**Title**
Ketamine enantiomers



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**Publication Date**
1981

Peer reviewed|Thesis/dissertation

# Ketamine Enantiomers: Comparative Pharmacology and Neurochemical Effects

by

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DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Pharmacology

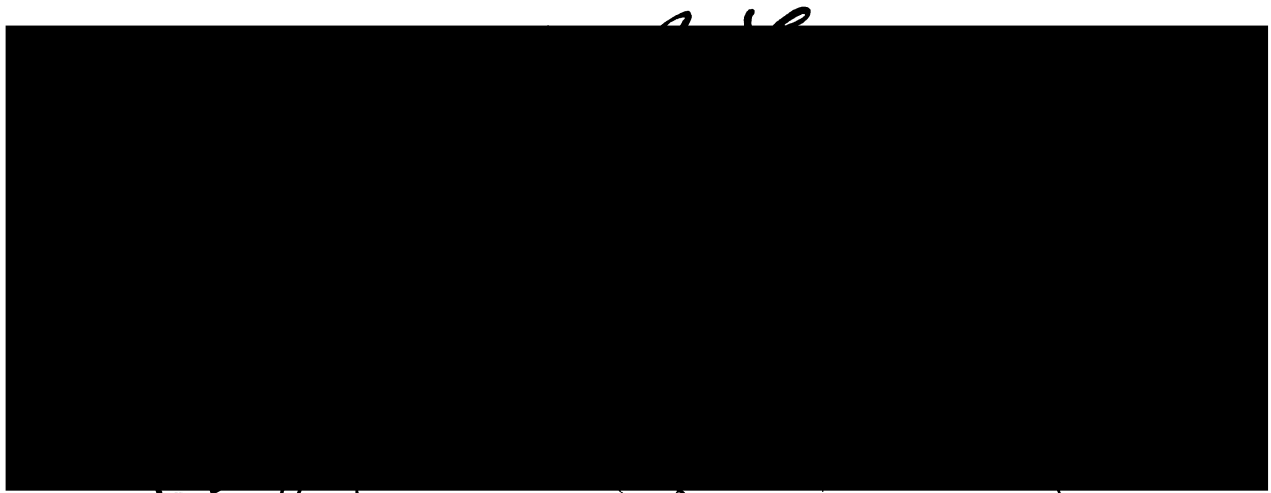
in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco



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# ACKNOWLEDGEMENTS

To Trevor, for his discussions and excellent advice during my research training, for his valuable suggestions in writing this dissertation and for his patience and moral support during my completion of this work on a part-time basis.

To Dr. Walter Way, for his interest in and encouragement of my research project, for assistance in the biodisposition studies and for serving on my dissertation committee.

To Dr. Horace Loh, for serving on my committee and for the use and helpful advice of his research group in performing the opiate and muscarinic binding assays.

To Dr. Ping-Yee Law, for his invaluable suggestions and discussions concerning the opiate receptor studies.

To Chuck Pudwill, for his valuable assistance in the gas chromatographic procedures and for his technical help in the biodisposition studies.

To Eva Soliven, for her helpful assistance with the receptor and reuptake assays.

To Beth Taylor, for her work on the dopamine binding experiments.

To Stan, for his interest in and enthusiasm for my research, and for his love and unending patience and encouragement during the final years of my doctoral training.

To the Department of Pharmacology, for giving me the opportunity and providing me with the facilities to undertake graduate study at U.C., San Francisco.

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## ABSTRACT

KETAMINE ENANTIOMERS: COMPARATIVE PHARMACOLOGY AND NEUROCHEMICAL EFFECTS

Relative pharmacological potencies of the optical isomers of ketamine were estimated in the ICR mouse. The (+)-enantiomer was 3 x more potent than (-)-ketamine as an analgesic using the phenylquinone writhing test, only 1.5 x more potent in the induction of hypnosis and 1.9 x more potent in increasing locomotor stimulation. The (-)-enantiomer elicited greater locomotor stimulation than (+)-ketamine following equianalgesic doses. The duration of anesthesia following i.v. administration of 25 mg/kg was 3 x longer with (+)-ketamine compared to (-)-ketamine. The analgesic action of ketamine was reversed by 10 mg/kg of naloxone while pretreatment with atropine did not reverse ketamine analgesia.

The levels of ketamine, norketamine and metabolite II (hydroxylated products) were measured at various time intervals in the brain and plasma following s.c. administration of a subanesthetic dose of racemic ketamine and each of its optical isomers. The concentrations of the three parent drugs were similar in the plasma and brain at the time intervals measured. Similarly, no differences in brain levels of norketamine were detected for the three ketamine agents (except at 10 minutes, (-)-norketamine was higher). Significantly lower levels of norketamine were measured in the plasma after the administration of (-)-ketamine than were found after admin-

istration of either racemic ketamine or the (+)-isomer. Metabolite II was formed in significantly greater amounts in the plasma following the injection of the (-)-isomer than was measured after the racemate or the (+)-isomer. Metabolite II was also detected in the brain in the case of the (-)-enantiomer.

An assessment of the relative potencies of the ketamine enantiomers to displace $^{3}$H-naloxone, $^{3}$H-QNB and $^{3}$H-spiroperidol from specific receptors was examined in brain homogenates. The ketamine enantiomorphs displayed competitive binding activities in the $^{3}$H-naloxone and $^{3}$H-QNB binding experiments. The respective $IC_{50}$ values for (+)- and (-)-ketamine were 0.12 and 0.22 mM in the $^{3}$H-naloxone studies and 0.12 and 0.11 mM in the $^{3}$H-QNB assays. Specific binding of $^{3}$H-spiroperidol in calf caudate nucleus was not inhibited to any significant degree by ketamine at concentrations up to 0.1 mM.

The effects of the ketamine enantiomers on acetylcholinesterase action in mouse homogenates and on reuptake of $^{3}$H-dopamine, $^{3}$H-norepinephrine and $^{3}$H-serotonin in mouse brain synaptosomes were investigated. (-)-Ketamine was approximately 3 x more potent than (+)-ketamine in the inhibition of acetylcholinesterase. On inhibition of $^{3}$H-dopamine and $^{3}$H-norepinephrine reuptake (+)-ketamine was about 4 x more potent than (-)-ketamine while (-)-ketamine showed twice the potency of (+)-ketamine in reducing serotonin uptake. Relative to their inhibition

of acetylcholinesterase and catecholamine uptake both isomers displayed greater potencies on the serotonergic reuptake system with $IC_{50}$ values of 0.17 and 0.35 mM for (-)- and (+)-ketamine respectively.

Racemic ketamine and its enantiomers reduced $^{3}H$-phencyclidine binding in mouse brain homogenates in an equivalent manner. The $IC_{50}$ values for (±)-ketamine, (+)-ketamine and (-)-ketamine were 0.25, 0.29 and 0.27 mM respectively while the $IC_{50}$ value for phencyclidine in the same experiments was 0.27 μM.

Inhibitory concentrations measured in the *in vitro* assays were related to *in vivo* brain ketamine levels achieved after analgesic and hypnotic doses of the ketamine isomers.

# CHAPTER ONE: GENERAL INTRODUCTION

## INTRAVENOUS ANESTHESIA: A HISTORICAL PERSPECTIVE

The first attempt at an intravenous injection in humans was made by means of a quill and bladder by the famous English architect, Christopher Wren, in the year 1656. With an intravenous injection of opium Wren incidently produced a state of unconsciousness. But it is Sigismund Elshiholtz who is attributed with the first genuine attempt at intravenous anesthesia, for in 1665 he injected an opium solution for the avowed purpose of "producing insensibility". Intravenous anesthesia, however, did not achieve clinical importance until the introduction of the rapid acting barbiturates in the 1930's. By alleviating some of the problems associated with the early inhalation anesthetics including need for cumbersome equipment, respiratory tract irritation, delayed onset and lengthy emergence from anesthesia, these parenteral agents gained widespread acceptance.

Since its introduction in 1934, thiopental has remained in wide use as an induction agent and is considered the prototype of the intravenous rapid acting barbiturates. Anesthesia by intravenous administration of thiopental is manifested by a rapid loss of consciousness, eventual disappearance of muscle tone and depressed respiration (Dundee, 1956; Conway and Ellis, 1969). With increasing depth of anesthesia by thiopental there appears to be a general depression of the central nervous system (Machne et al, 1955; Abrahamian et al, 1963) and with excessive doses, depression of the vital medullary centers may lead to respiratory and cardio-

vascular collapse (Dundee and Wyant, 1974). Rapid distribution of thiopental from the central nervous system to other tissue compartments appears to be responsible for its termination of action as metabolic degradation and renal excretion are much too slow to account for its short duration of action. Thus, a substantial amount of the drug remains in the body upon recovery from unconsciousness allowing for cumulative effects or potential adverse drug interactions. The ability of the rapid acting barbiturates to cause profound respiratory and circulatory depression, their slow elimination from the body and unsuitability for intramuscular administration are important features limiting their use.

Finally, an additional factor affecting the use of this group of agents is their inability to decrease the sensation of pain without significant impairment of consciousness. In fact, at subanesthetic doses, these drugs are known to increase pain (Dundee, 1960; Clutton-Brock, 1961) thus restricting their use as sole agents to prevent sensation of pain or to provide sleep in the presence of severe pain.

In more recent years techniques have been introduced with the aim at producing sensation or light sleep with profound analgesia. The first of these attempts, popularized in France in the 1950's, was a "lytic" cocktail comprising a mixture of various tranquillizers and analgesics which resulted in a state termed "artificial hibernation" (Laborit and Huguerno, 1954). Artificial hibernation was associated with cardiovascular instability and prolonged depression of conscious-

ness and in the late 1950's was replaced by a more effective state termed neuroleptanalgesia. This state was also based on combination drug therapy and was achieved by the intravenous administration of a mixture composed of a neuroleptic (usually a butyrophenone) and an analgesic (usually a narcotic). With this method a painless state is produced without hemodynamic instability and impairment of consciousness (Morrison, 1969). These parenteral agents have gained use in procedures not requiring full general anesthesia or where retention of consciousness is required for patient cooperation. However, respiratory depression, slow onset of induction as well as increased potential for drug interactions are some of the major drawbacks to the employment of the neuroleptic narcotic mixtures.

## DISSOCIATIVE ANESTHESIA

In the search for more desirable parenteral agents with analgesic and anesthetic properties without respiratory or cardiovascular depressive activity, attention was drawn to the phenylcyclohexylamines (figure 1-1). After demonstration by Chen and coworkers of promising actions in laboratory animals, phencyclidine was introduced for clinical evaluation in 1959 (Chen et al, 1959). However, preliminary clinical trials with phencyclidine by several groups revealed inadequate anesthesia and convulsive behavior in some patients and unmanageable mania in a large portion of individuals

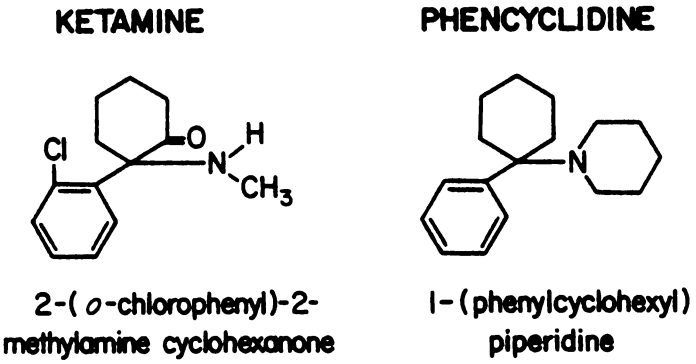



figure 1-1. Chemical structures of two dissociative anesthetic agents.

(Greifenstein et al, 1959; Johnstone and Baigel, 1959). These reports precluded the clinical use of phencyclidine. It is of interest that, in more recent times, phencyclidine has achieved illicit status and has become one of the most widely abused drugs in the United States (Snyder, 1980).

Animal studies in 1965 by McCarthy and coworkers showed another arylcyclohexylamine, ketamine (figure 1-1), possessed some of the advantages of phencyclidine including marked analgesia, lack of respiratory and cardiovascular depression. Furthermore, they indicated the psychic stimulatory component appeared less prominent after administration of ketamine (McCarthy et al, 1965). A clinical investigation by Domino and coworkers in the same year confirmed ketamine was more satisfactory as an anesthetic agent than its predecessor, phencyclidine (Domino et al, 1965). The early clinical studies showed ketamine maintained respiratory and cardiovascular function, provided adequate anesthesia and caused profound analgesia at subanesthetic doses while producing less convulsive and psychic emergence reactions (Domino et al, 1965; Corssen and Domino, 1966). Ketamine was approved for commercial use by the FDA in 1970.

This distinctive type of anesthesia caused by the arylcyclohexylamines characterized by catalepsy, amnesia and marked analgesia (Pender, 1971) was termed <u>dissociative anesthesia</u> (Domino et al, 1965). This term originated from studies based on electroencephalographic effects of ketamine which were interpreted as showing a depression of the thalamo-

neocortical systems while simultaneously activating the limbic system causing a "functional dissociation" between the two systems (Domino et al, 1965; Miyasaka and Domino, 1968). However, other investigators have interpreted the electroencephalographic effects of ketamine as a functional disorganization attributable to subliminal seizure activity in both the thalamus and limbic systems (Mori et al, 1971; Ferrar-Allado et al, 1973) and suggest "catalept-anesthesia" may be a more appropriate description of ketamine anesthesia (Winters et al, 1972). Irrespective of the term used to designate ketamine anesthesia,it is evident this agent exhibits pharmacological actions different from those caused by the barbiturates and lacks a number of the disadvantages associated with the use of the other parenterally administered agents and more conventional anesthetics.

## KETAMINE PHARMACOLOGY

Unlike the barbiturates, ketamine is suitable for both intravenous and intramuscular injection. Rapid loss of consciousness is achieved by intravenous administration of ketamine producing a trance-like cataleptic state accompanied by mild hypertonus, nystagmus, involuntary movements, maintenance of pharyngeal and laryngeal reflexes and increased salivation (Corssen and Domino, 1966; Morgan et al, 1971; Lanning and Harmel, 1975).

Some of the more distinctive properties associated with

ketamine induced anesthesia include cardiovascular stimulation, minimal respiratory depression and profound analgesia. With clinical doses of ketamine, cardiovascular stimulation invariably occurs characterized by increases in heart rate and arterial pressure (Dundee, 1974; Johnstone, 1976). Although a transient period of respiratory depression and apnea may occur after ketamine administration, maintenance of respiratory function is predominant and there may be a slight increase in respiratory rate in some individuals (Corssen and Domino, 1966; Pender, 1971).

