that it provided some relief (Jacob et al., 1975). Furthermore, no blockade by propranolol of the morphine-induced euphoria was found in man (Jacob et al., 1975). Results obtained in the chronic spinal dog indicate that propranolol neither antagonized nor mimics the effects of morphine

(Martin et al., 1974). Similarly, the morphine LD_{50} in mice is not altered by pretreatment with propranolol (Navarro et al., 1976).

c. Cholinergic Agonist and Antagonists

There is considerable evidence suggesting that acetylcholine (ACh) may be involved in some of the acute manifestations of morphine action and in the expression of certain withdrawal signs. Several investigators have indicated a possible role for cholinergic mechanisms in the withdrawal syndrome precipitated by narcotic antagonists. The changes in the jumping response appear to be related to brain levels of acetylcholine. It has been demonstrated the morphine dependent animals which jump after a naloxone injection exhibited a decrease in brain acetylcholine, whereas those that did not jump exhibited no change in brain acetylcholine (Bhargava and Way, 1975). Elevation of brain acetylcholine by the cholinesterase inhibitor physostigmine greatly inhibit naloxone-precipitated withdrawal jumping in mice (Bhargava and Way, 1972; 1976; Brase et al., 1974). Conversely, injection of hemicholinium intracerebrally to inhibit central nervous system acetylcholine synthesis, enhanced naloxone-precipitated withdrawal jumping (Bhargava and Way, 1976). The centrally active cholinergic agonist nicotine, tremorine, oxotremorine and arecoline have been found to significantly inhibit naloxone induced jumping behavior in mice (Brase et al., 1974). Whereas the cholinergic antagonist atropine, benztropine, pempidine and mecamylamine significantly

potentiate this jumping (Brase et al., 1974). However the effects elicited by the cholinergic antagonist atropine on precipitated withdrawal conflict since both an enhancement and an inhibition of naloxone-precipitated withdrawal jumping have been found. The critical factors in whether a stimulatory or inhibitory response is to be elicited by atropine appears to be the time of atropine administration and the brain level of acetylcholine. When naloxone is administered 10 min after atropine there is enhanced jumping response (Brase et al., 1974; Bhargava and Way, 1976). However, if naloxone is administered 30 min after atropine the jumping response is inhibited and after 2 hours no effect of atropine is apparent (Jhamandas and Dickinson, 1973; Iwamoto et al., 1973). During peak naloxone-precipitated withdrawal jumping there is a lowering of brain acetylcholine (Bhargava and Way, 1975). When atropine enhances naloxone precipitated withdrawal jumping, brain acetylcholine levels are also decreased. At those time intervals when the potentiating action of atropine is absent, brain acetylcholine levels are normal (Bhargava and Way, 1976).

Several studies exist which have examined the effects of cholinergic and anticholinergic agents on the expression of morphine dependence in rats (Frederickson and Pinsky, 1975; Hynes et al., 1976). The anticholinergic drugs atropine and mecomylamine were found to have a biphasic effect on the narcotic withdrawal syndrome by Frederickson and Pinsky (1975). Mecamylamine by itself potentiated the peak

severity and accelerated the subsequent decline in severity of the signs. Atropine by itself was found to have a similar tendency but none of its effects proved significant (Frederickson and Pinsky, 1975). However, when the two treatments were combined, there was no significant effect on the severity of withdrawal (Frederickson and Pinsky, 1975). Apparently the action of atropine antagonized the actions of mecamylamine. Atropine methylnitrate, a peripherally active anticholinergic, reduced the withdrawal severity (Frederickson and Pinsky, 1975). The anticholinergic atropine and dexetimide produced no significant effect on withdrawal wet shakes, weight loss, piloerection and diarrhea; however, there was a slight increase in the number of withdrawal body shakes (Hynes et al., 1976). Eserine sulfate, a tertiary anticholinesterase agent, when administered during the abstinence period was found to have little effect on the severity of the total withdrawal syndrome but decreased weight loss (Frederickson and Pinsky, 1975). Choline chloride was found to effectively reduce the overall withdrawal severity and weight loss throughout the withdrawal period (Frederickson and Pinsky, 1975). Hynes et al. (1976) found that pilocarpine, a drug which stimulates cholinergic receptors, was effective in reducing both wet dog-like body shakes and withdrawal aggression but increased diarrhea and body weight loss. Pretreatment with atropine blocked all of the effects of pilocarpine on withdrawal signs. While methylscopolamine, an anticholinergic drug unable to penetrate into the central

nervous system failed to block the pilocarpine-induced inhibition of withdrawal wet shakes but protected the animal against pilocarpine-induced diarrhea, suggesting that pilocarpine's reduction of withdrawal body shakes was centrally mediated (Hynes et al., 1976). The hyperinitability characteristics of the abstinence syndrome have been attenuated by the administration of the physostigmine (Grumbach, 1969).

Atropine and methylatropine have been also tested in rats for their ability to alter the reinforcing action of intravenous morphine sulfate. Morphine self-administration was blocked by atropine but not by methylatropine (Davis and Smith, 1975). Similarly, atropine but not methylatropine prevented the establishment of a conditioned reinforcer based on passive intravenous infusions of morphine (Davis and Smith, 1975). These results indicate that a central cholinergic system exerts an influence on the brain mechanisms which are affected by morphine to produce positive reinforcement (Davis and Smith, 1975).

d. 5-Hydroxytryptamine Agonist and Antagonist

Evidence suggesting that morphine and 5-hydroxytryptomine (5-HT) may interact in the peripheral and central nervous systems is mostly circumstantial. Molecular models of morphine exhibit some degree of complementariness to 5-HT and arguments can be made indicating that certain actions of morphine may be mediated by endogenous 5-HT. The compound, p-chlorophenylalanine (PCPA), which selectively inhibits the synthesis of 5-HT (Koe and Weissman, 1966) has been reported

to modify morphine effects on analgesia (Tenen, 1968; Fennessy and Lee, 1970) and on motor activity (Eidelberg and Schwartz, 1970; Cheney and Goldstein, 1971). Attempts have been made to modify the morphine abstinence syndrome with agents that might alter brain 5-HT, but no clear picture has emerged. Cyproheptadine, a 5-HT antagonist, when given in large doses prior to levallorphan precipitated withdrawal abolished teeth chattering, ejaculations, wet dog shakes, loose stools and reduced body weight loss (Opitz and Reimann, 1973). Huidobro et al.,(1963) reported that the administration of 5-HT or its metabolic precursors attenuated the abstinence syndrome. Collier (1965) in the development of his receptor theory of tolerance and dependence, pointed out that the abstinence signs in the dog and rat are very similar to those produced by the administration of the 5-HT precursor, 5-hydroxytryptophan.

Several attempts to link morphine with 5-HT in the tolerant and dependent state have been made by studying the effects of morphine on brain concentrations of 5-HT after repeated morphine administration and during the withdrawal state. The evidence indicates that steady state levels of 5-HT are affected only slightly, or not at all, by acute or chronic morphine administration or by morphine withdrawal (Way, 1971; Way and Shen, 1971). A comparison of the turnover of brain 5-HT in tolerant and non-tolerant mice revealed a mean increase in tolerant animals more than double

that in non-tolerant ones (Way, Loh and Shen, 1968; Loh, Shen and Way, 1969; Shen, Loh and Way, 1970). The increased rate of 5-HT synthesis resulting from chronic morphine administration was blocked by cycloheximide, an inhibitor of protein synthesis and this was accompanied by an inhibition of tolerance and development of physical dependence to morphine (Way et al., 1968; Loh et al., 1969). When the development of tolerance and physical dependence to morphine was prevented by concomitant administration of the narcotic antagonist, naloxone, the increased brain 5-HT levels was also prevented. The elevated turnover rate which existed during the tolerant state reverted to normal two weeks after withdrawal (Loh et al., 1969). When brain 5-HT synthesis was inhibited by PCPA, tolerance and physical dependence development to morphine was in part prevented (Way et al., 1968; Shen et al., 1970). On the basis of these findings it has been suggested that 5-HT turnover may be associated with morphine tolerance and physical dependence.

e. Endorphins

Intracerebroventricular administration of methionine-enkephalin or morphine sulfate prior to naloxone administration has been shown to inhibit the precipitated withdrawal jumping response in mice dependent on morphine by the pellet implantation method (Bhargava, 1977). Howevever, both methionine-enkephalin and morphine sulfate failed to inhibit withdrawal defecation and rearing behavior. Morphine sulfate was found to be 4 times as potent as methionine-

enkephalin, on molar basis, in inhibiting the abstinence syndrome (Bhargava, 1977).

f. Miscellaneous Drugs

Tetrahydroconnabinol when administered prior to naloxone precipitated abstinence in rats, blocked the appearance of wet shakes and escapes (Hine et al., 1975), and reduced withdrawal jumping, defecation and rearing in mice (Bhargava, 1976).

C. The Pharmacology of Naloxone

Naloxone or N-allylnoroxymorphone was synthesized by Lewenstein and Fishman in the early part of 1960 from oxymorphone, a potent analgesic (Blumberg and Dayton, 1973). Initial investigations demonstrated that naloxone was a potent narcotic antagonist in animals being about 10-20 times more active than nalorphine (Blumberg et al., 1961). Following the animal studies, Foldes et al. (1963) reported that naloxone was also a potent narcotic antagonist in man.

1. Narcotic Antagonism

Naloxone is at least 10-20 times more active than nalorphine and about 3-6 times more active than levallorphan when tested by prevention of the Straub tail erection in mice, by counteraction of narcosis in rats, and by counter action of narcotic depression of respiration in rabbits (Blumberg et al., 1965). In precipitating the withdrawal syndrome in morphine-dependent monkeys, naloxone is about 7 times more active than nalorphine (Deneau and Seevers, 1963).

Nalorphine is a narcotic antagonist that has several undesirable side effects, such as respiratory depression and psychotomimetic reactions (Blumberg and Dayton, 1973). Similarly, naloxone is 5-8 times more potent than nalorphine in precipitating the abstinence syndrome in morphine-dependent humans and is itself non-addicting (Jasinski et al., 1967).

Naloxone acts very rapidly and counteracts narcotic depression within 1-2 min when given intravenously (Blumberg and Dayton, 1973). Peak brain levels of naloxone occur within 15 min of injection and decline by 50 percent within 1 hour (Berkowitz et al., 1975). It is effective in counteracting various different narcotics, but is ineffective against non-narcotic depressants such as barbiturates (Blumberg and Dayton, 1973).

2. Counteraction of Narcotic Antagonist Analgesics

Unlike nalorphine and levallorphan, naloxone not only counteracts narcotics but also counteracts agonist effects of narcotic antagonist analgesics. Thus naloxone counteracts the depressant effects of cyclazocine on the flexor reflex of the spinal dog (McClane and Martin, 1967), the antinoriceptive activity of cyclazocine, cyclorphan, levallorphan, nalorphine and pentazocine in writhing test in mice and rats (Blumberg et al., 1966). The depressant action of pentazocine in rats and the respiratory depression caused by pentazocine in anaesthetized dogs are also reversed by naloxone (Blumberg and Dayton, 1973). In man, naloxone also counteracts undesirable side effects of

cycloazocine (Jasinski et al., 1968) and the respiratory depressent action of pentazocine (Kallos and Smith, 1968).

3. Other Pharmacological Properties of Naloxone

Naloxone is not a 'pure' antagonist for at high doses it does exhibit some agonist actions. For example, in rats a dose of 50 mg/kg, which is about 200 times the narcotic antagonist dose, will produce restlessness and salivation. At higher toxic doses clonic-tonic convulsions appear (Blumberg and Dayton, 1973). At doses well above the narcotic antagonist dose naloxone appears to manifest few or no side effects or agonist action in man or mammalian laboratory animals (Blumberg and Dayton, 1973). However, there is some evidence of agonist activity at non-toxic doses in behavior experiments in pigeons (McMillan et al., 1970).

Naloxone, although a very potent narcotic antagonist, appears to show no anti-nociceptive activity in animals (Blumberg et al., 1961; Blumberg et al., 1965) and little or no analgesic activity in man (Blumberg and Dayton, 1973). There is essentially no effect on respiration at therapeutic doses of naloxone in narcotic-free animals. However, at about 100 times the antagonist dose in rabbits the respiratory rate and minute volume begin to show a slight increase (Blumberg and Dayton, 1973). Naloxone by itself has essentially no effect on respiration in man (Foldes et al., 1963; Jasinski et al., 1967). Heart rate and blood pressure are not significantly affected by naloxone (Blumberg and Dayton, 1973). Naloxone likewise has little or no cardiovascular

effect in man (Jasinski et al., 1967). Naloxone causes no significant change in the body temperature of rabbits or dogs (Blumberg and Dayton, 1973).

Naloxone has been shown to modify the effects of d-amphetamine on continuous avoidance behavior in the rat (Holtzman and Jewett, 1973; Holtzman, 1974). The dose response curves for d-amphetamine stimulation of continuous avoidance and locomotor activity were shifted downward by the administration of naloxone (Holtzman, 1974). Additionally, naloxone has proven to be almost as effective as d-amphetamine in suppressing food intake in food-deprived rats (Holtzman, 1974). However, naloxone failed to affect food intake in the mouse, indicating that some effects of naloxone may be species dependent (Holtzman, 1974). Naloxone has also been shown to block apomorphine induced stereotyped behaviors in male guinea pigs (Moon and Margolin, 1977).

Haloperidol and morphine sulfate completely suppress bar-pressing for brain self-stimulation in rats implanted with electrodes in the lateral hypothalamus (Wauquier et al., 1974). Naloxone at a dose (5 mg/kg) which was ineffective when given alone, differentially reversed the morphine effects but was without any reversing influence on the actions of haloperidol (Wauquier et al., 1974). When the self-stimulating electrodes are in or just lateral to the pontine central gray the rate of self-stimulation was dose-relatedly decreased by naloxone administration (Belluzzi and Stein, 1977). Electrical stimulation of the periaque-

ductal grey matter in rats and humans has been found to produce a strong analgesia (Mayer and Liebeskind, 1974; Akil et al., 1976) which is antagonized by naloxone (Akil et al., 1976; Adams, 1976).

Several experiments have demonstrated that naloxone can restore normal peristaltic reflex activity in fatigued guinea pig preparations or after inhibition by adenine nucleotides (Van Neuten et al., 1976). Distention of the guinea pig ileum segment elicits peristaltic activity, however maintenance of this distended condition results in the gradual disappearance of peristaltic activity (Van Neuten et al., 1976). Naloxone has been found by Van Neuten et al. (1976) to restore normal activity in such preparations. Adenosine, adenosine monophosphate, adenosine diphosphate and adenosine triphosphate inhibit the perestaltic reflex activity of guinea pig ileum and this inhibitory effect is also reversed by naloxone (Van Neuten et al., 1976). In the guinea pig ileum segment, met-enkephalin, an endogenous ligand for the opiate receptor, causes a dose-dependent inhibition of the rhythmic peristaltic reflex activity induced by increasing the distending pressure (Van Neuten et al., 1977). This inhibitory effect of met-enkephalin is reversed by naloxone (Van Neuten et al., 1977).

Recent studies have revealed the existence of endogenous morphine-like compounds in the central nervous system (Teschemacher et al., 1975; Hughes et al., 1975). These endogenous morphine-like compounds have been reported to

produce analgesia when injected intraventricularly in rats and cats (Belluzzi et al., 1976) which is antagonized by naloxone. A morphine-like peptide obtained from the pituitary gland, endorphin, and two brain peptides, enkephalins, have been shown to have a high affinity for the opiate receptor demonstrated by displacement of naloxone from these receptors (Terenius and Wahlstrom, 1975; Pasternak et al., 1975; Bradbury et al., 1976). These findings have led to the hypothesis that these morphine-like compounds are partially responsible for pain thresholds. If this is true then an antagonism of the effects of the endogenous compounds by naloxone should affect pain perception. However, naloxone has been found not to alter the perception of experimentally induced pain in normal human subjects (El-Sobky et al., 1976). Rats treated with doses of naloxone sufficient to block morphine analgesia have been found to show no change in the threshold for escape from foot shock (Goldstein et al., 1976). However, naloxone has been shown to lower pain threshold in mice (Jacob et al., 1974), to block analgesia produced by electrical stimulation of the periaqueductal grey in rats (Akil et al., 1976), to antagonize acupuncture analgesia in man (Mayer and Price, 1976), and to antagonize nitrous oxide analgesia in mice (Berkowitz et al., 1976). Bell and Martin (1977) recorded C-fiber reflexes from an S, ventral root in the acute decerabrate low spinal cat following stimulation of the ipsilateral superficial peroneal nerve or application of radiant heat to the metacarpel footpad. Naloxone was found

to increase the electrically evoked C-fiber reflex and the radiant heat evoked ventral root reflex (Bell and Martin, 1977). The fact that naloxone facilitated these reflexes in doses too small to have non-specific excitatory effects is consistent with the hypothesis that these effects are due to antagonism of a naturally occurring opiate-like substance.

D. Opiate Receptors

1. Stereospecific Binding Sites for Opiates

It has been postulated for many years that opiates must bind to macromolecular sites or receptors located in, or on the surface of, nerve cells before they can produce their characteristic pharmacological responses. The reason for this receptor postulate was the high degree of structural and steric specificity which characterizes opiate action. Among the many classes of natural and synthetic morphine-like analgesics studied, it is usually the D-isomer that is active, while the L enantiomorph tends to have very low or no analgesic potency (Simon, 1976). Small structural alterations in the opiate molecule can lead to dramatic changes in potency. The search for opiate receptors has been underway in a number of laboratories for many years. However, success was limited until Goldstein et al. (1971) suggested using stereospecificity as a criterion of receptor binding. They incubated mouse brain homogenate with H^3-levorphanol. Stereospecific binding was defined as that portion of the binding that was prevented by levorphanol but not by

dextrorphan. In their experiments stereospecific binding amounted to only 2% of total binding. Using modifications of the Goldstein procedure Pert and Snyder (1973), Simon and his co-workers (1973) and Terenius (1973a) independently identified binding to brain tissue that was highly stereospecific by using labeled opiates and opiate antagonist of high specific activity.

Stereospecific binding sites have been found in the central nervous system of many species and the myenteric plexus of the guinea pig ileum. They are tightly bound to membranous elements of the cell and have been reported to be most concentrated in the synaptosomal fraction of brain and guinea pig ileum homogenates (Hitzeman et al., 1974; Pert et al., 1974; Terenius, 1973b) suggesting that they occur on membranes in the vicinity of synapses. Whether they are situated pre- or postsynaptically has not yet been established. Opiate binding sites have been found in the central nervous system of every vertebrate species so far examined, including man (Hiller et al., 1973) but not in that of any invertebrates (Pert et al., 1974).

Biochemical studies have shown that stereospecific binding of opiates is highly sensitive to proteolytic enzymes (Simon et al., 1973; Pasternak and Snyder, 1973) and various sulfhydryl reagents (Simon et al., 1973; Terenius, 1973a) suggesting the participation of protein. The binding of agonist and antagonist drugs has a broad pH optimum between 6.5 and 8, and is decreased by the presence of alkali,

alkaline earth, transition metal and organic cations. An exception is the sodium ion which at concentrations up to 200 mM enhances antagonist binding while depressing agonist binding (Simon, 1976).

Opiate binding sites have high affinity for narcotic analgesics and their antagonists. Dissociation constants range from 0.4 nM for etorphine to no detectable affinity for drugs which do not exhibit morphine-like narcotic analgesic effects (Simon, 1976). One of the most important convincing pieces of evidence regarding the pharmacological relevance of binding sites is the degree of correlation between binding affinity of a variety of ligands and their pharmacological potency. Excellent correlation has been found between the _in vivo_ potencies of opiates and antagonists and their affinities for the stereospecific binding sites _in vitro_ (Simon _et al._, 1973; Pert and Snyder, 1973; Terenius, 1974). The ability of opiates to inhibit electrically stimulated contractions of the isolated guinea pig ileum has also been shown to be very well correlated with their binding affinities for stereospecific sites in the myenteric plexus of the ileum (Creese and Snyder, 1975). Narcotic binding affinities, determined both in the presence and absence of sodium, showed excellent and closely similar correlation with the tail withdrawal analgesia test in rats after intravenous administration of drugs (Simon, 1976). Very high correlation was also obtained between binding affinities of drugs and their ability to inhibit the contractions of the

guinea pig ileum. These binding affinities show good correlation with the cod-liver oil test for antidiarrheal potency (Simon, 1976). The stereospecificity, saturability and high affinity of the stereospecific opiate binding sites and the excellent correlation between potency and binding affinity of opiates and their antagonist strongly support the hypothesis that these sites are pharmacologically relevant recognition and binding sites for opiates.

a. Distribution of Opiate Receptors in Different Regions of the Brain

The manner in which stereospecific binding sites are distributed throughout the brain is of considerable interest as a clue to the sites of opiate action. Receptor binding has been determined in numerous regions of the monkey brain where receptor binding varies by a greater than 30-fold range (Kuhar et al., 1973). The amygdala shows the greatest amount of receptor binding, with its anterior portion displaying almost twice as much as the posterior. Binding in the periaqueductal area of the midbrain is about the same as in the posterior amygdala. The hypothalamus and medial thalamus, the next highest areas, display only about 40 percent as much binding as the anterior amygdala. In the head of the caudate, receptor binding has been found to be 80 percent of that in the hypothalamus and medial thalamus. Marked regional variations have also been found within the cerebral cortex. Frontal cortex is the highest cortical area with precentral gyrus, postcentral gyrus and occipital pole showing considerably

less binding. Receptor binding is very low or not detectable in various white matter areas. The cerebellum, lower brain stem and the spinal cord show only very low levels of receptor binding (Kuhar et al., 1973). Stereospecific binding has also been demonstrated in human brain (Hiller et al., 1973). There are large differences in binding within the human brain ranging from 0.4 pmol/mg protein in the olfactory trigone, amygdala and septal nuclei to virtually no binding in cerebral white matter, dentate nucleus of the cerebellum, tegmentum, pineal and pituitary gland (Simon, 1976; Hiller, 1973). The most interesting conclusions reached from these studies is that most areas with high opiate binding are located in, or associated with, the limbic system.

The heterogenity of opiate receptor distribution is reminiscent of the large regional differences observed for concentrations of the neurotransmitters. There are similarities of opiate receptor distribution to the distribution of several neurotransmitters, such as acetylcholine, γ-aminobutyric acid, serotonin and the catecholamines (Snyder and Pert, 1975). However, there are some differences as well. Acetylcholine and opiate receptor binding are both high in the caudate nucleus, the putamen is quite rich in acetylcholine but contains only a moderate degree of opiate binding. The periaqueductal areas of the midbrain, which possesses the second highest levels of opiate receptors, has only moderate acetylcholine-synthesizing capacity (Snyder and Pert, 1975). Whereas the hypothalamus is rich in both

γ aminobutyric acid and opiate receptors, the globus pallidus which contains one of the highest γ -aminobutyric acid concentrations in the brain, is relatively low in opiate receptor binding (Snyder and Pert, 1975). The caudate nucleus is high in opiate receptor binding and tyrosine hydroxylase, the rate limiting enzyme in catecholamine synthesis. Additionally, the hypothalamus and periaqueductal gray are also rich in tyrosine hydroxylase and opiate receptor binding. However, while the medial thalamus contains much more opiate receptor binding than the lateral hypothalamus,the reverse is true for tyrosine hydroxylase (Snyder and Pert, 1975). Serotonin is found in the caudate, hypothalamus and amygdala just as the opiate receptor is. However the midbrain raphe area, which contains the cell bodies of serotonin neurons in the brain and one of the richest in serotonin content, is quite low in opiate receptor binding (Snyder and Pert, 1975). Lesions that selectively destroy norepinephrine, serotonin, acetylcholine, or dopamine containing pathways have no effect on opiate receptor binding in the areas of brain in which these pathways possess the greatest density of nerve terminals (Kuhar et al., 1973). These results indicate that the opiate receptors are not localized on or within the nerve terminals of the specific neurotransmitter pathways discussed here.

b. Interaction of Opiate Receptors with Agonist and Antagonist

Simon et al. (1973) reported that the presence of salt in the incubation mixture led to a dose-dependent

decrease in etorphine binding while no such effect was observed for naloxone binding by Pert and Snyder (1973). The effect of salt is a general one between the classes of agonist and antagonist drugs resulting in an actual enhancement of antagonist binding which in the case of naloxone and naltrexone is at least two-fold (Pert and Snyder, 1974). They have further demonstrated that the effect is not a salt or ionic strength effect but a unique property of sodium ions, exhibited to a lesser extent by lithium but by none of the other alkali metal ions. None of a large variety of other cations studied including alkaline earth and transition metals (Pert and Snyder, 1974) nor a series of organic cations (Simon et al., 1975) could mimic the selective action of sodium and lithium. This effect is remarkable both for the ability of an inorganic cation to distinguish between such close related structures as opiate agonist and antagonists and for the uniqueness of sodium in this regard.

The question of whether the changes produced by sodium are due to changes in the number of binding sites or in binding affinities has been explored by both the Simon and Snyder groups. However, on this point there exists disagreement between the experiments performed in the two laboratories which has yet to be resolved. Pert and Snyder (1974) have reported an increase in the number of high affinity binding sites for naloxone in the presence of sodium with no change in affinity. On the other hand, Simon et al. (1975) reported that sodium causes an increase in the affinity of naltrexone without any

change in the number of binding sites. Similarly, for agonist binding Simon et al. (1975) reported a decrease in affinity while Pert and Snyder (1974) reported a decrease in number of binding sites.

Unlabeled opiates have been allowed to compete with labeled antagonists in the presence and absence of sodium. When the unlabeled competitor was a relatively poor antagonist there was little or no change in the concentration able to displace 50 percent of the labeled antagonist (ED_{50}) in the presence of sodium while the ED_{50} of strong agonist was increased dramatically (Simon, 1976). Drugs with mixed agonist-antagonist properties acted in an intermediate fashion, their ED_{50} for displacement of ^{3}H-naloxone or ^{3}H-naltrexone being increased 2 to 7 fold in sodium (Simon, 1976). Shifts in ED_{50} are compatible with changes in affinity but not with changes in the number of binding sites (Simon, 1976).

In spite of the disparity of results between the Simon and Snyder groups they view the mechanism of the sodium effect in a similar manner and have independently proposed similar models (Simon, 1975; Pert and Snyder, 1975). The essence of these models is that the sodium ion acts as an allosteric effector, the binding of which to an allosteric site on the receptor molecule results in a conformational change in the opiate binding site. The new conformer, sodium dependent, exhibits a higher affinity for antagonist and a lower affinity for agonist than the conformer that exists in sodium-free media.

c. The Effect of Tolerance and Dependence on Opiate Receptors

There has been great interest in the way the development of tolerance and physical dependence may affect the number and properties of opiate receptors. It has been known for a long time that during chronic morphinization there is not only an increase in the dose of morphine required to produce analgesia and other central effect (tolerance) but there is also a dramatic decrease in the amount of antagonist required to produce precipitated withdrawal. Thus it is attractive to postulate that these changes result from alterations in the receptor which increase its affinity for antagonists and reduce its affinity for agonists (Simon, 1976). To date experiments in several laboratories have failed to provide direct evidence for either a qualitative or quantitative change in opiate receptors during chronic treatment of animals with opiates. Klee and Streaty (1974) reported that the binding of ^{3}H-dihydromorphine to particulate fractions derived from the brains of controls and morphine dependent rats is identical with respect to both number of sites and binding affinity. The displacement of bound dihydromorphine by either morphine or naloxone is unchanged in brain fractions from dependent rats (Klee and Streaty, 1974). The latter results provides evidence against a significant increase in the affinity of antagonist. Similar negative results have been obtained by Hitzeman *et al*. (1974) and Pert *et al*. (1973).

2. The Binding of Non-Narcotic Drugs to the Opiate Receptor Site

a. Butyrophenone Influences and Related Neuroleptics

The butyrophenones, neuroleptic drug useful in the treatment of schizophrenia, were developed as analogues of the opiate meperidine (Janssen, 1965). Recently, haloperidol, the prototype of the butyrophenones, was found to bind to the opiate receptor and inhibit binding of labeled naloxone (Clay and Brougham, 1975). The haloperidol binding is greatly reduced in the presence of 100 nM Na^+ (Clay and Brougham, 1975), and this finding is consistent with the data reported for other opiate agonists (Pert and Snyder, 1974). However, the affinity of haloperidol for the opiate receptor is markedly different from that of morphine. Clay and Brougham (1975) found that the concentration of morphine required to reduce the stereospecific binding of 8 nM [^{3}H]-naloxone by 50 percent was 12 nM while the concentration of haloperidol needed to achieve the same effect was 880 nM. Although both morphine and haloperidol are bound in a competitive manner to the opiate receptor, there is some indication that haloperidol may be bound in a different manner than morphine. This indication is provided by an analysis of the log-probit plots for the binding affinity of these drugs. Morphine has a profile that parallels that of a large number of opiate agonist and antagonists (Clay and Brougham, 1975; Pert and Snyder, 1973). Haloperidol appears to have a binding profile differing from that reported for morphine or other opiate agonist

and antagonists (Clay and Brougham, 1975). These results have been confirmed by Creese et al. (1976) who have also shown that several other butyrophenones also display substantial potency as inhibitors of ^{3}H naloxone binding. They have found benperidol and pimozide to be almost as potent as the opiate fentanyl in inhibiting ^{3}H-naloxone binding in the presence of sodium. The potency of these two drugs is decreased very little in the presence of sodium, suggesting that these pharmacological actions may resemble those of opiate antagonist (Pert et al., 1973; Pert and Snyder, 1974). On the other hand, spiperone was found to lose a great deal of potency in the presence of sodium, consistent with opiate agonist activity. The other butyrophenones tested by Creese et al. (1976) respond to sodium in a fashion resembling that of mixed agonist-antagonists.

The results achieved by Creese et al. (1976) for pimozide differ from those of Clay and Brougham (1975) who found pimozide to have no effect on opiate binding. Other studies have also shown pimozide to have no significant effect on the specific binding of the tritiated opiate antagonist, naloxone (Charalampous and Askew, 1974). Phenothiazine also have influences on the opiate receptor. Creese et al. (1976), have found that a number of the phenothiazine neuroleptics also inhibit ^{3}H-naloxone binding. Of the phenothiazines tested, thioridazine was the most potent with promethazine (a non-neuroleptic phenothiazine) and chlorpromazine being somewhat weaker. Fluphenazine, one of the most potent

neuroleptic phenothiazines, is one of the weakest in its influence on the opiate receptor binding of ^{3}H-naloxone (Creese et al., 1976). However, other investigators have found chlorpromazine to not affect opiate binding at concentrations up to 10^{-3} M (Clay and Brougham, 1975). Sodium decreases the potency of thioridozine by 1.5 fold, consistent with an opiate antagonist profile, while the sodium ratio for the other phenothiazines resemble opiate mixed agonist-antagonists (Creese et al., 1976). In light of the fact that these drugs have a very low affinity for the opiate receptor, this suggests that the sodium influences for these drugs do not have major pharmacological relevance.

b. Adrenergic Agents

Phenoxybenzamine, an alpha receptor antagonist, has been found to have no significant effect on the binding of naloxone (Charalampous and Askew, 1974). However, the beta adrenergic receptor antagonist, propanolol and the beta adrenergic agonist, isoproterenol, were found to significantly reduce the binding of naloxone to the opiate receptor (Charalampous and Askew, 1974). The inhibition of binding by propanolol was reconfirmed by in vivo administration of propanolol. Sodium was found not to affect binding of the beta antagonist and agonist as with specific opiate antagonists and agonists (Charalampous and Askew, 1974).

c. Cholinergic Agents

Atropine, a muscarinic cholinergic receptor antagonist, was found to not significantly affect specific

binding of the triated opiate antagonist, naloxone (Charalampous and Askew, 1974; Pert and Snyder, 1973).

E. A Comparison of Narcotic Analgesics with Neuroleptics

Narcotic analgesics bear certain behavioral and biochemical similarities to neuroleptic drugs.

1. Behavioral Similarities

a. Acute Actions

(1) Catalepsy

Catalepsy is a state of behavioral immobility accompanied by either muscular hypotonia as with neuroleptics, or by muscle rigidity as with narcotics. Both, haloperidol and morphine, cause catalepsy in most animal species and in human subjects, with the ED_{50} in rats, 1.5 and 20 umol/kg, respectively (Lal et al., 1975). The catalepsy induced either by haloperidol or by morphine has been effectively counteracted by apomorphine as benztropine (Lal et al., 1975). B-Endorphin, an endogenous peptide with morphine-like biological properties, has been found to also produce catalepsy when injected into the periaqueductal gray (Jacquet and Marks, 1976) and the cerebrospinal fluid (Bloom et al., 1976).

(2) Jumping

Dihydroxyphenylalanine (L-DOPA) injected in mice pretreated with amphetamine reliably elicits upward jumping (Lal, et al., 1975a). Haloperidol, pimozide, chlorpromazine, thionidazine and clozapine block the mouse jumping (Colpaer et al., 1975) suggesting that jumping behavior is a measure of dopaminergic stimulation. Morphine also

blocks mouse jumping in a dose related manner (Lal et al., 1975a). Dexitimide, a centrally acting anticholinergic drug, reversed the pimozide induced blockade of jumping (Van Neuten, 1962) without reversing the morphine induced blockade (Lal et al., 1975), suggesting that the narcotics block dopamine receptors but through a different brain site which does not involve a dopaminergic cholinergic interaction (Lal et al., 1975).