One of the most advantageous features of ketamine anesthesia is the occurrence of marked analgesia (Domino et al, 1965; Corssen and Domino, 1966; Sadove et al, 1971) which persists after the return of consciousness (Iwatsuki et al, 1967; Aguado-Matorras and Nalda-Felipe, 1970). This is in contrast to the action of the thiobarbiturates which cause an increase in sensitivity to pain at subanesthetic doses. Because of its dual hypnotic and analgesic properties ketamine may be used as the sole anesthetic in certain surgical procedures and painful manipulations (Wilson et al, 1967; Feingold and MacMahan, 1975).

Unfortunately, ketamine shares some of the post-hypnotic psychic reactions exhibited by its chemical congener, phencyclidine, varying in severity from pleasant dream states to restlessness, auditory and visual hallucinations, delerium, and nightmares (Corssen and Domino, 1966; Dundee et al, 1971; Morgan et al, 1971; Sussman, 1974). While early studies

reported minor or a low incidence of psychotomimetic effects upon emergence from ketamine anesthesia, it is these post anesthetic excitatory actions which have limited the use of ketamine and may be contributing factors to the problems of reported ketamine abuse (FDA Bulletin, 1979). Efforts to minimize ketamine induced psychic reactions have included the administration of neuroleptics, barbiturates, analgesics or benzodiazepines both prior and post anesthesia (Becksey et al, 1972; Coppel et al, 1973; Lilburn et al, 1978). In this regard, prior administration of diazepam has been shown to be effective in decreasing the severity and incidence of adverse emergence reactions associated with ketamine anesthesia as well as in reducing the cardiovascular stimulatory effects of ketamine (Kothary and Zsigmond, 1977; Kortila and Levanen, 1978).

Studies on the pharmacology of ketamine in laboratory animals show the effects of ketamine are manifested in a variety of ways including ataxia and loss of righting reflex (McCarthy et al, 1965; Cohen and Trevor, 1974; Balser, 1980), analgesia (Marietta et al, 1977) and increased spontaneous locomotor activity (Marietta et al, 1976; Meliska and Trevor, 1978). Additional behavioral studies in a variety of species at subhypnotic doses of ketamine have revealed stereotypic and circling (rotation) behavior (Myslobodsky et al, 1979a,b) and rate dependent effects on scheduled controlled behavior (Wenger, 1976; Wenger and Dews, 1976) which can be differentiated from those on spontaneous locomotor activity (Meliska

and Trevor, 1978). In rhesus monkeys ketamine maintains self-administration behavior similar to that of a number of drugs and conventional reinforcers (Moreton et al, 1977).

Development of tolerance has been reported for a number of actions of ketamine. Among these are decreased duration of hypnosis (Douglas and Dagirmanjian, 1975; Cumming, 1976; Livingston and Waterman, 1978), reduced analgesia and duration of ataxia (Marietta et al, 1976) following repeated administration of hypnotic doses of ketamine. A stimulatory action of ketamine has also been shown to be affected by prior drug experience evidenced by diminished spontaneous locomotor stimulation following subanesthetic doses of ketamine (Meliska and Trevor, 1978)

The metabolism of ketamine has been described in a variety of species (Cohen et al, 1973; Chang and Glazco, 1974; Lau and Domino, 1977) and its biodisposition has been studied in relation to its pharmacological effects. The identification in early studies of two metabolites, norketamine (metabolite I) and an enone, led to the scheme shown in figure 1-2 to describe the biotransformation pathways of ketamine (Chang et al, 1965). Recent studies, however, suggest this scheme oversimplified the metabolic fate of ketamine since additional metabolites arising from the hepatic hydroxylation at various positions on the cyclohexanonal ring of both the parent compound and norketamine have been identified (Adams et al, in press). Additionally, it is suggested that the enone product, commonly known as metabolite II, is the result

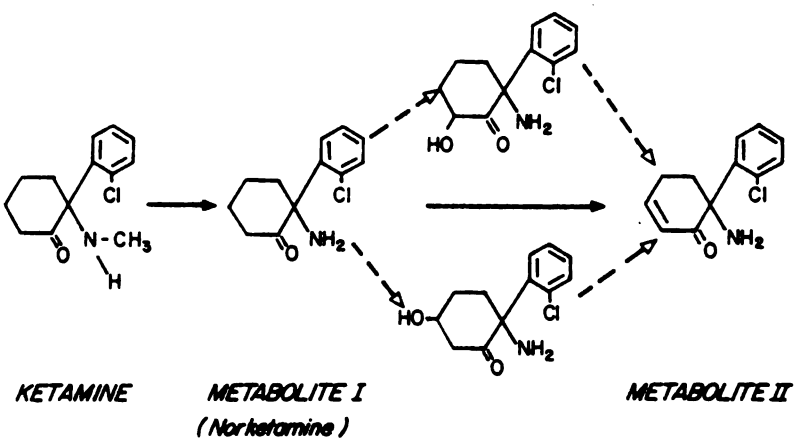



figure 1-2. Biotransformation pathways for ketamine proposed by Chang and Glazko (1974). Predominant metabolites are indicated with solid arrows while dashed arrows indicate postulated hydroxylated intermediates.

of a methodological artefact originating from the thermal dehydration of hydroxylated metabolites in the gas chromatographic column. Figure 1-3 represents a revised version of the biotransformation pathways for ketamine based on these new findings.

Biodisposition studies in rats show ketamine rapidly enters the brain after intravenous administration and achieves peak brain levels within one minute followed by redistribution into other tissues (Cohen and Trevor, 1974; White et al, 1976). Although the N-demethylated metabolite, norketamine, appears in the brain shortly after ketamine administration, it has been shown to be considerably less active than the parent compound (Cohen and Trevor, 1974; White et al, 1975). Redistribution appears to be the predominant factor in the termination of anesthesia while hepatic metabolism is important in the cessation of the post anesthetic actions of ketamine (Cohen and Trevor, 1974; Marietta et al, 1976). Hepatic metabolism also appears to play a role in the development of tolerance demonstrated by the observation that hepatic enzyme induction by ketamine pretreatment in rats occurs *in vitro* and *in vivo* accompanied by a decrease in duration of hypnosis (Livingston and Waterman, 1978) and a reduction of analgesia, ataxia, and locomotor activity (Marietta et al, 1976). In related studies, the shortening of ketamine anesthesia (Piel et al, 1969) and post-hypnotic effects (Marietta et al, 1976) also occur with phenobarbital pretreatment while SKF-525A, a potent inhibitor of hepatic microsomal enzymes, potentiates the

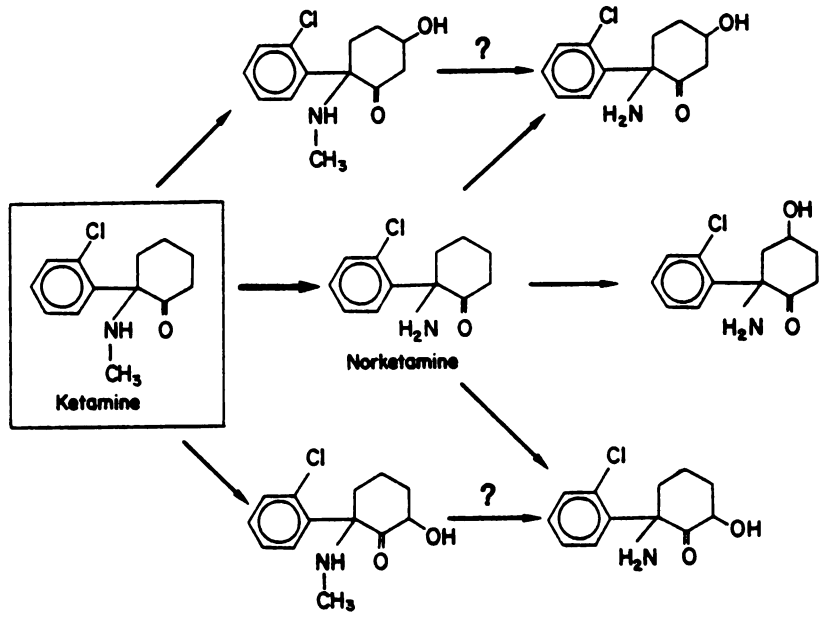



figure 1-3. Biotransformation pathways for ketamine proposed by Adams et al (in press). Heavy arrow denotes major metabolite formed.

effects of ketamine.

Attempts to relate the pharmacological actions of ketamine to underlying neurochemical events have dealt primarily with the central nervous system, although interest in the cardiovascular stimulatory properties of ketamine has led to investigation of peripheral sites of action. Biochemical studies have examined the influence of ketamine on a number of brain neurotransmitter systems, however the interaction of phencyclidine with central neurotransmitters perhaps has been more thoroughly studied because of interest in its psychotomimetic actions.

The study of the interaction of ketamine with the dopaminergic system include both behavioral and biochemical investigations. Efforts to link behavioral effects of ketamine to alterations in dopaminergic transmission rely on the observation that ketamine and its congener, phencyclidine, produce stereotypic and rotational behavior in rodents in an "amphetamine like" fashion (Wenger and Dews, 1976; Myslobodsky et al, 1979a, b; Martin et al, 1979; Murray and Horita, 1979). Moreover, prior administration of dopamine antagonists has been reported to reduce the phencyclidine induced stereotypic (Martin et al, 1979; Murray and Horita, 1979) and rotational (Finnegan et al, 1979) behavior. However, there are no published studies on the effect of antidopamine agents on stereotypic or circling behavior of ketamine.

Biochemical studies report ketamine, as well as phencyclidine, inhibit synaptosomal uptake of dopamine in rat

brain homogenates with $IC_{50}$ values in the molar range of $10^{-4}$ (Azzaro and Smith, 1977) and $10^{-7}$ (Smith et al, 1977) respectively. In vivo administration of hypnotic doses of ketamine either causes no change (Sung et al, 1973; Ylitalo et al, 1976) or decreases (Kari et al, 1978) dopamine levels in rat brain, depending on the rat strain. In turnover studies, brain homovanillic acid content was shown to increase following an anesthetic dose of ketamine in rats (Ylitalo et al, 1976) while a smaller anesthetic dose administered to primates caused no change in brain dopamine metabolites over the same time period (Bacopoulos et al, 1979). Phencyclidine administration has been shown to decrease accumulation of brain $^{14}C$-dopamine formed from intracerebrally administered $^{14}C$-tyrosine and to increase the accumulation of the O-methylated metabolite of dopamine in the mouse (Hitzemann et al, 1973).

Much of the investigation of ketamine interaction on the noradrenergic system has been directed toward the cardiovascular component of ketamine action by examining the sympathetic neuronal effects of ketamine. For example, ketamine has been found to increase levels of norepinephrine in the circulation (Baraka et al, 1973; Lundy et al, 1973), desensitize arterial baroreceptors (Dowdy and Kaya, 1968) and inhibit reuptake of norepinephrine into adrenergic neurons of isolated heart and vessel preparations (Miletich et al, 1973; Nedergaard, 1973). The direct action of ketamine on heart muscle has been demonstrated to be one of depression evidenced by decreased heart rate and contractility in perfused heart

preparations (Traber et al, 1968; Goldberg et al, 1970; Aronson and Hanno, 1978). The consensus appears to be that ketamine induced cardiovascular stimulation is due to increased sympathetic discharge by actions at both central and peripheral sites (Goldberg et al, 1970; Traber et al, 1970; Schwartz and Horwitz, 1975) but the underlying neurochemical mechanism remains unclear.

In the central nervous system, biochemical studies on the effect of ketamine on noradrenergic transmission report ketamine inhibits reuptake of norepinephrine at $10^{-4}$ molar concentrations in rat brain cortex slices (Taube et al, 1975; Smith et al, 1975) and synaptosomal fractions of various brain regions (Smith et al, 1975; Azzaro and Smith, 1977). In similar experiments, phencyclidine was found to be 100 times more effective than ketamine in inhibiting norepinephrine uptake into brain tissue (Taube et al, 1975; Smith et al, 1977). Much higher concentrations of ketamine and phencyclidine are required to release norepinephrine from the same preparations (Taube et al, 1975; Smith et al, 1975; Smith et al, 1977). Following in vivo administration of ketamine, norepinephrine content in rat brain has been found to increase (Ylitalo et al, 1976) or decrease (Sung et al, 1973) when measured at different time periods and following different modes of ketamine administration. Phencyclidine administration has been shown to decrease norepinephrine concentration in rat brain (Leonard and Tonge, 1969) and to decrease accumulation of $^{14}$C-norepinephrine formed from $^{14}$C-tyrosine while increasing levels of normetanephrine in mouse

brain (Hitzemann et al, 1973).

Studies on the interactions of ketamine with serotonergic function include examination of the action of ketamine on metabolism and neuronal uptake of brain serotonin (5-HT). Under similar experimental conditions but in different strains of animals, administration of ketamine has been shown to both increase 5-HT turnover (Vargiu et al, 1978) and decrease 5-HT turnover (Ylitalo et al, 1976) in rat brain evidenced by changes in content of 5-hydroxyindoleacetic acid (5-HIAA). Similarly, phencyclidine has been found to either increase or decrease 5-HIAA content in rat brain, depending on the rat strain (Tonge and Leonard, 1971). However, a smaller anesthetic dose of ketamine when administered to primates did not alter the brain concentration of 5-HIAA (Bacopoulos et al, 1979). In neuronal uptake experiments, ketamine in the $10^{-5}$ molar range was shown to inhibit transport of 5-HT into synaptosomal fractions of rat brain (Azzaro and Smith, 1977) while phencyclidine was about 100 times more potent in this regard (Smith et al, 1977).

There are few studies on the interaction of ketamine with the cholinergic system while the cholinergic action of phencyclidine and some of its derivatives has been better documented. Ketamine was found to inhibit bovine and rat brain acetylcholinesterase with apparent $k_i$ values in the range of $10^{-4}$ molar and to cause a transient (1 minute) increase in acetylcholine levels in rat brain following a hypnotic dose (Cohen et al, 1974). A measurement of acetylcholine turnover rate in different regions of rat brain

during ketamine anesthesia revealed a slight reduction in turnover rates in caudate nucleus and hippocampus but no change in cortex and hypothalamic and thalamic regions (Ngai et al, 1978). In the same study no change in acetylcholine levels occurred in any brain region examined during the time of anesthesia. Finally, ketamine has been shown to exert weak anticholinergic action in ganglia preparations (Mahmoodi et al, 1980) and smooth muscle preparations (Kloog et al, 1977). Some of the studies on the cholinergic component of phencyclidine and its derivatives include reports of inhibition of acetylcholinesterase ($k_i \sim 10^{-5}$ M) from cerebral and peripheral tissue (Maayani et al, 1973; Maayani et al, 1974; Pinchasi et al, 1977), mild anticholinergic activity in isolated smooth muscle and more potent anticholinergic activity in striated muscle (Maayani et al, 1974; Kloog et al, 1977).