(3) Stereotypy

Drugs which directly or indirectly stimulate dopamine receptors cause stereotypy in rats. Morphine, methadone and demerol are potent antagonists of apomorphine and amphetamine in the stereotypy test. Amphetamine and apomorphine induced stereotypies are also antagonized by neuroleptic drugs (Lal et al., 1975).

(4) Vomiting

The narcotic drugs, morphine, methadone and demerol are potent antagonist of apomorphine induced vomiting in dogs (Lal et al., 1975). Neuroleptics also show marked activity in antagonizing apomorphine induced vomiting (Lal et al., 1975).

(5) Aggression

Aggression can be elicited in laboratory animals by a variety of treatments. Apomorphine produces aggression in a dose dependent manner and both haloperidol and morphine effectively block this aggression (Lal et al., 1975). Additionally, haloperidol and morphine effectively

block the aggression elicited by amphetamine and L-DOPA treatment (Lal et al., 1975). Electric shock delivered to the paw is known to elicit aggression in paired rats. Haloperidol and morphine block shock induced aggression in a dose-dependent manner (Lal et al., 1975). Withdrawal from narcotic drugs produces marked irritability and aggression (Lal, 1975). This withdrawal aggression is blocked by narcotics and neuroleptics in relatively low doses (Puri and Lal, 1973).

(6) Neuroleptanalgesia

Various combinations of narcotic analgesics and butyrophenones have been employed for neuroleptanalgesia as a total or partial alternative to surgical anesthesia (De Castro and Mundeleer, 1959; Nilson and Janssen, 1961; Deligene, 1961; Edmonds-Seal and Prys-Roberts, 1970; Lewis and Jennings, 1972). The term neuroleptanalgesia implies the use of a butyrophenone in combination with a short-acting, potent analgesic (Edmonds-Seal and Prys-Roberts, 1970). Of the butyrophenones available those commonly used in neuroleptanalgesia are haloperidol and droperidol. The analgesics commonly used in neuroleptanalgesia are dextromoramide (Palfium), phenoperidine (Operidine) and fentanyl (Sublimaze) in order of increasing potency and decreasing duration of action (Edmonds-Seal and Prys-Roberts, 1970).

The effects of mixtures of neuroleptics and analgesic drugs may be predicted from a knowledge of the pharmacology of the constituents. The pharmacology of the mixture of fentanyl citrate and droperidol (Innovan) will be examined

since it is the most common of the neuroleptanalgesics. Droperidol is a butyrophenone type neuroleptic which produces sedation, tranquilization, adrenergic blockade and vasodilation (Janssen et al., 1963; Yelnosky et al., 1964), whereas studies on the pharmacology of fentanyl citrate alone indicate its principal actions are analgesia, central nervous system depression, respiratory depression and bradycardia (Janssen, 1962; Gardocki and Yelnosky, 1964). Each compound in the mixture exerts its own pharmacologic action without any well-defined antagonistic or potentiating interactions with one exception. Droperidol enhances the analgesic activity of fentanyl citrate (Yelnosky and Gardocki, 1964). Since droperidol appears to be devoid of analgesic activity, the enhancement of the analgesic action of fentanyl citrate is the result of a potentiating effect of droperidol, not an additive effect. The mechanism of potentiation is not known; however, it is doubtful that it is due to inference by droperidol with the catabolism of fentanyl citrate since the duration of analgesic action of the combination was no greater than that of fentanyl alone (Yelnosky and Gardocki, 1964). Other investigators have found no potentiation of the analgesic effect of fentanyl by droperidol (Corssen, Domino and Sweet, 1964; Prys-Roberts and Kelnan, 1967). The neuroleptics do not appear to augment the ventilatory depressant effects of the analgesics (Corssen, Domino and Sweet, 1964; Yelnosky and Gardocki, 1964; Prys-Roberts and Kelman, 1967; Harper et al., 1976). Nausea and vomiting have been found after the

administration of fentanyl (Harper et al., 1976). This is hardly surprising since this result occurs with most narcotics. However, the concomitant administration of droperidol reduced the incidence of nausea and vomiting (Harper et al., 1976). Fentanyl produces minimal circulatory changes as evidenced by the lack of orthostatic hypotension (Harper et al., 1976). This is in contrast to the combination of fentanyl plus droperidol which appears to predispose some patients to orthostatic hypotension (Harper et al., 1976). The hyperthermia normally seen after morphine administration in cats has been reported to be markedly potentiated by pimozide, a specific dopamine receptor blocker (French et al., 1976).

In the state of neuroleptanalgesia the patient appears calm, detached from his surroundings and immobile. The face is without expression and the closed eyes give an impression of sleep, though the patient will respond immediately to quiet command (Hayward-Butt, 1957; Edmonds-Seal and Prys-Roberts, 1970). The degree of analgesia is dose-dependent but limited by ventilatory depression although sufficient analgesia can be obtained with spontaneous ventilation for minor surgery (Edmonds-Seal and Prys-Roberts, 1970).

Neuroleptanalgesia has been mainly employed in minor surgical procedures, as an adjunct to local analgesics, as preanesthesia medication and in combination with light general anesthesia. Minor surgical procedures such as insertion of eyelid sutures, nerve blocking injections and dressing of burns have been performed under neuroleptanalgesia

(Edmonds-Seal and Prys-Roberts, 1970). Neuroleptanalgesia combinations have been found useful for supplementing local analgesic techniques such as retio-ocular block (Cameron, 1967), endobronchial intubation (Coppen and Fox, 1968), and bronchoscopy procedures (Berenyi et al., 1966). Neurolepti-analgesia is widely used as a preanesthetic medication. Fentanyl plus droperidol has been found to produce better pre-operative sedation than morphine, together with significantly less post-operative nausea and vomiting (Norris and Telfer, 1968). The concept of combining neuroleptanalgesics with light general anesthesia has been employed for a number of years. A mixture of fentanyl citrate and droperidol has been used successfully with nitrous oxide for general anesthesia in man (Holderness et al., 1963). In the field of cardiovascular surgery, the use of neuroleptanalgesic combination during cardiopulmonary bypass has supplanted the use of volatile anesthetic agents which are known to depress myocardial function (Corssen et al., 1965).

The combination of fentanyl plus droperidol has also been used with remarkable success in dogs (Yelnosky and William, 1964), guinea pigs (Lewis and Jennings, 1972), rats (Jones and Simmons, 1968; Garcia et al., 1975), mice (Lewis and Jennings, 1972), parakeets (Yelnosky and William, 1964), and rabbits (Lewis and Jennings, 1972) to produce analgesia, anesthesia, sediation and tranquilization for surgery.

b. Chronic Actions

(1) Tolerance to Cataleptic Action

Rats made tolerant to the effects of morphine by chronic treatment show tolerance to the catalepsy-inducing action of both morphine and haloperidol (Lal et al., 1975). However, rats chronically treated with haloperidol show no tolerance to either morphine or haloperidol (Lal et al., 1975).

(2) Withdrawal Signs

Withdrawal from chronic morphine produces wet-dog-like body shakes, ptosis, weight loss, hyperactivity and aggression (Lal, 1975). Rats withdrawn from chronic haloperidol exhibit body shakes and increased locomotor activity (Lal et al., 1975). In both cases, the aggression eliciting effects of apomorphine are markedly increased. However, administration of amphetamine to morphine-withdrawn rats elicits intense aggression (Lal et al., 1971) but fails to elicit aggression in haloperidol withdrawn rats (Lal et al., 1975).

2. Biochemical Similarities

a. Acute Actions

(1) Striatal Dopamine

Steady state concentrations of dopamine in the rat striatum have been found not to change after the injection of morphine sulfate or haloperidol. However, both morphine and haloperidol increase the rate of dopamine depletion after inhibition of its synthesis by alpha methyl-

paratyrosine (Lal et al., 1976). In the absence of changes in dopamine steady state levels, an alteration in the depletion rates of neurotransmitter can be considered as a measure of changes in the turnover rate of that transmitter (Costa and Neff, 1966). Both morphine and haloperidol produce a dose-dependent increase in striatal dopamine turnover, with haloperidol showing considerable more potency (Lal et al., 1976). The fact that both of these drugs produce increases in striatal dopamine turnover is indicated by the fact that they also increase striatal homovanillic acid, a dopamine metabolite (Lal et al., 1976). Naloxone block the action of morphine on striatal dopamine turnover; however, it did not affect the similar action of haloperidol.

The effects of morphine and haloperidol in causing stimulation of dopamine turnover coincides with their effect on catalepsy. Both morphine and haloperidol produce a dose-related catalepsy (Lal et al., 1975), with the cataleptic action of morphine being readily reversed by naloxone; however, naloxone treatment was ineffective in haloperidol catalepsy (Wauquier et al., 1974).

An important interaction between haloperidol and morphine in producing an elevation of dopamine turnover was noted when a subthreshold dose of haloperidol was combined with the ED_{50} dose of morphine. The subthreshold dose of haloperidol caused a significant increase in the effect of morphine so that the morphine ED_{50} produced as great an effect as was produced by the maximally-effective dose of

morphine and haloperidol were combined there was an increase in striatal dopamine turnover equivalent to that which was obtained by haloperidol alone (Lal, et al., 1976).

Apomorphine is known to directly stimulate dopamine receptors (Anden et al., 1967, Ernst, 1967; Costall and Naylor, 1973) and thereby reduce dopamine turnover. Lal et al., (1976) reasoned that if the actions of morphine and haloperidol involved blockade of dopamine receptors, then apomorphine might reverse the actions of both drugs by stimulating the same receptor. Similarly, it has been known for some time that anticholinergic type anti-Parkinsonian drugs interact with dopamine containing neurons in the central nervous system and thereby alter the activity of neuroleptics. Therefore, if certain actions of morphine overlap with those of haloperidol then benztropine may interact with both drugs in a similar manner. Treatment with morphine or haloperidol produces a significant increase in striatal dopamine turnover while apomorphine or benztropine reduce dopamine turnover (Lal, et al., 1975). Apomorphine on benztropine reversed the elevation of dopamine turnover elicited by an injection of morphine or haloperidol (Lal et al., 1976). A similar interaction was also observed for catalepsy. While neither apomorphine or benztropine cause catalepsy on their own, either drug effectively antagonized the catalepsy induced by haloperidol or morphine (Lal, et al., 1976).

The haloperidol-induced elevation of striatal dopamine turnover is markedly diminished after lesioning of the

nigrostriatal tract at the level of the lateral hypothalamus (Anden et al., 1973). For purposes of comparison the nigrostriatal tract was lesioned before the administration of morphine sulfate. Unilateral lesions of the nigrostriatal tract increased the dopamine content of the striatum (Hynes et al., 1975; Lal, et al., 1976). Following lesioning morphine still elevated dopamine turnover but the magnitude of this elevation was smaller than that on the intact side (Lal, et al., 1976). The effect of morphine on striatal dopamine turnover was completely eliminated by bilateral lesioning of the nigrostriatal tract (Lal et al., 1976).

(2) Adenylate Cyclase and Phosphodiesterase

Earlier studies have suggested that many actions of the catecholamines may be mediated by cyclic AMP (Greengard and Kebabian, 1974). An adenylate cyclase activated by low concentrations of dopamine have been found in homogenates of rat cuadate nucleus. This enzyme is stimulated by dopamine and l-norepinephrine and in the presence of optimal amounts of either dopamine or norepinephrine no further increase in adenylate cyclase activity can be obtained by the addition of the other catecholamine (Krugger et al., 1975). Like dopamine and norepinephrine, morphine sulfate added to the homogenate of the rat striatum also increased the formation of cyclic AMP (Lal et al., 1975; Puri et al., 1975). However, the morphine stimulation of adenylate cyclase was not antagonized by naloxone. The maximal stimulation of adenylate cyclase by either morphine or dopamine was about the same

(Puri et al., 1975). When submaximally effective concentrations of dopamine were combined with morphine, there was an additive effect. However, in the presence of an optimal concentration of either dopamine or morphine, no further increase in adenylate cyclase activity could be obtained by the addition of the other agent (Lal et al., 1976).

The effect of morphine on striatal phosphodiesterase activity in crude striatal homogenates has also been measured. The in vitro addition of morphine inhibited phosphodiesterase activity only when the highest concentrations of C AMP were employed. Similarly, the in vivo administration of morphine also inhibited phosphodiesterase activity when the three highest C AMP concentrations were employed as substrate (Lal, et al., 1976).

b. Chronic Actions

(1) Striatal Dopamine

Unlike its effects in naive rats an acute injection of morphine failed to produce an increase in the striatal dopamine turnover in morphine-dependent rats. In these morphine dependent rats, dopamine turnover was markedly lower in response to an acute morphine injection than the turnover rate obtained in naive or chronically haloperidol treated rats in response to morphine administration. Similarly, an acute dose of haloperidol, which increased striatal dopamine turnover in naive and chronically haloperidol treated rats was only marginally effective in morphine dependent rats (Lal, et al., 1976).

It has been previously demonstrated that supersensibility develops to the aggression-eliciting effects of apomorphine in rats withdrawn from chronic morphine or haloperidol (Puri and Lal, 1973; Gianutsos et al., 1974; Lal et al., 1975). After chronic morphine administration supersensitivity also develops to apomorphine's effect on striatal dopamine turnover (Lal, et al., 1976). Doses of apomorphine which were previously ineffective in naive rats cause a marked inhibition of striatal dopamine turnover in rats withdrawn from chronic morphine or haloperidol.

(2) Adenylate Cyclase Activity

The relationship between opiates and the cyclic AMP-adenylate cyclase system has been examined in a number of ways. Peripheral administration of cyclic AMP antagonized morphine analgesia and increased withdrawal signs in morphine-tolerant animals (Ho et al., 1972; 1973). The injection of cyclic AMP into the lateral ventricles of rats also increased withdrawal signs in tolerant animals (Collier and Francis, 1975).

An acute injection of morphine (60 mg/kg) has been found to produce significant increases in cyclic AMP levels in midbrain, cerebellum, striatum and cortex 15 to 30 minutes after the injection (Clouet and Iwatsubo, 1976). While in the hypothalamus, medulla and cerebellum, the levels of cyclic AMP were significantly lower than control values two hours after the injection of morphine (Clouet, et al., 1975). In morphine tolerant rats sacrificed two hours after the last

twice-daily injections for 10 days, the levels of cyclic AMP were at control levels or higher, indicating that tolerance to the decreased cyclic AMP had developed (Clouet et al., 1975).

Adenylate cyclase activity was increased in midbrain and striatum one hour after the injection of 60 mg/kg morphine or 15 mg/kg levorphonal, but not after 15 mg/kg dextrorphan (Clouet and Iwatsubo, 1976). The enzyme activity was, however, decreased by narcotic agonists in the cerebellum. In tolerant animals, cerebellar adenylate cyclase activity increased by a morphine challenge, as it was in midbrain and cortex, again indicating a tolerance in cerebellar cyclase (Clouet et al., 1975).

Dopamine-sensitive adenylate cyclase activity in shocked preparations of striatal synaptosomes was found to increase after acute morphine treatment above control dopamine-stimulation (Clouet and Iwatsubo, 1976). Although the basal cyclase activity was found to increase above control levels from 15 to 60 minutes after morphine injection, the absolute stimulation by 100 μM dopamine was significantly higher at 30 minutes to 2 hours after the injection of the opiate than stimulation by dopamine in preparations from untreated rats. The percent stimulation by dopamine was similar at each time point after acute morphine treatment (Iwatsubo and Clouet, 1975). The basal adenylate cyclase activity in striatal nerve endings was unchanged after rats were made tolerant by morphine pellet implantation. However,

when rats were made dependent by increasing doses of morphine a significant increase in the basal levels of adenylate cyclase was observed. The increase was seen from 1 to 72 hours after the last injection of morphine and was therefore not related to the acute effects of morphine. The elevated levels of adenylate cyclase activity could not be further stimulated by *in vitro* addition of dopamine (Lal *et al.*, 1976).

3. Endocrinological Similarities

a. Adrenocorticotropin Hormone

Current evidence indicates that adrenocorticotropin (ACTH) is under dual hypothalamic control (Fortier, 1966) and neurochemical findings have in addition led to the proposal of adrenergic-inhibitory and cholinergic-stimulatory regulation of ACTH secretion (Ganong, 1972; de Wied and de Jong, 1974, Marks *et al.*, 1970). In considering the effects of narcotics on pituitary function, it must be determined if morphine interferes with the basal secretion of ACTH, the release of ACTH in response to stress and the circadian rhythm of ACTH secretion.

Acute morphine administration in rats stimulates ACTH secretion (Briggs and Munson, 1955; George and Way, 1955; Nikidijevic and Maickel, 1967). Using the depletion of adrenal ascorbic acid as a measure of ACTH release it has been reported that a single injection of morphine enhances the secretion of ACTH in the unanesthetized rat (George and Way, 1955; Nasmyth, 1954; Van Peenen and Way, 1957; Briggs and Munson, 1955; Nikodijevic and Maichel, 1967; Lotti *et al.*,

1969). Similar findings have been reported following acute administration of (+) and (-) methadone (George and Way, 1955). Preadministration of nalorphine in small doses inhibits the adrenal ascorbic acid depletion effect of both morphine (George and Way, 1955; Burdette et al., 1961) and the isomers of methadone (George and Way, 1955). The effects of morphine on ACTH are also completely blocked by naloxone (Kakka and George, 1974). These results suggest that the ACTH releasing effects of these drugs are mediated via specific neural pathways and/or narcotic receptors. This ascorbic acid depleting effect of morphine and methadone is dependent upon an intact pituitary-adrenal axis since it is abolished by hypophysectomy (George and Way, 1955).

A more direct measure of pituitary-adrenal activity is reflected by changes in the plasma level of corticosteroids. The effect of acute morphine administration on adrenal cortical hormone secretion appears to be species dependent (George and Lomax, 1972). Several reports have clearly shown that a single injection of morphine is capable of elevating plasma corticosterone levels in conscious rats (Nikodijevic and Maickel, 1967; Lotti et al., 1969; Oliver and Troop, 1963; Shusher and Browning, 1961) and plasma 17-hydroxycorticosterone levels in dogs (Suzuki et al., 1959). However, this has not been confirmed in studies on the guinea pig (Sobel, et al., 1958) and man (Eisenman et al., 1958).

Although the mechanism by which morphine exerts these effects is not clear, it is however evident that it is

mediated by the central nervous system (George and Lomax, 1972; Kokka and George, 1974; Zimmermann and Critchlow, 1973). The studies of George and Way (1959) in rats with hypothalamic lesions that were challenged with morphine and those of Lotti et al., (1969) in which rats were injected intrahypothalamically with micro-quantities of morphine, have indicated that the integrity of the hypothalamic-pituitary axis is essential for these actions of morphine. It was found that hypothalamic lesions, primarily in the anterior medial eminence blocked the ascorbic acid depleting effect of morphine. Conversely, injections of microgram amounts of morphine into this hypothalamic region reduced adrenal ascorbic acid while elevating plasma corticosterone levels. Thus most of the available evidence indicates that morphine activation of ACTH secretion is mediated via a direct action on the rostral region of the hypothalamus and medial eminence (George and Way, 1959; Lotti et al., 1969; Zimmermann and Critchlow, 1973), although extrahypothalamic sites and peripheral mechanisms can not be excluded (Kokka and George, 1974).

The chronic administration of morphine to rats produces a marked adrenal cortical hypertrophy. This observation was first made in 1926 by McKay and McKay and has been confirmed by others (George and Way, 1959; Tanabe and Cafruny, 1958; Sloan et al., 1963; Sloan and Eisemann, 1968). Investigations in which different parameters of pituitary-adrenal activity were studied seem to indicate an opposite effect, for example chronic administration of morphine depresses the

basal level of adrenal cortical secretion. The injection of morphine into guinea pigs for 12 days was found to reduce the basal level of urinary 17-hydroxysteroids (Sobel et al., 1958). Similar findings have been observed in the rat and in man. Paroli and Melchiorri (1961) injected rats for periods ranging between 10 to 40 days and 40 to 100 days and noted a decrease in the urinary levels of hydroxysteroids. In a study on human addicts Eisenmann et al. (1961) found that two-to-four month cycles of morphine addiction reduced both plasma and urinary 17-hydroxycorticosteroid levels. They showed that this reduction in the basal secretory rate of adrenal cortical hormones was due chiefly to a decrease in the production of 17-hydroxycorticosteroid.

Chronic administration of morphine may also depress the pituitary adrenal response to a variety of stresses. In rats treated with morphine for a five-day period, the response to several stresses was blocked (Munson and Briggs, 1955). In other investigations on rats injected with morphine for 25 days (Paroli and Melchiorri, 1961) and guinea pigs injected for 12 days (Sobel et al., 1958) it was reported that the increase in urinary hydroxysteroids in response to cold stress was inhibited. On the basis of these findings it would appear that chronic morphine administration produces a depression in the basal secretion of adrenal cortical hormones and prevents pituitary-adrenal activation in response to numerous stresses. However, in a study with methadone-treated heroin addicts, Cushman et al. (1970)

found no differences in basal plasma 17-hydroxycorticosteroid levels of addicts from normal control valves, also chronic methadone treatment did not interfere with the metyrapone test, which produces an increase in urinary 17-ketogenic steroids,or to the stress of insulin hypoglycemia, as reflected by elevated plasma 17-hydroxycorticosteroid (17-OHCS) levels.

The normal diurnal pattern of 17-OHCS also may be altered by the chronic administration of morphine and methadone. Eisenmann et al. (1961) found that morphine interfered with the early morning rise of plasma 17-OHCS; they also noted the presence of a mid-day increase of plasma 17-OHCS during the period of morphine administration. The results of Cushman et al. (1970) in general, corroborate these findings. They were able to show that chronic methadone treatment in a group of 16 heroin addicts altered the normal diurnal variations of plasma 17-OHCS in 10 of the 16 subjects.

In both the rat and man, abrupt withdrawal of morphine resulted in increased adrenal cortical activity (George and Lomax, 1972). Rats given morphine chronically and then abruptly withdrawn from the drug or administered nalorphine show an increase in levels of urinary 17-OHCS, 17-ketosteroids and aldosterone (Paroli and Melchiorri, 1961). In man, also, the abrupt withdrawal of morphine produces a marked increase in plasma and urinary 17-OHCS and an elevation in urinary 17-ketosteroids (Eisenmann et al., 1961). The maximal rise in adrenal cortical levels correlates well with the peak physiological effects of the abstinence syndrome, approximately 48

hours after withdrawal.

Although there are numerous reports regarding the effect of chlorpromazine on pituitary adrenal function, it is not clear whether chlorpromazine stimulates or inhibits ACTH secretion. The discrepancies could arise from a number of factors, the dose of chlorpromazine, the time chlorpromazine was administered prior to stress challenge, the core temperature of the stressed animal and the parameter of pituitary adrenal activation employed.

In general, numerous studies in rats have indicated that small doses of chlorpromazine do not alter adrenal ascorbic levels to an appreciable degree, larger doses deplete ascorbic acid (de Wied, 1967; George and Lomax, 1972). Both small and large doses of chlorpromazine have been reported to have no effect; a partial blocking effect, or complete blocking effect on the ascorbic acid depletion response to several types of stress (de Wied, 1967; George and Lomax, 1972). Marks et al. (1970) have shown that a single injection of chlorpromazine (25 mg/kg) was capable of lowering hypothalamic corticotropin-releasing factor, while simultaneously elevating plasma corticosterone levels. When the corticotropin-releasing factor content of the ventral hypothalamus returned to control levels it was found to be closely related to the return to normal adrenal corticosterone levels. Marks et al. (1970) interpret the blockade of stresses following chlorpromazine-administration as being due to the possibility that corticotropin-releasing factor secretion has

become maximal, which is the result of removal of inhibitory neural influences by chlorpromazine.

The results obtained from other species are more consistent. Using plasma 17-OHCS as an index of pituitary adrenal activity, it has been shown that chlorpromazine increases ACTH secretions in the guinea pig (Sasaki, 1963) dog (Egdahl and Richards, 1956; Betz and Ganong, 1963) and monkey (Harwood and Maon, 1957). This effect of chlorpromazine is abolished by hypophysectomy (Egdahl and Richards, 1956), thereby placing the site of action at the pituitary or hypothalamus.

Chronic administration of chlorpromazine like morphine produces adrenal cortical hypertrophy, but, unlike morphine, does not consistently prevent adrenal activation in response to stress (de Wied, 1967). In patients treated with chlorpromazine, the ACTH response to the stresses of insulin (Christy et al., 1957) and typhoid vaccine (Fotherby et al., 1959) is inhibited.

b. Antidiuretic Hormone

It is well established that morphine and its surrogates produce an antidiuretic effect in certain animals (Fujimoto, 1971; Hayward, 1974). The bulk of evidence (Fujimoto, 1971; Hayward, 1974) favors the concept first proposed by de Bodo (1944) that the antidiuretic effect of morphine in the dog is due to release of antidiuretic hormone. However, many of the studies on the antidiuretic effect of morphine are contradictory, depending on the species and

dosage employed, route of administration, presence or absence of anesthetic agents, and the development of tolerance. Studies showing convincing antidiuretic hormone release by morphine were performed in the dog (Fujimoto, 1971; de Bodo, 1944; Duke et al. 1951; Handley and Keller, 1950), rat (George and Way, 1959) and monkey (Hayward, 1974) but studies in man show either a primary change in renal hemodynamics (Papper and Papper, 1964; Habif et al., 1951) or a diuretic effect (Fujimoto, 1971).

Neuroleptics have been found to induce antidiuretic as well as diuretic effects, and their action on antidiuretic hormone release are not clear (Gaunt et al., 1963; de Wied, 1967).

A dose response relationship has been reported for the antidiuretic action of chlorpromazine (Meier et al., 1955; Supek et al., 1960). In addition, in rats anesthetized with alcohol a very low dose of chlorpromazine elicited a marked antidiuresis while in rats with diabetes insipidus the antidiuretic effect of chlorpromazine was no longer demonstrable. This observation suggests that the antidiuretic effect of chlorpromazine is due to increased release of antidiuretic hormone (de Wied, 1967). High doses of chlorpromazine given over a several-day period may depress synthesis of antidiuretic hormone (de Wied, 1967) and in lower dose levels may inhibit antidiuretic hormone release in response to hypertonic salt loading or to painful stimuli (Shibusawa et al., 1955; de Wied and Jinks, 1958).

c. Gonadotropins

Morphine administration has been shown to effect the secretion of gonadotropins (George and Lomax, 1972; Barraclough and Sawyer, 1955). One of the earlier suggestions that morphine can alter pituitary gonadotropic hormone secretion was from a survey in which it was found that human female addicts exhibited decreased libido, amenorrhea and sterility during a period of morphine addiction. In many of these cases, even though the menstrual cycles returned to normal after withdrawal from the drug, sterility persisted (Menninger-Lerchenthal, 1934). Barraclough and Sawyer (1955) found that administration of morphine between 12 and 2 P.M. on the day of proestrus in rats prevents ovulation. Chronically injected morphine daily between 12 and 2 P.M. inhibits ovulation for a considerable period of time. Tolerance to the drug results in the recurrence of ovulation, although cycles remain irregular. The site of the morphine effect is near the medial eminence. Electrical stimulation in the medial eminence, but not in the posterior tuberal region, overcomes the morphine blockade of ovulation (Sawyer, 1963). Addicts have irregular menstrual cycles and decreased gonadotropin secretion as reflected by a diminished urinay excretion of 17-ketosteroids, while the response to exogenous gonadotropins is enhanced (Eisenmann et al., 1958; Hollister, 1973; Stoffer, 1968). Chronic administration of morphine to male rats has been shown to produce atrophic changes in their seminal vesicles and prostates (George and Lomax, 1972).

Although these data are indicative of a luteinizing hormone suppressing effect of morphine, there is also some evidence in favor of a follicle-stimulating hormone inhibiting action of morphine. Rennels (1961) found that two weeks of morphine administration produced changes in pituitary cytology and gonadotropin content which were indicative of follicle-stimulation hormone suppression and without effect on luteinizing hormone secretion. In summary, it is apparent that morphine administration, either acute or chronic, inhibits gonadotropin secretion. Although there is evidence for a morphine inhibitory effect on both follicle-stimulating hormone and luteinizing hormone secretion, most of the evidence points to an interference with luteinizing hormone release (George, 1971; George and Lomax, 1972; de Wied et al., 1974).

The endocrine system most uniformly affected by the phenothiazines is the pituitary-gonad axis. Numerous investigations in a variety of species have shown that either acute or chronic administration of phenothiazines inhibits secretion of pituitary follicle-stimulating hormone and luteinizing hormone. Phenothiazines have produced some of the following effects which are indirect indices of depression of follicle-stimulating hormone and luteinizing hormone secretion: failure of ovulation, delayed sexual maturation, reduction in weight of ovaries, uteri and vaginae, reduction in size of sex organs in the male, and inhibition of compensatory ovarian hypertrophy (de Wied, 1967).

While most of the evidence points to an inhibitory

effect of chlorpromazine on hypothalamic pituitary function, there are data that suggest the possibility of a blocking action of chlorpromazine at the target gland. In large doses chlorpromazine has been found to block the action of gonadotropins on target glands in both sexes (Meidinger, 1954; Zarrow and Brown-Grant, 1964). However, data contrary to these findings raise doubts regarding the target gland as the site of chlorpromazine action (de Wied, 1967; de Wied and de Jong, 1974).

The results reported from animal studies have also been observed in the human female. Chlorpromazine administered one to three days prior to ovulation delays menstruation for a period of 8 to 16 days (Wheklaw, 1956). Also, many reports show that chlorpromazine treatment may produce menstrual irregularities and amenonhea (de Wied, 1967). Butyrophenone type neuroleptics have many of the same endocrine effects as the phenothiazines (Byck, 1975).

d. Growth Hormone

Morphine and methadone have been found to dose dependently elevate plasma concentrations of growth hormone (Howard and Martin, 1971; Wakaboyaski et al., 1971; Kokka et al., 1972; 1973; Kokka and George, 1974). Nalorphine, which has many of the agonist properties of morphine, also produced increases in plasma concentrations of growth hormone (Kokka and George, 1974). Naloxone, a pure antagonist, was without effect on growth hormone. The morphine increase in plasma growth hormone was not blocked by naloxone (Kokka and

George, 1974). Chronic morphine administration does not result in tolerance to its effects on growth hormone (Kokka et al., 1973; Kokka and George, 1974). Bilateral electrolytic lesions of the ventromedial nucleus did not affect morphine-induced stimulation of growth hormone secretion. These results suggest that morphine stimulation of growth hormone secretion may be due to a direct effect on the anterior pituitary, but it is also possible that morphine acts at the level of the median eminence to inhibit release of growth hormone-inhibiting factor (Kokka and George, 1974).

The secretion of growth hormone is under the control of somatotropin release-inhibiting hormone (Brazeau et al., 1973) and growth hormone releasing hormone (Deuben and Meites, 1964). The influence of dopaminergic neurons on growth hormone release is unclear. Pimozide, a dopamine receptor blocker, elevates plasma growth hormone in rats (Mueller et al.,1973). While plasma growth hormone in rats is dramatically reduced by haloperidol, with a dose of 0.3 mg/kg causing levels to fall below 20 percent of control values (Muller et al., 1976). Haloperidol also blocked the apomorphine-induced stimulation of growth hormone secretion (Mueller et al., 1976). Chlorpromazine has also been found to inhibit secretion of growth hormone (Byck, 1975) and to block the L-DOPA-induced stimulation of growth hormone in man (Mims et al., 1975). Chronic chlorpromazine administration has been reported to have no effect on body growth in rats (Meidinger, 1954) or to inhibit growth in mice (Cranston and Segal, 1958).

e. Prolactin

Prolactin is a hormone produced by the anterior lobe of the pituitary gland. It is capable of initiating and sustaining lactation, but only when other essential hormones such as estrogen, progesterone and oxytocin are present. The effect of morphine and related compounds on prolactin release has not been studied extensively. Several years ago, Meites (1966) reported that acute administration of morphine stimulates lactation in the rat, indicating an increase in the discharge of prolactin. Recently, increased circulating levels of prolactin have been observed in rats following systemic (Zimmermann et al., 1974) or intraventricular (McCann et al., 1974) administration of morphine.

The effects of neuroleptics on prolactin levels have been more extensively studied. The administration of tranquilizers to some patients has long been known to cause breast enlargement and galactorrhea (MacLeod, 1976). Phenothiazine derivatives, especially perphenazine, have long been known to induce mammary development and initiate milk secretion in many species. Injection of perphenazine causes a prompt increase in serum prolactin levels (Ben-David et al., 1970); Lu et al., 1970; MacLeod and Lehmeyer, 1974) and increases synthesis of the hormone (MacLeod and Lehmeyer, 1972). Dickerman et al. (1972) showed that as little as 10 ug haloperidol per 100 grams body weight increased serum prolactin levels. The inhibition produced by dopamine of the in vitro secretion of both ^{3}H-prolactin and of radio-

immunoassayable prolactin was reversed by coincubation with 5×10^{-9} M haloperidol (MacLeod, 1976). The neuroleptic drug pimozide has also been found to be a potent stimulator of prolactin secretion. Injecting the drug into rats significantly increases the circulating level of prolactin and the *in vitro* capacity of the pituitary to synthesize the labeled hormone (MacLeod, 1976). Pimozide also rendered the rat pituitary gland refractory to the usual inhibitory *in vitro* action of dopamine (MacLeod and Lehmeyer, 1973; 1974) and thus has a pharmacological action similar to perphenazine and haloperidol.