The most recent attempts to elucidate the biochemical events underlying the action of ketamine and its analogue, phencyclidine, include examination of their interaction with putative brain neurotransmitter receptors. In competitive binding studies with receptors in a series of neuronal systems, phencyclidine and some of its piperidine analogues were found to compete for opiate as well as muscarinic binding sites in brain tissue (Vincent et al, 1978). Recognition of the opiate and muscarinic binding sites were seen in the concentration range of 1 and 100 μM for the series of phencyclidine compounds tested. None of the phencyclidine derivatives was

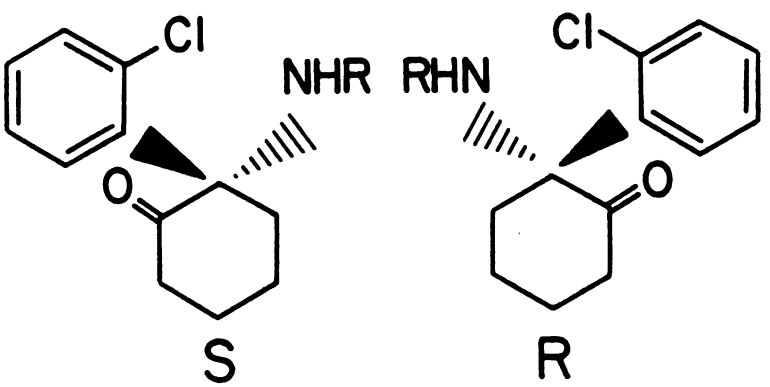
able to interact with other putative neurotransmitter receptors examined including glycine, gamma-butyric acid, glutamate, beta adrenergic, dopamine, and serotonin. Ketamine interaction with neurotransmitter receptors has been investigated at the level of the opiate receptor and was reported to inhibit specific naloxone binding at concentrations in the $10^{-5}$ molar range (Smith et al, 1980), although this finding has been disputed (Fratta et al, 1980). Recently, two independent laboratories have identified phencyclidine binding sites in the brain displaying relatively high affinity for ketamine (Vincent et al, 1979; Zukin and Zukin, 1979). However, subsequent studies suggest the phencyclidine binding sites are, in fact, artefactual reflecting methodological problems (Maayani and Weinstein, 1980).

The data compiled to date reveal ketamine perturbs a number of neuronal systems evidenced at the physiological, behavioral and biochemical level, but the relative contribution of these biochemical events to the pharmacological actions of ketamine is not clear. It would be valuable to identify the mechanism and potential sites of action of ketamine so that interaction between ketamine and other drugs may be avoided and the adverse effects of ketamine controlled. In addition, and more importantly, the elucidation of the neurochemical actions underlying the psychic effects of ketamine and other phencyclidine-like agents, reportedly reminiscent of schizophrenic psychosis (Snyder, 1980), might contribute to the understanding and subsequent

treatment of a serious mental disorder and societal problem.

## RESEARCH PROPOSAL AND ORGANIZATION

The ketamine molecule contains a chiral center at C2 of the cyclohexanone ring and as such exists as two optical isomers (figure 1-4). Clinically, ketamine is administered as a racemic mixture of the (+)- and (-)-enantiomorphs and all work cited thus far concerns the racemate. At the onset of this investigation, there was one published study in one animal species, the Sprague Dawley rat, which reported differences in the potency of the ketamine isomers in certain pharmacological actions (Marietta et al, 1977). The (+)-enantiomer was shown to be more potent as a hypnotic, had a higher therapeutic index, expressed as $LD_{50}$ over $ED_{50}$ (hypnosis), than the racemate or (-)-enantiomer, and at equihypnotic doses (+)-ketamine caused less locomotor stimulation than the (-)-isomer. In the same study, examination of the biodisposition of the ketamine compounds showed no differences between the brain levels of either of the individual isomers or the racemate after equimolar administration, suggesting potency differences were not due to pharmacokinetic factors. At the time of this report on the actions of the ketamine enantiomers there were few biochemical studies on the central neurochemical action of ketamine, apart from inhibition of reuptake of biogenic amines and inhibition of acetylcholinesterase, and no published studies on the neurochemical properties of the individual ketamine isomers.




R = $CH_3$ : Ketamine

R = H : Norketamine

figure 1-4. Enantiomeric forms of ketamine. Configuration about the asymmetric center is denoted by S and R as assigned by the Cahn-Ingold-Prelog convention (1956).

The possibility that one isomer may provide the beneficial actions of ketamine with less of the undesirable effects seemed of clinical value and worthy of further investigation. In addition, the fact that elucidation of the biodispositional and neurochemical properties of the individual isomers may help clarify the mechanism of action and relative importance of underlying biochemical events to certain pharmacological actions of ketamine provided the impetus for this research. Accordingly, the purpose of this present investigation was to study the pharmacological actions and relative potencies of the individual ketamine enantiomers and to examine the biodispositional properties and certain neurochemical events accompanying the central actions of the ketamine isomers.

Precedence for differential effects of optical isomers and the importance of stereochemical properties of drugs acting on the central nervous system are well established for agents such as narcotic analgesics (Portoghese, 1966), amphetamine (Segal, 1975), hallucinogens (Shulgin, 1973), and barbiturates (Huang and Barker, 1980), all having properties of relevance to certain pharmacological effects of ketamine. Furthermore, there is little doubt that centrally acting agents whose mechanisms of actions have been thoroughly documented, act primarily by altering some aspect of synaptic transmission be it synthesis, storage, release or inactivation of neurotransmitters or by blocking or stimulating post synaptic receptors.

The initial phase of this research involved the establishment of differences in pharmacological actions of the isomers in a species other than the rat as well as the measurement of brain and plasma levels of the parent compound, norketamine and metabolite II following administration of the individual ketamine enantiomers and the racemate. In the latter phase of this study the interaction of the isomers with synaptic transmission events was investigated at the level of the inactivation process of particular neurotransmitters and interaction with postsynaptic receptors associated with actions relevant to the central effects of ketamine. In this regard, biogenic amines have been implicated in CNS excitatory behavior, increased motor activity and psychotomimetic behavior (Schildkraut and Kety, 1967; Snyder et al, 1970; Snyder 1972); serotonin, in addition,is considered to play a role in pain perception and sleep (Costa et al, 1974). Cholinergic agents have been associated with behavioral disturbances and analgesia (Christian et al, 1971; Wray and Cowan, 1973; Maayani et al, 1974) while the employment of opiates for their analgesic actions is well established. Accordingly, the effects of the ketamine enantiomers on acetylcholinesterase activity and reuptake of the biogenic amines were examined and their interaction with opiate, muscarinic and dopamine receptors investigated in central nervous tissue. Finally, the culmination of this research included an investigation of specific binding of phencyclidine to brain tissue and an assessment of the relative potencies of the individual

ketamine enantiomers in their interaction with these putative phencyclidine receptor sites.

In summary, and more specifically, the objectives of this investigation were to: (1) determine if the reported differences in pharmacologic potency between the ketamine isomers occurred in another animal species, namely the mouse, (2) assess the relative analgesic properties of the (+)- and (-)-isomers which had not been examined previously, (3) compare the relative analgesic potencies of the enantiomers with their hypnotic and locomotor activities, (4) examine the influence of opiate and muscarinic antagonists on ketamine analgesia, (5) determine the possible role of biodisposition events in observed differences in the pharmacological potencies of the optical isomers of ketamine, (6) compare the interaction of the ketamine enantiomers with brain opiate, muscarinic and dopamine receptors, (7) determine the influence of the individual ketamine enantiomers on brain acetylcholinesterase activity and on reuptake of norepinephrine, dopamine and serotonin into mouse brain synaptosomes, (8) compare the ability of the ketamine isomers to interact with specific phencyclidine receptor sites in brain homogenates, and (9) relate brain concentrations of ketamine achieved *in vitro* with inhibitory concentrations determined *in vitro*.

# CHAPTER TWO: EXPERIMENTAL METHODS

## ASSESSMENT OF PHARMACOLOGICAL EFFECTS AND BIODISPOSITION OF KETAMINE

The pharmacological properties of the individual ketamine enantiomers and racemic ketamine were compared by examining their action on analgesia, hypnosis and locomotor activity. To determine the possible role of biodisposition events in observed differences in pharmacological potencies of the ketamine isomers, *in vivo* brain and plasma levels of ketamine and its major metabolite, norketamine, were measured following an analgesic dose of the individual isomers and racemate and following a hypnotic dose of racemic ketamine.

### Animals and Chemicals

Male ICR mice weighing 20-30g (Simonsen Laboratories, Gilroy, CA) housed six to a cage under automatically controlled humidity, temperature and lighting conditions (on 0600, off 1800) were used in all experiments. The animals were allowed free standard laboratory diet and water *ad libitum*. All studies were performed between 0800 and 1800 hours and the animals were placed in the experimental laboratory at least 30 minutes prior to testing each day.

All drug solutions were prepared the day of use. A 0.02% solution of phenylquinone (2-phenyl-1,4-benzoquinone, from Sigma Chemical Co., St. Louis, Mo.) was dissolved in a 5% ethyl alcohol aqueous solution and maintained at 37° C. Naloxone HCl (gift of Endo Laboratories Inc., Garden City, N.Y.), atropine sulfate (Sigma Chemical Co., St. Louis,Mo.), racemic ketamine HCl (gift of Parke, Davis and Co., Detroit,

Mich.) and the ketamine isomers (gift of Bristol Laboratories, Syracuse, N.Y.) were dissolved in saline and injected in a volume of 3 ml/kg. The purity of the crystalline salts of the ketamine isomers was determined using optical rotation with $[\alpha]_D^{25}$= +92.48 (c200, water) and $[\alpha]_D^{25}$ = -91.88 (c 200, water). Both compounds melted at 259-261° C. All other chemicals were of reagent grade. Sources of compounds for the gas chromatographic assay have been detailed by Cohen (1973). The concentrations of naloxone HCl and atropine sulfate are expressed in salt form while those of racemic ketamine and its isomers are in terms of the free base.

Assessment of Analgesia and Effect of Naloxone and Atropine

Ketamine analgesia was examined by use of the phenylquinone writhing test as described by Siegmund et al (1957). After intraperitoneal injection of phenylquinone, the mouse shows a response consisting of a wave of constriction and elongation passing caudally along the abdominal wall, followed by extension of the hind limbs. The ability of a substance to suppress this phenylquinone elicited abdominal constriction is considered a measure of its analgesic activity and correlates with analgesic potency in humans (Pearl and Harris, 1966; Collier et al, 1968).

Mice were injected i.p. with 0.25 ml of phenylquinone and 1.5 - 2 minutes later were injected s.c. in the neck region with either (±)-ketamine, one of its isomers or saline (3 ml/kg). The number of writhes was recorded for each animal over a 10 minute period 5 minutes after administration

of phenylquinone. This test was performed with one mouse per cage and one observer per maximum of three cages with the observer uninformed of the test agents administered. At least 9 animals were employed per dose; animals which did not writhe within an hour after administration of phenylquinone were not used in the calculations. To control for variations in response throughout the day and differences in sensitivity with different batches of animals paired experiments were always performed using a saline injected group as the control.

In experiments to determine the effect of naloxone on ketamine analgesia the same procedures were used as described above except that naloxone or saline (3 ml/kg) was injected s.c. in the hindquarters immediately following administration of phenylquinone. Similar procedures were used to examine the effect of atropine on ketamine analgesia except atropine (or saline) was administered 10 minutes prior to phenylquinone. A minimum of 7 animals was observed for each test drug procedure and writhing was observed as noted previously.

Assessment of Hypnosis

The loss of the righting reflex for a minimum of 10 seconds was used as the measurement of hypnosis for drugs studied in this investigation. To determine the median effective hypnotic dose all drugs were administered s.c. and a total of 5 doses and 10 animals per dose were used for each test drug. The duration of loss of the righting reflex was defined as the time interval between the end

of drug administration and recovery of the righting reflex.

Assessment of Locomotor Activity

Locomotor activity was recorded with a photocell-type electronic motility meter (Motron Produkter Model Fc40, Stockholm, Sweden). Each mouse was injected with the test drug or saline and immediately placed in an individual clear plastic cage on the motility meter and the locomotor activity was recorded at 5 minute intervals for 30 minutes following injection. The doses and drugs were administered randomly throughout the day with each drug injected animal either preceded or followed by a saline injected animal. At least 6 animals per dose were utilized for each test drug.

In Vivo Assay for Ketamine, Norketamine and Metabolite II

A highly sensitive assay for ketamine, the N-demethylated metabolite (norketamine) and Metabolite II in plasma was developed by Chang and Glazko (1972) based upon gas-liquid chromotography of a heptafluorobutyryl derivative utilizing an electron capture detector. Cohen et al (1973) adapted and modified this procedure for estimating ketamine and norketamine in plasma as well as cerebral tissue. It is this modified version which was utilized in the present study.

Following administration of the individual ketamine isomers or the racemate the animals were decapitated at various time intervals. Upon decapitation heparin treated blood samples were obtained by exsanguination and whole brains were removed and homogenized in 0.1N HCl (10% w/v).

Plasma samples were obtained by centrifuging the blood samples at 1000 x g for 10 minutes and brain supernatant samples were collected by centrifuging the brain homogenates for 90 minutes at 40,000 x g. Both samples were assayed for ketamine and its metabolites as described below.

Aliquots of plasma and brain supernatant were placed in glass stoppered conical centrifuge tubes and 0.1N HCl and deionized water were added to a final volume of 2 ml and pH equivalent to 0.5N HCl. After the addition of the internal standard (2-amino-2-(o-bromophenyl)-2-(methylamino)-cyclohexanone) to a final concentration of 0.25 g/ml, the tubes were vortexed. Five ml of benzene and 0.1 ml of a 10% borax buffer were added and hand shaken for 20 seconds and the tubes were centrifuged for 30 minutes at 1750 rpm. Ketamine, norketamine, Metabolite II and the internal standard partitioned into the benzene layer and this layer was pipetted into clean glass stoppered conical centrifuge tubes for derivatization. Following the addition of heptafluorobutyric anhydride (0.1 ml) and pyridine (0.1 ml) to the benzene extract, the stoppered tubes were hand shaken vigorously for 5 seconds and allowed to stand at room temperature for 30 minutes with additional vortexing every 10 minutes. Two ml of 0.5N NaOH was added to each tube to remove excess reagent and the tubes were shaken for 5 minutes. The aqueous phase was removed with a pipette, 2 ml of 0.25N HCl added and the resulting mixture shaken for 5 minutes. The aqueous phase was again removed after centrifugation

at 1750 rpm for 5 minutes. One gram of anhydrous sodium sulfate was added to the remaining solution, the tubes were vortexed and 0.5 to 2 μl of each tube was injected into a GLC column for analysis of ketamine and its metabolites.