Chronic morphine treatment has been found to decrease the release of prolactin (de Weid *et al.*, 1974). However, prolactin levels in methadone-treated males have been found to be within normal limits (Cushman and Kosek, 1974). Withdrawal from chronic treatment with morphine has been found to result in lower circulating levels of serum prolactin in male rats (Lal *et al.*, 1977).

Rats receiving chronic daily injections of perphenazine demonstrate an increase in prolactin synthesis, and in male rats the drug caused a fivefold increase in *in vitro* prolactin secretion (MacLeod, 1976). The pituitary glands from these perphenazine-treated rats when incubated with dopamine, failed to exhibit the usual prolactin inhibiting action of dopamine (MacLeod, 1976). An implant of pimozide in the pituitary gland or in the median eminence-arcuate region produces a gradual increase in serum prolactin (Ojeda

et al., 1974). Withdrawal from chronic haloperidol treatment has been found to result in lower circulating levels of serum prolactin in male rats (Lal et al., 1977). These results suggest hyperactivity of the tubero-infundibular dopamine system (Lal et al., 1977). The tubero-infundibular dopamine neurons are responsible for a tonic inhibitory effect on the release of prolactin (de Wied and de Jong, 1974; Kamberi, 1973; Collu et al., 1973).

f. Thyroid Hormone

Nearly all of the studies regarding the effects of narcotic analgesics on pituitary-thyroid function indicate that these agents inhibit basal thyroid secretion (George and Lomax, 1972). Single intravenous injections of morphine, meperidine, levorphanal, dextrorphan and dihydromorphinone, have been reported to stimulate thyrotropin (TSH) release as measured by the release of thyroidal ^{131}I (George and Lomax, 1972). The chronic injection of all of these compounds for a period of five-day inhibited thyroid activity (Redding et al., 1966). Codeine administered to rats for 14 days has been found to lower basal thyroid function; however, codeine did not prevent hypersecretion of TSH since it did not block the thyroid hypertrophy induced by methylthiouracil (Schreiber, 1968).

Chronic morphine administration for three weeks decreased pituitary TSH content (Hohlweg et al., 1961). Injection of morphine for periods of three or five days inhibits release of ^{131}I-labeled thyroid hormone (George and

Lomax, 1965), depresses ^{131}I uptake by the thyroid and reduces thyroid and pituitary weights (Samel, 1958). These data show that morphine may have a dual action on the pituitary-thyroid axis (George and Lomax, 1972). A single injection of morphine accelerates the release of ^{131}I-labeled thyroid hormone in mice, whereas administration of morphine for a period of three days or longer inhibits several parameters of thyroid activity in rats (George and Lomax, 1972).

Although the mechanism through which this inhibition occurs is not known, there are several lines of evidence which implicate hypothalamo-hypophysial involvement. Hypothalamic control of TSH is well established. Anterior hypothalamic lesions block the goitrogenic effects of propylthiouracil (Greer, 1952) whereas electrical stimulation of this area enhances TSH secretion (Harris and Woods, 1958). On the basis of these findings, Lomax and George (1966) studied the effect of morphine on thyroid activity in rats with hypothalamic lesions. They found that lesions in the caudal region of the hypothalamus completely blocked the morphine-induced thyroidal inhibition, but animals with lesions in the anterior hypothalamus displayed normal ^{131}I thyroidal release rates. Lomax and George (1966) suggest that morphine might be activating an inhibitory hypothalamic area rather than depressing a facilitatory one for the secretion of TSH. In a subsequent study Lomax *et al*. (1970) found that intrahypothalamic administration of microquantities of morphine (5 µg) produced inhibition of thyroid activity when the drug

was injected into both rostal and caudal regions of the hypothalamus. The extent of inhibition was found to vary with the injection site. Injections of morphine into the caudal hypothalamus inhibited release of ^{131}I-labeled thyroid hormone throughout the period of drug administration, while injection of morphine into the rostal site produced a transient inhibition, suggesting the development of tolerance in this area. Since there is some evidence that electrical stimulation of the caudal hypothalamus may inhibit TSH secretion (Vertes et al., 1965) and since injection of morphine into the caudal hypothalamus produces hyperactivity and hyperthermia (Lotti et al., 1965), Lomax et al. (1970) suggested that morphine was producing its thyroid inhibiting effect primarily via stimulation of inhibitory neurons in the caudal hypothalamus.

Conclusions drawn from experiments on the effect of neuroleptics on thyroid function vary considerably. This is caused by the various measures used to determine thyroid activity, the different dosages and the duration of the treatment employed, and differences in experimental circumstances, especially with regard to environmental temperature. Using ^{131}I-uptake by the thyroid as a measure of pituitary-thyroid activity, several workers observed a decrease in uptake after a relatively high dose of chlorpromazine (Arvay et al., 1960; Wiseman, 1962; Ksycki and Lockett, 1965; de Wied, 1967). Doses of 10 mg of chlorpromazine per kg and lower generally do not depress ^{131}I-uptake by the thyroid (Wiseman, 1962;

Wright, 1958), but Milcou et al., (1955) found a depression with about 6 mg/kg.

Inhibitory effects on ^{131}I-uptake may be the result of a central effect or of a direct action of chlorpromazine either on iodine trapping or on peripheral mechanisms. Direct effects of chlorpromazine on synthesis of thyroid hormone in a dose of about 10 mg/kg have been observed by Ksychki and Lockett (1965). They found that incubation of thyroids from mice treated with chlorpromazine exhibited reduced organic binding of ^{131}I. Evidence for a direct action of chlorpromazine on the thyroid has been also derived from studies by Mayer et al. (1956). Incubation of chlorpromazine with thyroid slices depressed ^{131}I trapping by the gland, while later stages of the iodine cycle proceeded abnormally only if high doses were used.

The rate of release of ^{131}I by the thyroid has for some time been regarded as the most reliable indirect parameter of pituitary-thyroid activity (Brown-Grant et al., 1954; de Wied, 1967). It has been employed by a few investigators for the study of chlorpromazine on thyroid function (Foldes et al., 1959; George and Lomax, 1965; Milcou et al., 1955; Wright, 1958). George and Lomax (1965) found that 5 mg/kg of chlorpromazine decreased ^{131}I-release by the thyroid in intact as well as adrenalectomized rats. Since TSH accelerated ^{131}I-release in chlorpromazine treated animals, it seemed likely that thyroid activity was not directly blocked by the drug (de Wied, 1967).

The effects of chronic treatment with chlorpromazine on thyroid function have been studied with a variety of functional and morphological parameters. A reduction in ^{131}I-uptake by the thyroid in rats chronically treated with chlorpromazine (2 to 100 mg/kg/day) has been demonstrated (Arvay et al., 1960; Reiss, 1958; Wiseman, 1962; Wright, 1958). The release of ^{131}I from the thyroid is similarly depressed by chronic treatment (Foldes et al., 1959; Samuel, 1958; Wright, 1958). These results suggest that thyroid function is blocked in animals on long-term basis with chlorpromazine. However, other studies (de Wied, 1967; Arvay et al., 1960; von Brauchitsch, 1961) have shown a biphasic effect on ^{131}I-uptake by the thyroid after long-term administration of high doses of chlorpromazine. Initially, during the first two to three days, these investigators have found an inhibition of ^{131}I-uptake which is then followed 9 to 10 days later by an increased uptake of ^{131}I. Long-term administration of chlorpromazine also has been noted to produce goiter in the rat (Sulman, 1959), although chlorpromazine paradoxically partly inhibits the goitrogenic effect of thiouracil derivatives (Aleshin and Us, 1960; Meidinger, 1954). The effects of chronic chlorpromazine administration in man are difficult to interpret since most patients receiving chlorpromazine have psychiatric disorders. Most of the studies in man have failed to show changes in thyroid activity (de Wied, 1967), although it has been reported that chlorpromazine may increase thyroidal ^{131}I-uptake without interfering with other

parameters of thyroid functions (Blumberg and Kelin, 1969); George and Lomax, 1972). Although the mechanism by which chlorpromazine interferes with the action of TSH on thyroid tissue has not clearly been established, there is some evidence which favors an interference at the adenyl cyclase - cyclic AMP level (Yamashita et al., 1970; Wolff and Jones, 1970) as well as the lysosomal membrane level (Onaya, 1969; Williams and Wolff, 1971).

In summary, there is evidence that acute administration of the phenothiazines may inhibit both the uptake and release of thyroid ^{131}I, indicative of reduced TSH secretion, but that chronic administration reverses these effects. In vitro experiments suggest that chloropromazine interferes with thyroid function by interfering with the action of TSH on the thyroid.

F. Methods for the Production of Physical Dependence

A large number of methods have been devised for inducing narcotic tolerance and dependence in laboratory animals. These different techniques may be distinguished by the route, frequency and method of drug delivery to the animal. The objectives of these experimental maneuvers are to minimize acute toxicity while effecting a sufficiently high narcotic intake so that a high degree of tolerance and dependence will rapidly develop. Some of the more extensively and thoroughly investigated models for producing narcotic dependence are daily parenteral injections, oral intake via water or food, implantation of pellets and infusion.

1. Parenteral Injections

Laboratory animals have traditionally been made dependent on morphine through intraperitoneal or subcutaneous injections of the drug on a regular basis for relatively long periods of time (Himmelsbach et al., 1935; Martin et al., 1963; Akera and Brody, 1968; Buckett, 1964; Hynes et al., 1976). The procedure of Martin et al. (1963) is a typical example of the intraperitoneal method, which is the most common of the injection regimens for addicting rats. The initial dose of morphine was 5 mg/kg administered intraperitoneally twice daily at 8 A.M. and 3 P.M. The dose was increased biweekly until the rats were receiving 320 mg/kg/day by the thirty-fifth day of addiction. The rats were stabilized at this dose level for a week and the morphine was withdrawn abruptly. Other investigators have employed one daily dose of narcotic (Wikler et al., 1963) while others have employed three (Akera and Brody, 1968 ; Hynes et al., 1976) and even four daily narcotic injections (Puri and Lal, 1973). A variety of terminal morphine doses have also been employed. Terminal morphine doses have ranged from 30 mg/kg/day (Akera and Brody, 1968) to over 400 mg/kg/day (Buckett, 1964; Puri and Lal, 1973; Hynes et al., 1976). Although many investigators administer the narcotic by the intraperitoneal route (Martin et al., 1963; Buckett et al., 1964; Puri and Lal, 1973; Hynes et al., 1976) others have used subcutaneous injections (Akera and Brody, 1968).

Mice have also been rendered tolerant to and physically dependent on morphine by daily subcutaneous injections of increasing doses of morphine (Loh et al., 1969; Marshall and Grahame-Smith, 1971). Morphine has been injected three times a day in these procedures. Loh et al. (1969) used a starting dose of 10 mg/kg which was gradually increased over a three-week period until 200 mg/kg was given every 8 hours, while a maximum of only four days of morphine administration was employed by Marshall and Grahame-Smith (1971).

Both abrupt withdrawal and precipitated withdrawal have been employed after the chronic injection of morphine. During abrupt withdrawal loss of body weight, body shakes, hypothermia, piloerection, ptosis, loose stools and aggression have been noted (for review see Granutsos et al., 1975). After the precipitation of withdrawal by a narcotic antagonist, loss of body weight, jumping, diarrhea, piloerection and body shakes have been observed (Marshall and Grahame-Smith, 1971; Blasig et al., 1976).

The administration of narcotics by daily injection is a reliable method for the induction of physical dependence that is widely employed. There are, however, several drawbacks to this technique. For example, it requires that the researcher spend part of his time injecting animals on a regular basis. There is also a possibility of producing skin inflammation, injury and infection. Large amounts of narcotics are needed to establish dependence according to this method.

2. Oral Consumption

The oral route of narcotic administration is another method which has been employed by several investigators to induce dependence in laboratory rats (Risner and Khavari, 1973; Stolerman and Kumar, 1970; Shuster et al., 1963; Wikler et al., 1963). Although the oral ingestion method is simple and economical, there are some difficulties associated with it. The amount of morphine solution ingested by rats is apparently limited by at least two factors. First, the highly bitter taste of the opium alkaloid appears to be aversive to animals even at low concentrations. Secondly, the animals consume relatively constant quantities of water to satisfy bodily fluid requirements (Khavari and Risner, 1973).

One method used to overcome these problems has been to force animals to drink narcotic solutions in order to relieve their thirst. Wikler et al., (1960) found that normal rats deprived of water for twenty-two hours drank an aqueous solution of etonitazene methane sulfonate avidly. Within seven minutes after starting to drink this potent narcotic the rats exhibited exophthalmos, tail rigidity and periods of stupor and/or hyperactivity (Wikler et al., 1960). After twenty-four hours of abstinence similarly water-deprived addicted rats (maintained on a single subcutaneous dose of morphine, 200 mg/kg/daily) also drank a solution of etonitazene avidly. These rats showed not only morphine-like effects but also the disappearance of withdrawal body shakes (Wikler et al., 1960; Wikler et al., 1963). Further investigation revealed that

rats drink an aqueous solution of etonitazene in substantial quantities even without prior water-deprivation (Wikler et al., 1963). When only the etonitazene solution is available for drinking during abstinence, morphine-addicted rats consume significantly greater quantities of the etonitazene solution than they do of water during the same period and at the end of etonitazene consumption period body shakes are reduced (Wikler et al., 1963). Stolerman and Kuman (1970) also forced rats to drink morphine solutions in order to relieve their thirst. They found that these rats overcame their aversion for morphine solutions and eventually preferred them to water.

Narcotics have been administered in milk or sucrose solutions in order to reduce the adversive taste of the opiates. Mice have been reported to become tolerant and dependent by drinking large quantities of dilute evaporated milk containing dihydromorphinone (Shuster et al., 1963). When these mice were withdrawn they lost body weight indicating that physical dependence had been established. The addicted mice showed no clear preference for milk containing the narcotic when allowed a choice between plain milk and dihydromorphinone milk (Shuster et al., 1963). A procedure for a highly effective and reliable method for oral morphine administration has been developed by Khavari and Risner (1972; 1973). Specifically, they found that rats would ingest large quantities of morphine when the drug was presented in a sucrose medium and that they would prefer sucrose morphine over the

sucrose vehicle in subsequent two-bottle choice test (Khavari and Risner, 1972). The morphine-sucrose solution was effective in producing morphine dependence in rats. This conclusion was confirmed by the presence of withdrawal signs such as body weight loss, anorexia and adipsia when the morphine was withheld or nalorphine was administered (Khavari and Risner, 1973).

The possibility of adding morphine directly to the animals food as a method for inducing physical dependence has not been extensively investigated. This method was used by Sollman (1924) in a limited way but he reported that dependence did not develop as measured by the effects of subsequent removal of morphine from the food. Sollman's experiment represented the only attempt to induce dependence using morphine-adulterated food until Madinaveitia's (1969) examination of this method. He also observed that in rats which for several weeks had morphine added to their food in concentrations up to 1 mg/g there was no significant changes seen upon withdrawal. More recently Khavari and Risner (1973) investigated the ingestion of morphine adulterated food in rats. They found that those animals maintained on concentrations of 1 mg/g morphine in the food showed a preference for morphine-adulterated food over regular food in a choice test. Those rats given concentrations of 3 or 4 mg/g morphine showed severe withdrawal signs when they failed to maintain their morphine intake in the food choice test. These signs varied in intensity as a direct function of the animals'

prior morphine intake and indicated they were morphine dependent (Khavari and Risner, 1973). Therefore, it seems that either morphine dependence or forced treatment seems to be essential for the rat to show preference for morphinized food.

3. Pellet Implantation

In recent years, a morphine pellet implantation procedure has been employed to produce tolerance and physical dependence in the mouse, rat and guinea pig. The implantation technique was originated by Maggiolo and Huidobro (1961) who made a pellet by compressing 75 mg of morphine base under high pressure. Such a pellet proved cumbersome to make and its absorption is not rapid enough to produce physical dependence of a magnitude that can be measured by abrupt withdrawal, although striking abstinence signs could be precipitated by injection of nalorphine (Maggiolo and Huidobro, 1961). Gibson and Tingstad (1970) made improvements in this pellet and this new formulation has been widely used for the quantitative assessment of tolerance and physical dependence (Way et al., 1969; Ho et al., 1972; Wei et al., 1973; Wei, 1973). The implantation procedure has gained considerable popularity because of its ease and suitability for rapidly inducing tolerance and physical dependence. The pellets are implanted under light anesthesia subcutaneously in the back or in the lower abdominal wall (Wei and Way, 1975). Between twenty-five and fifty percent of the morphine in the pellet is absorbed in the first two days after implantation, after which the rate of absorption of morphine plateaus (Way et al., 1969;

Blasig et al., 1973; Goldstein and Schultz, 1973). After implantation of the morphine pellet, animals exhibit the characteristic signs of acute morphine effects (Wei and Way, 1975). The acute effects of the morphine pellet largely subside within twenty-four hours, while the optimum time for obtaining a tolerant and dependent state after implanting a morphine pellet is three days (Wei and Way, 1975). In the mouse, tolerance and dependence were noted to be maximum three to four days after implanting the pellet, after which there is a decrease in these parameters because the pellet becomes encapsulated with fibrous tissue (Way et al., 1969). Similarly, in the rat tolerance and precipitated withdrawal signs became maximal three to five days after a single pellet implant (Wei, 1973; Cicero and Meyer, 1973). Dependence was maximal three days after morphine implantation in the guinea pig (Goldstein and Schultz, 1973).

The withdrawal syndrome is represented by a constellation of behavioral signs after pellet implantation. The following withdrawal signs have been observed in the rodent, body weight loss, jumping, wet shakes, vocalizations, fighting behavior and piloerection (Wei and Way, 1975). Most investigators have found the abrupt withdrawal syndrome difficult to quantify because the protracted course of abstinence requires extended periods of continuous observation after pellet removal (Ho et al., 1972). The delayed onset and protracted course of abrupt morphine withdrawal have led to the increasing use of antagonist-precipitated withdrawal for the

assessment of physical dependence. The withdrawal syndrome precipitated by opiate antagonists is a rapid event that appears to condense, in a short period, the abstinence signs of abrupt withdrawal (Wei and Way, 1975).

The pellet implantation technique provides a simple and rapid method for inducing morphine tolerance and dependence in a wide variety of laboratory animals. However, after a pellet is in place the daily dose of morphine cannot be controlled directly. Abrupt morphine withdrawal may require surgical intervention or may not be possible. The effectiveness of the pellet method for inducing primary physical dependence on narcotics other than morphine has not been reported.

4. Infusion

Recently, several investigators have demonstrated that physical dependence on narcotics can be rapidly induced in the rat by either continuous or discontinuous injections of morphine solutions (Coussens et al., 1973; Teiger, 1974; Numan et al., 1975). A high degree of physical dependence on morphine has been produced in the rat with two to six days of continuous intraperitoneal infusion (Teiger, 1974). In this model, rats are prepared with an indwelling intraperitoneal catheter, which is led out through a stab wound in the back. They are then fitted with a harness and allowed to roam freely within their home cage by use of a spring and swivel arrangement. The catheter is attached to a Harvard pump, and morphine or other drugs are infused

continuously at a slow rate (Teiger, 1974; Harris, 1976). On abrupt withdrawal or administration of an antagonist, the typical signs of abstinence are seen. After two days of morphine infusion (100 mg/kg/day) as little as 0.1 mg/kg of naloxone or 1 mg/kg of nalorphine precipitated abstinence, while after six days of morphine infusion, abrupt withdrawal tests showed a high degree of physical dependence. Weight loss and hyperirritability were the signs used to follow the time course and severity of the withdrawal syndrome (Teiger, 1974; Harris, 1976). This method also allowed Teiger (1974) to demonstrate physical dependence on codeine and merperidine by abrupt withdrawal as well as precipitated withdrawal. Harris (1976) has used this technique to study the concomitant administration of narcotics and narcotic antagonists.

Intermittent injections of morphine sulfate in increasing doses has been found to produce reliable narcotic dependence in rats (Coussens et al., 1973; Numan et al., 1975; Smith and Davis, 1975). In this model rats are implanted with an indwelling jugular catheter and fitted with a harness. The catheter is connected by way of a swivel to an injection system located outside the chamber (Smith and Davis, 1973; 1975; Numan et al., 1975). Discontinuation of injections resulted in the appearance of a withdrawal syndrome characterized by ptosis, piloerection, writing, body shakes, hypothermia and weight loss (Coussens et al., 1973; Numan et al., 1975). Rats withdrawn from these injections acquired high rates of operant responding for morphine self-administration (Numan et al., 1975).

The short duration of time needed for infusion methods to induce physical dependence on morphine as well as other narcotics makes it a useful method. However, surgery is required along with expensive infusion equipment.

III

METHODS AND MATERIALS

A. Animals

Male hooded rats of the Long-Evans strain, random-bred, weighing 250-350 grams were obtained from Charles River Breeding Farms, Wilmington, Massachusetts. Prior to their use in this investigation the rats were housed in colony cages and allowed free access to food and water at all times.

B. Drugs

Azaperone, benperidol, haloperidol, spiramide, oxipermide, spiperone, pipamperone, pimozide, fentanyl, and dexetimide were obtained through the courtesy of McNeil Laboratories, Inc., Fort Washington, Pennsylvania and Janssen Pharmaceutica, Beerse, Belgium. Chlorpromazine, trifluoperazine, phenoxybenzamine, tranylcypromine and amphetamine were obtained from Smith Kline and French Laboratories of Philadelphia, Pennsylvania. Eli Lilly and Company of Indianapolis, Indiana supplied the methadone, propoxyphene, apomorphine and fluoxetine. The naloxone was provided by the Endo Laboratories, Inc. of Garden City, New York. Pilocarpine, dihydroxyphenyl-l-alanine, atropine, scopolamine, oxotremorine, and DL-5-hydroxytryptophan were purchased from the Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. Chlordiazepoxide, flurozepam, diazepam, Ro4-4602/1 and physostigmine were generously supplied by Hoffmann-La Roche, Inc., Nutley, New Jersey.

The Merck Sharp and Dohme Company of West Point, Pennsylvania supplied the methysergide, benztropine and amitriptyline. The d and l isomers of butaclamol were supplied by the Ayerst Laboratories of Montreal, Canada. Loxapine was supplied by Lederle Laboratories of Pearl River, New York. The morphine sulfate was purchased from the Merck Chemical Company, Rahway, New Jersey. Winthrop Laboratories provided the pentazorine. Deanol was provided by the Riker Laboratories of Northridge, California. The Eastman Kodak Company of Rochester, New York provided the choline chloride. Desmethylimipramine was obtained from the Geigy Pharmaceutical Company of Ardsley, New York. The alpha-methyl-p-tyrosine was purchased from the Sigma Chemical Company, St. Louis, Missouri. Boehringer Ingelheim, Ltd. of Elmsford, New York provided the clonidine. Sodium pentabarbital was obtained from the Mallinckrodt Chemical Works, St. Louis, Missouri.

Azaperone, benperidol, haloperidol, spiramide, oxipermide, pipamperone, spiperone and pimozide were dissolved in 0.3% tartaric acid. Alpha-methyl-p-tyrosine, L-DOPA and DL-5-hydroxytryptophan were suspended in 0.5% carboxymethyl cellulose. All other drugs were dissolved in physiological saline.

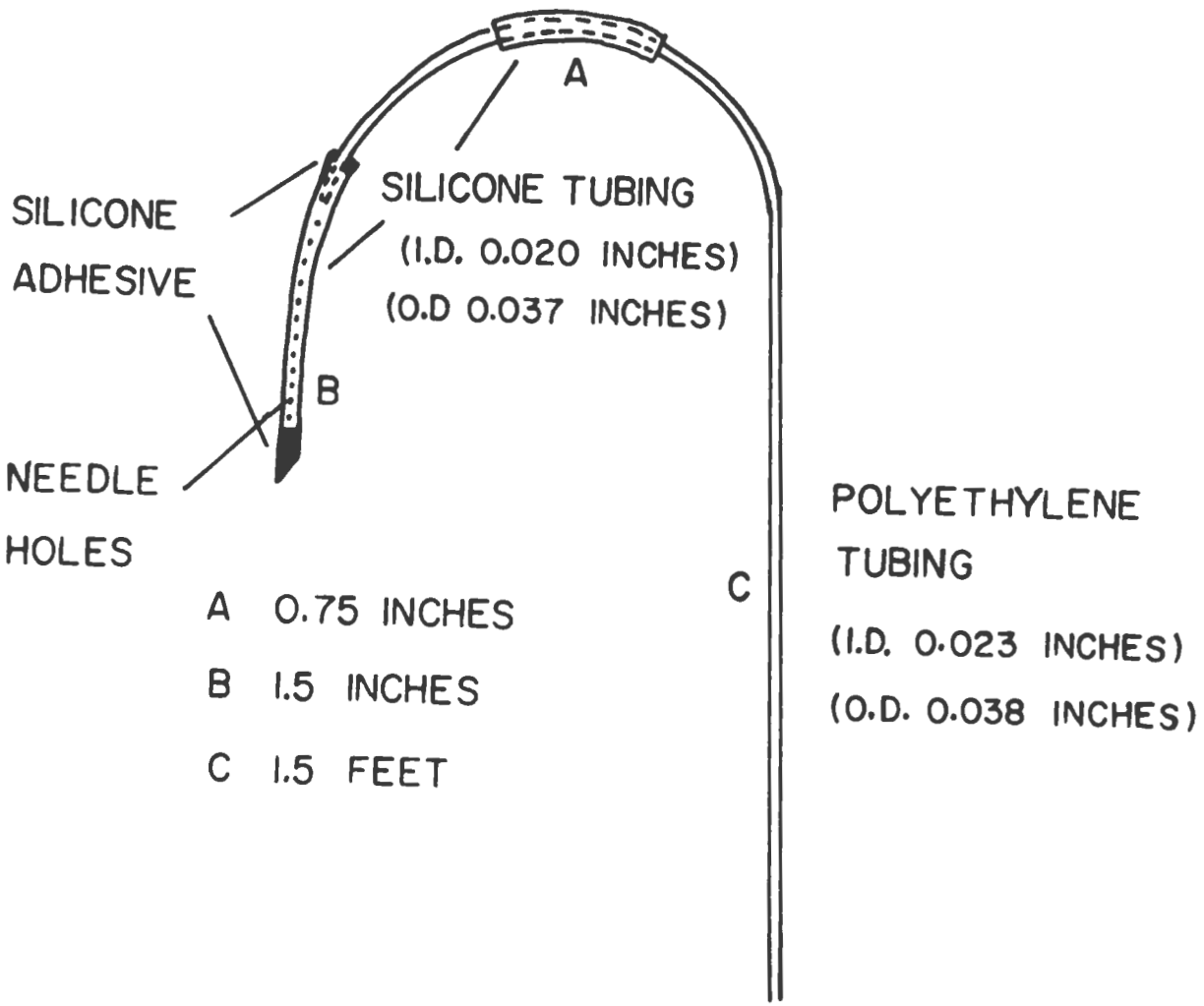
C. Chronic Intravenous Morphine Administration

1. Cannula Construction

The cannulas employed for chronic intravenous infusion were constructed from polyethylene and silicone tubing. The intramedic polyethylene tubing, I.D. 0.023 in. x

O.D. 0.0 38 in., was obtained from Clay Adams, Parsippany, New Jersey. Silastic brand medical grade silicone tubing, I.D. 0.020 in. x O.D. 0.037 in., was obtained from the Dow Corning Corporation, Midland, Michigan. The polyethylene tubing was cut into 1.5 ft segments while the silicone tubing was cut into either 0.75 in. or 1.5 in. pieces. The two pieces of silicone tubing were then placed in ether for 15 seconds resulting in expansion of the tubing. The polyethylene was first inserted through the 0.75 in. silicone tubing until it was about 1 in. from the end of the polyethylene tubing. Secondly, the 1.5 in. segment of silicone tubing was placed about 0.25 in. over the end of polyethylene tubing closest to the 0.75 in. silastic piece. The tip of the silicone tubing not overlapping the polyethylene tubing was sealed with Silastic brand medical adhesive made by the Dow Corning Corporation, Midland, Michigan. A small amount of silicone adhesive was also placed around the junction between the polyethylene and silicone tubing. The cannula was then bent to form a U-shape in a hot water bath. The bend was made at the middle of the 0.75 in. piece of silicone tubing. The cannulas were allowed 24 hours to dry prior to use. Many small holes made by needle pricks were placed in the 1.5 in. segment of silicone tubing for the drug solution to flow through before being implanted in the jugular vein. The cannula is shown in Figure 1.

FIGURE 1. Diagram of the Cannula Employed for Chronic Intravenous Morphine Administration



A 0.75 INCHES

B 1.5 INCHES

C 1.5 FEET

2. Cannula Implantation

The rats were anesthetized with 50 mg/kg of sodium pentobarbital administered intraperitoneally.

As soon as the rat was unconscious, the back of the neck behind the ears, the upper chest and lower neck were shaved with small animal clippers. As much hair as possible was removed from these sites. These areas were then wiped with gauze soaked in 70% alcohol solution.

An incision was made through the skin above the right jugular vein which is approximately 1-2 cm from the midline. The right external jugular vein was exposed by using blunt dissection.

A small cut was then placed in the vein through which the cannula was inserted. The cannula was filled with 0.9% saline and attached to a 3 ml syringe also filled with saline. The silicone tip of the cannula was inserted into the vein as far as the polyethylene tubing, so that the entire 1.5 in. segment of silicone tubing was in the vein. The cannula was then tied in the vein with silk suture. Additional sutures were employed to sew the cannula to the neck muscles.

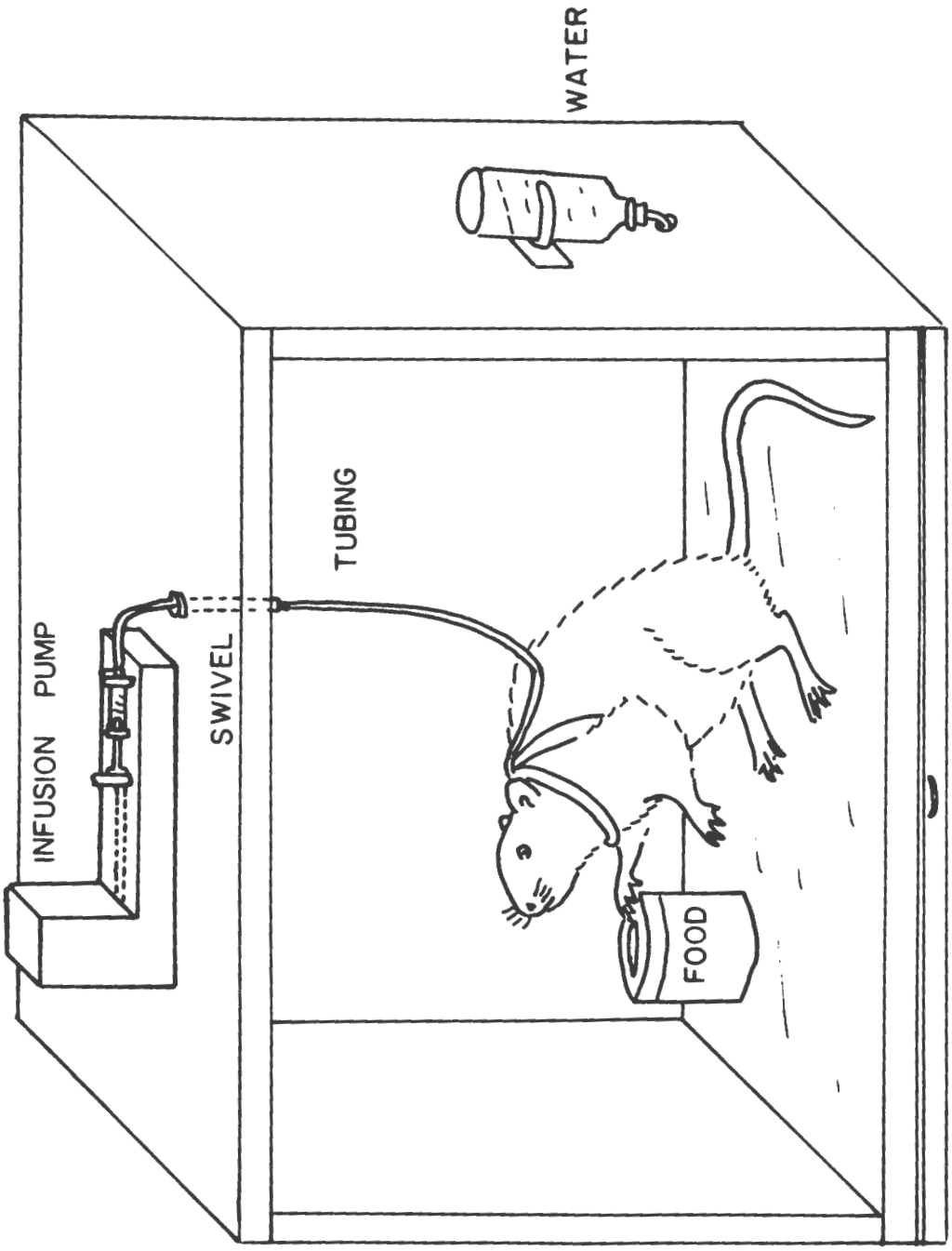
A 10 cm sterile piece of 15 gauge stainless steel tubing with a sharpened point at one end was inserted beneath the skin at the site of the incision. The tubing was pushed carefully underneath the skin until the sharpened end was brought through the shaved skin on the rat's back. The cannula was then inserted into the stainless steel tubing until it came out the other end. The entire length of cannula was pulled through the tubing prior to its removal.