Gas chromatographic apparatus and conditions were similar to those described in previous publications (Marietta, 1976; Cohen, 1973). Standard curves based on peak height ratios of standards to internal standard were linear for concentrations of ketamine ranging from 25 to 300 ng per ml and concentrations of norketamine and Metabolite II from 12.5 to 150 ng per ml. Subsequent samples were diluted such that the concentration of ketamine, norketamine and Metabolite II were within the linear portion of their respective standard curves. Recovery of ketamine and its metabolites from known standard solutions in plasma and brain homogenates ranged from 95-99% and duplicate assays of samples varied by less than 3%.

Data Analysis

Log (dose) response curves were plotted on probability paper and the median analgesic dose ($AD_{50}$) and median hypnotic dose ($HD_{50}$) and their 95% confidence limits (C.L.) were estimated by the method of Litchfield and Wilcoxon (1949). The potency ratios (P.R.) and 95% confidence limits of the isomers for analgesia and hypnosis as well as the equi-analgesic doses ($AD_{80}$) of the isomers were also determined by this method. A least squares linear regression was utilized to graph the 30 minute cumulative locomotor activity

data of each isomer. The regression lines were tested for parallelism using a modification of the Student's t test for regression lines as described by Zar (1974). The estimation of potency ratio (P.R.) for locomotor activity was calculated by a procedure described by Finney (1964) for comparison of regression equations and the 95% confidence limits were found by applying Fieller's theorem. Statistical differences between the means of two groups were evaluated by the Student's unpaired t test and P values less than 0.05 were judged significant.

RECEPTOR BINDING ASSAYS

Comparative binding studies were undertaken to compare the possible interaction of the ketamine enantiomers with a series of central nervous system receptors. Rapid filtration methods were employed to assess the effect of the isomers on the specific binding of $^{3}$H-naloxone (opiate), $^{3}$H-quinuclidinyl benzilate (muscarinic) and $^{3}$H-spiroperidol (dopaminergic) to brain tissue. In addition, the specific binding of $^{3}$H-phencyclidine to rat and mouse brain homogenates and the effect of the ketamine agents on this radioligand binding was investigated.

Chemicals

$^{3}$H-naloxone (15.2 Ci/mmol), $^{3}$H-dl-quinuclidinyl benzilate (29.4 Ci/mmol), $^{3}$H-spiroperidol (23.6 Ci/mmol) and $^{3}$H-phencyclidine (48.0 Ci/mmol) were purchased from New England Nuclear (Boston, Mass.), were used without further

purification and were stored at -4°C in ethanol solution. The drugs were obtained as follows: atropine (Sigma Co.), oxotremorine (Aldrich Chem. Co.), carbamylcholine (Sigma Co.), phencyclidine HCl (Philips Roxane, Inc., St. Joseph, Mo.), racemic ketamine HCl (gift of Parke, Davis and Co., Detroit, Mich.), ketamine isomers (gift of Bristol Lab., Syracuse, N.Y.), levorphanol and dextrorphan (gift of Roche), and apomorphine (Merck and Co.). The purity of the ketamine isomers was determined as described previously in the chemicals section of assessment of pharmacological effects. All other chemicals were obtained commercially and were reagent grade.

$^{3}$H-Naloxone Binding Assays

In 1973, Pert and Snyder described the specific binding of $^{3}$H-naloxone to brain homogenates and demonstrated that the stereospecific binding of $^{3}$H-naloxone was inhibited by opiates with potencies relative to their pharmacological activities *in vivo*. The opiate binding assays in this investigation were done as described by Pert and Snyder (1973) with minor modifications, using naloxone as the radiolabelled ligand.

Male ICR mice (25-30g) were decapitated, brains rapidly removed and the cerebella were excised. The brains were homogenized in a 30 to 1 volume of 50 mM Tris, pH = 8 at room temperature, by 8 strokes of a motor driven Teflon pestle in a Thomas glass homogenizer. After the homogenate was centrifuged at 18,000 x g for 20 minutes, the supernatant

fluid was discarded and the pellets were reconstituted in a 30 to 1 volume of Tris buffer by 4 strokes of the motor driven pestle.

The binding assay was performed by placing the following aliquots in one and one-half ml polyethylene microcentrifuge tubes: 0.8 ml of freshly prepared homogenate (added last), 0.1 ml $^{3}$H-naloxone (final concentration, 6.7 nM), 0.1 levorphanol or drug or buffer. After the tubes were incubated at 4°C for 2 hours, the contents of the tubes were filtered under vacuum through a glass fiber filter (GF/B, Whatman, 2.4 cm). The filters were washed three times under vacuum with 5 ml ice cold buffer. The filters were placed in plastic scintillation vials, 8 ml of ScintiVerse was added to each vial, each sample was vortexed for 10 seconds and the vials were stored at 25°C for 8 to 12 hours. The radioactivity of the samples was determined by a liquid scintillation spectrometer (Packard, Tri-Carb, Model 3375) at a counting efficiency of 20 %. Each determination was done in triplicate and the experiments were repeated at least three times.

The protein in each homogenate was measured by the method of Lowry et al (1951) using bovine serum albumin as the standard. The protein concentration was estimated to be 1.7 to 2.4 mg/ml. Specific binding of $^{3}$H-naloxone was defined as the difference between the radioactivity bound in the absence of levorphanol and the radioactivity bound in the presence of 4 μM levorphanol.

## $^{3}$H-Quinuclidinyl Benzilate Binding Assays

Quinuclidinyl benzilate (QNB) is a potent muscarinic antagonist in the central (Albanus, 1970; Meyerhoffer, 1972) and peripheral (Becker, 1964) nervous system. This compound has been shown to bind with high affinity and specificity in brain homogenates (Yamamura and Snyder, 1974) and was used as the ligand for the muscarinic binding assays in this investigation.

The $^{3}$H-QNB binding assays were performed as described by Yamamura and Snyder (1974) using mouse brain homogenates. Male ICR mice (24-30g) were decapitated and their brains rapidly removed. The cerebella were excised and the brains were homogenized in a 10 to 1 volume of ice cold 0.32M sucrose by 10 strokes of a motor driven Teflon pestle in a Thomas glass homogenizer. The homogenate was centrifuged at 1000 x g for 10 minutes. The pellets were discarded and the supernatant was homogenized in a final volume of 30 to 1 of 0.05M potassium phosphate buffer, pH = 7.4, by 5 strokes of the motor driven pestle.

The following aliquots were placed in one and one-half ml polyethylene microcentrifuge tubes: 0.2ml of fresh brain homogenate (added last), 0.1 ml $^{3}$H-QNB (final concentration, 0.4nM), 0.1 ml oxotremorine or drug or buffer and additional buffer to bring total volume to 1 ml. After a 60 minute incubation period at 25$^{o}$C the contents of each tube were filtered under vacuum through a glass fiber filter (GF/B Whatman, 2.4 cm). The filters were washed three times

under vacuum with 4 ml ice cold buffer. The filters were placed in liquid scintillation vials, 7 ml of ScintiVerse was added, each vial was vortexed for 10 seconds and the samples were stored at 25°C for 8 to 12 hours. The radioactivity of the samples was determined by liquid scintillation spectrometry (Packard, Model 3375, Tri-Carb Scintillation Spectrometer) at a counting efficiency of 20%. Each determination was done in triplicate and the experiments were repeated at least 4 times.

The protein in each homogenate was measured by the Lowry method (1951) using bovine serum albumin as the standard. The protein concentration was estimated to be 0.18 to 0.27 mg/ml. Specific binding of $^{3}$H-QNB was defined as the total amount of $^{3}$H-QNB bound minus the amount of $^{3}$H-QNB bound in the presence of 100 μM oxotremorine.

$^{3}$H-Spiroperidol Binding Assays

In order to investigate the interaction of ketamine with the dopamine receptor, spiroperidol was the ligand selected for the competitive binding experiments. Spiroperidol, a potent butyrophenone neuroleptic, is reported to have the highest affinity for dopamine receptors in brain tissue than any other known drug (Burt et al, 1976). Inhibition studies have shown that the relative potencies of numerous dopamine agonists and antagonists in competing for $^{3}$H-spiroperidol binding correlate with their pharmacological profile in intact animals (Fields et al, 1977; Creese et al, 1977; Ladura et al, 1978).

$^{3}$H-Spiroperidol binding experiments were done on crude homogenates of calf caudate nucleus as described by Seeman et al (1976). Calf brains were obtained from a local packer's plant. The caudates were removed within 2 hours after death, pooled, sliced into cubes and suspended in buffer (TEAN) at an approximate 50 mg concentration (wet weight) per ml of buffer. TEAN buffer contained 15 mM Tris HCl, 5 mM $Na_2EDTA$, 1.1 mM ascorbic acid, and 12.5 uM nialamide at pH = 7.4. A preliminary crude homogenate was made using 20 strokes of a motor driven Teflon pestle in a Thomas glass homogenizer. The crude homogenate was incubated at 37$^{o}$C for 60 minutes and stored in 3 ml aliquots at -20$^{o}$C for up to 2 months. Immediately prior to each assay, the samples were thawed, resuspended in a ground-glass homogenizer (10 strokes) by hand, and centrifuged at 24,500 x g for 15 minutes. The supernatant was discarded and the pellets resuspended in 5 ml of TEAN buffer. The suspension was finally homogenized by 5 strokes of the motor driven pestle.

The following aliquots were placed in one and one-half ml polyethylene microcentrifuge tubes: 0.2ml $^{3}$H-spiroperidol (final concentration = 0.8 nM), 0.1 ml of drug or apomorphine or buffer, 0.1 ml buffer and 0.2 ml brain homogenate (added last) to bring total volume to 0.6 ml. After 30 minutes incubation at 25$^{o}$C, a 0.5 ml aliquot was removed from each vial and filtered under vacuum through a glass fiber filter (GF/B Whatman, 2.4 cm). The filter was washed twice with

7.5 ml of ice cold TEAN buffer and the filters were placed in liquid scintillation vials, 8 ml of ScintiVerse was added and the samples were stored at 25°C for 6 or more hours. Each determination was done in triplicate and repeated three or more times. The radioactivity of the samples was measured by liquid scin tillation spectrometry (Packard, Model 3375, Tri Carb Scintillation Spectrometer) at a counting efficiency of 20%.

The protein in each suspension was estimated by the Lowry method using bovine serum albumin as the standard and was approximately 1.5 mg/ml. Specific binding was defined as the total amount of $^{3}$H-spiroperidol bound minus the amount of $^{3}$H-spiroperidol bound in the presence of 5 mM apomorphine.

$^{3}$H-Phencyclidine Binding Assays

The effects of phencyclidine, racemic ketamine and the ketamine isomers on $^{3}$H-phencyclidine binding in rodent brain homogenates were examined by use of the method described by Zukin and Zukin (1979). Male Sprague Dawley rats (150-200 g) or male ICR mice (24-30 g) were decapitated, brains rapidly removed and homogenized in 33 to 1 volume of 50 mM Tris HCl, pH = 7.4 (4°C) with a Brinkmann Polytron (setting 5 for 45 sec). After the homogenates were centrifuged for 15 minutes at 30,000 x g, the pellet was resuspended in 100 volumes of 50 mM Tris HCl and centrifuged again at 30,000 x g for 15 minutes. The pellet was reconstituted in 100 volumes of the same buffer and the protein was measured by the method of Lowry et al (1951) using bovine serum

albumin as the standard. The protein concentration was estimated to be 0.45-0.60 mg protein per ml.

Aliquots of freshly prepared homogenate, 7.0 nM $^{3}$H-phencyclidine and 0.1 mM cold phencyclidine or drug or buffer were incubated in triplicate at 4°C for 45 minutes. A 1 ml aliquot was then filtered under vacuum through a glass fiber filter (GF/B Whatman, 2.4 cm) and the filter was rapidly washed with two aliquots of 10 ml of ice cold Tris HCl (pH=7.4, 4°C). The filters were placed in liquid scintillation vials, Aquasol was added and samples were counted in a Packard, Model PL, PRIAS, Liquid Scintillation Spectrometer after 8-12 hours. Each determination was repeated at least twice.

Data Analysis

$IC_{50}$ values were determined using logarithmic-probability paper (Litchfield and Wilcoxon, 1949) employing at least 5 concentrations of each drug. Student's t test for unpaired data was used to evaluate the difference between the means and P values less than 0.05 were judged significant.

ACETYLCHOLINESTERASE INHIBITION EXPERIMENTS

The inhibitory properties of the individual ketamine isomers on mouse brain acetylcholinesterase action were studied by employing a photometric method developed by Ellman et al. (1961). Acetylcholinesterase activity was followed by measuring the increase in color caused by a product of the enzymic hydrolysis of acetylthiocholine

represented by the following reaction:

acetylthiocholine $\xrightarrow{\text{(enzyme)}}$ thiocholine + acetate

thiocholine + dithiobisnitrobenzoate $\longrightarrow$ yellow color

Chemicals

Acetylthiocholine and 5',5'-dithio-bis-2-nitrobenzoic acid were purchased from Sigma Co. Phencyclidine HCl was obtained from Philips Roxane, Inc., St. Joseph, Miss. and racemic ketamine was a gift of Parke, Davis and Co. The ketamine enantiomers were synthesized by a procedure involving recrystallization of the (+)- and (-)-tartaric acid salts as described by U.K. Patent Specification #1,330,878, Bristol Meyers Corp., Ketamine Resolution, 1973. The purity of the crystalline salts thus formed was determined using optical rotation with $[\alpha]_D^{25}$ = + 90.50 (c=2.00, water), a melting point of 260-261°C and $[\alpha]_D^{25}$ = - 94.50, melting point of 263-264°C. All other chemicals were of reagent grade.

Enzyme Assay

Acetylcholinesterase activity was studied in the unpurified mouse brain homogenate using acetylthiocholine as the substrate. Male ICR mice (23-30 g) were decapitated, brains rapidly removed and homogenized in 30 volumes of ice cold 0.1M phosphate (Na) buffer, pH = 7.0, by 10 strokes of a motor driven Teflon pestle in a Thomas glass homogenizer. The homogenate was further diluted 1 : 2 with the phosphate buffer and 0.2 ml aliquots of this enzyme solution were used in the standard assay. The reaction mixture contained 0.01N 5'5'-dithiobis-2-nitrobenzoic acid (DTNB),

acetylthiocholine (ATC) at varying concentrations, drug at varying concentrations, enzyme solution, and 0.1M phosphate buffer to a final volume of 3 ml. Tubes containing DTNB, drug, enzyme and buffer were allowed to equilibrate at 25°C for 10 minutes and the reaction was then started by adding ATC. The rate of the reaction was determined in duplicate in four or more experiments on a Gilford (Stasar II) spectrophotometer at 412 mu. Specific activity is expressed as moles ATC hydrolyzed per mg protein per minute after subtraction of non-enzymatic hydrolysis of acetylthiocholine. Protein was estimated by the Lowry method using bovine serum albumin as the standard and ranged from 2.9 mg to 3.4 mg/ml.

Data Analysis

The kinetics of the reaction was analyzed by Lineweaver-Burk plots using initial velocities obtained over a substrate concentration range of 0.031 mM - 1.0 mM (Lineweaver-Burk, 1934). The kinetic constants were calculated by least squares regression analysis. The apparent $K_i$ for each ketamine enantiomer was determined by use of slope ratios at three inhibitory concentrations by a method described by Webb (1963) and was confirmed by direct observation of Hunter and Downs plots (1945). The Dixon method was employed to estimate the apparent $K_i$ for phencyclidine. The apparent $IC_{50}$ values were calculated by logarithmic-probability (% inhibition) paper using at least four concentrations of each drug in the presence of 0.1 mM substrate.

## SYNAPTOSOMAL UPTAKE EXPERIMENTS

Experiments were performed to examine the action of the ketamine enantiomers on the accumulation of norepinephrine, dopamine and serotonin in synaptosomal preparations of mouse cerebral tissue. In general, the method used in this study involved incubating the *in vitro* brain preparations with radiolabeled substrates for short periods of time and subsequently measuring the amount of substrate taken into the synaptosomes in the presence and absence of the test drug.

### Chemicals

$^{3}$H-Dopamine (15.4 Ci/ mmol), $^{3}$H- dl-norepinephrine (15.2 Ci/ mmol) and $^{3}$H-serotonin binoxalate (29.7 Ci/mmol) were obtained from New England Nuclear (Boston, Mass.). The isomers of ketamine were synthesized and tested for purity as described previously in the acetylcholinesterase experiments. Other chemicals were obtained from commercial sources and were of reagent grade.

### Reuptake Assay

Synaptosomal uptake activity was measured by a modification of the method of Coyle and Snyder (1969). Male ICR mice (23 - 30 g) were decapitated and brains rapidly removed. The brains were homogenized in 10 volumes of ice cold 0.32M sucrose by 10 strokes of a motor driven Teflon pestle in a Thomas glass homogenizer. The homogenates were centrifuged at 1000 x g for 10 minutes (0 - 4$^{o}$C) to sediment debris and nuclei (Whittaker, 1965). The precipitate was discarded

and the supernatant (synaptosomal suspension) was gently stirred and put on ice. A 0.2 ml aliquot of this synaptosomal suspension was added to a Krebs-Henseleit bicarbonate solution (Krebs and Henseleit, 1932) in 15 ml Cores (#8411) centrifuge tubes in an ice water bath. The Krebs-Henseleit solution was prepared fresh daily and gassed 15 minutes prior to use (95% $O_2$, 5% $CO_2$). The Krebs-Henseleit solution included mM/l: 118 NaCl, 4.69 KCl, 1.3 $CaCl_2 \cdot 2H_2O$, 1.18 $MgSO_4$, 1.19 $KH_2PO_4$, 25.0 $NaHCO_3$, 0.04 $Na_2EDTA$, as well as glucose ( 2 mg/ml), ascorbic acid (0.2 mg/ml) and nialamide (final concentration = $7.5 \times 10^{-5}$ M).

Varying concentrations of the ketamine enantiomers were added to the synaptosomal buffer solutions and pre-incubated for 5 minutes at 37°C in a Dubnoff metabolic shaker. At the end of this period varying concentrations of $^3$H-dopamine (5 - 40 nM), $^3$H-norepinephrine (5 - 40 nM) and $^3$H-serotonin (1 - 20 nM) were added to the tubes in the incubation bath to make a final volume of 4 ml and the incubation continued for 5 minutes. The incubation was terminated by placing the samples in an ice-water bath for a few minutes, followed by centrifugation at 10,000 x g for 15 minutes in a refrigerated (0 - 4°C) centrifuge. The incubation medium in each tube was aspirated and the surface of the pellet was washed with 5 ml of 0.9% ice cold saline. After 0.5 ml Soluene-100 (Packard Instrument Co., Inc., Downers Grove, ILL.) was added to each tube, the samples were placed in an oven (40°C) until the pellets were dissolved. Five ml of DIMILUME

(Packard Instrument Co.) was added to each tube, the samples were vortexed and placed in glass scintillation vials. Radioactivity was measured in a Packard, Model PL, PRIAS, Liquid Scintillation Spectrometer.

All assays were performed in duplicate and repeated four to six times. The non-specific uptake of the radioactive amines was determined by keeping matched control samples on ice. The specific amine uptake was calculated by subtracting the radioactive uptake (0 - $4^{o}$) from the uptake at $37^{o}$. The protein in each homogenate was estimated by the Lowry method using bovine serum albumin as the standard and ranged from 0.58 to 0.68 mg/kg.

Data Analysis

$IC_{50}$ values of the ketamine enantiomers were determined by using logarithmic-probability paper (Litchfield and Wilcoxon, 1949) using four to five concentrations of each isomer in the presence of 10 nM of each biogenic amine. The kinetic nature of the inhibitory action of ketamine on the uptake of the amines was analyzed by Lineweaver-Burk plots (Lineweaver and Burk, 1934). Kinetic constants were obtained by least squares regression analysis. The inhibitory constants ($k_i$) of the isomers were determined with the method of Dixon (1954) by examining the uptake of two concentrations of the biogenic amines in the presence of four to five concentrations of each isomer.

# CHAPTER THREE: EXPERIMENTAL RESULTS

Analgesia and Hypnosis

The analgesic action of racemic ketamine and its isomers was determined by the phenylquinone writhing test and the dose-response relationships for each drug are shown in figure 3-1. The potency of the racemic mixture was intermediate between the individual isomers with the (+)-isomer showing the greater analgesic potency. The analgesic doses necessary to inhibit writhing by 50% ($AD_{50}$) for the (+)-isomer, racemic ketamine and (-)-isomer were 3.7, 6.5 and 11 mg/kg s.c. respectively (Table 3-1). A comparison of doses necessary to cause hypnosis in 50% of the animals indicated the same rank order of potency between the three drugs but a calculation of the potency ratios shows the (+)-enantiomer to be 3 x more potent in terms of analgesia and only 1.5 x more potent in the induction of hypnosis than the (-)-enantiomer (Table 3-1). Hypnosis was evaluated by observing the loss of the righting reflex for a minimum of 10 seconds and, typically, the onset of hypnosis occurred between 2 and 6 minutes after subcutaneous injection of the drugs. It is apparent that significant analgesia can be achieved at doses well below those required to attain hypnosis with the (+)-isomer giving the widest margin for such effects. Accordingly, in calculating the $HD_{50}/ AD_{50}$ ratios for each drug, the margin can be characterized by values of 10, 6.9 and 5.1 for the (+)-isomer, racemic ketamine and the (-)-isomer respectively.

It should be noted that in an attempt to determine

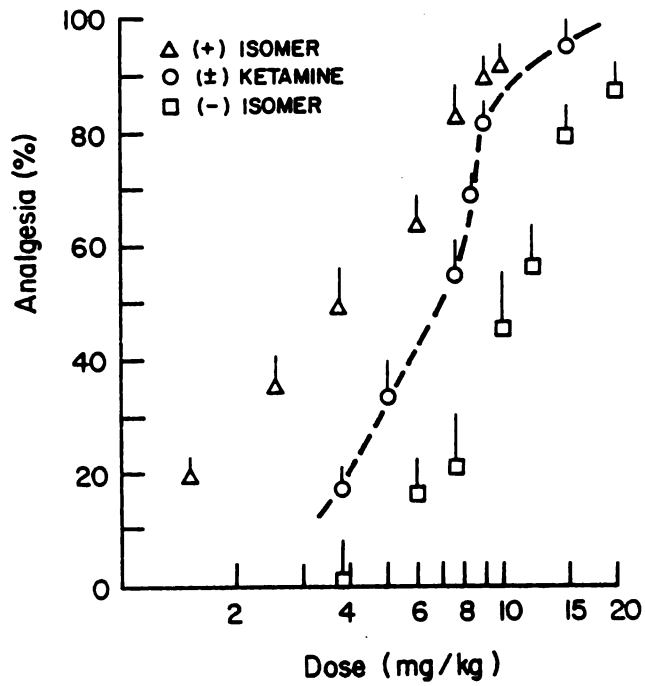



figure 3-1. The analgesic actions of (±)-ketamine and its enantiomers in mice measured by the phenylquinone writhing test. Beginning 5 min after injection of phenylquinone, the number of writhes for each animal was recorded over a 10 min period. Either saline or (±)-ketamine or one of its isomers was administered s.c. 1.5 - 2 min after phenylquinone. Percent analgesia was determined by calculating the % inhibition of writhes compared to the controls. Each point represents the mean (± S.E.) of at least 9 experiments.

TABLE 3-1

(A) The median effective analgesic and hypnotic doses of (±) ketamine and its isomers in mice[1].

(B) The potency ratios for analgesia and hypnosis of the isomers[1].

| (A) Drug | $AD_{50}$[2] mg/kg s.c. (95% C.L.) | $HD_{50}$[3] mg/kg s.c. (95% C.L.) |
|---|---|---|
| (-)-isomer | 11 (9.2-13) | 56 (46-68) |
| (±)-ketamine | 6.5 (5.2-8.1) | 45 (41-49) |
| (+)-isomer | 3.7 (2.6-5.2) | 38 (35-41) |

| (B) | Analgesia | Hypnosis |
|---|---|---|
| Potency ratio[4] | 3.0 (2.1-4.3) | 1.5 (1.2-1.9) |

[1] The median effective doses, confidence limits and potency ratios were determined by the Litchfield-Wilcoxon test (1949).

[2] Analgesic dose necessary to inhibit writhing by 50% in the phenylquinone writhing test. 6-7 doses and at least 9 animals per dose were used for each drug.

[3] Hypnotic dose necessary to cause loss of the righting reflex in 50% of the animals. 5 doses and 10 animals per dose were used for each drug.

[4] Potency ratio is the relative potency of the (+)-isomer compared to the (-)-isomer.

the lethal dose by subcutaneous administration of these drugs, it became apparent that the dosages necessary to achieve lethality were too high for the amount of the isomers available for our experimental purposes. In the case of racemic ketamine no lethalities were observed in 10 animals at 250 mg/kg s.c., while one lethality in 8 animals occurred after the administration of each isomer using the same dose. Further experimentation with racemic ketamine indicated a $LD_{50}$ greater than 400 mg/kg s.c. for this drug.

An assessment of duration of hypnosis by measuring the duration of the loss of the righting reflex following equimolar doses (25 mg/kg) by intravenous administration of each isomer and the racemate revealed hypnosis was almost 3 x longer after (+)-ketamine administration than that caused by (-)-ketamine. The length of hypnosis in minutes $\pm$S.E.M. was 1.9 $\pm$ .33, 4.0 $\pm$ .49 and 5.3 $\pm$ .72 for (-)-ketamine, racemate and (+)-ketamine respectively.

Locomotor Activity

In comparing locomotor activity of the two isomers the (+)-enantiomorph was shown to be the most potent. Five dosages selected for each isomer for comparison of activity were dosages which were found to cause 50 - 90% analgesia in the writhing test. A dose dependent increase in locomotor activity was observed after subcutaneous administration of each drug. After subtraction of the paired saline controls a least squares linear regression was utilized to graph 30 minute cumulative activity data (figure 3-2). The slopes

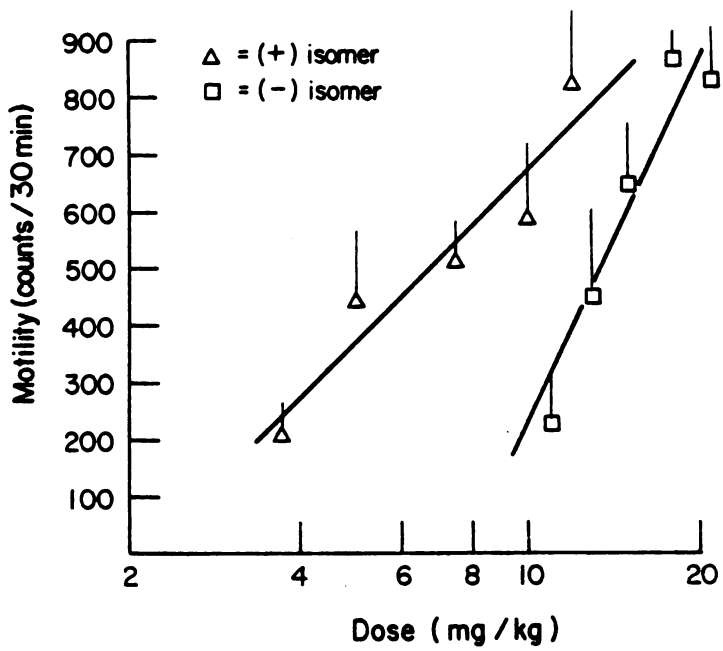



figure 3-2. Locomotor activity following s.c. adminstration of (+)- and (-)-ketamine. Motility meter activity was recorded for individual mice for 30 min subsequent to injection of the drug. Activity is expressed as total counts per 30 min after subtraction of the activity of a paired saline control. The equation of the regression line for the (-)-isomer is $y = 2192 \log x - 1987$, $r = 0.96$; for the (+)-isomer the equation is $y = 990 \log x - 331$, $r = 0.94$. Each point represents the mean ($\pm$S.E.) of at least 6 experiments.

of the regression lines were not significantly different ( .10 < P < .20 ) and the potency ratio was estimated to be 1.8 ( 1.4 - 2.3 C.L. ).

Considering the three-fold difference in analgesia between the isomers it seemed plausible that the (+)-isomer could elicit less locomotor stimulation than the (-)-isomer during an equivalent analgesic response. The results of such an experiment are represented in figure 3-3 where the locomotor activity at an equianalgesic dose ( $AD_{80}$ ) of the enantiomers is plotted; in this case saline control activity is not subtracted from the drug response. (+)-Ketamine caused significantly less locomotor stimulation at two time intervals. Additionally, the (+)-enantiomer was observed to cause less total stimulation of locomotor activity over the 30 minute observation period ( 766 ± 77 vs. 1128 ± 48, P < .01 ).

Effect of Naloxone and Atropine on Ketamine Analgesia

Subcutaneous administration of 10 mg/kg naloxone, 1-2 minutes prior to the injection of (±)-ketamine, reversed the analgesic action of ketamine in the phenylquinone writhing test (Table 3-2). A lower dose of naloxone (5 mg/kg) did not significantly antagonize ketamine analgesic activity and naloxone alone did not affect the writhing response.