The incision was then sutured closed. The muscle was sutured back together with absorbable sutures while silk suture was employed to close the skin. Following surgery 75,000 units of procaine penicillin G was injected intramuscularly in the hind paw. The rat was then placed in a saddle. The saddle was constructed from a small square of brass shim stock to which a curved piece of 13 gauge hypodermic needle tubing was soldered for the purpose of attaching a metal spring leash. A copper wire collar for the rat's neck was also soldered to the brass square. A piece of Velcro^{R} nylon closure was cemented to the top of the saddle and a mating Velcro^{R} strap was employed to pass around the rat's thorax just behind the forelegs. The inner parts of both the saddle and the strap were padded with 1/4-inch polyurethane foam. Each rat was placed individually in plexiglass chambers for the duration of the experiment. The plexiglass chambers were located in air-conditioned rooms thermostatically maintained at 20-22° with room lighting turned off between 8 P.M. and 7 A.M. The cannula was passed from the rat's saddle through a long metal spring to the top of the plexiglass chamber where it was connected to an injection system located outside the chamber. This apparatus is shown in Figure 2.

3. Injection System

The injection system consisted of a Harvard Apparatus Compact Infusion Pump Model 975 which was modified to hold 4 or 8 syringes having a 10-ml capacity. The syringes

FIGURE 2. Rat Housed in Plexiglass Chamber Fitted with Cannula and Saddle for Chronic Intravenous Morphine Administration


WATER
TUBING
SWIVEL
INFUSION PUMP
FOOD

were then filled with either a solution of morphine sulfate or normal saline which was infused according to the addiction schedule.

D. Addiction Procedure

The syringes in the infusion pumps were filled with morphine sulfate and infused according to the schedule shown in Table 1. The starting dose of 36 mg/kg/day morphine sulfate was gradually increased until a terminal dose of 100 mg/kg/day was reached. A minimum of five days on the terminal dose of morphine was allowed before the infusion pumps were turned off to produce withdrawal.

E. Measurement of Withdrawal Body Shakes

Morphine withdrawal body shakes were measured 8 to 12 hours after the morphine infusion was discontinued. The infusion pumps were stopped at midnight so that the pretreatment withdrawal observation could be made at 8 A.M. the following morning. To observe withdrawal body shakes the doors of the plexiglass chambers were opened and food cups removed. Each rat was observed for a period of 30 min during which the occurrence of withdrawal body shakes were counted. Body shakes were defined as violent shaking movements of the head and/or trunk of the rat which resemble the action of an animal that has been drenched with water (for a description and review, see Gianutsos et al., 1975a). The test drug was then injected, food cup replaced, cage door closed, and a prede-

Table 1. Morphine Concentration and Infusion Schedule to Establish Morphine Dependence in the Rat

	Infusion Flow Rate[1]		Morphine Infusion/Day	
Day	Pump Setting	ml/day[2]	Total mg	mg/kg
1	30	1.08	12	36
2	29	1.51	17	50
3	28	2.16	24	71
4-10	27	3.02	33	100

1. Harvard Apparatus Infusion Pump Model No. 975. Syringes with a 10-ml capacity.

2. Morphine sulfate dissolved in saline at a concentration of 11 mg/ml.

determined period for onset of drug action was allowed. Following this treatment the rats were observed again for 30 min for the occurrence of body shakes, as before the cage door was opened and food cup removed.

After the post-drug observation the infusion pump was turned on again to continue infusion of morphine solution until the next withdrawal.

F. Statistical Analysis

The effectiveness of the test drug was determined by comparing the withdrawal body shakes observed on the same day before and after the drug treatment. Therefore, the pre-drug shakes served as a sign of withdrawal against which the effectiveness of the test drug was evaluated. This was accomplished by calculating the average number of body shakes exhibited during the pre-drug observation period by those withdrawn rats reaching criteria. Withdrawn rats had to show a minimum of 8 withdrawal body shakes in the 30 min. observation period to be employed for drug testing. Criteria was established at 8 body shakes since 97 percent of the rats withdrawn from morphine showed this number of shakes while only 13 percent of the rats infused chronically with saline exhibited 8 or more body shakes. The average number of body shakes occurring during the pre-drug observation was then divided into the number of withdrawal shakes observed for each individual subject after the test drug administration. A mean and standard error was then calculated from these individual percentage values.

Regression analysis was employed to test for the existence of a dose response relationship. The Student's 't' test was employed to establish significant differences between treatment groups, with the level of statistical significance defined as a P value of less than 0.05. The dose of test drug that reduced withdrawal body shakes by 50 percent (ID_{50}) was calculated from the regression equation Y=MX+b. Where the slope is M and the Y intercept is b. The equation was then solved for X where Y was designated as 50 percent inhibition.

IV

RESULTS

A. The Occurrence of Morphine Withdrawal Body Shakes

Rats implanted with indwelling cannula and perfused with saline showed only negligible withdrawal body shakes (Table 2). These results are in agreement with previous reports (Numan et al., 1975; Lal and Numan, 1976). However, termination of morphine infusion in rats who had been continuously perfused with morphine for several days resulted in the reliable and frequent occurrence of withdrawal body shakes as can be seen in Table 2. These dependent rats showed an average of 25 withdrawal body shakes in a 30-min observation period which was significantly ($p < 0.001$) greater than the number of body shakes observed in saline infused rats. The frequency distribution for the occurrence of withdrawal body shakes upon the cessation of morphine or saline infusion is shown in Table 3. Rats withdrawn from morphine exhibit more than 8 withdrawal body shakes 97 percent of the time while saline controls show less than 7 shakes 87 percent of the time in a 30-min observation period. Fifty-two percent of the time withdrawn rats show between 8 and 21 body shakes while only 13 percent of saline infused rats exhibited shakes at this frequency. More than 21 shakes were observed in the remaining 45 percent of withdrawn rats while saline infused rats show no shakes at this frequency.

Table 2. The Incidence of Withdrawal Body Shakes in Dependent and Non-Dependent Rats

Chronic Treatment[1]	N[2]	Withdrawal Body Shakes[3] (Mean + S. E.)
Saline	54	2.6 ± 0.5
Morphine	54	25.1 ± 1.7*

1. Intravenously infused for several days as described in the text.

2. The number of rats observed after specified chronic treatment.

3. Pretreatment withdrawal shakes were observed for a 30 min period 8 hours after the termination of morphine or saline infusion.

* Significantly ($p < 0.001$) different from saline group according to Student's "t" test, two-tailed.

Table 3. The Incidence of Withdrawal Body Shakes in the Rat

Number of Withdrawal Shakes	Percent of Rats Showing Shakes of Stated Frequency[1]	
	Morphine Withdrawal[2]	Saline Controls[3]
0	0	44
1-7	3	43
8-14	30	9
15-21	22	4
22-28	14	0
29-35	14	0
36-42	8	0
43-49	4	0
≥ 50	5	0

1. Withdrawal shakes were observed for a 30-min period 8 hours after the termination of morphine or saline infusion.

2. Based upon 505 withdrawal observations of morphine dependent rats.

3. Based upon 54 withdrawal observations of saline perfused rats.

Before initiating the drug studies it was first established that rats once made dependent upon morphine exhibited a fairly consistent number of shakes even upon repeated withdrawal. This information was obtained by withdrawing dependent rats 12 consecutive times and observing the occurrence of withdrawal body shakes after 8 hours of withdrawal. At least 14 hours of morphine infusion was instituted between withdrawal observations. The data given in Table 4 show that an average of 18 body shakes occurred during the first withdrawal session after 5 days of maintenance at the terminal concentration of morphine. The number of shakes was significantly greater than those observed before withdrawal ($p < 0.0001$) or those observed in rats withdrawn from saline infusion ($p < 0.001$). The rate of withdrawal body shakes was somewhat higher on day 3 and then remained fairly constant throughout the period of consecutive withdrawal trials until the experiment was terminated after completion of 12 withdrawal observations.

B. The Effect of Narcotics on Morphine Withdrawal Body Shakes

1. The Effect of Narcotic Agonists

Upon the termination of morphine infusion, withdrawal body shakes began to occur within 3 hours but were not fully developed until 6 hours after which time they remained constant for up to 24 hours if the morphine infusion was not reinstated. The administration of morphine, methadone or fentanyl, potent narcotic agonists, reliably abolished the

Table 4. The Frequency of Withdrawn Body Shakes During Twelve Consecutive Withdrawal Sessions

Withdrawal[1] Sessions	N[2]	Withdrawal Shakes[3] (Mean + S.E.)
1	36	18.3 ± 1.4
2	29	20.0 ± 1.5
3	22	24.7 ± 3.1
4	18	25.3 ± 4.6
5	16	27.9 ± 4.3
6	15	24.5 ± 3.2
7	12	26.8 ± 4.7
8	13	25.7 ± 3.1
9	12	24.6 ± 3.5
10	11	32.2 ± 3.6
11	10	27.4 ± 3.3
12	8	25.3 ± 4.5

1. Dependent rats were withdrawn on 12 consecutive days. Withdrawal shakes were counted after 8 hours of withdrawal. At least 14 hours of morphine infusion were instituted between withdrawal observation sessions.

2. The number of rats observed during each withdrawal session.

3. Withdrawal shakes were observed for a 30-min period 8 hours after the termination of morphine infusion.

occurrence of withdrawal body shakes 8 hours after the termination of morphine infusion. These results are summarized in Table 5 which shows that the regression coefficient for each of these narcotics was significant ($p < 0.05$). Calculation of ID_{50} concentrations on a mg/kg basis revealed fentanyl to be the most potent with methadone being the next most potent and morphine having the lowest potency for those narcotics tested.

2. The Effect of Narcotic Antagonist

The injection of either naloxone or pentazocine 8 hours after the cessation of morphine infusion did not cause any further increase in the rate of withdrawal shakes as is shown in Table 5. There was a slight tendency towards an increase in the number of withdrawal body shakes, however, this increase was not significant.

3. The Interaction Between Narcotics and Naloxone

Morphine (40 mg/kg) administration reduced withdrawal body shakes by about 90 percent. Naloxone antagonizes this morphine-induced blockade of withdrawal shakes in a dose dependent manner (Table 6). A naloxone dose of 1.25 mg/kg completely reversed the blockade produced by morphine given at a dose of 40 mg/kg. Increasing the naloxone dose to 5 mg/kg did not increase the withdrawal shakes beyond those seen before morphine injection.

C. The Effect of Dopaminergic Agents on Morphine Withdrawal Body Shakes

1. The Effect of Dopaminergic Antagonists

The effect of the various dopaminergic antagonists

Table 5. The Effect of Narcotic Agonist and Antagonist on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[3]	ID_{50} (mg/kg)	Correlation Coefficient
Saline	-	64	110.2 ± 9.6		
Fentanyl	0.04	5	116.4 ± 70.0		
	0.16	16	107.0 ± 22.7		
	0.64	6	4.8 ± 4.8		
	2.5	6	0 ± 0	0.3	-0.88*
Methadone	2.5	7	53.4 ± 14.4		
	10	8	14.7 ± 9.4		
	40	5	0 ± 0	4.1	-0.96*
Morphine	10	14	67.6 ± 21.7		
	40	10	11.6 ± 5.0	14.4	-0.99*
Naloxone	0.16	5	169.8 ± 43.9		
	0.64	8	112.4 ± 26.5		
	1.25	11	128.6 ± 11.4		
	5.00	8	105.1 ± 14.5	-	-0.33
Pentazocine	10	6	111.5 ± 36.9		
	40	5	133.4 ± 52.1	-	+0.88

1. All drugs were administered i.p. 30 min prior to observation with the exception of fentanyl and naloxone which were given 10 mins prior to post treatment observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

*Significant ($p < 0.05$) regression coefficient.

Table 6. Reversal of Morphine Induced Blockade of Withdrawal Body Shakes by Naloxone

Naloxone[1] (mg/kg)	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[3]	Correlation Coefficient
0	10	11.6 ± 5.0	
0.04	7	23.8 ± 11.7	
0.16	7	65.0 ± 13.9	
0.64	8	49.3 ± 17.5	
1.25	8	86.3 ± 13.4	
5.00	8	97.8 ± 32.0	+0.93*

1. Morphine sulfate (40 mg/kg) was administered i.p. to all rats 30 min prior to post treatment observation. Naloxone was then injected i.p. 20 min after the morphine administration which was 10 min prior to observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal body shakes were observed for a 30 min period after 8 hours of morphine abstinence.

* Significant regression coefficient ($P < 0.05$).

test on withdrawal shakes are shown in Table 7. Benperidol dose dependently reduced withdrawal shakes and this effect was significant ($r = 0.93$, $p < 0.05$) by regression analysis. A dose of 0.01 mg/kg of benperidol was without effect but increasing the dose to 0.64 mg/kg reduced withdrawal body shakes by 95 percent. (+)Butaclamol produced a dose dependent reduction in body shakes with a dose of 0.64 mg/kg reducing shakes by approximately 80 percent. The correlation coefficient for the relationship between the log dose of (+)butaclamol and percent reduction in body shakes was ($r = 0.98$, $p < 0.05$). (-)Butaclamol did not reduce withdrawal shakes. The phenothiazine neuroleptic, chlorpromazine, at a dose of 0.16 mg/kg reduced shakes to 50 percent of pretreatment. The degree of reduction remained at approximately 50 percent even when the dose of chlorpromazine was increased to 0.64 or 2.5 mg/kg. However, further increasing the dose to 10 mg/kg did completely reduce the occurrence of withdrawal shakes. The correlation coefficient for chlorpromazine ($r = 0.83$) was not significant suggesting that the effect was not dose dependent. Haloperidol reduced withdrawal shakes in a dose dependent manner. A reduction of 80 percent was achieved by the administration of haloperidol at a dose of 0.64 mg/kg. Regression analysis demonstrated a significant ($r = 0.98$, $p < 0.05$) dose effect relationship. The dopamine antagonist loxapine produced a similar reduction in withdrawal body shakes. A near total blockade of withdrawal shakes was achieved by the administration of 2.5 mg/kg of loxapine.

Table 7. The Effect of Various Neuroleptics on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[3]	ID_{50} (mg/kg)	Correlation Coefficient
Saline	-	64	110.2 ± 9.5		
Benperidol	0.01	15	116.1 ± 15.7		
	0.04	8	48.5 ± 13.2		
	0.16	12	50.8 ± 6.8		
	0.64	5	4.8 ± 3.7	0.08	-0.93*
+Butaclamol	0.04	4	66.2 ± 18.6		
	0.16	7	40.2 ± 10.8		
	0.64	7	21.1 ± 7.7	0.12	-0.98*
-Butaclamol	0.64	9	128.4 ± 14.2	-	
Chlorpromazine	0.16	12	45.7 ± 10.5		
	0.64	4	25.2 ± 20.0		
	2.50	5	50.4 ± 15.7		
	10.00	2	0 ± 0	0.49	-0.83
Haloperidol	0.04	9	83.4 ± 14.8		
	0.16	8	41.5 ± 9.5		
	0.64	13	19.4 ± 5.3	0.17	-0.98*
Loxapine	0.04	6	53.8 ± 7.2		
	0.16	9	38.8 ± 13.6		
	0.64	7	44.7 ± 17.9		
	2.50	9	0.8 ± 0.5	0.16	-0.92*
Oxiperomide	0.14	7	90.0 ± 19.1		
	0.16	9	30.0 ± 7.7		
	0.64	9	18.2 ± 6.1		
	2.50	10	3.8 ± 2.2	0.16	-0.96*

Table 7 (continued)

Pimozide	0.04	7	55.5 ± 16.4		
	0.16	7	68.1 ± 23.4		
	0.64	12	82.7 ± 18.7		
	2.50	13	35.8 ± 12.2	1.64	-0.68
Pipamperone	2.50	6	38.6 ± 13.2		
	10.00	5	79.8 ± 14.4		
	40.00	4	22.2 ± 14.5	11.02	-0.71
Spiperone	0.01	10	72.0 ± 14.3		
	0.04	9	50.2 ± 16.9		
	0.16	7	22.0 ± 11.3		
	0.64	15	14.7 ± 5.2	0.04	-0.97*
Spiramide	0.04	6	82.6 ± 22.5		
	0.16	7	43.5 ± 9.3		
	0.64	9	34.5 ± 16.8		
	2.50	6	0.6 ± 0.6	0.19	-0.98*
Trifluoperazine	0.16	3	80.3 ± 15.1		
	0.64	6	36.3 ± 14.6		
	2.50	4	38.7 ± 29.6		
	10.00	5	0 ± 0	0.74	-0.97*

1. All drugs were administered i.p. 30 min prior to observation with the exception of haloperidol and pimozide which were given 120 min prior to post-treatment observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

*Significant regression coefficient ($p < 0.05$).

Dose effect analysis demonstrated a significant ($r = 0.92$, $p < 0.05$) correlation coefficient. A dose dependent reduction in withdrawal body shakes was also produced by the administration of oxiperomide. Withdrawal shakes were reduced by 95 percent by the administration of oxiperomide at a dose of 2.5 mg/kg. Regression analysis revealed a significant ($r = 0.96$, $p < 0.05$) correlation coefficient. Pimozide did not reduce withdrawal body shakes in a dose dependent manner. Even with highest dose employed, 2.5 mg/kg, withdrawal shakes were only reduced by approximately 65 percent. Pipamperone failed to reduce withdrawal body shakes in a dose dependent manner ($r = 0.71$). A dose of 2.5 mg/kg reduced shakes by 60 percent; however, when the dose was increased to 10 mg/kg the reduction was only 20 percent. Withdrawal shakes were reduced by 80 percent when a 40 mg/kg dose of pipamerone was employed. Spiperone did reduce the frequency of withdrawal shakes in a dose related fashion. At a dose of 0.04 mg/kg spiperone reduced shakes by about 50 percent while increasing the dose to 0.64 mg/kg reduced shakes by 85 percent. The correlation coefficient for the relationship between the log dose of spiperone and percent reduction in body shakes was significant ($r = 0.97$, $p < 0.05$). A similar dose related decrease in the frequency of withdrawal shakes was produced by the administration of the neuroleptic spiramide. A total reduction in shakes was achieved when sipramide was administered at a dose of 2.5 mg/kg. Regression analysis show a significant ($r = 0.98$, $p < 0.05$) correlation coefficient.

Trifluoperazine, a phenothiozine neuroleptic, also reduced withdrawal shakes but only at relatively high doses. A 10 mg/kg dose of trifluoperazine was needed to completely abolish the occurrence of withdrawal body shakes. The dose dependent effect was found to be significant ($r = 0.97$, $p < 0.05$) by regression analysis. Consideration of the ID_{50} values for these neuroleptics showed that on a mg/kg basis the order of potency for those neuroleptics tested to be as follows: spiperone, benperidol, +butaclamol, loxapine, oxiperomide, haloperidol, spiraimide, chlorpromazine, trifluoperazine, pimozide and pipamperone.

2. The Effect of Dopaminergic Agonists.

The dopaminergic agonists tested for their effects on withdrawal shakes all reduced shakes as can be seen in Table 8. Amphetamine produced a dose-related decrease in the frequency of withdrawal shakes which was significant ($r = 0.96$, $p < 0.05$) by regression analysis. When a 2.5 mg/kg dose of amphetamine was employed shakes were reduced by 85 percent. In a similar fashion withdrawal shakes were dose dependently reduced by the dopaminergic agonist, apomorphine. A 95 percent reduction was demonstrated when apomorphine was given at a dose of 0.64 mg/kg. The correlation coefficient for the dose-effect relationship was ($r = 0.99$, $p < 0.05$). The administration of L-DOPA also produced a dose dependent reduction in withdrawal shakes. Analysis of the L-DOPA effect demonstrated a significant

Table 8. Reduction of Morphine Withdrawal Body Shakes Induced by Dopamine Receptor Stimulants

Treatment[1]	Dose (mg/kg)	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[3]	ID_{50} (mg/kg)	Correlation Coefficient
Saline	-	64	110.2 ± 9.6		
Amphetamine	0.04	7	106.3 ± 24.4		
	0.16	8	53.6 ± 11.9		
	0.64	8	20.7 ± 4.6		
	2.50	9	13.0 ± 6.0	0.26	-0.96*
Apomorphine	0.01	3	86.6 ± 28.1		
	0.04	5	49.2 ± 13.9		
	0.16	7	35.3 ± 8.7		
	0.64	8	5.0 ± 2.5	0.06	-0.99*
L-DOPA[4]	10.0	9	66.6 ± 17.3		
	40.0	6	41.0 ± 20.9		
	160.0	5	23.2 ± 14.4	32.47	-0.97*

1. All drugs were administered i.p. 30 mins prior to observation with the exception of apomorphine which was given 10 mins prior to post-treatment observation.
2. The number of rats employed per dose.
3. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.
4. These rats were injected with 25 mg/kg of R04-4602/1 30 mins prior to L-DOPA administration.

*Significant regression coefficient ($p < 0.05$).

($r = 0.97$; $p < 0.05$) correlation coefficient. The ID_{50} values for amphetamine, apomorphine and L-DOPA were 0.26, 0.06 and 32.47 mg/kg, respectively.

3. The Interaction Between Dopaminergic Agents and Naloxone

When administered after a large dose of haloperidol, naloxone caused a considerable reversal of the haloperidol effect in blocking withdrawal shakes as can be seen in Tables 9 and 10. Haloperidol in a dose of 0.64 mg/kg reduced body shakes to 20 percent of pretreatment but when administered in conjunction with naloxone body shakes were reduced to only about 50 percent of pretreatment. Naloxone, at a dose of 0.64 mg/kg produced a maximum effect which could not be further potentiated by increasing the naloxone dose to 5 mg/kg. However, only the 5 mg/kg dose of naloxone produced an effect which was significantly ($p < 0.05$) different from haloperidol alone (Table 10).

The data summarized in Table 10 show the interaction between the various neuroleptics found to reduce withdrawal body shakes and naloxone. The maximum effect dose of each neuroleptic was combined with a 5 mg/kg dose of naloxone. Benperidol, oxipermide, haloperidol and trifluoperazine were the only neuroleptics to have their effects significantly ($p < 0.05$) antagonized by the administration of naloxone (5 mg/kg). The interaction between benperidol and naloxone proved to be significant ($p < 0.002$) in that benperidol alone (0.64 mg/kg) reduced withdrawal shakes by 95 percent

Table 9. The Effect of Naloxone on the Haloperidol Induced Reduction in Morphine Withdrawal Body Shakes

Naloxone[1] (mg/kg)	N[2]	Withdrawal Shakes[3] % Pretreatment (Mean ± S.E.)	Correlation Coefficient
0	13	19.4 ± 5.3	
0.64	9	45.5 ± 11.5	
1.25	9	35.5 ± 9.7	
5.00	8	45.0 ± 9.7	+0.77

1. Haloperidol (0.64 mg/kg) was administered i.p. to all rats 120 mins prior to post treatment observation. Naloxone was then injected i.p. 10 mins prior to observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

Table 10. The Interaction Between Neuroleptics and Naloxone on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	Withdrawal Shakes[2] % Pretreamtnet (Mean ± S.E.) Saline[3]	Naloxone[3]
Saline		110.2 ± 9.6 (64)	105.1 ± 14.5 (8)
Benperidol	0.64	4.8 ± 3.7 (5)	60.0 ± 7.9 (8)*
+Butaclamol	0.64	21.2 ± 7.7 (7)	29.4 ± 5.2 (5)
Chlorpromazine	10.00	0 ± 0 (2)	5.3 ± 5.3 (3)
Haloperidol	0.64	19.4 ± 5.3 (13)	45.0 ± 9.7 (8)*
Loxapine	2.50	0.8 ± 0.5 (9)	8.3 ± 4.9 (6)
Oxiperomide	2.50	3.8 ± 2.2 (10)	18.9 ± 6.8 (10)*
Spiperone	0.64	14.7 ± 5.2 (15)	28.1 ± 8.4 (10)
Spiramide	0.64	34.5 ± 16.8 (9)	48.8 ± 8.2 (10)
Trifluoperazine	10.00	0.3 ± 0.3 (5)	19.6 ± 7.3 (16)*

1. All drugs were administered i.p. 30 mins prior to observation with the exception of haloperidol which was given 120 mins prior to post treatment observation.

2. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

3. Saline or naloxone (5 mg/kg) was administered 10 mins prior to observation.

4. The number of rats employed per dose is indicated in parentheses.

* Significantly ($p < 0.05$) different from saline by Student's 't' test.

but when administered in conjunction with naloxone (5 mg/kg) body shakes were reduced by only 40 percent. Naloxone failed to antagonize the effects of spiperone, spiramide, loxapine, chlorpromazine and +butaclamol to any significant extent.

The data obtained when naloxone was administered in conjunction with dopaminergic agonist is shown in Table 11. The effect of apomorphine was significantly ($p < 0.05$) antagonized by the administration of naloxone (5 mg/kg). However, the administration of naloxone in conjunction with amphetamine failed to produce any significant antagonism of the amphetamine effect.

D. The Effect of Adrenergic Agents on Morphine Withdrawal Body Shakes

1. The Effect of Adrenergic Antagonist

The data in Table 12 demonstrates a blockade of withdrawal shakes by azaperone, a butyrophenone neuroleptic possessing high alpha noradrenergic potency. The effect of azaperone was significantly ($r = 0.96$, $p < 0.05$) dose dependent by regression analysis with a dose of 0.64 mg/kg reducing body shakes by about 95 percent. The alpha noradrenergic blocker phenoxybenzamine failed to produce any significant effect on withdrawal body shakes. Similarly, the beta blocker propronalol also failed to reduce withdrawal shakes to any significant extent. A dose of 10 mg/kg of propronalol only reduced shakes by about 30 percent.

Table 11. The Interaction Between Dopamine Receptor Stimulants and Naloxone on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	Withdrawal Shakes[2] % Pretreatment (Mean ± S.E.) Saline[3]	Naloxone[3]
Saline		110.2 ± 9.6 (64)	105.1 ± 14.5 (8)
Amphetamine	2.50	13.0 ± 6.0 (9)	2.5 ± 1.6 (8)
Apomorphine	0.64	5.0 ± 2.5 (8)	43.3 ± 17.1 (6)*

1. All drugs were administered i.p. 30 mins prior to observation with the exception of apomorphine which was given 10 mins prior to post treatment observation.

2. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

3. Saline or naloxone (5 mg/kg) was administered 10 mins prior to observation.

4. The number of rats employed per dose is indicated in parentheses.

* Significantly ($p < 0.05$) different from saline by Student's 't' test.

Table 12. The Effect of Adrenergic Blockers on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[3]	ID_{50} (mg/kg)	Correlation Coefficient
Saline	-	64	110.2 ± 9.6		
Azaperone	0.01	4	78.7 ± 15.3		
	0.04	7	33.1 ± 15.9		
	0.16	6	10.6 ± 8.9		
	0.64	13	7.8 ± 3.2	0.04	-0.96*
Phenoxybenzamine	0.64	7	74.4 ± 14.8		
	2.5	20	51.9 ± 11.2		
	10.0	9	68.5 ± 16.1	8.66	-0.76
Propranolol	2.5	10	89.2 ± 11.8		
	10.0	6	70.0 ± 13.1	38.45	-0.99*

1. All drugs were administered i.p. 30 mins prior to post treatment observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

* Significant regression coefficient ($p < 0.05$).

2. The Effect of Adrenergic Agonist

The adrenergic agonist amitriptyline, clonidine, desmethylimipramine and tranylcypromine were tested for their effects on withdrawal body shakes. The effect of these agents on body shakes are shown in Table 13. Desmethylimipramine ($r = 0.98$, $p < 0.05$) and clonidine ($r = 0.95$, $p < 0.05$) produced dose dependent decreases in morphine withdrawal body shakes which were significant. Clonidine proved to be very effective in that a dose of 0.16 mg/kg completely abolished the occurrence of withdrawal body shakes. Amitriptyline and tranylcypromine also reduced body shakes to a limited extent, regression analysis show no significant correlations between dose and effect. Clonidine had an ID_{50} value of 0.02 mg/kg while the value for desmethylimipramine was considerably higher, 1.74 mg/kg.

3. The Interaction Between Adrenergic Agents and Naloxone

The administration of naloxone after azaperone (0.64 mg/kg) caused a considerable reversal of the azaperone effect in reducing withdrawal shakes as can be seen in Tables 14 and 15. Azaperone when administered at a dose of 0.64 mg/kg reduced body shakes by 95 percent but when administered in conjunction with naloxone body shakes were reduced by only about 40 percent. A dose of 0.64 mg/kg of naloxone produced a maximum effect which could not be further potentiated by increasing the naloxone dose to 5 mg/kg (Table 14). The effects of azaperone were significantly ($p < 0.01$) antagonized by the administration of naloxone at a dose of 5.0 mg/kg

Table 13. The Effect of Adrenergic Agonists on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[3]	ID_{50} (mg/kg)	Correlation Coefficient
Saline	-	64	110.2 ± 9.6		
Amitriptyline	2.5	4	50.3 ± 14.4		
	10.0	6	49.6 ± 13.9	6.13	-0.87
Clonidine	0.01	10	44.1 ± 8.6		
	0.04	6	22.3 ± 8.1		
	0.16	6	0 ± 0	0.02	-0.95*
Desmethylimipramine	0.64	8	78.7 ± 13.8		
	2.5	8	30.2 ± 11.2		
	10.0	7	13.7 ± 4.7	1.74	-0.98*
Tranylcypromine	2.5	9	40.0 ± 9.9		
	10.0	8	22.0 ± 8.0	3.16	-0.94

1. All drugs were administered i.p. 30 mins prior to post treatment observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

* Significant regression coefficient ($p < 0.05$).

Table 14. Reversal of Azaperone Induced Blockade of Withdrawal Body Shakes by Naloxone

Naloxone[1]	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[3]	Correlation Coefficient
0	13	7.8 ± 3.2	
0.64	7	61.2 ± 16.3	
1.25	8	52.5 ± 25.9	
5.00	8	71.6 ± 21.3	+0.88

1. Azaperone (0.64 mg/kg) was administered i.p. to all rats 30 mins prior to post treatment observation. Naloxone was then injected i.p. 20 mins after the azaperone administration which was 10 mins prior to observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal body shakes were observed for a 30 min period after 8 hours of morphine abstinence.

Table 15. The Interaction Between the Adrenergic Agonist Clonidine and the Narcotic Antagonist Naloxone on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[2]	
		Saline[3]	Naloxone[3]
Saline		110.2 ± 9.6 (64)[4]	105.1 ± 14.5 (8)
Azaperone	0.64	7.8 ± 3.2 (13)	81.6 ± 21.3 (8)*
Clonidine	0.16	0.0 ± 0.0 (6)	12.3 ± 4.1 (11)

1. All drugs were administered i.p. 30 mins prior to post treatment observation.

2. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

3. Saline or naloxone (5 mg/kg) was given 10 mins prior to post treatment observation.

4. The number of rats employed per dose is indicated in parentheses.

* Significantly ($p < 0.01$) different from saline by Student's 't' test.

(Table 15). The antiwithdrawal effect of clonidine was also reduced by the administration of naloxone at a dose of 5 mg/kg. These data are shown in Table 15. The naloxone antagonism of the clonidine effect was not nearly as large as the naloxone antagonism of azaperone.

E. The Effect of Agents that Influence the Synthesis and Storage of Catecholamines on Morphine Withdrawal Body Shakes

The results achieved with alpha-methyl-p-tyrosine and reserpine are given in Table 16. Alpha-methyl-p-tyrosine, an inhibitor of catecholamine synthesis, reduced withdrawal shakes only when a dose of 160 mg/kg was employed. Regression analysis failed to reach significance for this effect. Reserpine, which interferes with the storage of catecholamines, dose-dependently reduced withdrawal body shakes. A dose of 10 mg/kg of reserpine reduced shakes by approximately 60 percent. Regression analysis show a significant ($r = 0.96$, $p < 0.05$) dose effect relationship for reserpine.

F. The Effect of Serotoninergic Agents on Morphine Withdrawal Body Shakes

The data for the effect of serotoninergic agents on withdrawal body shakes is shown in Table 17. Fluoxetine, which increases the concentration of serotonin at its receptor site by blocking its uptake, reduced body shakes by 50 percent at a dose of 0.64 mg/kg. This effect could not be further intensified by increasing the dose to 2.5 mg/kg. The administration of 5-HTP was similarly without significant

Table 16. The Effect of Agents that Influence the Synthesis and Storage of Catecholamines on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)	ID_{50} (mg/kg)	Correlation Coefficient
Saline		64	110.2 ± 9.6		
Alpha-methyl-p-Tyrosine	40	6	91.3 ± 16.9		
	160	5	50.6 ± 19.7	189.6	-0.97
Reserpine	0.16	8	84.5 ± 16.5		
	0.64	8	53.0 ± 12.6		
	2.50	10	48.5 ± 9.0		
	10.0	10	37.7 ± 12.4	2.2	-0.96*

1. All drugs were administered i.p. 60 mins prior to observation with the exception of alpha-methyl-p-tyrosine which was given 120 mins prior to post treatment observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

* Significant regression coefficient ($p < 0.05$).