Similar procedures were employed to evaluate the effect of atropine on ketamine analgesia, except atropine was injected 10 minutes prior to phenylquinone. Atropine pretreatment

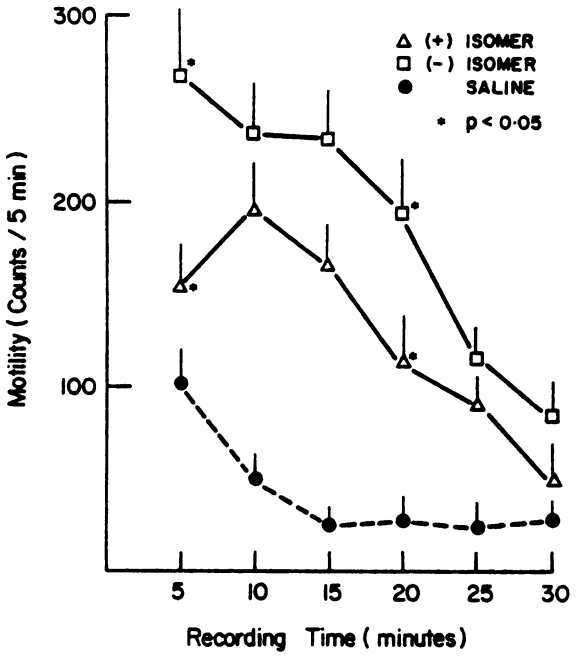



figure 3-3. Locomotor activity following equianalgesic ($AD_{80}$), s.c. administration of (+)-ketamine (7.5 mg/kg), (-)-ketamine (18 mg/kg) and saline (3 ml/kg). Motility meter activity was recorded for individual mice during each of 6 consecutive 5 min intervals subsequent to injection of the drug or saline. Activity is expressed as counts per 5 min interval. Each point represents the mean (±S.E.) of 6 experiments. *P< 0.05.

TABLE 3-2

Effect of naloxone on (±)-ketamine analgesia in mice measured by the phenylquinone writhing test[1].

| Dose (mg/kg) naloxone | Saline + saline | Saline + ketamine (% analgesia) | Naloxone + ketamine (% analgesia) | Naloxone + saline |
|---|---|---|---|---|
| 5 | 42 ± 3 | 14 ± 2(67) | 17 ± 2(60) | 43 ± 1 |
| 10 | 33 ± 3 | 12 ± 3(64) | 28 ± 4[2](14) | 32 ± 5 |

[1] The means (±S.E.M.) represent number of writhes over a 10 min period for at least 7 animals. Ketamine dose was 10 mg/kg s.c. Significant difference of means was determined by Student's unpaired t-test. See Experimental Methods Chapter for description of procedure.

[2] Significantly different from ketamine + saline, $P < 0.01$, and not significantly different from control (saline + saline), $P=0.35$.

did not affect the writhing activity or ketamine analgesic action in a significant manner at any of the doses of atropine examined (Table 3-3).

Brain and Plasma Levels of Ketamine, its Isomers and Metabolites

The brain and plasma levels of the three parent compounds, metabolite I (norketamine) and metabolite II were ascertained at various time intervals after subcutaneous administration of 7 mg/kg of (±)-ketamine and its isomers. Metabolite II comprises the sum total of hydroxylated products arising from ketamine and norketamine metabolism (see Introduction). In order to relate the disposition data to analgesia it was necessary to eliminate the possibility that the phenylquinone treatment was influencing the disposition or metabolism of racemic ketamine or the optical isomers. Therefore, animals were injected with either saline or phenylquinone prior to administration of ketamine in the same manner used in the analgesia testing experiments. The results of these studies indicated no differences in brain and plasma levels of the test drugs or metabolites in the presence or absence of phenylquinone. The results shown in figures 3-4 through 3-7 are from the saline treated animals.

With the exception of the 10 minute time point where the level of the (-)-isomer was elevated in the brain, the levels of the three parent drugs were similar in the plasma and brain at the time intervals measured and the peak levels occurred near 5 minutes after administration of each agent (figures 3-4, 3-5). This is in contrast to the pharmaco-

TABLE 3-3

Effect of atropine on (±)-ketamine analgesia in mice measured by the phenylquinone writhing test[1]

| Atropine (mg/kg) | (n) | Saline + saline | (n) | Saline + ketamine | (n) | Atropine + ketamine | (n) | Atropine + saline |
|---|---|---|---|---|---|---|---|---|
| 5 | (8) | 45 ± 7 | (7) | 13 ± 3 | (7) | 23 ± 9 | (7) | 43 ± 9 |
| 10 | (11) | 45 ± 2 | (11) | 17 ± 4 | (11) | 28 ± 7 | (6) | 43 ± 7 |
| 15 | (8) | 37 ± 3 | (11) | 15 ± 4 | (12) | 25 ± 7 | (8) | 41 ± 7 |

[1] The mean (± S.E.M.) represent number of writhes over a 10 minute period. Ketamine dose was 10 mg/kg s.c. Significant differences of means were tested by Student's unpaired t-test. See Experimental Methods Chapter for description of procedure.

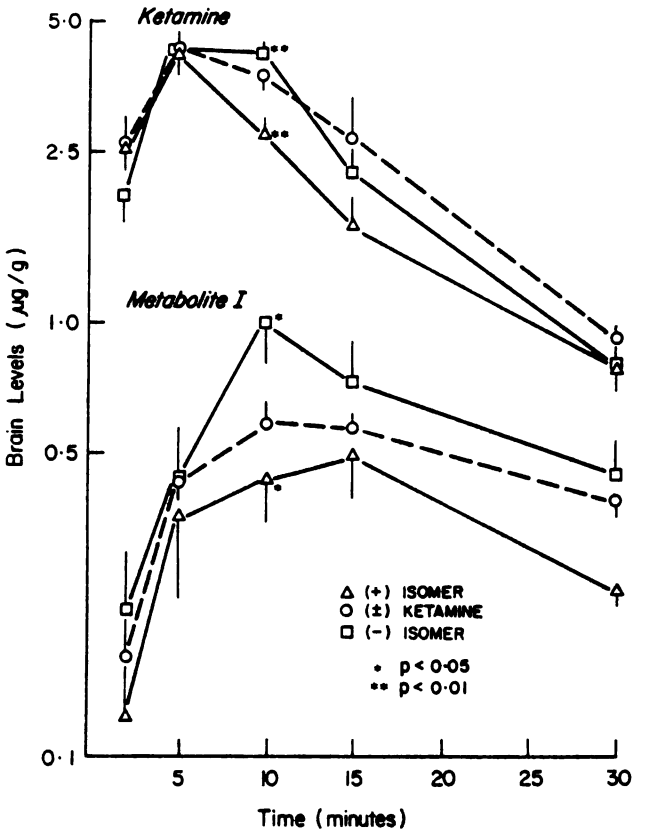



figure 3-4. Brain levels of the parent ketamine agents and metabolite I (norketamine) formed after s.c. administration (7 mg/kg) to mice. Animals were sacrificed at the indicated times after administration of racemic ketamine or one of its enantiomers and samples of whole brain were assayed for enantiomeric and racemic ketamine levels and their metabolites. Each point represents the mean of 5 experiments (±S.E.). *P < 0.05, **P < 0.01.

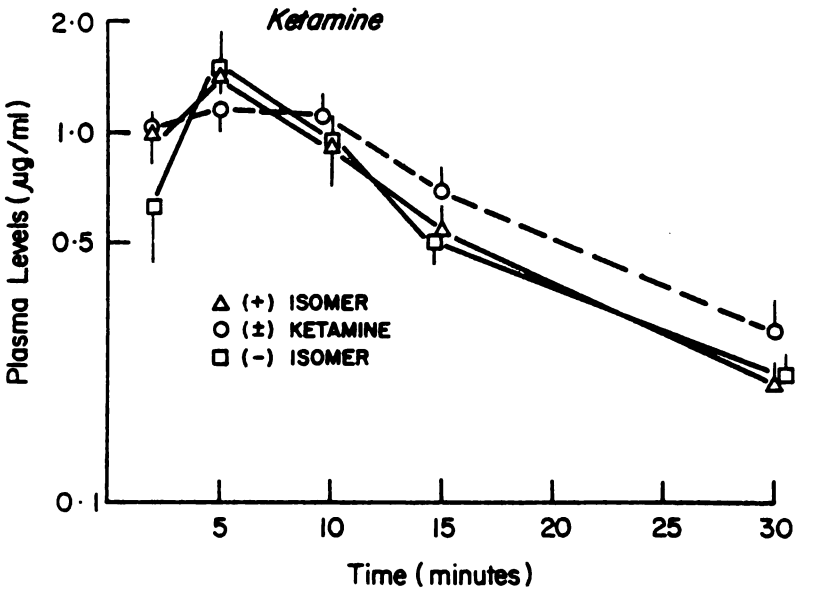



figure 3-5. Plasma levels of racemic ketamine and its enantiomers following s.c. administration (7 mg/kg) to mice. Animals were sacrificed at the indicated times and samples of the plasma were assayed for isomeric and racemic ketamine levels. Each point represents the mean of 5 experiments.

kinetics of the metabolites of the three drugs where, for example, there was a significantly greater amount of metabolite II (i.e. hydroxylated metabolites) formed in the plasma after administration of the (-)-isomer (figure 3-7). Metabolite II was also found in the brain at concentrations of 0.05 (±0.02), 0.10 (±0.02) and 0.18 (±0.03) µg/g at 10, 15 and 30 minutes respectively in the case of the (-)-enantiomer while no metabolite II was detected in the brain following the injection of either racemic ketamine or the (+)-enantiomer at any of the time intervals measured. Despite the significantly lower levels of metabolite I in the plasma after the administration of (-)-ketamine the levels of metabolite I of this isomer were similar or greater in the brain than after the administration of either racemic ketamine or the (+)-isomer (figures 3-4 and 3-6).

The brain and plasma levels of ketamine and its two metabolites were also measured following the intravenous administration of a hypnotic dose (25 mg/kg) of racemic ketamine and are represented in Table 3-4. Peak brain levels lf ketamine were achieved near one minute and loss of righting reflex observed within 30 seconds after injection. At the time of recovery of the righting reflex brain levels of the parent compound and metabolite I were 22 and 4 µg per gram of tissue respectively. With this higher dose of racemic ketamine metabolite II was detected in the brain within 5 minutes after administration.

Apparent molarity was estimated, assuming a specific

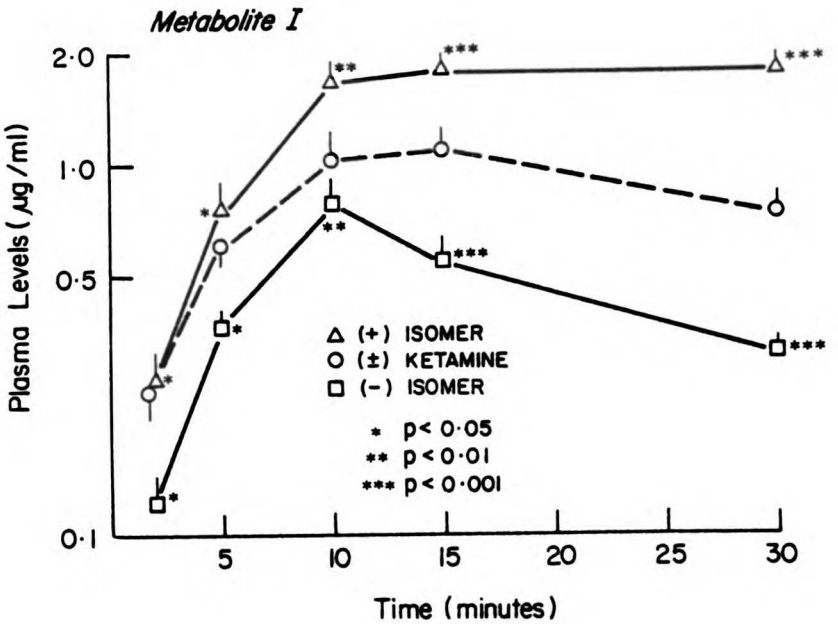



figure 3-6. Plasma levels of metabolite I (norketamine) of racemic ketamine and its two enantiomers following s.c. administration (7 mg/kg) of the parent drugs to mice. Animals were sacrificed at the indicated times and the samples of the plasma were assayed for isomeric and racemic metabolite I levels. Each point represents the mean of 5 experiments. *P<0.05; **P<0.01; ***P<0.001.

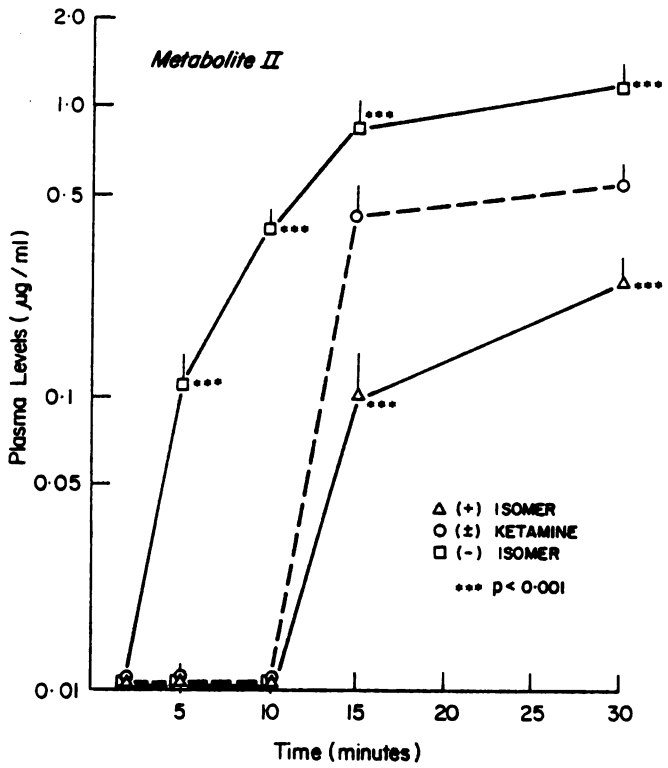



figure 3-7. Plasma levels of metabolite II of racemic ketamine and its two enantiomers following s.c. administration (7 mg/kg) of the parent drugs to mice. Animals were sacrificed at the indicated times and samples of the plasma were assayed for isomeric and racemic metabolite II levels. Each point represents the mean of 5 experiments (± S.E.). No metabolite II within the level of detectability of the gas chromatographic assay (0.01 μg/ml) was found 2 min following administration of any of the drugs or 5 or 10 min in the case of racemic ketamine or the (+)-enantiomer. ***P< 0.001.