Table 17. The Effect of Serotoninergic Agents on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[3]	Correlation Coefficient
Saline	-	64	110.2 ± 9.6	
Fluoxetine	0.64	6	51.8 ± 7.1	
	2.50	7	63.0 ± 17.7	-0.76
5-HTP[4]	2.50	6	153.2 ± 25.5	
	10	7	128.0 ± 24.9	
	40	8	88.6 ± 37.5	-0.43
Methysergide	0.64	4	105.2 ± 32.1	
	2.50	6	81.6 ± 27.1	-0.93

1. All drugs were administered i.p. 30 mins prior to post treatment observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

4. These rats were injected with 25 mg/kg of R04-4602/1 30 mins prior to 5-HTP administration.

effect on the frequency of withdrawal body shakes. At a dose of either 2.5 or 10 mg/kg of 5-HTP there was a slight increase in withdrawal body shakes. Methysergide, a serotonin antagonist, was without effect on the occurrence of withdrawal body shakes at the doses tested.

G. The Effect of Cholinergic Agents on Morphine Withdrawal Body Shakes

1. The Effect of Anticholinergic Agents

Atropine, benztropine, dexetimide and scopolamine, all well established anticholinergic drugs, were tested for their effects on morphine withdrawal body shakes. The data for these anticholinergic agents are summarized in Table 18. Atropine at a dose of 2.5 mg/kg had no effect on body shakes, however, increasing the dose to 10 mg/kg resulted in a slight but nonsignificant increase in the intensity of withdrawal shakes. Benztropine was without significant effect on withdrawal body shakes at those doses tested. No significant result was achieved for the effect of dexetimide on withdrawal shakes. However, there was a slight increase in shake frequency at a dexetimide dose of 0.64 mg/kg. Similarly, scopolamine was without significant effect on withdrawal body shakes. Although at a dose of 0.64 mg/kg scopolamine did increase withdrawal shakes to 169 percent of pretreatment, this effect failed to achieve significance.

2. The Effect of Cholinergic Agonist

A number of cholinergic agonist were studied for

Table 18. The Effect of Anticholinergics on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[3]	Correlation Coefficient
Saline	-	64	110.2 ± 9.6	
Atropine	2.5	15	97.8 ± 20.0	
	10.0	9	132.1 ± 23.7	-0.63
Benztropine	2.5	8	109.6 ± 12.7	
	10.0	8	90.8 ± 19.1	-0.88
Dexetimide	0.04	7	93.6 ± 15.6	
	0.16	7	96.6 ± 20.3	
	0.64	5	140.6 ± 8.0	
	2.50	6	118.3 ± 12.8	+0.52
Scopolamine	0.16	7	75.4 ± 30.8	
	0.64	8	169.1 ± 36.8	+0.62

1. All drugs were administered i.p. 30 mins prior to post treatment observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

their effect on body shakes and the results from these studies are summarized in Table 19. Deanol and choline chloride produced decreases in withdrawal body shakes, however, regression analysis failed to show significance. The calculated ID_{50} values for deanol and choline chloride were 1,016 and 86 mg/kg respectively. Oxotremorine failed to have any significant effect on the occurrence of withdrawal shakes. The ID_{50} for oxotremorine was calculated from the regression line to be 23 mg/kg which is well above the lethal dose for this drug. A significant dose-effect relationship was established by regression analysis for physostigmine ($r = 0.99$, $p < 0.05$) and pilocarpine ($r = 0.99$, $p < 0.05$). The ID_{50} values for physostigmine and pilocarpine, 0.1 and 3.8 mg/kg, respectively, were well within the pharmacological range of these drugs. Pilocarpine at a dose of 10 mg/kg reduced withdrawal shakes by 90 percent; this was the largest reduction in body shakes produced by any of the cholinergic agonists.

3. The Interaction Between Cholinergic Agents and Naloxone

A 10 mg/kg dose of pilocarpine which reduced withdrawal shakes by 90 percent was interacted with a 5 mg/kg dose of naloxone. The results of this interaction are given in Table 20. Naloxone failed to antagonize the pilocarpine induced decrease in withdrawal body shakes. This is evidenced by the fact that withdrawal body shakes were still reduced by 90 percent after the administration of both pilocarpine and naloxone.

Table 19. The Effect of Cholinergic Agonists on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[3]	ID_{50} (mg/kg)	Correlation Coefficient
Saline	-	64	110.2 ± 9.6		
Deanol	640	4	57.7 ± 16.5		
	2560	5	25.2 ± 11.4	1,016.6	-0.98
Choline Chloride	10	6	82.2 ± 17.6		
	40	6	42.3 ± 7.6		
	160	7	54.7 ± 12.3	86.7	-0.88
Oxotremorine	0.04	5	93.0 ± 43.1		
	0.16	5	101.4 ± 21.3		
	0.64	14	75.1 ± 24.2	23.3	-0.84
Physostigmine	0.04	6	72.6 ± 7.7		
	0.16	5	39.6 ± 11.3	0.10	-0.99*
Pilocarpine	2.5	5	76.2 ± 15.7		
	10.0	7	9.7 ± 4.8	3.87	-0.99*

1. All drugs were administered i.p. 30 mins prior to observation with the exception of oxotremorine and physostigmine which were given 10 mins prior to post treatment observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

* Significant regression coefficient ($p < 0.05$).

Table 20. The Interaction Between Pilocarpine and Naloxone on Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	Withdrawal Shakes[2] % Pretreatment (Mean ± S.E.)	
		Saline[3]	Naloxone
Saline		110.2 ± 9.6 (64)	105.1 ± 14.5 (8)
Pilocarpine	10	9.7 ± 4.7 (7)	9.1 ± 4.5 (7)

1. All drugs were administered i.p. 30 mins prior to post treatment observation.

2. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

3. Saline or naloxone (5 mg/kg) was administered 10 mins prior to observation.

4. The number of rats employed per dose is indicated in parentheses.

H. The Effect of Gabaminergic Agents on Morphine Withdrawal Body Shakes

The effects of bicuculline, depakene and picrotoxin, on morphine withdrawal body shakes are shown in Table 21. Bicuculline tested at doses of 0.16 and 0.64 mg/kg was without effect on withdrawal body shakes. A biphasic effect was observed on withdrawal shakes after the administration of depakene. A slight reduction in shakes was observed when depakene was administered in doses between 2.5 and 40 mg/kg; however, as the dose was further increased to 160 or 320 mg/kg, withdrawal shakes increased in frequency. The highest dose employed, 640 mg/kg, yielded a 60 percent decrease in withdrawal shakes. Picrotoxin, a blocker of γ-aminobutyric acid (GABA) at post-synaptic receptors, reduced the frequency of body shakes by about 70 percent at a dose of 0.64 mg/kg. Further increasing the dose of picrotoxin to 2.5 mg/kg was lethal in those rats tested.

I. The Effect of Sedative-Hypnotic Agents on Morphine Withdrawal Body Shakes

The data summarized in Table 22 show the effect of the sedative-hypnotic agents tested on withdrawal body shakes. Chlordiazepoxide had no effect on withdrawal shakes at either 2.5 or 10 mg/kg. When the dose was increased to 40 mg/kg there was a near total reduction in body shakes. The results achieved with flurazepam were similar. A dose of 40 mg/kg of flurazepam was necessary to reduce withdrawal body shakes

Table 21. The Effect of Gabaminergic Agents on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[3]	Correlation Coefficient
Saline		64	110.2 ± 9.6	
Bicuculline	0.16	7	99.8 ± 41.0	
	0.64	6	124.8 ± 57.3	+0.55
Depakene	2.5	8	66.4 ± 19.2	
	10.0	19	42.2 ± 9.2	
	40.0	8	76.6 ± 17.4	
	160.0	5	113.0 ± 31.4	
	320.0	9	280.2 ± 64.7	
	640.0	6	39.8 ± 34.7	+0.28
Picrotoxin	0.64	8	33.4 ± 12.6	
	2.50	2	lethal	

1. All drugs were administered i.p. 30 mins prior to observation with the exception of picrotoxin which was given 15 mins prior to post treatment observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

Table 22. The Effect of Sedative-Hypnotic Agents on Morphine Withdrawal Body Shakes

Treatment[1]	Dose (mg/kg)	N[2]	Withdrawal Shakes % Pretreatment (Mean ± S.E.)[3]	ID_{50} (mg/kg)	Correlation Coefficient
Saline		64	110.2 ± 9.6		
Chlordiazepoxide	2.5	5	106.4 ± 21.0		
	10.0	6	80.0 ± 24.6		
	40.0	4	6 ± 6	14.2	-0.91
Flurazepam	2.5	7	76.7 ± 16.9		
	10.0	5	124.0 ± 49.2		
	40.0	6	42.0 ± 22.6	144.8	-0.54
Pentobarbital	0.64	6	74.5 ± 17.9		
	2.50	5	96.4 ± 16.6		
	10.00	6	50.0 ± 23.3	21.4	-0.77

1. All drugs were administered i.p. 30 mins prior to post treatment observation.

2. The number of rats employed per dose.

3. Pretreatment withdrawal shakes were observed for a 30 min period after 8 hours of morphine abstinence.

by about 60 percent. Pentobarbital was without effect on withdrawal shakes until a dose of 10 mg/kg was administered. Withdrawal shakes were reduced to 50 percent of pretreatment when a 10 mg/kg dose of pentobarbital was administered. The highest dose of each sedative hypnotic employed produced a substantial degree of sedation. Regression analysis showed no significant correlation between dose and effect for these drugs.

V. DISCUSSION

A. Morphine Withdrawal Body Shakes

Morphine withdrawal body shakes were reliably observed upon the termination of continuous morphine infusion. These shakes were similar to those previously described by several authors (for review see Gianutsos et al., 1975) in rats withdrawn from periodic injections of narcotic drugs. However, their rate of occurrence in the continuously infused rats was higher than that usually seen in rats made dependent by intraperitoneal (Gianutsos et al., 1975) subdermal (Herz et al., 1974) or oral (Lal et al., 1975) administration of narcotics. Body shakes are related to narcotic withdrawal because they are not seen in rats similarly infused with saline or in morphine-infused rats which were not withdrawn (Table 2). Additionally, these shakes were abolished by narcotic drugs and the reinstatement of narcotic infusion (Table 5). This shake reducing action of narcotic drugs was antagonized by the administration of the narcotic antagonist naloxone (Table 6). Recently, it has been demonstrated that many morphine effects may be brought under stimulus control through various procedures of conditioning (Lal et al., 1976). Like many of the other effects of morphine, the shake mitigating action of this narcotic could be elicited by conditional stimuli previously paired with morphine injections (Numan et al., 1975).

Withdrawal body shakes were selected as a sign of abstinence since they are a consistent, reliable and objective measure. Rats withdrawn from morphine for an eight-hour period emitted an average of 25 body shakes in a 30 min observation period. The frequency of withdrawal shakes remained constant over many withdrawal sessions (Table 3). These violent shaking movements were easily detectable. The reliability for detection of these body shakes was very high between independent observers. For the purpose of establishing inter-observer reliability two trained observers recorded withdrawal body shakes during the same withdrawal session. While observing the same withdrawal session these independent observers recorded essentially the same number of body shakes. In fact they were always within 2 percent of each other for the total number of shakes occurring during the withdrawal session. The intense and violent nature of these shaking movements make them an objective measure of withdrawal that is easily distinguishable from body tremors or jerking type movements.

Withdrawal observation was always started 8 hours after the termination of morphine infusion. There were several reasons for selecting 8 hours of withdrawal as the time interval to start the withdrawal observation. By the eighth hour of withdrawal the shaking movements were fully developed and occurring at a high rate. Body shakes were found to start to occur 3 hours after the morphine infusion was terminated and to have reached a fairly constant frequency by 6 to 7

hours of withdrawal. Once the withdrawal shakes were fully developed they remained at a fairly constant frequency for up to 24 hours if the morphine infusion was not reinstated. Thus the pretreatment shakes could be observed 8 hours after the termination of morphine infusion, a test drug could then be injected and given sufficient time to exert its action with a high degree of certainty that the rate of occurrences of withdrawal shakes would remain unchanged except for drug action. The fact that withdrawal shakes remained at a constant intensity following 8 hours of abstinence is demonstrated by the failure of a saline injection to influence the occurrence of this withdrawal sign.

Morphine, methadone and fentanyl, potent narcotics, blocked the occurrence of withdrawal shakes (Table 5). The narcotic antagonist naloxone and pentazocine increased the rate of withdrawal shakes slightly but not significantly (Table 5). However, naloxone effectively antagonized the morphine induced blockade of withdrawal shakes in a dose dependent manner (Table 6).

In addition to the blockade of withdrawal shakes produced by the narcotic agonist all the dopamine receptor blockers investigated were found to mitigate the rate of shaking with the exception of pimozide and pipamperone (Table 7). These results are in agreement with previous findings (Lal et al., 1971; Lal and Numan, 1976). However there appeared to be dramatic potency differences between those neuroleptics employed. The butyrophenone neuroleptics, benperidol,

haloperidol, oxiperomide, spiperone and spiramide, were specific in blocking withdrawal shakes in that they reduced them at low doses which are well within the range of those producing the usual pharmacological actions of these drugs. (+)Butaclamol, a derivative of benzocycloheptapyridoisoquinolinol, exerted its antiwithdrawal effect at a low dose, that is, in the dose range within which it produces its other psychopharmacological activities (Voith and Herr, 1975). Similarly, the member of the dibenzoxazepine series employed, loxapine, also mitigated withdrawal shake behavior at doses within its pharmacological range for antidopamine activity (Coupet et al., 1976). The two phenothiazine neuroleptics employed in this study were only effective in blocking withdrawal shakes at very high doses which also produce hypotonia and nonspecific sedation, while pimozide, of the diphenylbutylpiperidine series, and pipamperone did not totally block withdrawal shakes even at the highest dose tested. From this comparison it is clear that the butyrophenone, benzocycloheptapyridoisoquinolinol and dibenzoxazepine type neuroleptics are more potent and specific in blocking withdrawal body shakes than are the phenothiazines and diphenylbutylpiperidine type neuroleptics.

The dopamine receptor agonists tested for antiwithdrawal potency also reduced the rate of withdrawal body shakes (Table 8). Amphetamine, a drug which causes the release of dopamine (McKenzie and Szerb, 1968; Carr and Moore, 1970) dose dependently reduced body shakes as did the direct acting

dopamine receptor agonist apomorphine (Ernst, 1967; Anden et al., 1967). L-DOPA, which is converted centrally into dopamine (Carlsson, 1971) reduced body shakes in a similar fashion.

With the exception of azaperone, all the adrenergic blockers tested for anti-abstinence properties were without effect (Table 12). Azaperone is a sedative neuroleptic of the butyrophenone series which is a potent alpha-noradrenergic blocking drug (Niemegeers et al., 1974). The adrenergic agonists also seem to mitigate the occurrence of withdrawal shakes. However, only desmethylimipramine and clonidine exhibited a significant dose dependent decrease in the occurrence of this withdrawal sign (Table 13). Inhibition of catecholamine synthesis by alpha-methyl-p-tyrosine (Nagatsu et al., 1964) or storage by reserpine (Haggendal and Dahlstrom, 1972) decreased occurrence of withdrawal body shakes (Table 16).

Agents that affect serotonergic mechanisms by either increasing or decreasing the concentration of transmitter present at its receptor site were without significant effect on the occurrence of withdrawal body shakes (Table 17). Manipulation of gabaminergic mechanism also failed to influence this withdrawal sign to any significant extent (Table 21).

The results achieved with the utilization of cholinergic agents provided a more consistent picture. A cursory examination of the data indicates that anticholinergics increase withdrawal shakes while cholinergic decrease. Although

the anticholinergics used, atropine, benztropine, dexetimide and scopolamine, did tend to increase body shakes, these increases failed to achieve significance (Table 18). With the exception of oxotremorine, all the cholinergics employed decreased body shakes (Table 19). This effect achieved dose related significance for only physostigmine and pilocarpine. The reduction in body shakes induced by pilocarpine is in agreement with previous results (Hynes et al., 1976).

The involvement of nonspecific sedation in the reduction of withdrawal shakes was investigated by studying the effect of several hypnotic drugs on this index of withdrawal. Regression analysis revealed no significant correlation between the dose of chlordiazepoxide, flurazepam or pentobarbital and their effects on withdrawal shakes (Table 22). However, a 40 mg/kg dose of chlordiazepoxide did completely reduce the occurrence of this withdrawal sign.

Body shakes measured here as an index of withdrawal originate within the central nervous system. There are several lines of evidence which point to that conclusion. Naloxone when injected into the brain of morphine dependent animals elicits body shakes (Way et al., 1973; Laschka et al., 1976a). Narcotic withdrawal has also been attenuated by lesion of the central nervous system (Pouzolo and Kerr, 1972). Pilocarpine, a cholinergic agonist, is known to reduce both withdrawal aggression and body shakes (Hynes et al., 1976). The antiwithdrawal effect of pilocarpine is centrally mediated. Its antiwithdrawal effects are not blocked by methyl-

scopolamine, a peripherally active anticholinergic which does not penetrate into the brain (Hynes et al., 1976). These lines of evidence clearly point to the central origins of withdrawal body shakes. It is therefore assumed that the drugs which reduce body shakes are acting on the central nervous system. All drugs employed in this study have well established central mechanisms of action. However, it is possible that they could have peripheral actions which might influence the expression of withdrawal body shakes. It is conceivable that a drug could inhibit withdrawal by preventing the muscular expression of withdrawal. That is to say, a drug could prevent the expression of a withdrawal sign without having any effect on the underlying pathology. D-tubocurare would be an example of a drug that would produce this type of effect, since it would produce muscular paralysis and thereby prevent the expression of withdrawal with little or no effect on the underlying neuropathology. None of the drugs employed in this study are known to act in this fashion, thus it can be reasonably assumed that they are exerting their antiwithdrawal effects on the central nervous system.

B. The Neurotransmitter Hypothesis of Antiwithdrawal Activity

Neuroleptics have many important and well demonstrated effects on neurotransmitters such as dopamine, norepinephrine, serotonin, γ aminobutyric acid and acetylcholine. The antiwithdrawal actions of neuroleptics may be mediated by their

effects on the aforementioned neurotransmitters.

Previously, it was hypothesized that the antiwithdrawal effectiveness of butyrophenones was the result of their dopamine receptor blocking action. This hypothesis was based upon the supersensitivity of dopamine-receptors seen during morphine withdrawal (for review see Lal, 1975; Lal et al., 1976) and the effectiveness of haloperidol in reducing narcotic withdrawal in laboratory animals (Lal et al., 1971; 1971; Puri and Lal, 1973; Gianutsos et al., 1974; Lal and Numan, 1976; Martin et al., 1974) and human patients (Karkalas and Lal, 1973; Lal and Hynes, 1977). The antiwithdrawal potencies of the additional butyrophenone type neuroleptics tested support this hypothesis. The effective doses of those neuroleptics with antiwithdrawal potency in producing other pharmacological actions are given in Table 23. As can be seen from this table there is a correlation between the dose of these drugs which antagonize the effect of apomorphine and the antiwithdrawal dose. Generally, those neuroleptics which inhibit the effects of apomorphine at low concentrations also reduced withdrawal body shakes at low doses. There are several exceptions, however; azaperone which is the most potent neuroleptic in terms of its antiwithdrawal effect is the least potent apomorphine antagonist. Pimozide is another exception in that it is a very poor inhibitor of withdrawal body shakes but is a relatively potent apomorphine antagonist. Even with these exceptions there appears to be a relationship between antiwithdrawal and anti-apomorphine activity.

Table 23. A Comparison of the Various Properties of Those Neuroleptics Tested for Anti-Withdrawal Potency

	ID_{50} (mg/kg)	ED_{50} Values in the Rat (mg/kg)[1]				
	Anti-Withdrawal	Ampetamine Antagonism	Norepinephrine Antagonism	Catalepsy	Ptosis	Apomorphine Antagonism
Azaperone	0.03	2.5	0.33	8.0	1.5	0.98
Spiperone	0.04	0.02	1.2	0.036	0.27	0.0002
Benperidol	0.08	0.012	0.30	0.18	1.2	0.0005
+Butaclamol	0.12	6.31	10.0	-	-	.052
Loxapine	0.16	0.12	1.25	0.25	-	0.3
Oxiperomide	0.16	0.03	5.0	40.0	2.5	0.014
Haloperidol	0.17	0.038	2.1	0.18	1.0	0.018
Spiramide	0.19	0.016	9.5	0.34	1.5	0.0021
Chlopromazine	0.49	0.60	0.60	2.3	2.3	0.71
Trifluoperazine	0.74	0.08	7.2	0.6	2.5	0.034
Pimozide	1.64	0.1	40.0	0.18	4.5	0.011
Pipamperone	11.02	2.5	3.0	16.5	2.7	0.97

[1]Values were derived from Janssen and Van Bever (1975).

Apomorphine antagonism is a well established index of the ability of a drug to block brain dopamine receptors. The antiwithdrawal activity of neuroleptics may result from the inhibition of brain dopamine receptors. However, this hypothesis is not supported by the fact that agents which stimulate dopamine receptors also reduce withdrawal wet shakes. The three dopamine receptor stimulants employed in this study all dose dependently reduce withdrawal body shakes. Although both dopamine receptor agonist and antagonist have the same effect on body shakes, they differ with respect to their effects on other withdrawal signs. Butyrophenones, for example, have previously been shown to block many other signs of narcotic withdrawal. These include hypothermia (Lal et al., 1971; Cox et al., 1975) aggression (Puri and Lal, 1973; Gianutsos et al., 1974; Lal, 1975); jumping (Takemori et al., 1976) and responding for self-administration of narcotics (Hanson and Cimini-Venema, 1972; Lal and Hynes, 1977). Whereas dopaminergic agonists have been found to intensify many other signs of narcotic withdrawal, pretreatment with L-DOPA, amphetamine or apomorphine enhances morphine withdrawal aggression (Lal et al., 1971; Lal, 1974) and withdrawal jumping (Gunne, 1965; Herz, 1975). Thus it is conceivable that the action of dopaminergic agonists on withdrawal body shakes are not related to dopaminergic activity. Drugs that stimulate dopamine receptors are known to induce stereotyped behaviors which consist of sniffing, licking and biting movements (Ernst, 1967; Gianutsos et al., 1974). The possibility

exists that the induction of these stereotyped behaviors and the occurrence of withdrawal shakes are not compatible. Thus the withdrawn rat treated with a dopamine receptor agonist would only be able to emit stereotyped movement and not body shakes.

The marked potency of azaperone in blocking shakes does not support this hypothesis. Azaperone is one of the least potent anti-dopamine drugs tested yet the most potent butyrophenone in blocking withdrawal shakes (Table 12). However, azaperone is a potent alpha-noradrenergic blocking drug (Niemegeers et al., 1974). It is thus possible that the antiwithdrawal action of neuroleptics may be the result of antinoradrenergic activity. However, chlorpromazine which is approximately an equally potent blocker of alpha adrenergic receptors (Janssen and Van Bever, 1975), is one of the weakest blockers of withdrawal shakes (Table 7). Additionally, the alpha blocker phenoxybenzamine (Nickerson and Grump, 1949) failed to reduce body shakes as did the beta adrenergic blocker propranolol (Nickerson and Collier, 1975). The data summarized in Table 23 do not support the hypothesis that the antiwithdrawal activity of neuroleptics is the result of antiadrenergic activity. As can be seen from this table there is no correlation between the antiwithdrawal activity of neuroleptics and antiadrenergic potency. Further evidence against this particular hypothesis comes from the data on adrenergic agonist drug. One of the most potent drugs in reducing withdrawal body shakes is clonidine, an alpha receptor

stimulant (Anden et al., 1970). The tricyclic antidepressant agents amitriptyline and desmethylimipramine, which block the re-uptake of norepinephrine into adrenergic nerve terminals (Byck, 1975), also decreases the occurrence of withdrawal body shakes (Table 13).

Since the discovery that the symptoms of Parkinsonism could be relieved by drugs which act on either the cholinergic or the dopaminergic neuronal system, considerable evidence has suggested a possible reciprocal cholinergic-dopaminergic interaction in the central nervous system. Neuroleptic drugs have been found to increase the release of acetylcholine (Stadler et al., 1973; 1974; Lloyd et al., 1973). The possibility arises that neuroleptic drugs may be exerting their antiwithdrawal effects through cholinergic mechanisms. If this is true then cholinergic drugs should reduce withdrawal body shakes. The results achieved here indicate that cholinergic agonists tend to reduce withdrawal shakes (Table 19). However, this effect was significant for only two of the five cholinergics studied. Cholinergic agonist do possess some of the other antiwithdrawal effects seen after the administration of neuroleptics. They decrease withdrawal aggression (Hynes et al., 1976) and inhibit antagonist precipitated morphine withdrawal jumping (Grumbach, 1969; Bhargava and Way, 1972; Brase et al., 1974). Thus it is conceivable that the antiwithdrawal effects of neuroleptics are in part mediated by cholinergic mechanisms. However, if stimulation of cholinergic receptors is responsible for inhibition of

certain withdrawal signs then anticholinergics would be expected to increase withdrawal intensity or have no effect. The results obtained in general conform with this expectation. Those anticholinergic drugs tested produced some increase in withdrawal shakes but this effect failed to achieve significance (Table 18). The possibility exists that these withdrawn rats were showing the near maximum number of shakes physiologically possible, thus it may not have been possible to significantly increase their rate of occurrence by the administration of anticholinergics. This hypothesis does not explain the antiwithdrawal body shake effect produced by the administration of dopaminergic agonist. Apomorphine, amphetamine and L-DOPA have been found to increase acetylcholine levels in the caudate (Sethyl and Van Woert, 1974) which suggests a decreasedlevel of acetylcholine at its receptor site. In line with the above hypothesis, these agents would be expected to increase or have no effect on withdrawal shakes which is the direct opposite of what was found. Thus a dopamine-acetylcholine hypothesis of withdrawal shakes is not totally sufficient to explain all of the results.

In addition to the interaction between dopamine and acetylcholine, it has also been postulated that dopamine interacts with serotonin (Cools, 1974) and GABA (Ladinsky et al., 1976) in the central nervous system. The results obtained here do not support a role for either serotonin or GABA in morphine withdrawal body shakes. The administration of serotonin receptor agonist or antagonist were without

significant effect on the occurrence of body shakes observed in morphine withdrawal (Table 17). Similarly, those agents employed to modify GABA activity had no significant effect on this index of withdrawal (Table 21). Picrotoxin, a blocker of GABA receptors (Galindo, 1969), however, did reduce body shakes by about 70 percent at a dose of 0.64 mg/kg.

Neuroleptics in addition to their complex effects on the various neurotransmitters, also produce a considerable degree of sedation when given initially. Sedation then may be responsible for the antiwithdrawal activity of neuroleptic drugs. To test this possibility, several drugs of the sedative hypnotic type were administered to withdrawn rats. Neither chlordiazepoxide, flurazepam or pentobarbital had any significant dose related effects on withdrawal body shakes (Table 22). The highest dose of these drugs employed did reduce body shakes. The reduction was accompanied with a considerable degree of sedation much greater than observed with the various neuroleptics. The degree of sedation produced by the various neuroleptics does not correlate with the ability to reduce withdrawal body shakes. For example, haloperidol has less of a sedative effect than chlorpromazine (Byck, 1975) but is a more potent inhibitor of withdrawal shakes. Sedation does not appear to be an important factor in the reduction of withdrawal shakes produced by neuroleptics.

Of the neurotransmitters effected by neuroleptic drugs it appears that effects on dopamine systems are the most important in terms of antiwithdrawal activity. A relatively

good correlation exists between antiwithdrawal activity and antagonism of brain dopamine receptors for those neuroleptics tested. However, the antiwithdrawal effect of neuroleptics may be mediated at least in part through cholinergic mechanisms. This possibility arises because neuroleptics are known to effect cholinergic mechanisms and the manipulation of cholinergic systems was found to alter the occurrence of withdrawal body shakes. Administration of cholinergic agonists was found to decrease withdrawal body shakes and neuroleptics, which cause the release of acetylcholine (Stadler et al., 1973; 1974) similarly reduce this index of withdrawal.

C. The Opiate Receptor Hypothesis of Antiwithdrawal Activity

It has been recently recognized (Clay and Brougham, 1975; Creese et al., 1975; Leysen et al., 1976) that butyrophenone type neuroleptics are among many non-narcotic drugs which show binding affinity with opiate-binding sites in the central nervous system. It is possible t t the withdrawal blocking action of butyrophenones is related to this binding ability. Besides the availability of actual binding data, support for this hypothesis comes from studies with naloxone. The administration of naloxone after the maximally effective dose of azaperone, benperidol, oxipermide, haloperidol and trifluoperazine in reducing withdrawal shakes produced a significant reversal of the antiwithdrawal effect of these neuroleptics (Table 10). Unlike the complete antagonism of

morphine's action produced by naloxone, the only neuroleptic to be completely antagonized by naloxone was azaperone (Table 15). Naloxone antagonism in the case of benperidol, oxipermide, haloperidol and trifluoperazine although significant, was far from complete. Naloxone failed to antagonize the antiwithdrawal body shake effect of spiperone, spiramide, loxapine, chlorpromazine and +butaclamol to any significant extent (Table 10). These results do not support the idea that the binding of neuroleptics to the opiate receptor is responsible for their antiwithdrawal effects. Further evidence against this theory comes from the fact that pimozide which shows affinity for the opiate receptor (Creese et al., 1975) does not block withdrawal wet shakes.

Opiate receptor binding is believed to be responsible for many opiate actions, such as analgesia. We have been unable to detect any activity of these compounds in prolonging tail-withdrawal latency, a reliable and sensitive test for analgesia (Janssen et al., 1963; Niemegeers et al., 1976). However, it has not yet been established that binding with opiate receptor sites necessarily correlates with analgesia in the case of nonnarcotic drugs. It is possible that there are different opiate receptors responsible for different actions of narcotic and nonnarcotic drugs.

Of the other drugs found to have antiwithdrawal potency only one was antagonized to a significant extent by naloxone. The reduction in withdrawal shakes produced by apomorphine was reversed by the administration of naloxone (Table 11).

This result is not unexpected in light of the fact that apomorphine is a narcotic. However, the antiwithdrawal effects of clonidine, amphetamine or pilocarpine were not significantly antagonized by naloxone.

These results suggest that some neuroleptics, but not all, exert their antiwithdrawal effects to some extent by binding with opiate receptors. Although this explanation is highly speculative, the resemblance between butyrophenones with opiates in many other pharmacological actions (for review see Lal et al., 1975; 1976) provides some support for this hypothesis. It is difficult then to explain why some butyrophenone neuroleptics which reduce withdrawal shakes are not antagonized by naloxone. The naloxone effect may be the result of an increase in the severity of the withdrawal syndrome and not a real antagonism of the neuroleptic. Those neuroleptics which are seemingly antagonized by naloxone may not be able to reduce this withdrawal syndrome of increased intensity. The fact that the dose of naloxone, 5 mg/kg, employed in these experiments, does not produce an increase in the number of body shakes fails to support this explanation of the results. It thus appears that naloxone is able to antagonize the antiwithdrawal activity of some neuroleptics in a specific manner. However, even for those neuroleptics that were significantly antagonized by naloxone that antagonism was well below the total reversal level. This fact would indicate that in addition to the binding of these neuroleptics with the opiate receptor that they also reduce shakes by an

additional mechanism that is not antagonized by naloxone. It appears that the binding of neuroleptics to opiate receptors is in part responsible for the antiwithdrawal activity of neuroleptics; however, this mechanism is not totally sufficient to explain the antiwithdrawal activity of these drugs.

Although the binding of nonnarcotic drugs to the opiate receptor does not totally explain the antiwithdrawal activity of these compounds, a role for the opiate receptor cannot be ruled out. It is possible that morphine or opiate-like compounds present in the body could react with the opiate receptor to reduce withdrawal after nonnarcotic drug administration.

Withdrawal body shakes were observed eight hours after the termination of morphine infusion which may be insufficient time for the complete metabolism of all morphine present in the system. This concentration of morphine is insufficient to exert any significant pharmacological effects by itself. Since neuroleptics are known to potentiate some of the effects of morphine (Yelnosky and Gardocki, 1964; Edmonds-Seal and Prys-Roberts, 1970) it is possible that those neuroleptics with antiwithdrawal activity potentiate the morphine remaining in the system after eight hours of withdrawal. This potentiation of previously ineffective concentrations of morphine by the administration of neuroleptics may be responsible for the reduction in withdrawal intensity. If this mechanism is operating to reduce withdrawal then naloxone would be expected to antagonize the reduction in body shakes. The neuroleptic induced reduction in body shakes is, however, only

partially antagonized in some cases, while in others unaffected by naloxone administration, indicating that this mechanism does not fully account for the neuroleptic induced reduction in body shakes, although it may operate to a limited extent in the antiwithdrawal activity of certain neuroleptics. A similar mechanism may also be proposed to explain the amphetamine induced reduction in body shakes. Amphetamine has been found to augment the analgesia induced by morphine (Forrest et al., 1977). Administration of amphetamine to withdrawn rats could potentiate the morphine still present after eight hours of withdrawal. Again it would seem logical to expect naloxone to antagonize the antiwithdrawal effect of this combination; however, no such antagonism was observed, suggesting that amphetamine's reduction in body shakes is mediated by another mechanism. Although the actions of morphine can be potentiated by a variety of drugs such as neuroleptics and amphetamines, this potentiation does not appear to be responsible for the antiwithdrawal activity of these drugs.

Much recent evidence has suggested that the body contains endogenous morphine-like compounds having many properties similar to those of narcotic drugs (Hughes, 1975; Goldstein, 1976). These endogenous morphine-like compounds could be responsible for the antiwithdrawal activity of those nonnarcotic drugs tested. For example, neuroleptics may cause the body to release these endogenous morphine-like substances which in turn reduce withdrawal intensity. The activity of these endogenous morphine-like substances (endorphins) is

reversed by the narcotic antagonist naloxone (Hughes, 1975; Goldstein, 1976). If the endorphins are mediating the reduction in withdrawal intensity induced by neuroleptics then naloxone would be expected to antagonize this effect. Since naloxone is not able to antagonize the antiwithdrawal effect of all neuroleptics this explanation is not very tenable. This hypothesis is also not supported by the fact that neuroleptics do not produce analgesia which would be expected upon endorphin release (Belluzzi et al., 1976; Hill et al ., 1976; Unca et al., 1977). This data fails to establish a role for endorphins in the neuroleptic induced reduction of withdrawal body shakes.

D. The Multiple Receptor Site Hypothesis of Antiwithdrawal Activity

The probability exists that there is no single mechanism by which neuroleptic drugs inhibit the expression of morphine withdrawal body shakes. Neuroleptics are known to have many complex effects on the central nervous system and any combination of these effects may be responsible for the reduction in this withdrawal sign. It is conceivable that the reduction in body shakes induced by neuroleptics is the result of an effect on several different neurotransmitters and opiate receptors. From the data presented here the most likely combination of neurotransmitters are dopamine and acetylcholine. Naloxone was able to reverse that portion of antiwithdrawal activity that was the result of opiate

receptor binding but not that which was the result of neurotransmitter receptor binding. It is also possible that in addition to affecting opiate, dopamine and cholinergic mechanisms neuroleptics also bind with additional neurotransmitter receptor sites to reduce narcotic withdrawal body shakes.

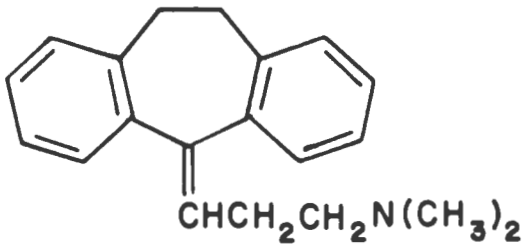
Many of the drugs that reduced withdrawal body shakes have very different mechanisms of action suggesting that they may share a common structural feature which is responsible for antiwithdrawal activity. The chemical structures of all those compounds employed in this study are shown in Figure 3. A cursory examination of these structures to ascertain a common structural feature in those drugs that inhibited withdrawal failed to show any apparent similarity. This does not rule out the existence of any structural similarity between antiwithdrawal agent. To demonstrate the presence of a common feature would necessitate an in-depth computer analysis for structure activity relationship.

The data obtained in this study suggest that neuroleptics reduce morphine withdrawal body shakes through activity on several different receptor sites in the central nervous system. Both narcotic and neurotransmitter receptor sites appear to be involved. The neurotransmitter receptor sites which appear to be the most important are those for dopamine and acetylcholine. The blockade of dopamine receptors and the release of acetylcholine resulting from the administration of a neuroleptic is one possible mechanism to explain antiwithdrawal activity. Other transmitters influenced by neuro-

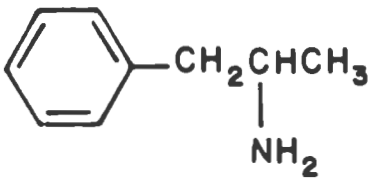
leptics can not be totally ruled out at this time but from the data achieved in these experiments it is not highly probable that they are involved to any major extent.

In addition to the effects of neuroleptics on the various neurotransmitters it is conceivable that an action on opiate receptors is in part responsible for the reduction in withdrawal body shakes. Neuroleptics may reduce withdrawal by binding with both the dopamine and opiate receptor. This could explain why naloxone did not fully antagonize the effect of any neuroleptic.

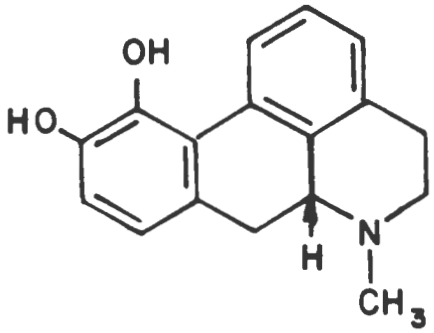
FIGURE 3 Chemical Structures of Those Drugs Investigated for Antiwithdrawal Potency



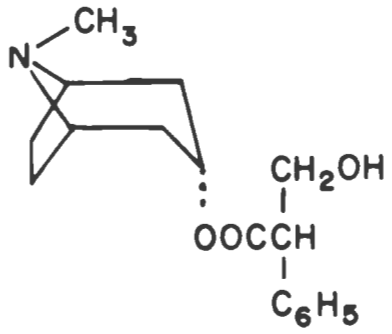
AMITRIPTYLINE



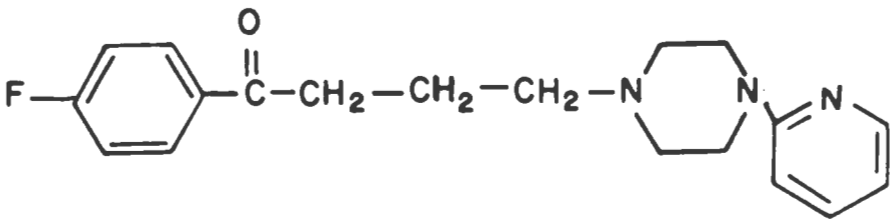
AMPHETAMINE



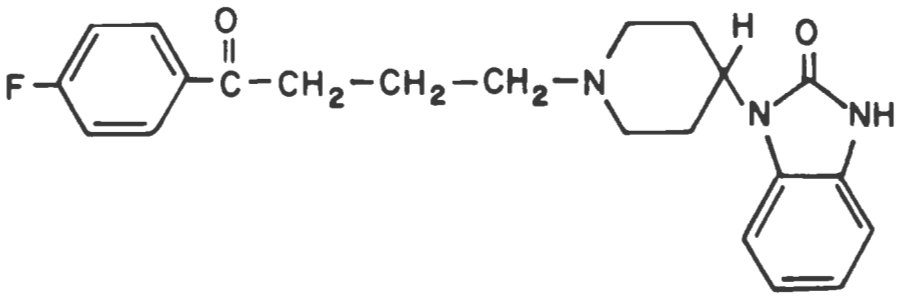
APOMORPHINE



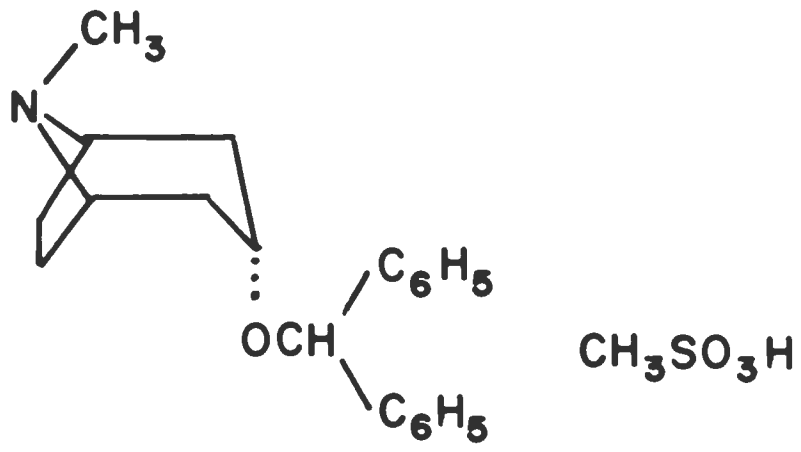
ATROPINE



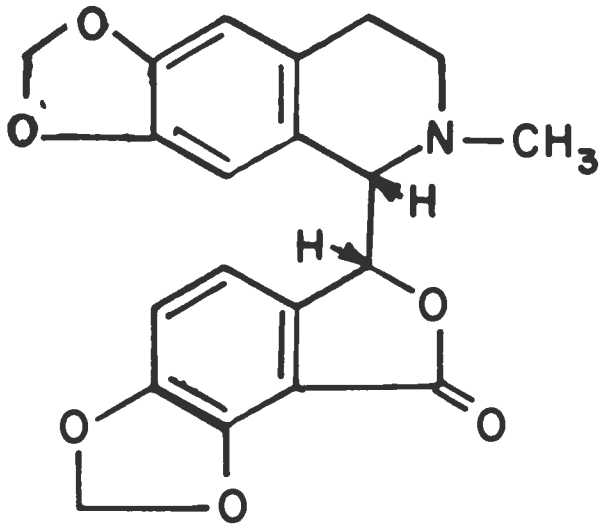
AZAPERONE



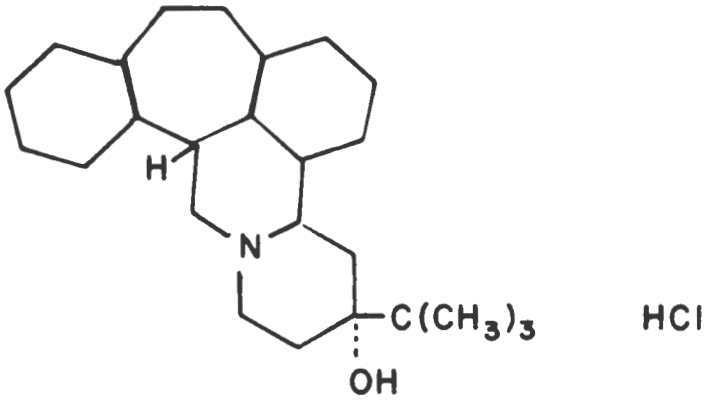
BENPERIDOL



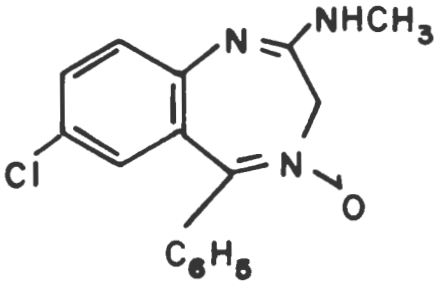
BENZTROPINE



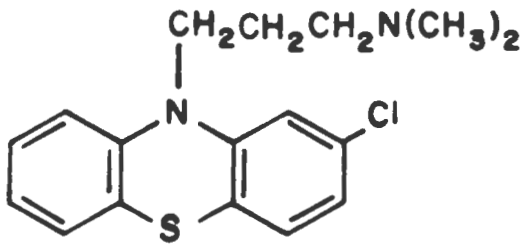
BICUCULLINE



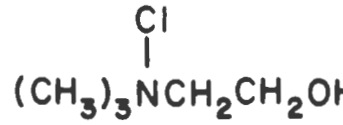
BUTACLAMOL



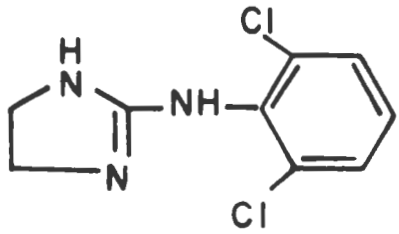
CHLORDIAZEPOXIDE



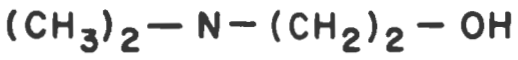
CHLORPROMAZINE



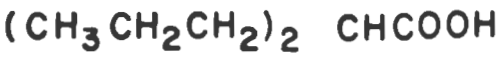
CHOLINE CHLORIDE



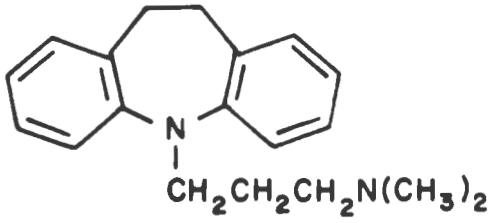
CLONIDINE



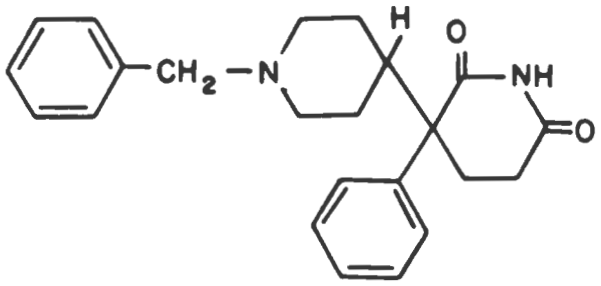
DEANOL



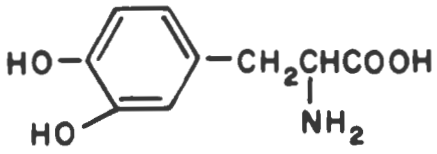
DEPAKENE (VALPROIC ACID)



DESMETHYLIMPRAMINE

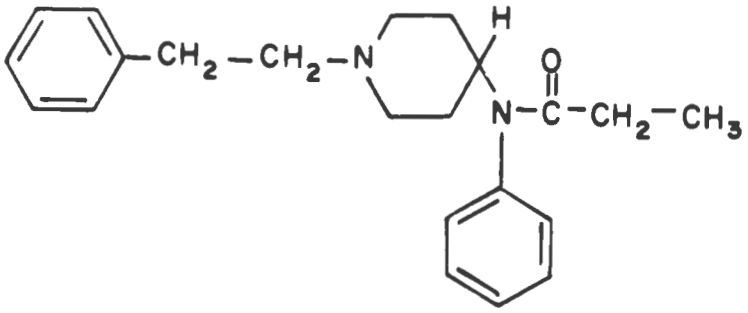


DEXETIMIDE

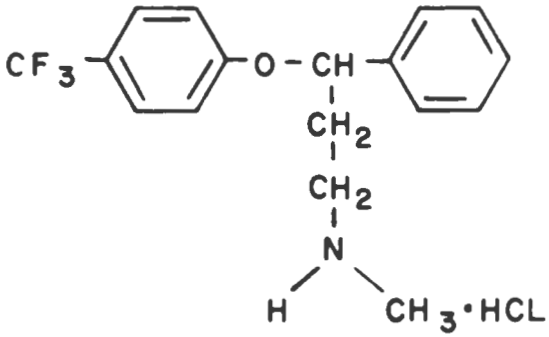


3-(3,1-DIHYDROXYPHENYL)ALANINE

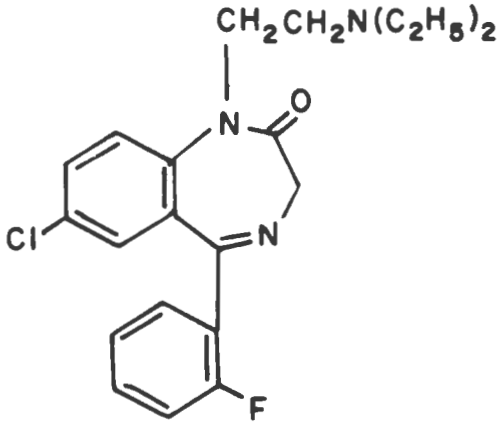
(DOPA)



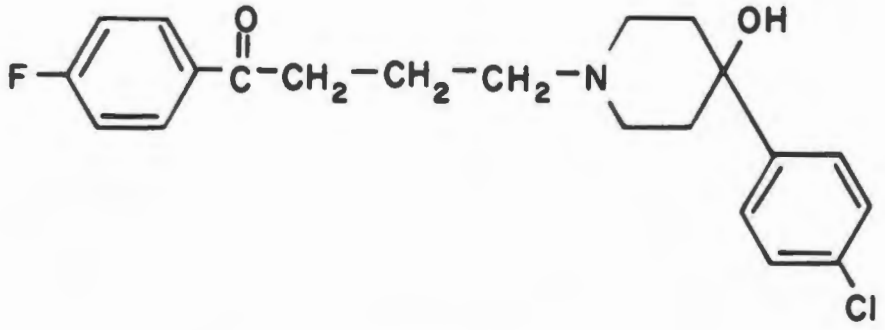
FENTANYL



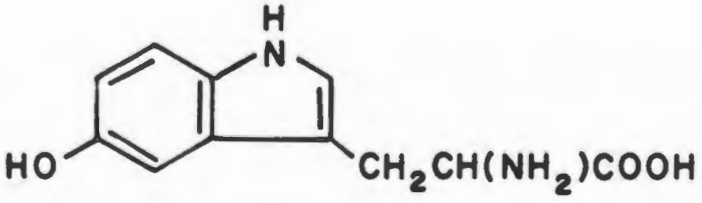
FLUOXETINE



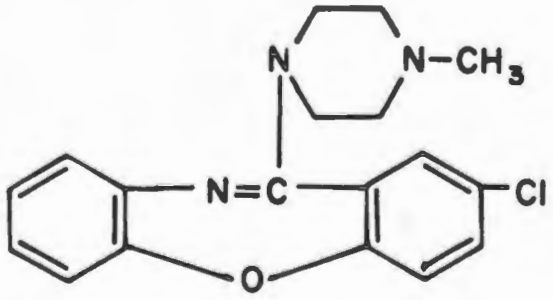
FLURAZEPAM



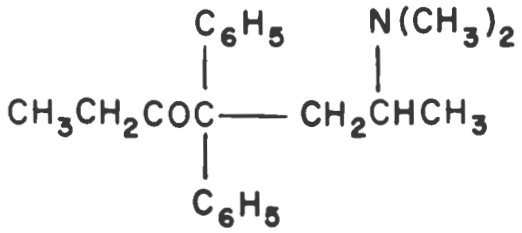
HALOPERIDOL



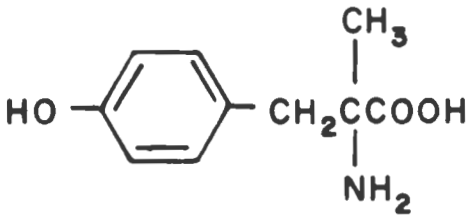
5-HYDROXYTRYPTOPHAN



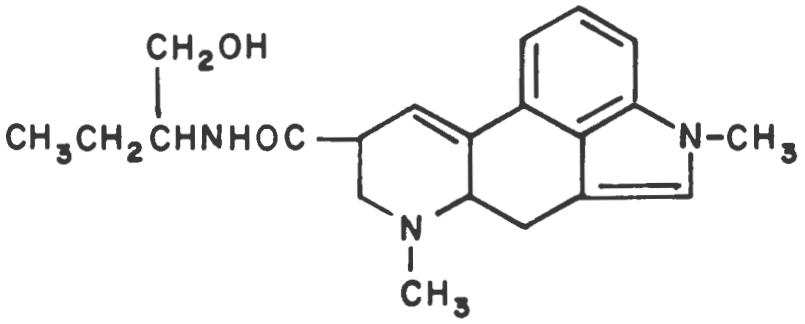
LOXAPINE



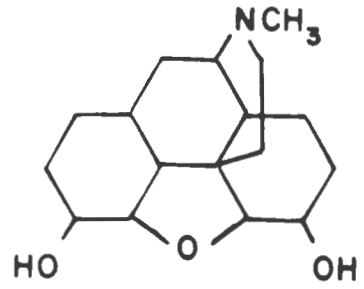
METHADONE



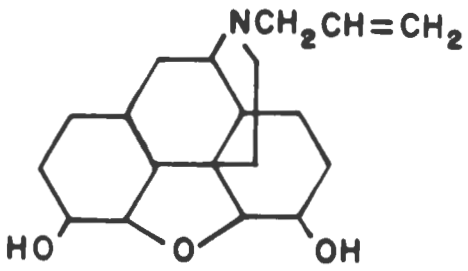
α — METHYL–P–TYROSINE



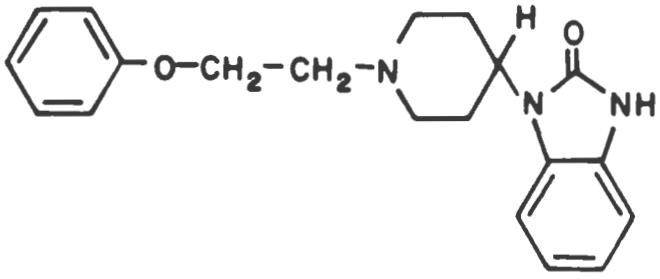
METHYSERGIDE



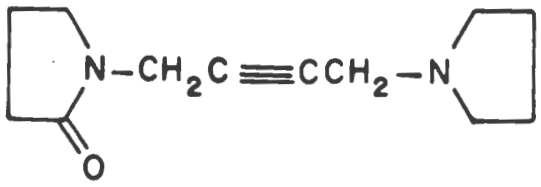
MORPHINE



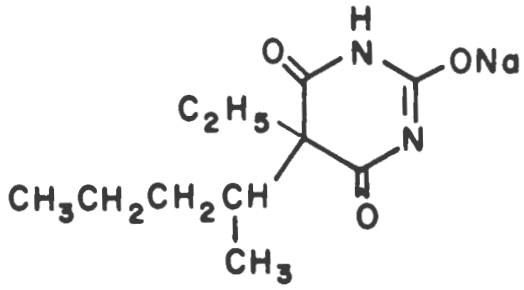
NALOXONE



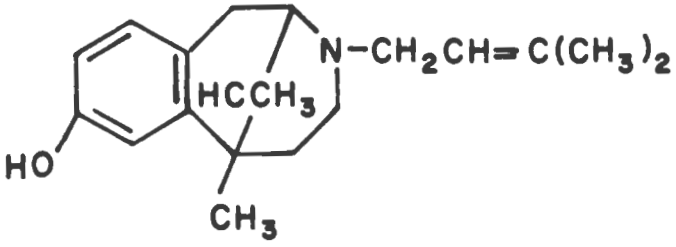
OXIPEROMIDE



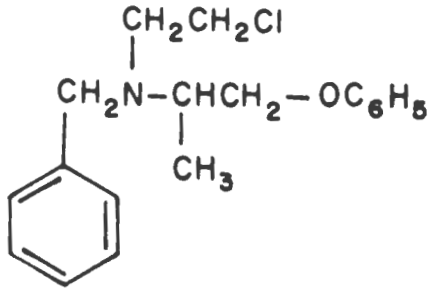
OXOTREMORINE



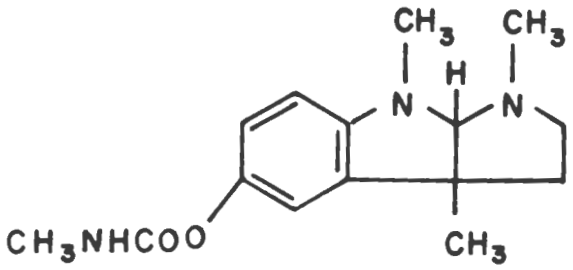
PENTOBARBITAL



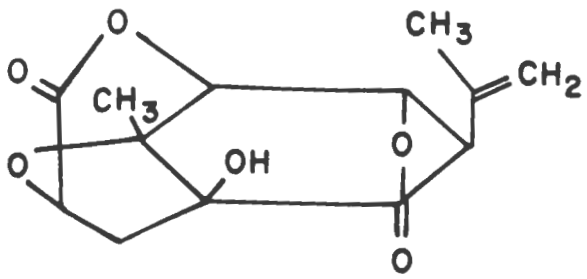
PENTAZOCINE



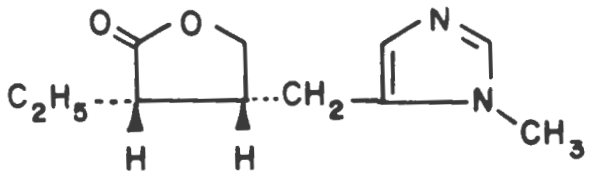
PHENOXYBENZAMINE



PHYSOSTIGMINE

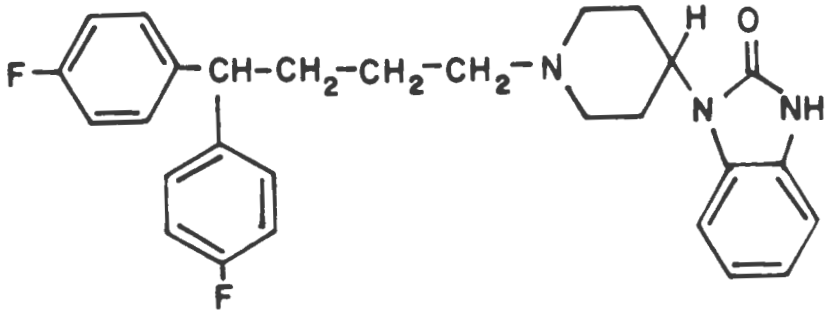


PICROTOXIN

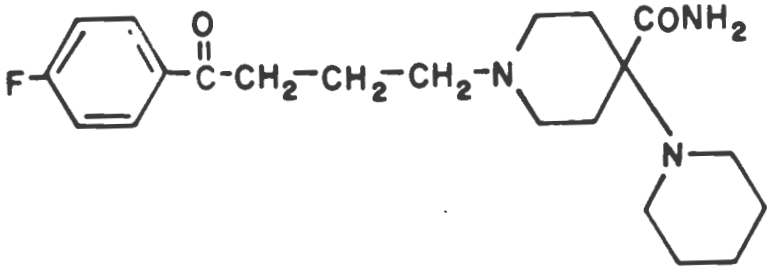


$C_{11}H_{16}N_2O_2$

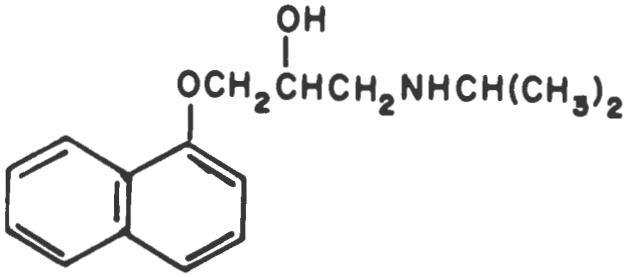
PILOCARPINE



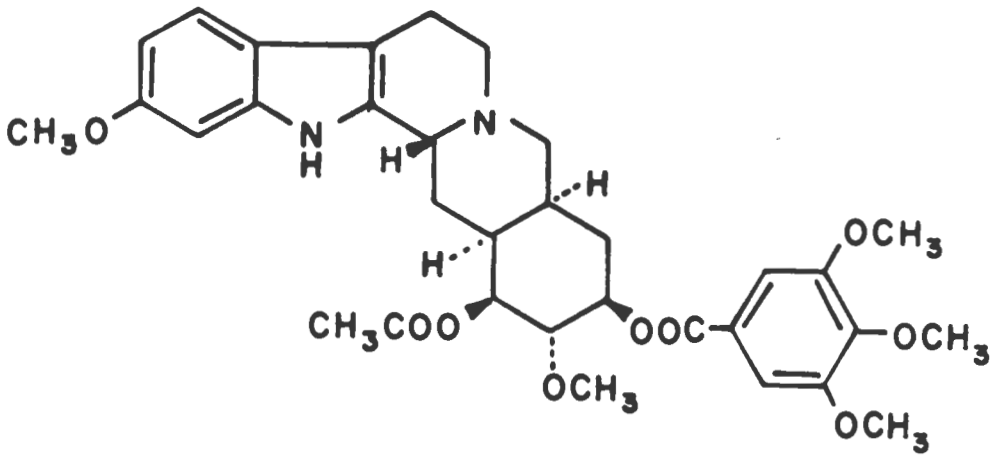
PIMOZIDE



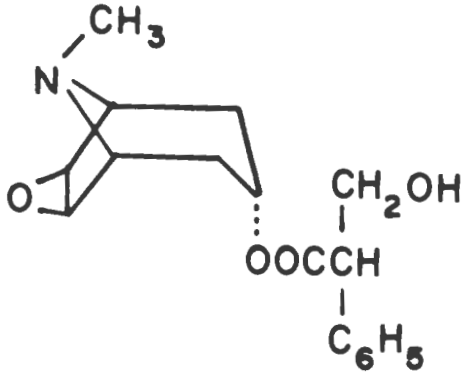
PIPAMPERONE



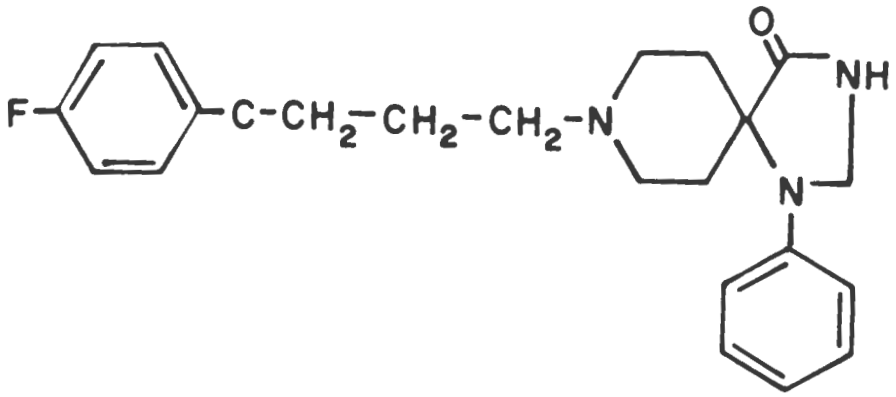
PROPRANOLOL



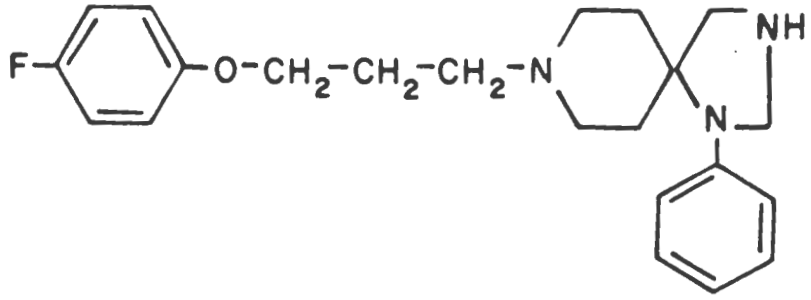
RESERPINE



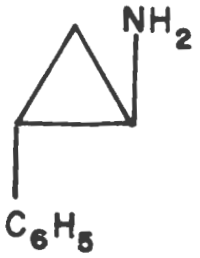
SCOPOLAMINE



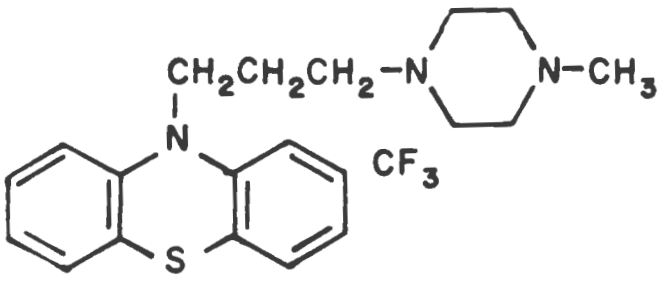
SPIPERONE



SPIRAMIDE



TRANYLCYPROMINE



TRIFLUOPERAZINE

VI

SUMMARY AND CONCLUSIONS

Termination of continuous morphine infusion resulted in the relabile occurrence of withdrawal body shakes (Table 2). These body shakes were abolished by the administration of narcotic drugs (Table 5). Morphine withdrawal body shakes were also dose dependently reduced by the administration of a variety of neuroleptics (Table 7). This reduction appears to be related to inhibition of brain dopamine receptors. Manipulation of other transmitter systems affected by neuroleptics points to a role for some of these neurotransmitters in the reduction of body shakes. Acetylcholine may play an important role in the antiwithdrawal activity of neuroleptics. The administration of cholinergic agents was found to produce dose related decreases in withdrawal body shakes (Table 19). It is highly possible that neuroleptics reduce withdrawal by acting on both dopaminergic and cholinergic mechanisms. The reversal of the antiwithdrawal activity of some neuroleptics by naloxone suggest a role for narcotic receptors (Table 10). The possibility that neuroleptics bind with narcotic receptors thereby reducing withdrawal is not totally consistent with all of the available data. Naloxone was not able to antagonize the antiwithdrawal activity of all neuroleptics and even for those neuroleptics which were antagonized by naloxone that antagonism was not complete, suggesting that narcotic receptors are involved to a limited extent in the

antiwithdrawal effects of some neuroleptics. It is possible that neuroleptic binding with both dopamine and narcotic receptor sites mediates the reduction in withdrawal body shakes, with some neuroleptics having more of an effect on the dopamine receptor rather than narcotic receptors thus being less likely to be reversed by naloxone. Neuroleptic effects on other neurotransmitters and receptor sites in conjunction with narcotic receptors can not be totally discounted.

The data suggest neuroleptics reduce withdrawal body shakes by complex effects on several different mechanisms within the central nervous system. Dopaminergic, cholinergic and narcotic mechanisms, appear to be the most relevant.

BIBLIOGRAPHY

Adams, J.E.: Naloxone reversal of analgesia produced by brain stimulation in the human. Pain 2, 161-166, 1976.

Akera, T. and Brody, T.M.: The addiction cycle to narcotics in the rat and its relation to catecholamines. Biochem. Pharmacol. 17, 675-688, 1968.

Akil, H., Mayer, D.J. and Liebeskind, J.C.: Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. Science 191, 961-962, 1976.

Anden, N.E., Carlson, A., Dahlstrom, A., Fuxe, K., Hillarp, N.A. and Larsson, K.: Demonstration and mapping out of nigroneostriatal dopamine neurons. Life Sciences 3, 523-530, 1964.

Anden, N.E., Corrodi, H., Fuxe, F., Hokfelt, B., Hokfelt, T., Rydin, C., Svensson, T.: Evidence for a central noradrenaline receptor stimulation by clonidine. Life Sci. 9, 513-523, 1970.