TABLE 3-4

Levels of ketamine and metabolites in brain and plasma of mice following a hypnotic dose of racemic ketamine[1].

| | COMPOUND | | | | | |
|---|---|---|---|---|---|---|
| | Ketamine | | Norketamine | | Metabolite II | |
| Minutes | Brain ug/g | Plasma ug/ml | Brain ug/g | Plasma ug/ml | Brain ug/g | Plasma ug/ml |
| 1 Min. | 50 ± 8 | 8.3 ± .6 | 1.7 ± .1 | 1.5 ± .5 | -- | -- |
| 5 Min. | 19 ± 1 | 4.4 ± .2 | 4.9 ± .9 | 3.5 ± .5 | -- | .73 ± .1 |
| 15 Min. | 7.6 ± .2 | 1.7 ± .3 | 4.4 ± .5 | 4.4 ± .4 | 1.6 ± .8 | 1.3 ± .1 |
| 30 Min. | 1.5 ± .3 | .46 ± .1 | 1.4 ± .2 | 3.3 ± .5 | 1.1 ± .2 | 2.6 ± .4 |
| 60 Min. | .59 ± .2 | .18 ± .06 | .61 ± .05 | 2.1 ± .3 | .60 ± .1 | 1.8 ± .32 |

[1] Following caudal vein administration of hypnotic dose (25 mg/kg) of the racemate, levels of ketamine, norketamine and metabolite II were measured at the indicated times by a procedure described in the Experimental Methods Chapter. Four or more animals were used at each time period.

gravity of 1.0 for brain tissue, for brain levels of ketamine following an anesthetic and analgesic dose of ketamine (Table 3-5). The concentration of ketamine in the brain was $9 \times 10^{-5}$ M at the recovery of the righting reflex.

$^{3}$H-Naloxone, $^{3}$H-QNB and $^{3}$H-Spiroperidol Binding Assays

The effects of various drugs on the stereospecific binding of $^{3}$H-naloxone to opiate receptors in mouse brain homogenates are shown in figure 3-8. Racemic ketamine (not shown), the ketamine enantiomers and phencyclidine showed a concentration dependent inhibition of $^{3}$H-naloxone binding. However, the concentrations necessary to displace radioactive naloxone by ketamine, its enantiomers and phencyclidine were much greater than that required by the potent opiate, levorphanol, or even its non analgesic enantiomer, dextrorphan. Analysis of the log-probit profiles of the inhibition data for each drug revealed parallel lines, suggesting ketamine, phencyclidine and the opiates were competing for the same binding sites (figure 3-9).

Comparison of the $IC_{50}$ values indicated a difference ($P<.05$) in the ability of the ketamine enantiomers to recognize the opiate receptor (Table 3-6). (+)-Ketamine was about twice as effective as (-)-ketamine in inhibiting $^{3}$H-naloxone binding in this assay. Phencyclidine was more potent than any of the ketamine compounds, causing 50% inhibition of radioactive naloxone binding at approximately 40 $\mu$M.

The specific binding of $^{3}$H-QNB to muscarinic cholinergic

TABLE 3-5

In vivo brain levels of ketamine[a].

| Time after injection (Min.) | Anesthetic Dose[b] Ketamine (ug/g tissue) | "Apparent Molarity" (M) | Time after injection (Min.) | Analgesic Dose[c] Ketamine (ug/g tissue) | "Apparent Molarity" (M) |
|---|---|---|---|---|---|
| 1 | 50 ± 7.7 | $2.1 \times 10^{-4}$ | 2 | 2.5 ± .32 | $1.1 \times 10^{-5}$ |
| 5 | 19 ± .96 | $7.8 \times 10^{-5}$ | 5 | 4.2 ± .34 | $1.8 \times 10^{-5}$ |
| 15 | 7.6 ± 1.6 | $3.2 \times 10^{-5}$ | 15 | 2.2 ± .22 | $.92 \times 10^{-5}$ |
| 30 | 1.5 ± .32 | $.63 \times 10^{-5}$ | 30 | .77 ± .12 | $.32 \times 10^{-5}$ |

[a] Brain tissue was assayed for ketamine at indicated times by the gas chromatographic procedure noted in Methods Chapter. Ketamine levels shown are mean values from 4 or 5 separate animals ± S.E.M.

[b] 25 mg/kg ketamine was administered intravenously via tail vein of mice.

[c] 7 mg/kg ketamine was administered subcutaneously in mice.

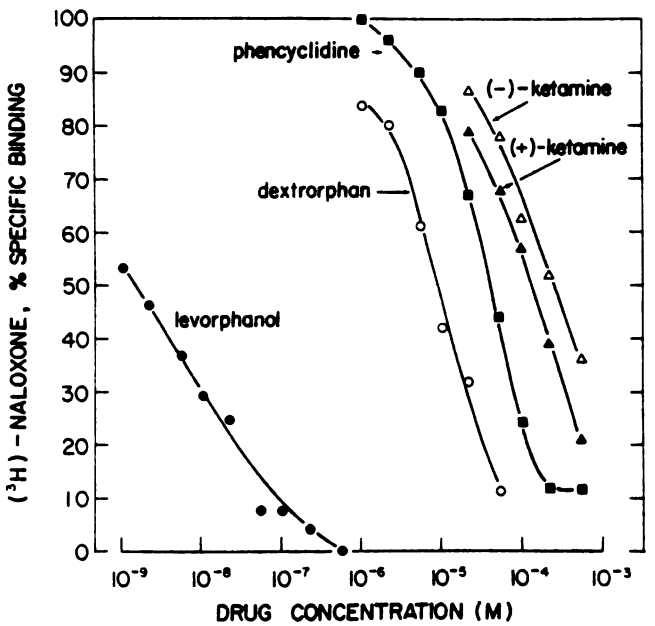



figure 3-8. Displacement of specific $^3$H-naloxone binding in mouse brain homogenates by various drugs. $^3$H-Naloxone (6.7 nM) was incubated for 2 hours at 4°C with brain homogenates in the presence of the indicated concentrations of drugs. Each determination was done in triplicate and the experiments were repeated at least 3 times. Specific binding of $^3$H-naloxone was defined as the difference between the radioactivity bound in the absence of levorphanol and radioactivity bound in the presence of 4 μM levorphanol.

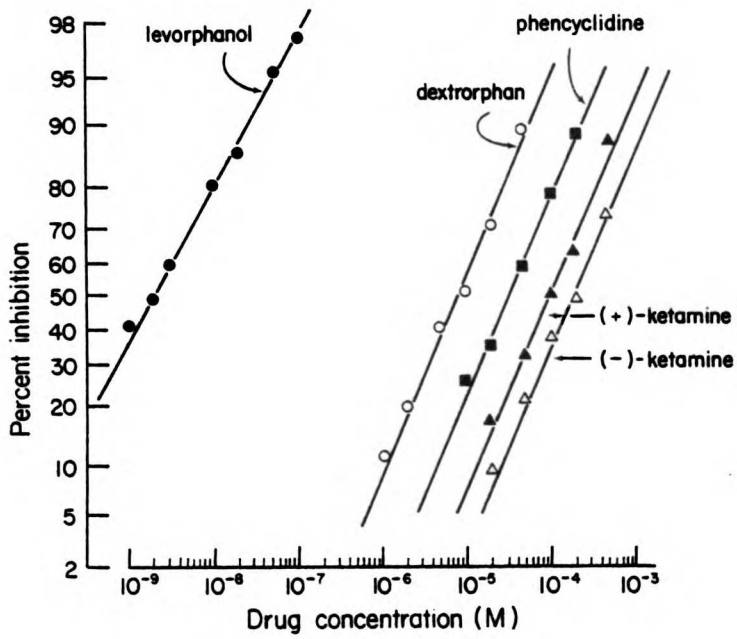



figure 3-9. Log-probit analysis of inhibition of stereospecific $^{3}$H-naloxone binding of various drugs. Five to seven concentrations of each drug were incubated for 2 hours at 4°C with mouse brain homogenates (1.7 to 2.4 mg prot/ml) in the presence of 6.7 nM $^{3}$H-naloxone. Percent inhibition of control stereospecific $^{3}$H-naloxone binding was computed for each concentration of drug after subtraction of nonspecific binding from all experimental points. Each determination was done in triplicate and repeated at least 3 times.

TABLE 3-6

Concentrations of drugs causing 50% inhibition of specific $^{3}$H-naloxone and $^{3}$H-QNB binding in mouse brain homogenates[a].

| Drug | $^{3}$H-naloxone $IC_{50}$ (uM) | Drug | $^{3}$H-QNB $IC_{50}$ (uM) |
|---|---|---|---|
| Levorphanol | .002 | Atropine | .005 |
| Dextrophan | 9 | Oxotremorine | 1.5 |
| Phencyclidine | 38 | Phencyclidine | 13 |
| (+)-ketamine | 120[b] | Carbamylcholine | 50 |
| (±)-ketamine | 150 | (+)-ketamine | 120 |
| (-)-ketamine | 220[b] | (-)-ketamine | 110 |

[a] $IC_{50}$ values represent means from 3 or more log-probit determinations each using 6 or more concentrations of drugs. The determinations varied by less than 18%.

[b] Means were significantly different ($p < .05$)

receptors in mouse brain was reduced in a concentration dependent fashion by the ketamine enantiomers and phencyclidine (figure 3-10). The slopes of the log-probit plots were equivalent for all the compounds tested (figure 3-11) and the $IC_{50}$ values estimated from these plots are given in Table 3-6. The $IC_{50}$ values showed the ketamine enantiomorphs to be similar in their ability to inhibit $^{3}$H-QNB binding while phencyclidine exhibited 10 times the potency of the ketamine isomers in reducing $^{3}$H-QNB binding.

The ability of racemic ketamine, phencyclidine and apomorphine to displace specific $^{3}$H-spiroperidol binding to dopamine receptors was examined in the calf caudate nucleus. Neither racemic ketamine nor phencyclidine inhibited radioactive spiroperidol binding to any significant degree at concentrations up to $10^{-4}$ M (figure 3-12). The biphasic pattern of inhibition exhibited by apomorphine has been shown to occur with a number of dopaminergic agonists in competing for spiroperidol binding, suggesting that spiroperidol is labeling two heterogeneous sets of receptors (Leysen et al, 1978; Titeler et al, 1978). The inhibitor concentration of apomorphine which reduced $^{3}$H-spiroperidol binding by 50% was 0.5 $\mu$M.

Effects of (+)-Ketamine and (-)-Ketamine on Inhibition of Mouse Brain Acetylcholinesterase

Measurement of initial velocities of acetylcholinesterase hydrolysis of acetylthiocholine in the presence and absence of the ketamine enantiomers disclosed differences in action

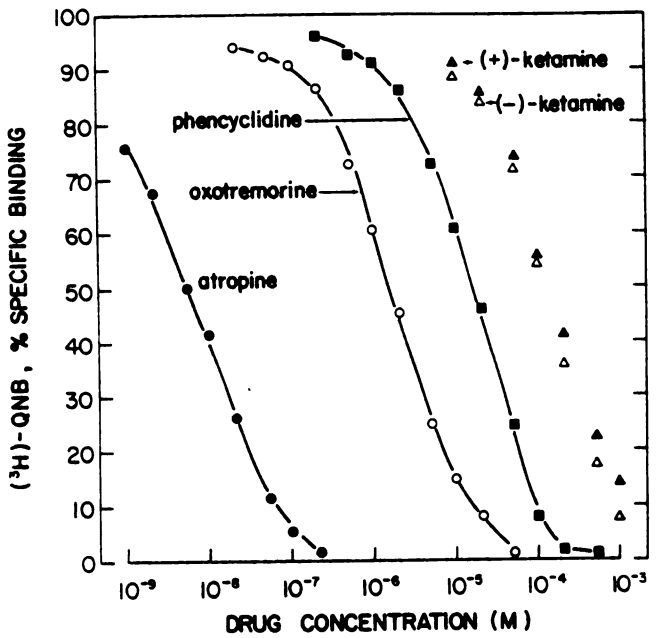



figure 3-10. Displacement of specific $^{3}$H-QNB binding in mouse brain homogenates by various drugs. $^{3}$H-QNB (0.4nM) was incubated at 25°C for 1 hour with brain homogenates in the presence of indicated concentrations of drugs. Each determination was done in triplicate and repeated at least 4 times. Specific binding of $^{3}$H-QNB was defined as the difference between the radioactivity bound in the absence of oxotremorine and radioactivity bound in the presence of 100 μM oxotremorine.

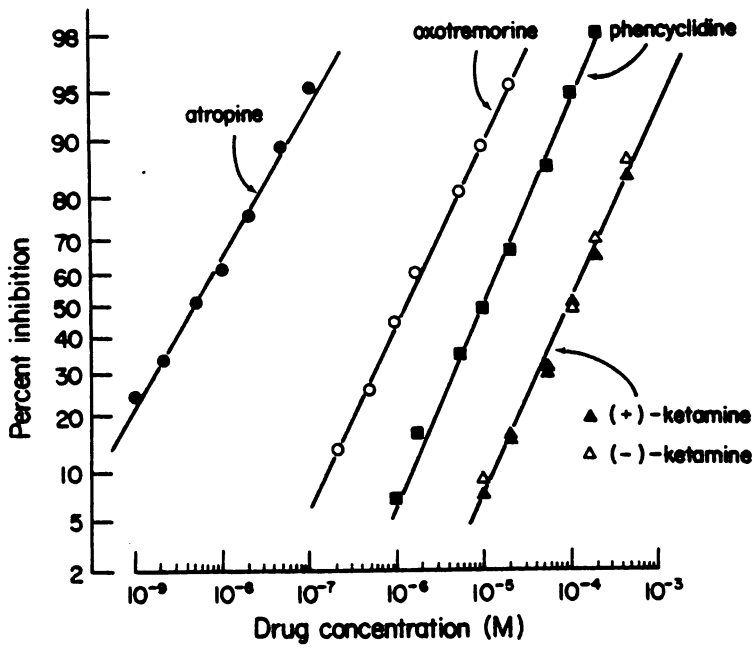



figure 3-11. Log-probit analysis of inhibition of specific $^3$H-QNB binding of various drugs. Six to eight concentrations of each drug were incubated for 1 hour at 25°C with mouse brain homogenates (0.18 to 0.27 mg prot/ ml) in the presence of 0.4 nM $^3$H-QNB. Percent inhibition of control of specific $^3$H-QNB binding was computed for each concentration of drug after subtraction of nonspecific binding from all experimental points. Each determination was done in triplicate and repeated four or more times.