Anden, N.E., Magnusson, T. and Stock, G.: Effect of drugs influencing monoamine mechanisms on the increase in brain dopamine produced by axatomy treatment with gamma hydroxybutyric acid. Nauyn. Schiedeberg's Archives. Pharmac. 278, 363-372, 1973.

Anden, N.E., Rubenson, A., Fuxe, K. and Hokfelt, T.: Evidence for dopamine receptor stimulation by apomorphine. J. Pharm. Pharmacol. 19, 627-629, 1967.

Arvay, A., Lampe, L., Kertesz, L. and Medveczky, L.: Changes of thyroid function in response to severe nervous stimulation. Acta Endocrinol. (Copenh.) 35, 469-480, 1960.

Barraclough, C.A. and Sawyer, C.H.: Inhibition of the release of pituitary ovulatory hormone in the rat by morphine. Endocrinology 57, 329-337, 1955.

Bell, J.A. and Martin, W.R.: The effect of the narcotic antagonists naloxone, naltrexone and nalorphine on spinal cord fiber reflexes evoked by electrical stimulation or radiant heat. European J. Pharmacol. 42, 147-154, 1977.

Belluzzi, J.D., Grant, N., Garsky, V., Sarantakis, D., Wise, C.D., and Stein, L.: Analgesia induced in vivo by central administration of enkephalin in rat. Nature 260, 625-626, 1976.

Belluzzi, J.D. and Stein, L.: Enkephalin may mediate euphoria and drive-reduction reward. Nature 266, 556-558, 1977.

Ben-David, M., Danon, A. and Sulman, F.G.: Acute changes in blood and pituitary prolactin after a single injection of perphenozine. Neuroendocrinology 6, 336-342, 1970.

Berenyi, K.J., Sakoya, I. and Snow, J.C.: Innovar-nitrous oxide anesthesia in oto-laryngology. Laryngoscope 76, 772-777, 1966.

Berkowitz, B.A., Ngai, S.H. and Finck, A.D.: Nitrous oxide "analgesia": Resemblance to opiate-action. Science 194, 967-968, 1976.

Berkowitz, B.A., Ngai, S.H., Hempstead, J. and Spector, S.: Disposition of naloxone: Use of a new radioimmunoassay. Europ. J. Pharmacol. 36, 259-262, 1976.

Bhargava, H.N.: Inhibition of naloxone-induced withdrawal in morphine dependent mice by 1-trans-$\Delta^{1,9}$-tetrahydrocannabinol. Europ. J. Pharmacol. 36, 259-262, 1976.

Bhargava, H.N.: Opiate-like action of methionine-enkephalin in inhibiting morphine abstinence syndrome. European J. Pharmacol. 41, 81-84, 1977.

Bhargava, H.N. and Way, E.L.: Acetylcholinesterase inhibition and morphine effects in morphine tolerant and dependent mice. J. Pharmacol. Exp. Ther. 183, 31-40, 1972.

Bhargava, H.N. and Way, E.L.: Brain acetylcholine and choline following acute and chronic morphinization and during withdrawal in mice and rats. J. Pharmacol. Exp. Therap. 194, 65, 1975.

Bhargava, H.N. and Way, E.L.: Morphine tolerance and physical dependence: Influence of cholinergic agonists and antanogists. European J. of Pharmacology 36, 79-88, 1976.

Black, W.C. and Grosz, H.J.: Propranolol antagonism of morphine-influenced behavior. Brain Res. 65, 362-367, 1974.

Blasig, J., Herz, A., Reinhold, K. and Zieglgansberger, S.: Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats. Psychopharmacologia 33, 19-38, 1973.

Blasig, J., Hollt, V., Herz, A. and Paschelke, G.: Comparison of withdrawal precipitating properties of various morphine antagonists and partial agonists in relation to their stereospecific binding to brain homogenates. Psychopharmacoloiga (Berl.) 46, 41-51, 1976.

Bloom, F., Segal, D., Ling, N., and Guillemin, R.: Endorphins: Profound behavioral effects in rats suggest new etiological factors in mental illness. Science 194, 630-632, 1976.

Blumberg, H. and Dayton, H.B.: Naloxone and related compounds. In: Agonist and Antagonist Actions of Narcotic Analgesic Drugs, ed. by H.W. Kosterlitz, H.O.J. Collier and J.E. Villarreal, pp. 110-119. University Park Press, Baltimore: 1973.

Blumberg, H., Dayton, H.B., George, M. and Rapaport, D.N.: N-allylnoroxymorphine: a potent narcotic antagonist. Fed. Proc. 20, 311, 1961.

Blumberg, H., Dayton, H.B. and Wolf, P.S.: Narcotic antagonist activity of naloxone. Fed. Proc. 24, 676, 1965.

Blumberg, H., Dayton, H.B. and Wolf, P.S.: Counteraction of narcotic antagonist analgesics by the narcotic antagonist naloxone. Proc. Soc. Exp. Biol. Med. 123, 755-758, 1966.

Boshka, S.C., Weisman, H.M. and Thor, D.H.: A technique for inducing aggression in rats utilizing morphine withdrawal. Psychol. Rec. 16, 541-543, 1966.

Bradbury, A.F., Feldberg, W.F., Smyth, D.G. and Snell, C.R.: Lipotropin C-fragment: An endogenous peptide with potent analgesic activity. In: Opiates and Endogenous Opioid Peptides, ed. by H.W. Kosterlitz, pp. 9-16. North Holland, Amsterdam, 1976.

Brase, D.A., Tseng, L.F., Loh, H.H. and Way, E.L.: Cholinergic modification of naloxone-induced jumping in morphine dependent mice. European J. Pharmacol. 26, 1-8, 1974.

Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., River, J. and Guillemin, R.: Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179, 77-79, 1973.

Briggs, F.N. and Munson, P.L.: Studies on the mechanism of stimulation of ACTH secretion with the aid of morphine as a blocking agent. Endocrinology 57, 205-219, 1955.

Brown-Grant, K., Harris, G.W. and Reichlin, S.: The effect of emotional and physical stress on thyroid activity in the rabbit. J. Physiol. (London) 126, 29-40, 1954.

Buckett, W.R.: A new test for morphine-like physical dependence (addiction liability) in rats. Psychopharmacologia 6, 410-416, 1964.

Burdette, B.H., Leeman, S. and Munson, P.L.: The reversal by nalorphine of the inhibitory effect of morphine on the secretion of adrenocorticotrophic hormones in stress. J. Pharmacol. 132, 323-328, 1961.

Byck, R.: Drugs and the treatment of psychiatric disorders. In: The Pharmacological Basis of Therapeutics. ed. by L. Goodman and A. Gilman, pp. 152-200, Macmillan Publishing Co., New York, 1975.

Cameron, F.M.: Neurolept-analgesia for cataract surgery. Brit. J. Anaesth. 39, 605-607, 1967.

Carlsson, A.: Basic concepts underlying recent development in the field of Parkinsons Disease. In: Recent Advances in Parkinsons Disease. ed. E. McDowell and J. Markam, pp. 1-31, Davis, Philadelphia, 1971.

Carr, L.A. and Moore, K.E.: Effects of amphetamine on the contents of norepinephrine and its metabolites in the effluent of perfused cerebral ventricles of the cat. Biochem. Pharmacol. 19, 2361-2374, 1970.

Charalampous, K.D. and Askew, W.E.: Effect of non-opiate antagonists on stereospecific opiate receptor binding of tritiated naloxone. Res. Comm. Chem. Path. Pharmacol. 8, 615-622, 1974.

Cheney, D.L. and Goldstein, A.: The effect of p-chlorophenylalanine on opiate-induced running, analgesia, tolerance and physical dependence in mice. J. Pharmac. Exp. Ther. 177, 309-315, 1971.

Chiosa, L., Dumitrascu, S. and Banaru, A.: The influence of imipramine on the "abstinence syndrome" to morphine in mice. Intern. J. Neuropharmacol. 7, 161, 1968.

Cicero, T.J. and Meyer, E.R.: Morphine pellet implantation in rats: Quantitative assessment of tolerance and dependence. J. Pharmacol. Exp. Ther. 184, 404-408, 1973.

Cicero, T.J., Meyer, E.R. and Bell, R.D.: Effects of phenoxybenzamine on the narcotic withdrawal syndrome in the rat. Neuropharmacology 13, 601-607, 1974.

Cicero, T.J., Wilcox, L.E., Meyer, E.R. and Michael, H.: Influence of catecholaminergic agents on narcotic binding in brain. Arch. Int. Pharmacodyn. 218, 221-230, 1975.

Clay, G.A. and Brougham, L.R.: Haloperidol binding to an opiate receptor site. Biochem. Pharmacol. 24, 1363-1367, 1975.

Clouet, D.H. and Iwatsubo, K.: Opiates and the brain adenylate cyclase system. In: Tissue Responses to Addictive Drugs, ed. by D.H. Ford and D.H. Clouet, pp. 297-313, Spectrum Publications, New York, 1976.

Clouet, D.H., Gold, G.J. and Iwatsubo, K.: Effects of narcotic analgesics on the cyclic AMP-adrenylate cyclase system in rat brain. Brit. J. Pharmacol. 54, 541-548, 1975.

Colasanti, B.K., Kosa, J.E. and Craig, C.R.: Appearance of wet dog shake behavior during cobalt experimental epilepsy in the rat and its suppression by reserpine. Psychopharmacologia 44, 33-36, 1975.

Collier, H.O.J.: A general theory of the genesis of drug dependence by induction of receptors. Nature 205 , 181-182, 1965.

Collier, H.O.J. and Francis, D.L.: Morphine abstinence is associated with increased brain cyclic AMP. Nature 255, 159-162, 1975.

Collier, H.O.J., Francis, D.L., and Schneider, C.: Modification of morphine withdrawal by drugs interacting with humoral mechanisms: Some contradictions and their interpretation. Nature (London) 237, 220-223, 1972.

Collu, R., Fraschini, F. and Martini, L.: Role of indoleamines and catecholamines in the control of gonadotrophin and growth hormone secretion. Progr. Brain Res. 39, 289-299, 1973.

Colpaert, F.C., Wauquier, A., Niemegeers, C. and Lal, H.: Reversal by a central antiacetylcholine drug of pimozide-induced inhibition of mouse jumping in amphetamine-DOPA treated mice. J. Pharma. Pharmac. 27, 536-537, 1975.

Cools, A.R.: The trans-synaptic relationship between dopamine and serotonin in the caudate nucleus of cats. Psychopharmacologia 36, 17-28, 1974.

Coppen, J.E. and Fox, J.W.: Endobronchial intubation under neuroleptanalgesia for a patient with severe haemotptysis. Anesth. Anal. Curr. Res. 47, 70-73, 1968.

Corssen, G., Chodoff, P., Domino, E.F. and Kehn, D.R.: Neuroleptanalgesia and anesthesia for open heart surgery. J. Thorac. Cardiovas. Surg. 49, 901-909, 1956.

Corssen, G., Domino, E.F. and Sweet, R.B.: Neuroleptanalgesia and anesthesia. Anesth. Anal. Curr. Res. 43, 748-752, 1964.

Costall, B. and Naylor, R.J.: The role of telencephalic dopaminergic systems in the mediation of apomorphine stereotyped behavior. Europ. J. Pharmacol. 24, 8-24, 1973.

Coupet, J., Szucs, V.A. and Greenblatt, E.N.: The effect of 2-chloro-11-(4-methyl-1-piperazinyl)-dibenz b,f 1,4 oxazepine (loxapine) and its derivatives on the dopamine-sensitive adenylate cyclase of rat striatal homogenates. Brain Res. 116, 177-180, 1976.

Coussens, W.R., Crowder, W.F. and Smith, S.G. Acute physical dependence upon morphine in rats. Behavioral Biol. 8, 533-543, 1973.

Cox, B., Aey, M. and Lomax, P.: Dopaminergic mechanism in withdrawal hypothermia in morphine dependent rats. Life Sci. 17, 41-42, 1975.

Cranston, E.M. and Segal, M.S.: Effect of tranquilizer and other agents on sexual cycle of mice. Proc. Soc. Exp. Biol. Med. 98, 320-327, 1958.

Creese, I., Feinberg, P.A. and Snyder, S.H.: Butyrophenone influences on the opiate receptor. Eur. J. Pharmacol. 36, 231-235, 1976.

Creese, I. and Snyder, S.H.: Receptor binding and pharmacological activity of opiates in the guinea pig intestine. J. Pharmacol. Exp. Ther. 194, 205-219, 1975.

Cushman, P., Jr., Bordier, B. and Hilton, J.G.: Hypothalamic pituitary adrenal axis in methadone-treated heroin addicts. J. Clin. Endocr. 30, 24-28, 1970.

Cushman, P., Jr., and Kreek, M.J.: Some endocrinologic observations in narcotic addicts. In: Narcotics and the Hypothalamus ed. by E. Zimmermann and R. George, pp. 161-172, Raven Press, New York, 1974.

Davis, W.M. and Smith, S.G.: Central cholinergic influence on self-administration of morphine and amphetamine. Life Sci. 16, 237-246, 1975.

DeBodo, R.C.: The antidiuretic action of morphine and its mechanism. J. Pharmacol. Exp. Therap. 82, 74-85, 1944.

De Boer, T., Metselaar, H.J. and Bruinvels, J.: Suppression of GABA-induced abstinence behavior in naive rats by morphine and bicuculline. Life Sci. 20, 933-942, 1977.

De Castro, J. and Mundeleer, P.: Anesthesie sans barbituriques La neuroleptanalgesie·Anesthesie Analgesie. Reanimation 16, 1022-1056, 1959.

Deligne, P.: Nouveaux neuroleptiques dans differents types d'anesthesie sans narcose ou d'anesthesie vigile. Agressologie 2, 363-370, 1961.

Deneau, G.A. and Seevers, M.H.: Evaluation of morphine-like physical dependence in the rhesus monkey (Macaca mulatta). Rep. Committee on Drug Addiction and Narcotics 25th Meeting. Addendum 1, 1-25, 1963.

Deuben, R.R. and Meites, J.: Stimulation of pituitary growth hormone release by hypothalamic extract in vitro. Endocrinology 74, 408-412, 1964.

De Wied, D.: Chlorpromazine and endocrine function. Pharmacol. Rev. 19, 251-288, 1967.

De Wied, D. and De Jong, W.: Drug effects and hypothalamic-anterior pituitary function. In: Annual Review of Pharmacology ed. by H.W. Elliott, R. Okun, R. George, pp. 389-412, Annual Reviews Inc., Palo Alto, 1974.

De Wied, D. and Jinks, R.: Effect of chlorpromazine on antidiuretic response to noxious stimuli. Proc. Soc. Exp. Biol. Med. 99, 44-45, 1958.

De Wied, D., van Ree, J.M. and De Jong, W.: Narcotic analgesics and the neuroendocrine control of anterior pituitary function. In: Narcotics and the Hypothalamus ed. by E. Zimmerman and R. George. pp. 251-264; Raven Press, New York, 1974.

Dickerman, S., Clark, J., Dickerman, E. and Meites, J: Effects of haloperidol on serum and pituitary prolactin and on hypothalamic PIF in rats. Neuroendocrinology 9, 332-340, 1972.

Dufficy, R.G.: Use of psychotherapeutic drugs in the acute detoxification of heroin addicts. Military Med. 138, 748-750, 1973.

Duke, H.N., Pickford, M., and Watt, J.A. The antidiuretic action of morphine: Its site and mode of action in the hypothalamus of the dog. Quart. J. Exp. Physiol. 36, 149-158, 1951.

Edmonds-Seal, J. and Prys-Roberts, C.: Pharmacology of drugs used in neurolept-analgesia. Brit. J. Anaesth. 42, 207-216, 1970.

Eidelberg, E. and Barstow, C.A.: Morphine tolerance and dependence induced by intraventricular injection. Science 174, 74-76, 1971.

Eidelberg, E. and Schwartz, A.S.: Possible mechanism of action of morphine on brain. Nature 225, 1152-1153, 1970.

Eisenman, A.J., Fraser, H.F. and Brooks, J.W.: Urinary execution and plasma levels of 17-hydroxy-corticosteroids during a cycle of addiction to morphine. J. Pharmacol. 132, 226-231, 1961.

Eisenman, A.J., Fraser, H.F., Sloan, J. and Isbell, H.: Urinary 17-ketosteroid excretion during a cycle of addiction to morphine. J. Pharmacol. Exp. Ther. 124, 305-311, 1958.

El-Sobky, A., Dostrovsky, J.O., and Wall, P.D.: Lack of effect of naloxone on pain perception in humans. Nature 263, 783-784, 1976.

Ernst, A.M.: Mode of action of apomorphine and dextroamphetamine on gnawing compulsion of rats. Psychopharmacologia (Berl.) 10, 316-323, 1967.

Fennessy, M.R. and Lee, J.R.: Modification of morphine analgesia by drugs affecting adrenergic and tryptaminergic mechanism. J. Pharm. Pharmac. 22, 930-935, 1970.

Fielding, S., Wilker, J., Hynes, M., Szewczak, M., Novick, W. and Lal, H.: Antinociceptive and antiwithdrawal actions of clonidine: A comparison with morphine. Fed. Proc. 36, 1024, 1977.

Florea, J. and Thor, D.H.: Drug withdrawal and fighting in rats. Psychon. Sci. 12, 33, 1968.

Foldes, F.F., Lunn, J.N., Moore, J. and Brown, I.M.: N-allylnoroxymorphone: A new potent narcotic antagonist. Am. J. Med. Sci. 245, 23-30, 1963.

Foldes, J., Nagy, J., Kertai, P. and Takacs, E.: Effect of chlorpromazine on thyroid activity. Acta Med. Acad. Sci. Hung. 14, 371-378, 1959.

Fortier, C.: Nervous control of ACTH secretion. In: The Pituitary Gland, Vol II, ed. by G.W. Harris and B.T. Donovan, pp. 195-234, Butterworth, London, 1966.

Forrest, W.H., Brown, B. Wm., Brown, C.R., Defalque, R., Gold, M., Gordon, H.E., James, K.E., Katz, J., Mahler, D.L., Schroff, P., and Teutsch, G.: Dextroamphetamine with morphine for the treatment of postoperative pain. N. Engl. J. Med. 296, 712-715, 1977.

Frederickson, R.C.A. and Pinsky, C.: Effects of cholinergic and anticholinergic drugs and a partial cholinergic agonist on the development and expression of physical dependence on morphine in rat. J. Pharmacol. Exp. Ther. 193: 44-55, 1975.

French, E.D., Vasquez, S.A., and George, R.: Potentiation of morphine hyperthermia in cats by pimozide and fluoxetine, hydrochloride (Lilly 110140). Fed. Proc. 35, 263, 1976.

Fujimoto, J.M.: The kidney. In: Narcotic Drugs: Biochemical Pharmacology, edited by D.H. Couet, pp. 366-393, Plenum Press, New York, 1971.

Galindo, A.: GABA-picrotoxin interaction in the mammalian central nervous system. Brain Res. 14, 763-767, 1969.

Ganong, W.F.: Evidence for a central noradrenergic system that inhibits ACTH secretion. In: Brain-Endocrine Interaction, ed. by K.M. Knigge, D.E. Scott, and A. Weindle, pp. 254-266, Karger, Basel, 1972.

Garcia, D.A., Wrenn, C.E., Jansons, D. and Marie, K.E.: Deep anesthesia in the rat with the combined action of droperidol-fentanyl and pentobarbital. Lab. Anim. Sci. 25, 585-587, 1975.

Gardocki, J. and Yelnosky, J.: A study of some of the pharmacologic actions of fentanyl citrate. Toxicol. Appl. Pharmacol. 6, 48-62, 1964.

Gaunt, P., Chart, J.J. and Renzi, A.A.: Interactions of drugs with endocrines. Annu. Rev. Pharmacol. 3, 109-128, 1963.

George, R.: Hypothalamus: Anterior pituitary gland. In: Narcotic Drugs, Biochemical Pharmacology ed. by D.H. Clouet, pp. 283-299. Plenum Press, New York, 1971.

George, R. and Lomax, P.: The effect of morphine, chlorpromazine and reserpine on pituitary-thyroid activity in rats. J. Pharmacol. Exp. Therap. 150, 129-134, 1965.

George, R. and Lomax, P.: Hormones. In: Chemical and Biological Aspects of Drug Dependence. ed. by S.J. Mule and H. Brill, pp. 523-543. CRC Press, Cleveland, Ohio, 1972.

George, R. and Way, E.L.: Studies on the mechanism of pituitary adrenal activation by morphine. Brit. J. Pharmacol. 10, 260-264, 1955.

George, R. and Way, E.L.: The role of the hypothalamus in pituitary-adrenal activation and antidiuresis by morphine. J. Pharmacol. Exp. Therap. 125, 111-115, 1959.

Gianutsos, G., Drawbaugh, R., Hynes, M.D. and Lal, H.: The narcotic withdrawal syndrome in the rat. In: Methods in Narcotic Research, Eds. S. Ehrenpris and A. Neidle, pp. 293-309, Marcel Dekker, Inc., New York, 1975a.

Gianutsos, G., Hynes, M.D., Drawbaugh, R.B., and Lal, H.: Morphine-withdrawal aggression during protracted abstinence. Role of latent dopaminergic supersensitivity. Pharmacologist 15, 218, 1973.

Gianutsos, G., Hynes, M.D., Drawbaugh, R.B.,and Lal, H.: Paradoxical absence of aggression during naloxone precipitated morphine withdrawal. Psychopharmacologic (Berl.) 43, 43-46, 1975b.

Gianutsos, G., Hynes, M.D., Puri, S.K., Drawbaugh, R.B. and Lal, H.: Effect of apomorphine and nigrostriatal lesions on aggression and striatal dopamine turnover during morphine withdrawal. Evidence for dopaminergic supersensitivity in protracted abstinence. Psychopharmacologia (Berl.) 34, 37-44, 1974.

Gibson, R.D. and Tingstad, J.E.: Formulation of a morphine implantation pellet suitable for tolerance-physical dependence studies in mice. J. Pharma. Sci. 59, 526-427, 1970.

Glass, L.: Narcotic withdrawal in the newborn infant. Nat. Med. Assoc. 66, 117-120, 1974.

Goldstein, A.: Opioid peptides (Endorphins) in pituitary and brain. Science 193, 1081-1086, 1976.

Goldstein, A., Lowney, K.I., and Pal, B.K.: Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain. Proc. Natl. Acad. Sci. U.S.A. 68, 1742-1747, 1971.

Goldstein, A., Pryor, G.T., Otis, L.S. and Larsen, F.: On the role of endogenous opioid peptides: Failure of naloxone to influence shock escape threshold in the rat. Life Sci. 18, 599-604, 1976.

Goldstein, A. and Schultz, R.: Morphine tolerant longitudinal muscle strip from guinea pig ileum. Brit. J. Pharmacol. 48, 655-666, 1973.

Greengard, P. and Kebbian, J.W.: Role of cyclic AMP in synaptic transmission in the mammalian peripheral nervous system. Fed. Proc. 37, 1059-1067, 1974.

Greer, M.A.: THe role of the hypothalamus in the control of thyroid function. J. Clin. Endocr. 12, 1259-1268, 1952.

Grosz, H.J.: Narcotic withdrawal symptoms in heroin users treated with propranolol. Lancet 2, 564-566, 1972.

Grumbach, L.: The effect of cholinergic and cholinergic blocking drugs on the abstinence syndrome in the rat. Committee on Problems of Drug Dependence, National Research Council pp. 5773-5785, 1969.

Habif, D.V., Papper, E.M., Fitzpatrick, H.F., Lawrence, P., Smythe, C. McC., and Bradley, S.F.: The renal and hepatic blood flow glomerular filtration rate and urinary output of electrolytes during cyclopropane, ether, and thiopental anesthesia, operation and immediate postoperative period. Surgery 30, 241-255, 1951.

Haggendal, J. and Dahlstrom, A.: The recovery of the capacity for uptake-retention of ^{3}H noradrenaline in rat adrenergic nerves after reserpine. J. Pharm. Pharmacol. 24, 565-574, 1972.

Handley, C.A., and Keller, A.D.: Changes in renal functions produced by morphine in normal dogs and dogs with diabetes insipidus. J. Pharmacol. Exp. Therap. 99, 33-37, 1950.

Hanson, H.M. and Cimini, Venema, C.A.: Effects of haloperidol on self-administration of morphine in rats. Fed. Proc. 31, 503, 1972.

Harper, M.H., Hickey, R.F., Cromwell, T.H. and Linwood, S.: The magnitude and duration of respiratory depression produced by fentanyl and fentanyl plus droperidol in man. J. Pharmacol. Exp. Ther. 199, 464-468, 1976.

Harris, L.S.: Interaction of narcotic antagonists. In: Interactions of Drugs of Abuse ed. by E.S. Vesell and M.C. Braude, pp. 288-296, New York Academy of Science, New York, 1976.

Harris, G.W. and Woods, J.W.: The effect of electrical stimulation of the hypothalamus or pituitary gland on thyroid activity. J. Physiol. (London) 143, 246-274, 1958.

Hayward, J.N.: Effect of drugs of abuse on motivated behavior and magnocellular neuroendocrine cells. In: Narcotics and the Hypothalamus ed. by E. Zimmerman and R. George, pp. 83-92, Raven Press, New York, 1974.

Hayward-Butt, J.T.: Ataralgesia: Operations without anesthesia. Lancet 273, 972-974, 1957.

Herz, A.: Significance of catecholamines in opiate dependence and withdrawal. In: Opiate Receptor Mechanisms eds. S.H. Snyder and S. Matthysse,pp 118-123, MIT Press, Cambridge, 1975.

Herz, A., Blasig, J. and Papeschi, R.: Role of catecholaminergic mechanisms in the expression of the morphine abstinence syndrome in rats. Psychopharmacologia 39, 121-143, 1974.

Herz, A., Teschemacher, H., Albus, K. and Zieglgandsberg, S.: Morphine abstinence syndrome in rabbits precipitated by injection of morphine antagonists into the ventricular system and restricted parts of it. Psychopharmacologia (Berl.) 26, 219-235, 1972.

Hill, R.G., Pepper, C.M. and Mitchell, J.F.: Depression of noriceptive and other neurones in the brain by iontophoretically applied met-enkephalin. Nature 262, 604-606, 1976.

Hiller, J.M., Pearson, J. and Simon, E.J.: Distribution of stereospecific binding of the potent narcotic analgesic etorphine in the human brain: Predominance in the limbic system. Res. Commun. Chem. Pathol. Pharmacol. 6, 1052-1062, 1973.

Himmelsbach, C.K.: Studies on relation of drug addiction to autonomic nervous system: results of tests of peripheral blood flow. J. Pharmacol. Exp. Therap. 80, 343, 1944.

Himmelsbach, C.K., Gerlach, G.H. and Stanton, E.J.: A method for testing addiction, tolerance and abstinence in the rat. Results of its application to several morphine alkaloids. J. Pharmacol. Exp. Ther. 53, 179-188, 1935.

Hine, B., Friedman, E., Torrelio, M. and Gershon, S.: Morphine dependent rats: Blockade of precipitated abstinence by tetrahydrocannabinol. Science 187, 1443-445, 1975.

Hitzeman, R.J., Hitzeman, B.A. and Loh, H.H.: Binding of ^{3}H-naloxone in the mouse brain: effect of ions and tolerance development. Life Sci. 14, 2393-2404, 1974.

Ho, I.K., Loh, H.H., and Way, E.L.: Effect of cAMP on morphine analgesia, tolerance and dependence. Nature 238, 397-398, 1972.

Ho, I.K., Loh, H.H. and Way, E.L.: Cyclic adenosine monophosphate antagonism of morpine analgesia. J. Pharmacol. Exp. Therap. 185, 336-346, 1973.

Ho, I.K., Lu, S.E.,Stolman, S., Loh, H.H. and Way, E.L.: Influence of p-chloro-phenylalanine on morphine tolerance and physical dependence and regional brain serotonin turnover studies in morphine tolerant dependent mice. J. Pharm. Exp. Ther. 182, 155-165, 1972.

Hohlweg, V.W., Knoppe, G. and Dorner, G.: Tierexperimentelle untersuchungen uber den einfluss von morphin auf die gonadotrope und thyretrope hypophysenfunktion. Endokr. Bd. 40, 152-159, 1961.

Holderness, M.C., Chase, P.E., and Dripps, R.D.: Use of a narcotic analgesic and a butyrophenone with nitrous oxide for general anesthesia. Anesthesiology 24, 336-340, 1963.

Hollister, L.E.: Human pharmacology of drugs of abuse with emphasis on neuroendocrine effects. Progr. Brain Res. 39, 373-381, 1973.

Holtzman, S.G.: Behavioral effects of separate and combined administration of naloxone and d-amphetamine. J. Pharmacol. Exp. Ther. 189, 51-60, 1974.

Holtzman, S.G. and Jewett, R.E.: Stimulation of behavior in the rat by cyclazoline: Effects of naloxone. J. Pharmacol. Exp. Ther. 187, 380-390, 1973.

Howard, N. and Martin, J.M.: A stimulatory test for growth hormone release in the rat. Endocrinology 88, 497-500, 1971.

Hughes, J.: Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. Brain Res. 88, 295-308, 1975.

Hughes, J., Smith, T.W., Kosterlitz, H.W., Fothergill, L.A., Morgan, B.A. and Morris, H.R.: Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature 258, 577-579, 1975.

Huidobro, F., Contreras, E. and Croxatto, R.: Studies on morphine III. Action of metabolic precursors to serotonin and noradrenaline and related substances in the abstinence syndrome to morphine in white mice. Archs Int. Pharmacodyn. Ther. 146, 444-454, 1963.

Hynes, M.D.: Interaction between nigrostriatal lesions and drugs affecting dopamine receptors. Unpublished Master's Thesis, University of Rhode Island, 1975.

Hynes, M.D., Gianutsos, G. and Lal, H.: Effects of cholinergic agonists and antagonists on morphine-withdrawal syndrome. Psychopharmacology 49, 191-195, 1976.

Iwamoto, E.T., Ho, I.K., and Way, E.L.: Elevation of brain dopamine during naloxone precipitated withdrawal in morphine-dependent mice and rats. J. Pharmacol. Exp. Therap. 187, 558-565 , 1973.

Iwamoto, E.T., Shen, F.H., Loh, H.H. and Way, E.L.: The effects of pargyline on morphine tolerant-dependent mice. Fed. Proc. 30, 278, 1971.

Iwatsubo, K. and Clouet, D.H.: Dopamine-sensitive adenylate cyclase of the caudate nucleus of rats treated with morphine or haloperidol. Biochem. Pharmacol. 24, 1499-1503, 1975.

Jacob, R.G., Holmstrand, J., Eriksson, J.H. and Anggard, E.: Propranolol in the treatment of opiate dependence - A controlled study. Psychopharmacologia (Berl.) 41, 71-73, 1975.

Jacob, J.J., Tremblay, E.C. and Colombel, M.C.: Facilitation de reactions nociceptives par la naloxone chez la souris et chex le rat. Psychopharmacologia 37, 217-222, 1974.

Jacquet, Y.F. and Marks, N.: The C-fragment of B - lipotropin: An endogeneous neuroleptic or antipsychotogenesis. Science 194, 632-634, 1976.

Jaffe, J.H. and Martin, W.R.: Narcotic analgesics and antagonists. In: The Pharmacological Basis of Therapeutics, 5th ed. (L.S. Goodman and A. Gilman eds.) pp.245-284, Macmillan Publishing Co., Inc., New York, 1975.

Jahn, U. and Mixich, G.: Benzylidenaminooxy-carbonsaure-derivate als ausloser von "wet dog shake"-verhalten bei normalen ratten. Psychopharmacologia 46, 191-196, 1976.

Janssen, P.A.J.: The evolution of the butyrophenones, haloperidol and trifluperidol, from meperidine-like 4-phenylpiperidines. Inter. Rev. Neurobiol. 8, 221, 1965.

Janssen, P.A.J.: The pharmacology of haloperidol. Int. J. Neuropsychiat. 3 Supp., 10-17, 1967.

Janssen, P.A.J., Niemegeers, C.J.E., and Dony, J.G.H.: The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneimittel-Forsch. 13, 502-507, 1963a.

Janssen, P.A., Niemegeers, C.J., Schellekins, K.H., Verbruggen, F.J. and Van Neuten, J.M.: The pharmacology of dehydrobenzperidol (R-4749), a new potent and short-acting neuroleptic agent chemically related to haloperidol. Arzneimittel-Forsch 13, 205-211, 1963b.

Janssen, P.A.J. and Van Bever, W.F.M.: Advances in the search for improved neuroleptic drugs. In: Current Developments in Psychopharmacology. Vol 2 ed. by W.B. Essman and L. Valzelli, pp. 167-184, Spectrum, New York, 1975.

Jasinski, D.R., Martin, W.R., and Haertzen, C.A.: The human pharmacology and abuse potential of N-allylnoroxymorphone (Naloxone). J. Pharmac. Exp. Ther. 157, 420-426, 1967.

Jasinski, D.R., Martin, W.R. and Sapira, J.D.: Antagonism of the subjective, behavioral, pupillary and respiratory depressant effects of cyclazocine by naloxone. Clin. Pharmac. Ther. 9, 215-222, 1968.

Jhamandas, K. and Dickinson, G.: Modification of precipitated morphine and methadone abstinence in mice by acetylcholine antagonists. Nature Neuro. Biol. 245, 219-221, 1973.

Jones, J.B. and Simmons, M.L.: Innovar-Vet as an intramuscular anesthetic for rats. Lab. Animal Care 18, 642-643, 1968.

Kallos, T. and Smith, T.C.: Naloxone reversal of pentazocine-induced respiratory depression. J. Am. Med. Assn. 204, 932, 1968.

Kamberi, I.A.: The role of brain monoamines and pineal indoles in the secretion of gonadotrophins and gonodotropin-releasing factors. Progr. Brain. Res. 39, 261-278, 1973.

Kandall, S.R.: Managing neonatal withdrawal. Drug. Ther. 6, 117-125, 1976.

Karkalas, J. and Lal, H.: A comparison of haloperidol with methadone in blocking heroin-withdrawal symptoms. Int. Pharmacopsychiat. 8, 248-251, 1973.

Khavari, K. and Risner, M.: Establishment of morphine preference in the rat. Psychon. Sci. 26, 141-142, 1972.

Khavari, K.A. and Risner, M.E.: Concentration-ingestion relations of Morphine-adulterated food and morphine solution. Psychopharmacologia (Berl.) 30, 45-60, 1973.

Khazan, N. and Colasanti, B.: EEG correlates of morphine challenge in post-addict rats. Psychopharmacologia 22, 56-63, 1971.

Khazan, N., Weeks, J.R. and Schroeder, L.A.: Electro-encephalographic electro-myographic and behavioral correlates during a cycle of self-maintained morphine addiction in the rat. J. Pharmacol. Exp. Ther. 155, 521-531, 1967.

Klee, W.A. and Streaty, R.A.: Narcotic receptor sites in morphine-dependent rats. Nature 248, 61-63, 1974.

Koe, B.K. and Weissman, A.: p-Chlorophenylalanine: A specific depletor of brain serotonin. J. Pharmac. Exp. Ther. 154, 499-516, 1966.

Kokka, N., Garcia, J.F. and Elliott, H.W.: Effects of acute and chronic administration of narcotic analgesics on growth hormone and corticotrophin (ACTH) secretion in rats. Prog. Brain Res., 39, 347-360, 1973.

Kokka, N., Garcia, J.F., George, R., and Elliott, H.W.: Growth hormone and ACTH secretion: Evidence for an inverse relationship in rats. Endocrinology 90, 735-743, 1972.

Kokka, N. and George, R.: Effects of narcotic analgesics, anesthetics, and hypothalamic lesions on growth hormone and adrenocorticotropine hormone secretion in rats. In: Narcotics and the Hypothalamus, ed by E. Zimmerman and R. George, pp. 137-157, Raven Press, New York, 1974.

Ksycki, S.M.J. and Lockett, S.M.C.: Effects of chlorpromazine on radioiodine uptake and metabolism in C-57 brown mice. Life Sci. 4, 1877-1886, 1965.

Kuhar, M.J., Pert, C.B. and Snyder, S.H.: Regional distribution of opiate receptor binding in monkey and human brain. Nature 245, 447-450, 1973.

Ladinsky, H., Consolo, S., Bianchi, S. and Jori, A.: Increase in striatal acetylcholine by picrotoxin in the rat: Evidence for a gabergic-dopaminergic-cholinergic link. Brain Res. 108, 351-361, 1976.

Lal, H.: Morphine withdrawal aggression. In: Methods in Narcotic Research, Eds. S. Ehrenpris and A. Neidle 149-171, Marcel Dekker, New York, 1975a.

Lal, H.: Narcotic dependence, narcotic action and dopamine receptors. Life Sci. 17, 483-495, 1975b.

Lal, H., Brown, G., Drawbaugh, R., Hynes, M.D. and Brown, C.: Enhanced prolactin inhibition following chronic treatment with haloperidol and morphine. Life Sci. 20, 101-106, 1977.

Lal, H., Colpaert, F.C. and Laduron, P.: Narcotic-withdrawal like mouse jumping produced by amphetamine and L-DOPA. Eur. J. Pharmac. 30, 113-116, 1975a.

Lal, H., Gianutsos, G. and Puri, S.K.: A comparison of narcotic analgesics with neuroleptics on behavioral measures of dopaminergic activity. Life Sci. 17, 29-34, 1975b.

Lal, H. and Hynes, M.D.: Effectiveness of butyrophenones and related drugs in narcotic withdrawal. Proceedings of the 10th Congress of the Collegium Internationale Neuro-Psychopharmacologicum (in press), 1977.

Lal, H., Miksic, S., Drawbaugh, R., Numan, R. and Smith, N.: Alleviation of narcotic withdrawal syndrome by conditional stimuli. Pavl. J. Biol. Res. 11, 251-262, 1976a.

Lal, H. and Numan, R.: Blockade of Morphine-withdrawal body shakes by haloperidol. Life Sci. 18, 163-168, 1976.

Lal, H., O'Brien, J. and Puri, S.K.: Morphine-withdrawal aggression sensitization by amphetamines. Psychopharmacologia 22, 217-223, 1971a.

Lal, H., Puri, S.K. and Karkalas, Y.: Blockade of opioid-withdrawal symptoms by haloperidol in rats and humans. Pharmacologist 13, 263, 1971b.

Lal, H., Puri, S.K. and Volicer, L.: A comparison between narcotics and neuroleptics: Effects on dopamine turnover, cyclic AMP, adenylate-cyclose. In: Tissue Responses to Addictive Drugs, Eds. D. Ford and Clouet, D. pp. 187-207. Spectrum Publications, Inc., New York, 1976b.

Lal, H., Wauquier, A. and Niemegeers, C.J.E.: Characteristics of narcotic dependence by oral ingestion of fentanyl solution. Pharmacologist 17, 237, 1975c.

Laschka, E., Herz, A. and Blasig, J.: Sites of action of morphine involved in the development of physical dependence in rats. I. Comparison of precipitated morphine withdrawal after intraperitoneal and intraventricular injection of morphine antagonists. Psychopharmacologia (Berl.) 46, 133-139, 1976a.

Laschka, E., Teschemacher, Hj., Mehraein, P. and Herz, A.: Sites of action of morphine involved in the development of physical dependence in rats. II. Morphine withdrawal precipitated by application of morphine antagonists into restricted parts of the ventricular system and by microinjection into various brain areas. Psychopharmacology (Berl.) 46, 141-147, 1976b.

LeCompte, G. and Friedman, J.J.: Chemotherapy in the treatment of heroin addiction: An alternative to methadone. J. Drug Issues 4, 332-341, 1974.

Lewis, G.E. and Jennings, P.B.: Effective sedation of laboratory animals using Innovar-Vet. Lab. Animal Sci. 22, 430-432, 1972.

Leysen, J., Gommeren, W. and Laduron, P.: Discrimination of narcotic drugs on ^{3}H-fentanyl receptor binding. Archives internationales de Pharmacodynamic et de Therapie 220, 335-328, 1976.

Lloyd, K.G., Stadler, H. and Bartholini, G.: Dopamine and acetylcholine neurons in the striatal and limbic structures: Effect of neuroleptic drugs. In: Frontiers in Catecholamine Research ed by E. Usdin and S. Snyder, pp. 777-779, Pergamon Press, New York, 1973.

Loh, H.H., Shen, F. and Way, E.L.: Inhibition of morphine tolerance and physical dependence development and brain serotonin synthesis by cycloheximide. Biochem. Pharmac. 18, 2711-2721, 1969.

Lomax, P. and George, R.: Thyroid activity following administration of morphine in rats with hypothalamic lesions. Brain Res. 2, 361-367, 1966.

Lomax, P., Kokka, N. and George, R.: Thyroid activity following intracerebral injection of morphine in the rat. Neuroendocrinology 6, 146-152, 1970.

Lotti, V., Kokka, N., and George, R.: Pituitary-adrenal activation following intra-hypothalamic microinjection of morphine. Neuroendocrinology 4, 326-332, 1969.

Lotti, V.J., Lomax, P. and George, R.: Temperature responses in the rat following intracerebral microinjection of morphine. J. Pharmacol. 150, 135-139, 1965.

Lu, K.H., Amenomori, Y., Chen, C.L. and Meites, J.: Effects of central acting drugs on serum and pituitary prolactin levels in rats. Endocrinology 87, 667-670, 1970.

MacKay, E.M. and MacKay, L.L.: Resistance to morphine in experimental uremia. Proc. Soc. Exp. Biol. Med. 24, 129, 1926.

MacLeod, R.M.: Regulation of prolactin secretion. In: Frontiers in Neuroendocrinology, Vol 4, ed. by L. Martini and W.F. Ganong, pp. 169-194, Ravens Press, New York, 1976.

MacLeod, R.M. and Lehmeyer, J.E.: Regulation of the synthesis and release of prolactin. In: Lactogenic Hormones, ed. by G.E.W. Wolstenholme and J. Knight, pp. 53-82. Churchill Livingstone, London, 1972.

MacLeod, R.M. and Lehmeyer, J.E.: Pituitary gland alpha-adrenergic receptors and their function in prolactin secretion. Endocrinology 92: A-50, 1973.

MacLeod, R.M. and Lehmeyer, J.E.: Studies on the mechanism of dopamine-mediated inhibition of prolactin secretion. Endocrinology 94, 1077-1085, 1974.

Maggiolo, C. and Huidobro, F.: Administration of pellets of morphine to mice: abstinence syndrome. Acta Physiol. Latinoamer. 11, 70-78, 1961.

Marks, B.H., Hall, M.M. and Bhattaeharya, A.N.: Psychopharmacological effects and pituitary-adrenal activity. Prog. Brain Res. 32, 57-70, 1970.

Marshall, I. and Grahame-Smith, D.G.: Evidence against a role of brain 5-hydroxy-tryptamine in the development of physical dependence upon morphine in mice. J. Pharmacol. Exp. Ther. 179, 634-641, 1971.

Martin, W.R.: Pathophysiology of narcotic addiction: Possible roles of protracted abstinence in relapse. In Drug Abuse Proceedings of the International Conference ed by C.J.D. Zarafonetis, pp. 153-159. Lea and Febiger, Philadelphia, 1972.

Martin, W.R., Eades, C.G., Thompson, W.O., Thompson, J.A. and Flanary, H.G.: Morphine physical dependence in the dog. J. Pharmacol. Exp. Ther. 189, 759-771, 1974.

Martin, W.R. and Sloan, J.W.: The pathophysiology of morphine dependence and its treatment with opioid antagonists. Pharmakopsychiat. Neuro-Psychopharmakol. 1, 260-270, 1971.

Martin, W.R., Wikler, A., Eades, C.G. and Pescor, F.T.: Tolerance to and physical dependece on morphine in rats. Psychopharmacologia 4, 247-260, 1963.

Mayer, S.W., Kelly, F.H. and Morton, M.E.: The direct antithyroid action of reserpine, chlorpromazine and other drugs. J. Pharmacol. Exp. Therap. 117, 197-201, 1956.

Mayer, D.J. and Liebeskind, J.C.: Pain reduction by focal electrical stimulation of the brain: An anatomical and behavioral analysis. Brain Res. 68, 73-93, 1974.

Mayer, D.J. and Price, D.D.: Central nervous system mechanisms of analgesia. Pain 2, 379-404, 1976.

McCann, S.M., Ojeda, S.R., Libertun, C., Harms, P.G. and Krulich, L.: Drug-induced alterations in gonadotropin and prolactin release in the rat. In: Narcotics and the Hypothalamus, ed. by E. Zimmermann and R. George, pp. 121-136, Raven Press, New York, 1974.

McClane, T.K. and Martin, W.R.: Antagonism of the spinal cord effects of morphine and cyclazocine by naloxone and thebaine. Int. J. Neuropharmacol. 6, 325-327, 1967.

McDonald, R.K., Evans, F.T., Weise, V.K. and Patrick, R.W.: Effect of morphine and nalorphine on plasma hydrocortisone levels in man. J. Pharmacol. 125, 241-247, 1959.

McKenzie, G.M. and Szerb, J.C.: The effect of dihydroxyphenylalanine, pheniprozine and dextroamphetamine on the *in vivo* release of dopamine from the caudate nucleus. J. Pharmacol. Exp. Ther. 162, 302-308, 1968.

McMillan, D.E., Wolf, P.S. and Carchman, R.A.: Antagonism of the behavioral effects of morphine and methadone by narcotic antagonists in the pigeon. J. Pharmac. Exp. Ther. 175, 443-458, 1970.

Meidinger, F.: Action comparee sur l'hypophyse d'antihistaminiques, de neuroplegiques et d'anti-inflammatories. C.R. Soc. Biol. (Paris) 148, 1086-1090, 1954.

Meier, R., Bruni, C. and Tripod, J.: Differenciation pharmacodynamique de lapresoline, du serpasil et de la chlorpromazine lors de leur actionsur la retention hydrique du rat. Arch. Int. Pharmacodyn. Therap. 104, 137-146, 1955.

Meites, J.: Control of mammary growth and lactation. In: Neuroendocrinology, Vol 1, ed. by L. Martini and W.F. Ganong, pp. 669-707. Academic Press, New York, 1966.

Menninger-Lerchenthal, E.: Schwanguschaft und geburt morphinistischer frauen. Zentrabl. Gynaekol. 58, 1044-1051, 1934.

Meyer, D.R. and Sparber, S.B.: Clonidine antagonizes body weight loss and other symptoms used to measure withdrawal in morphine pelleted rats given naloxone. Pharmacologist 18, 236, 1976.

Milcou, S.M., Negoescou, I., Radian, N., Bojinescou, A., Petrescou, C., Cocou, F. and Stancou, H.: The effect of chlorphenothiazine (chlorpromazine) on the concentration of radioiodine I^{131} in the thyroid of the albino rat. Proceeding International Conference Peaceful Uses of Atomic Energy Geneva 10, 331-333, 1955.

Mims, R.B., Scott, C.L., Modebe, O. and Bethune, J.E.: Inhibition of L-DOPA-induced growth hormone stimulation by pyridoxine and chlorpromazine. J. Clinical Endocrinol. Metabol. 40, 256-259, 1975.

Mueller, G.P., Simpkins, J., Meites, J. and Moore, K.E.: Differential effects of dopamine agonist and haloperidol on release of prolactin, thyroid stimulating hormone, growth hormone and luteinizing hormone in rats. Neuroendorcrinology 20, 121-135, 1976.

Muller, E.E., Cocchi, D., Jalando, H. and Udeschini, G.: Antagonistic role for norepinephrine and dopamine in the control of growth hormone secretion in the rat. Endocrinology 92, A248, 1973.

Nagatsu, T., Levitt, M. and Udenfriend, S.: Tyrosine hydroxylase: The initial step in norepinepherine biosynthesis. J. Biol. Chem. 239, 2910-2917, 1964.

Nasmyth, P.A.: Factors influencing the effect of morphine sulfate on the ascorbic acid content of rats adrenal glands. Brit. J. Pharmacol. 9, 95-99, 1954.

Navarro, G., Richardson, R. and Zuban, A.T.: Propranolol and morphine. Psychopharmacology 51, 39-42, 1976.

Nickerson, M. and Collier, B.: Drugs inhibiting adrenergic nerves and structures innervated by them. In: The Pharmacological Basis of Therapeutics ed. by L.S. Goodman and A. Gilman, pp 533-574. MacMillan, New York, 1975.

Nickerson, M. and Gump, W.S.: The chemical basis for adrenergic blocking activity in compounds related to dibenamine. J. Pharmacol. Exp. Ther. 89, 167-185, 1949.

Niemegeers, C., Lenaerts, F. and Awouters, F.: In vivo pharmacology of antidiarrheal drugs, In: Synthetic Antidiarrheal Drugs Van Bever and H. Lal, eds.) p. 65-114, Marcel Dekker, Inc., New York, 1976.

Niemegeers, C.J.E., Van Nueten, J.M. and Janssen, P.A.J.: Azaperone, a sedative neurleptic of the butyrophenone series with pronounced anti-aggressive and anti-shock activity in animals. Arzneimittel-Forsch. 24, 1798-1806, 1974.

Nikidijevic, O. and Maickel, R.P.: Some effects of morphine on pituitary-adrenocortical function in the rat. Biochem. Pharmacol. 16, 2137-2142, 1967.

Nilsson, E. and Janssen, P.: Neurolept-analgesia, an alternative to general anesthesia. Acta Anaesthesial. Scand. 5, 73-84, 1961.

Norris, W. and Kelman, G.R.: The influence of drugs used in neuroleptanalgesia on cardiovascular and ventilatory function. Brit. J. Anaesth. 39, 134-142, 1967.

Norris, W. and Telfer, A.B.M.: Thalamonal as a preoperative sedative. Brit. J. Anaesth. 40, 517-521, 1968.

Numan, R., Smith, N. and Lal, H.: A versatile procedure for rapid induction of narcotic addiction in the rat utilizing intravenous injections. Physiological Psychology 3, 261-262, 1975.

Ojeda, S.R., Harms, P.G. and McCann, S.M.: Effect of blockade of dopaminergic receptors on prolactin and LH release: Median eminence and pituitary sites of action. Endocrinology 94, 1650-1657, 1974.

Oliver, J.T. and Troop, R.C.: Plasma corticosterone levels in stressed rats following the administration of pentobarbital, morphine and dephenylhydantoin Steroids 1, 670-677, 1963.

Opitz, K. and Reimann, I.: Suppression of the drug-induced morphine withdrawal syndrome by cyproheptadine. Psychopharmacology (Berl.) 28, 165-170, 1973.

Papper, S. and Papper, E.M.: The effects of preanesthetic, anesthetic and post-operative drugs on renal function. Clin. Pharmacol. Ther. 5, 205-215, 1964.

Paroli, E. and Melchioni, P.: Urinary excretion of hydroxysteroids, 17-keto-steroids and aldosterone in rats during a cycle of treatment with morphine. Biochem. Pharmacol. 6, 1-17, 1961.

Pasternak,G.W., Goodman, R. and Snyder, S.H.: An endogenous morphine-like factor in mammalian brain. Life Sci. 16, 1765-1769, 1975.

Pasternak, G.W. and Snyder, S.H.: Opiate receptor binding: Effects of enzymatic treatment. Mol. Pharmacol. 10, 183-193, 1973.

Pert, C.B., Aposhian, D. and Snyder, S.H.: Phylogenetic distribution of opiate receptor binding. Brain Res. 75, 356-361, 1974a.

Pert, C.B., Pasternak, G. and Snyder, S.H.: Opiate agonists and antagonists discriminated by receptor binding in brain. Science 182, 1359-1361, 1973.

Pert, C.B., Snowman, A.M. and Snyder, S.H.: Localization of opiate receptor binding in synaptic membranes of rat brain. Brain Res. 70, 184-188, 1974b.

Pert, C.B. and Snyder, S.H.: Opiate receptor: Demonstration in nervous tissue. Science 179, 1011-1014, 1973.

Pert, C.B. and Snyder, S.H.: Opiate receptor binding of agonists and antagonists affected differentially by sodium. Mol. Pharmacol. 10, 868-879, 1974.

Pert, C.B. and Snyder, S.H.: Differential interactions of agonists and antagonists with the opiate receptor. Neurosci. Res. Program Bull. 13, 73-79, 1975.

Pozuelo, J. and Kerr, F.W.C.: Suppression of craving and other signs of dependence in morphine-addicted monkeys by administration of alpha-methyl-para-tyrosine. Mayo Clinic Proceedings 47, 621-628, 1972.

Puri, S.K., Cochin, J. and Volicer, L.: Effect of morphine sulfate on adenylate cyclase and phosphodiesterase activity in rat corpus striatum. Life Sci. 16, 759-768, 1975.

Puri, S.K. and Lal, H.: Effect of dopaminergic stimulation or blockade on morphine-withdrawal aggression. Psychopharmacologia 32, 113-120, 1973.

Redding, T.W., Bowers, C.Y. and Schally, A.V.: The effects of morphine and other narcotics on thyroid function in mice. Acta Endocrinol. (Kbh.) 51, 391-399, 1966.

Rennels, E.G.: Effect of morphine on pituitary cytology and gonadotrophic levels in the rat. Tex. Rep. Biol. Med. 19, 646-657, 1961.

Reiss, M.: Influence of chlorpromazine on endocrine function. In: Psychoendocrinology, pp. 182-197, Grune and Stratton, New York, 1958.

Risner, M.E. and Khavari, K.A.: Morphine dependence in rats produced after five days of ingestion. Psychopharmacologia 28, 51-62, 1973.

Samel, M.: Blocking of the thyrotrophic hormone secretion by morphine and chlorpromazine in rats. Arch. Int. Pharmacodyn. Therap. 117, 151-157, 1958.

Sawyer, C.H.: Neuroendocrine blocking agents: Discussion. In: Advances in Neuroendocrinology ed. by A.V. Nalbandor, pp. 444-457. University Illinois Press, Urbana, 1963.

Schreiber, V., Zbuzek, V. and Zbuzkova-Kmentova, V.: Effect of codeine on thyroid function in the rat. Physiol. Bohemoslov. 17, 253-258, 1968.

Schuster, C.R.: Psychological approaches to opiate dependence and self-administration by laboratory animals. Fed. Proc. 29, 2-5, 1970.

Schwartz, A.S. and Marchok, P.L.: Depression of morphine-seeking behavior in the rat by haloperidol. Committee on Problems of Drug Dependence, 579-591, 1974.

Sethy, V.H. and Van Woert, M.H.: Modification of striatal acetylcholine concentration by dopamine receptor agonists and antagonists. Res. Comm. Chem. Path. Pharmacol. 8, 13-28, 1974.

Shen, F.H., Loh, H. and Way, E.L.: Brain serotonin turnover in morphine tolerant-dependent mice. J. Pharm. Exp. Ther. 175, 427-434, 1970.

Shibusawa, K., Saito, S., Fukuda, M., Kawai, T., Yamada, H. and Tomizawa, K.: Inhibition of the hypothalamo-neurohypophyseal neurosecretion by chlorpromazine. Endocrinol. Jap. 2, 189-194, 1955.

Shuster, L., Hannam, R.V. and Boyle, W.E.: A simple method for producing tolerance to dihydro-morphinone in mice. J. Pharmacol. Exp. Ther. 140, 149-154, 1963.

Simon, E.J.: Opiate receptor binding with ^{3}H-etorphine. Neurosci. Res. Program Bull. 13, 43-50, 1975.

Simon, E.J.: The opiate receptors. Neurochemical Research 1, 3-28, 1976.

Simon, E.J., Hiller, J.M. and Edelman, I.: Stereospecific binding of the potent narcotic analgesic ^{3}H etorphine to rat-brain homogenate. Proc. Nat. Acad. Sci. 70, 1947-1949, 1973.

Simon, E.J., Hiller, J.M., Groth, J. and Edelman, I.: Further properties of stereospecific opiate binding sites in rat brain: On the nature of the sodium effect. J. Pharmacol. Exp. Ther. 192, 531-537, 1975.

Singh, M.D. and Singh, J.M.: Methadone-induced aggressive behavior. Toxicol. Appl. Pharmacol. 25, 452, 1973.

Sloan, J.W., Brooks, J.W., Eisenman, A.J. and Martin, W.R.: The effect of addiction to and abstinence from morphine on rat tissue catecholamine and serotonin levels. Psychopharmacologia 4, 261-270, 1963.

Sloan, J.W. and Eisenman, A.J.: Long persisting changes in catecholamine metabolism following addiction to and withdrawal from morphine. Res. Publ. Assn. Ment. Dis. 46, 96-105, 1968.

Slusher, M.A. and Browning, B.: Morphine inhibition of plasma corticosteroid levels in chronic venous-catheterized rats. Amer. J. Physiol. 200, 1032-1034, 1961.

Smith, S.G. and Davis, W.M.: Behavioral control by stimuli associated with acquisition of morphine self-administration. Behavioral Biol. 9, 777-780, 1973.

Smith, S.G. and Davis, W.M.: A method for chronic intravenous drug administration in the rat. In: Methods in Narcotic Research Eds. S. Ehrenpreis and A. Neidle, pp. 3-331. Marcel Dekker, Inc., New York, 1975.

Snyder, S.H. and Pert, C.B.: Regional distribution of the opiate receptor. In: Opiate Receptor Mechanisms, ed. by S.H. Snyder and S. Matthysse, pp. 35-39, MIT Press, Cambridge, 1975.

Sobel, H., Schapiro, S. and Marmorston, J.: Influence of morphine on urinary corticoid excretion by guinea pigs following exposure to cold and administration of Pitressin. Amer. J. Physiol. 195, 147-149, 1958.

Stadler, H., Lloyd, K.G. and Bartholini, G.: Dopaminergic inhibition of striatal cholinergic neurons: Synergistic blocking action of γ-butyrolactone and neuroleptic drugs. Arch. Pharmacol. 283, 129-134, 1974.

Stadler, H., Lloyd, K.G., Gadea-Ciria, M. and Bartholini, G.: Enhanced striatal acetylcholine release by chlorpromazine and its reversal by apomorphine. Brain Res. 55, 476-480, 1973.

Stoffer, S.S.: A gynecologic study of drug addicts. Am. J. Obst. Gynec. 101, 779-783, 1968.

Stolerman, I.P. and Kumar, R.: Preferences for morphine in rats: Validation of an experimental model of dependence. Psychopharmacologia 17, 137-150, 1970.

Supek, Z., Keckes, S. and Voivodic, S.: The action of chlorpromazine on water and chloride excretion in rats. Arch. Int. Pharmacodyn. Therap. 123, 253-259, 1960.

Suzuki, T., Yamashita, K., Zinnouchi, S. and Mitamura, T.: Effect of morphine upon the adrenal 17-hydroxycorticosteroid secretion in the dog. Nature 183, 825, 1959.

Takemori, A.E., Huang, J.T. and Yano, I.: Effect of various central biogenic amine-modifiers on the naloxone-induced jumping in morphine-dependent mice. Factors Affecting the Action of Narcotics 8, 1976. Paper presented at international symposium on factors affecting the action of narcotics. Milan, Italy, July 13-16, 1976.

Tanabe, T. and Cafruny, E.J.: Adrenal hypertrophy in rats treated chronically with morphine. J. Pharmacol. 122, 148-153, 1958.

Teiger, D.G.: Induction of physical dependence on morphine, codeine and meperidine in the rat by continuous infusion. J. Pharmacol. Exp. Ther. 190, 408-415, 1974.

Tenen, S.S.: Antagonism of analgesic effect of morphine and other drugs by p-chlorophenylalanine, a serotonin depletor. Psychopharmacologia 12, 278-285, 1968.

Terenius, L.: Characteristics of the "receptor" for narcotic analgesics in synaptic plasma membrane traction from rat brain. Acta Pharmacol. Toxicol. 33, 377-384, 1973a.

Terenius, L.: Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. Acta Pharmacol. Toxicol. 32, 317-320, 1973b.

Teranius, L.: Contribution of "receptor" affinity to analgesic potency. J. Pharm. Pharmacol. 26, 146-148, 1974.

Terenius, L. and Wahlstrom, A.: Morphine-like ligand for opiate receptors in human CSF. Life Sci. 16, 1759-1764, 1975.

Teschemacher, H., Opheim, K.E., Cox, B.M. and Goldstein, A.: A peptide-like substance from pituitary that acts like morphine. Life Sci. 16, 1771-1776, 1975.

Tseng, L.F., Loh, H.H. and Wei, E.T.: Effects of clonidine on morphine withdrawal signs in the rat. Europ. J. Pharmacol. 30, 93-99, 1975.

Urca, G., Hanan, F., Liebeskind, J.C. and Taylor, A.N.: Morphine and enkephalin: Analgesic and epileptic properties. Science 197, 83-86, 1977.

Van Neuten, J.M.: THe study of the effects of phenothiazine and butyrophenone derivatives on the action of amphetamine in rats. Doctor of Science Thesis, Paris University, 1962.

Van Neuten, J.M., Janssen, P.A.J. and Fontaine, J.: Unexpected reversal effects of naloxone on the guinea pig ileum. Life Sci. 18, 803-810, 1976.

Van Neuten, J.M., Van Ree, J.M., and Vanhoutle, P.M.: Inhibition by met-enkephalin of peristaltic activity in the guinea pig ileum, and its reversal by naloxone. European J. Pharmacol. 41, 341-342, 1977.

Van Peenen, P.F.D. and Way, E.L.: Effect of certain central nervous system depressants on pituitary-adrenal activating agents. J. Pharmacol. 120, 261-267, 1957.

Vertes, M., Vertes, Z. and Kouacs, S.: Effect of hypothalamic stimulation on pituitary-thyroid function. Acta. Physiol. Acad. Sci. Hung. 27, 229-235, 1965.

Villarreal, J.E.: The effect of morphine agonists and antagonists on morphine-dependent rhesus monkeys. In: Agonist and Antagonist Actions of Narcotic Analgesic Drugs eds. H.W. Kosterlitz, H.O.J. Collier, J.E. Villarreal, pp. 74-93, University Park Press, Baltimore, 1973.

Voith, K. and Herr, F.: The behavioral pharmacology of butaclamol hydrochloride (AY-23,025), a new potent neuroleptic drug. Psychopharmacologia 42, 11-20, 1975.

Wakabayashi, I., Arimura, A. and Schally, A.V.: Effects of dexamethasone and pentobarbital on plasma growth hormone levels in rats. Neuroendocrinology 8, 340-346, 1971.

Watanabe, H.: The development of tolerance to and of physical dependence on morphine following intraventricular injection in the rat. Jap. J. Pharmacol. 21, 383-391, 1971.

Wauquier, A., Niemegeers, L.J.E., Lal, H.: Differential antagonism by naloxone of inhibitory effects of haloperidol and morphine on brain self-stimulation. Psychopharmacologia 37, 303-310, 1974.

Way, E.L.: The role of serotonin in morphine effects. Fed. Proc. 31, 113-120, 1971.

Way, E.L., Loh, H. and Shen, F.: Morphine tolerance, physical dependence and synthesis of brain 5-hydroxytryptamine. Science 162, 1290-1292, 1968.

Way, E.L., Loh, H.H. and Shen, F.H.: Simultaneous quantitative assessment of morphine and physical dependence. J. Pharmacol. Exp. Ther. 167, 1-8, 1969.

Way, E.L. and Shen, F.: Interaction of morphine with the catecholamines and 5-hydroxytryptamine. In: Narcotic Drugs. Biochemical Pharmacology. ed. Clouet, D.H., pp. 229-253, New York, Plenum Press, 1971.

Weeks, J.R. and Collins, R.J.: Patterns of intravenous self-injection by morphine-addicted rats. In: The Addictive States ed by A. Wikler, pp. 288-298, Williams and Wilkins Company, Baltimore, 1968.

Wei, E.: Assessment of precipitated abstinence in morphine-dependent rats. Psychopharmacologia 28, 35-44, 1973.

Wei, E., Loh, H.H. and Way, E.L.: Neuroanatomical correlates of morphine dependence. Science 177, 616-617, 1972.

Wei, E., Loh, H.H. and Way, E.L.: Brain sites of precipitated abstinence in morphine-dependent rats. J. Pharmacol. Exp. Ther. 185, 108-115, 1973a.

Wei, E., Loh, H.H. and Way, E.L.: Neuroanatomical correlates of wet shake behavior in the rat. Life Sci. 12, 489-496, 1973b.

Wei, E., Loh, H.H. and Way, E.L.: Quantitative aspects of precipitated abstinence in morphine-dependent rats. J. Pharmacol. Exp. Ther. 184, 398-403, 1973c.

Wei, E. and Way, E.L.: Application of the pellet implantation technique for the assessment of tolerance and physical dependence in the rodent. In Methods in Narcotic Research ed. by S. Ehrenpreis and A. Neidle, pp. 244-256, Marcel Dekker, New York, 1975.

Whitelaw, M.J.: Delay in ovulation and menstruation induced by chlorpromazine. J. Clin. Endocr. 16, 972-984, 1956.

Wikler, A., Green, P.C., Smith, H.D. and Pescor, F.T.: Use of a benzimidazole derivative with potent morphine-like properties orally as a presumptive reinforcer in conditioning of drug. (See King Behavior of Rats.) Fed. Proc. 19, 22, 1960.

Wikler, A., Martin, W.R., Pescor, F.T. and Eades, C.G.: Factors regulating oral consumption of an opioid (etonitazene) by morphine addicted rats. Psychopharmacologia 5, 55-76, 1963.

Wiseman, R. Jr.: The effect of acute and chronic administration of chlorpromazine on the I^{131} distribution in normal rats. J. Pharmacol. Exp. Therap. 138, 269-276, 1962.

Wright, E.: Use of chlorpromazine in the measurement of radioactive iodine in the thyroid gland of rat. New Zeal. J. Agr. Res. 1, 455-461, 1958.

Yelnosky, J. and Gardocki, J.F.: A study of some of the pharmacologic actions of fentanyl citrate and droperidol. Toxicol. Appl. Pharmacol. 6, 63-70, 1964.

Yelnosky, J., Katz, R. and Dietrich, E.V.: A study of some of the pharmacologic actions of droperidol. Toxicol. Appl. Pharmacol. 6, 37-47, 1964.

Zarrow, M.X. and Brown-Grant, K.: Inhibition of ovulation in the gonadotrophin-treated immature rat by chlorpromazine. J. Endocr. 30, 87-96, 1964.

Zimmerman, E. and Critchlow, V.: Inhibition of morphine-induced pituitary-adrenal activation by dexamethasone in the female rat. Proc. Soc. Exp. Biol. Med. 143, 1224-1226, 1973.

Zimmerman, E., Pang, C.N. and Sawyer, C.H.: Morphine-induced prolaction release and its suppression by dexamethasone in male rats. Endocr. Soc. Abstr. 1974.