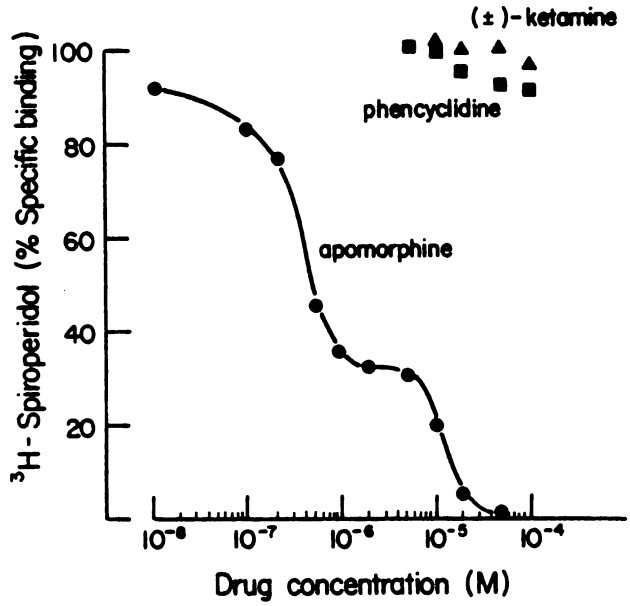



figure 3-12. Displacement of specific $^3$H-spiroperidol binding in calf caudate homogenates by apomorpine, phencyclidine and (±)-ketamine. $^3$H-Spiroperidol (0.8 nM) was incubated for 30 minutes at 25°C with brain homogenates (1.5 mg prot/ ml) in the presence of the indicated concentrations of drugs. Each determination was done in triplicate and repeated 3 or more times. Specific binding of $^3$H-spiroperidol was defined as the total amount of $^3$H-spiroperidol bound minus the amount of $^3$H-spiroperidol bound in the presence of 5 mM apomorphine.

of the optical isomers on acetylcholinesterase activity. (-)-Ketamine exhibited a potency 3.4 times that of (+)-ketamine as an inhibitor of acetylcholinesterase action, when $IC_{50}$ values were used as a basis for comparison. In the presence of 0.1 mM substrate the $IC_{50}$ values were $2.6 \times 10^{-4}$ M and $8.8 \times 10^{-4}$ M for the (-)- and (+)-isomer respectively while phencyclidine displayed an $IC_{50}$ of $5.2 \times 10^{-5}$ M in the assay (Table 3-7).

Kinetic analysis of Lineweaver-Burk graphs demonstrated mixed inhibition at two inhibitor concentrations in the case of each enantiomer (figures 3-13 and 3-14) and this inhibitory nature of ketamine action was confirmed by characteristic downward turning of Hunter and Downs plots in each case. However, in similar studies, phencyclidine acted in a competitive fasion as an inhibitor of acetylcholinesterase activity, based on Lineweaver-Burk analysis (figure 3-15).

The apparent inhibitory constants ($k_i$) were found to be $1.6 \times 10^{-4}$ M and $5.8 \times 10^{-4}$ M for (-)- and (+)-ketamine respectively, when estimated by slope ratios at three inhibitory concentrations by a method described by Webb for mixed inhibition systems. Similar values were obtained by direct observation of Hunter and Downs plots (Table 3-7). Phencyclidine exhibited an inhibitory constant ($k_i$) of $2.4 \times 10^{-5}$ M when measured by the Dixon method.

Effects of (+)-Ketamine and (-)-Ketamine on Uptake of $^3$H-Dopamine and $^3$H-Norepinephrine in Mouse Synaptosomes

Stereoselective inhibition of catecholamine reuptake

TABLE 3-7

Comparison of inhibition of whole mouse brain acetylcholinesterase by the ketamine isomers and phencyclidine.

| Drug | $IC_{50}$ (molar)[a] | Apparent $k_i$ (molar)[b] |
|---|---|---|
| (+)-ketamine | $8.8 \times 10^{-4}$ | $5.8 \times 10^{-4}$ |
| (-)-ketamine | $2.6 \times 10^{-4}$ | $1.6 \times 10^{-4}$ |
| Phencyclidine | $5.2 \times 10^{-5}$ | $2.4 \times 10^{-5}$ |

[a] The $IC_{50}$ values were calculated from logarithmic-probability plots of percentage inhibition using four concentrations of each drug in the presence of .1mM substrate. Each experiment was repeated four or more times, with a standard error of less than 5% in each case.

[b] The inhibitory constants ($k_i$) were estimated as described in the Experimental Methods Chapter.

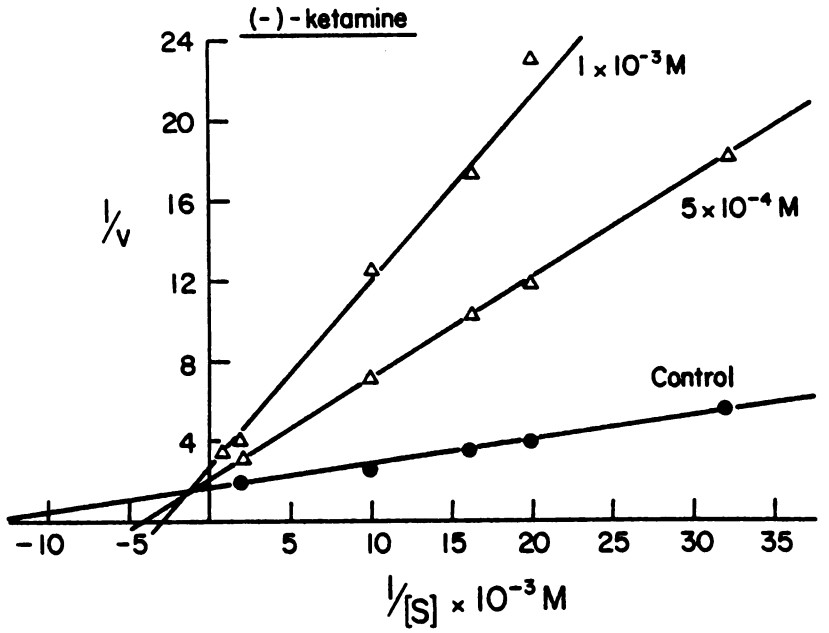



figure 3-13. Inhibition of acetylcholinesterase in whole mouse brain by (-)-ketamine. Initial velocity was measured at substrate (ATC) concentrations varying from 0.031 to 1 mM in the presence or absence of indicated concentrations of (-)-ketamine. Points represent mean values from at least 4 experiments and differed by less than 5 %. Velocity (V) is expressed as moles ATC hydrolyzed per mg protein per min after subtraction of non-enzymatic hydrolysis of ATC.

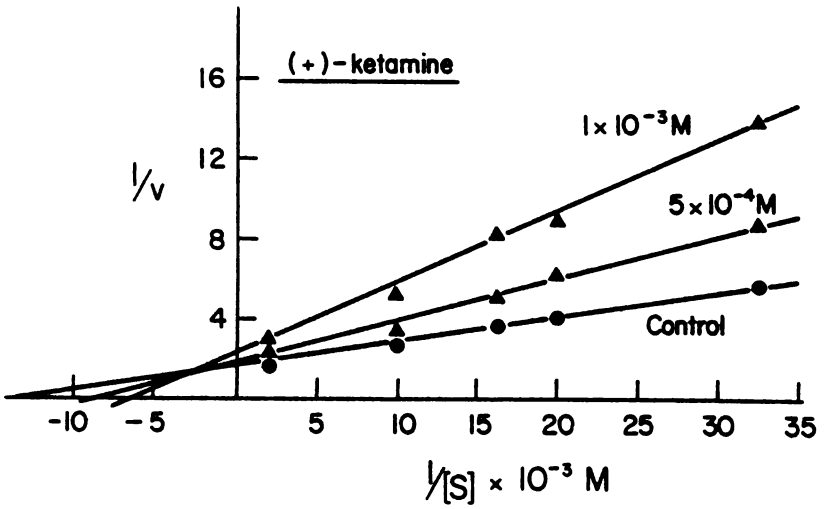



figure 3-14. Inhibition of acetylcholinesterase in whole mouse brain by (+)-ketamine. Initial velocity was measured at substrate (ATC) concentrations varying from 0.031 to 1 mM in the presence or absence of indicated concentrations of (+)-ketamine. Points represent mean values from at least 4 experiments and differed by less than 5 %. Velocity (V) is expressed as moles ATC hydrolyzed per mg protein per min after subtraction of non-enzymatic hydrolysis of ATC.

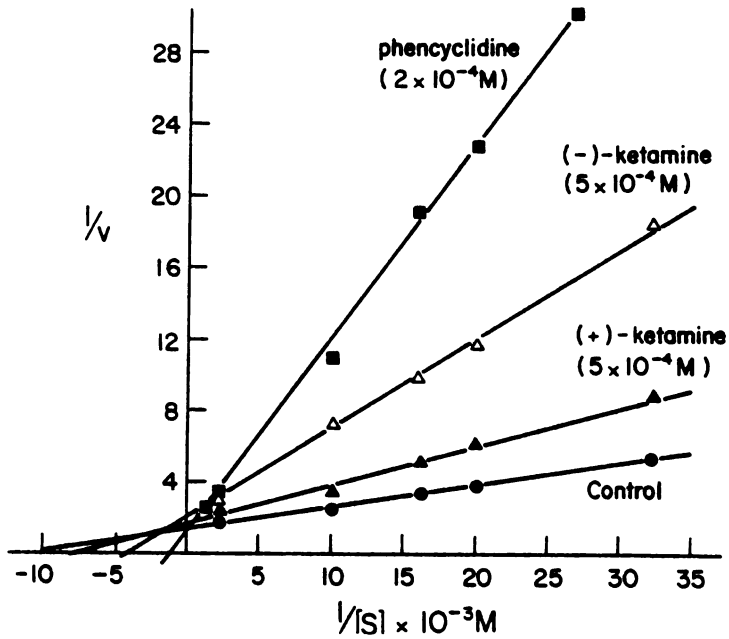



figure 3-15. Inhibition of acetylcholinesterase in whole mouse brain by phencyclidine and the ketamine enantiomers. Initial velocity was measured at substrate (ATC) concentrations varying from 0.031 to 1 mM in the presence or absence of the different concentrations of phencyclidine, (+)- and (-)-ketamine.Points represent mean values from at least 4 experiments and differed by less than 5 %. Velocity (V) is expressed as moles ATC hydrolyzed per mg protein per min after subtraction of non-enzymatic hydrolysis of ATC.

by the ketamine enantiomers was revealed in the $^{3}$H-neurotransmitter reuptake experiments with synaptosomal preparations where the (+)-enantiomer was shown to be more potent in this regard. The inhibitory concentrations ($IC_{50}$) required to produce 50% reduction in the accumulation of 10 nM $^{3}$H-dopamine and $^{3}$H-norepinephrine for (+)- and (-)-ketamine are shown in Table 3-8. A comparison of the $IC_{50}$ values shows the (+)-isomer to be approximately 4 times more potent than the (-)-isomer in inhibiting the uptake of $^{3}$H-dopamine and $^{3}$H-norepinephrine.

Lineweaver-Burk plots of inhibition of $^{3}$H-dopamine and $^{3}$H-norepinephrine reuptake indicated both isomers were probably competitive inhibitors evidenced by changes in $K_m$ and no change in $V_{max}$ values in the presence of each ketamine enantiomer. Figures 3-16 and 3-17 show the effect of 50 $\mu$M of (-)- and (+)-ketamine on $^{3}$H-dopamine and $^{3}$H-norepinephrine synaptosomal transport. A calculation of $K_m$ and $V_{max}$ values from the reciprocal plots indicated $8.4 \times 10^{-8}$ M and 3.8 pmol/mg/min respectively for $^{3}$H-dopamine reuptake and $9.0 \times 10^{-8}$ M and 1.2 pmol/mg/min in the case of $^{3}$H-norepinephrine uptake.

The competitive inhibitory activities of the ketamine enantiomers on $^{3}$H-dopamine and $^{3}$H-norepinephrine uptake were further characterized by estimating their inhibitory constants ($k_i$) from Dixon plots (figures 3-18 through 3-21). $K_i$ values measured by the point of intersection of these lines were 0.10 and 0.34 mM for (+)- and (-)-ketamine respectively

TABLE 3-8

Comparison of $IC_{50}$ values for isomers of ketamine on the inhibition of biogenic amine uptake into synaptosomes of whole mouse brain.

| | Mean $IC_{50}$ Values ± S.E.M.[a] | | |
|---|---|---|---|
| Drug | $^3$H-DA | $^3$H-NE | $^3$H-5HT |
| (-)-ketamine | $(2.6 \pm .10) \times 10^{-4}$M | $(2.3 \pm .10) \times 10^{-4}$ M | $(1.7 \pm .09) \times 10^{-5}$M |
| (+)-ketamine | $(.61 \pm .03) \times 10^{-4}$M | $(.60 \pm .03) \times 10^{-4}$M | $(3.5 \pm .20) \times 10^{-5}$M |
| RP[b] | 4.1 | 3.8 | .49 |

[a] The $IC_{50}$ values were calculated from logarithmic-probability plots of percentage inhibition using five concentrations of each isomer. Incubation was performed at 37°C for five minutes at 10nM of $^3$H-dopamine, $^3$H-norepinephrine and $^3$H-serotonin. The results are based on four to six experiments.

[b] RP is the relative potency of the (+)-isomer compared to the (-)-isomer.

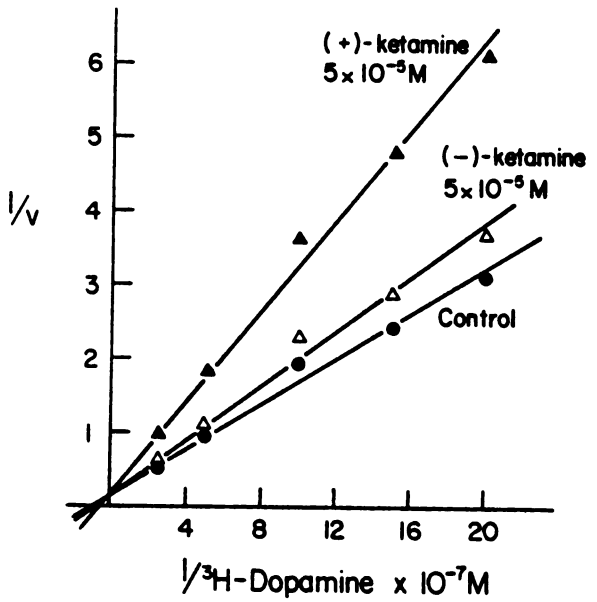



figure 3-16. Lineweaver-Burk plot of inhibition of $^{3}H$-dopamine uptake into synaptosomes of whole mouse brain by (+)- and (-)-ketamine. Synaptosomes were incubated (37°C) in duplicate for 5 minutes with $^{3}H$-dopamine concentrations varying from 5 nM to 40 nM in the presence of absence of 0.05 mM (+)- or (-)-ketamine. Points represent mean values of at least 4 experiments at each substrate concentration, differing by less than 6%. 1/V = (moles/mg prot/min)$^{-1}$ x $10^{-12}$.

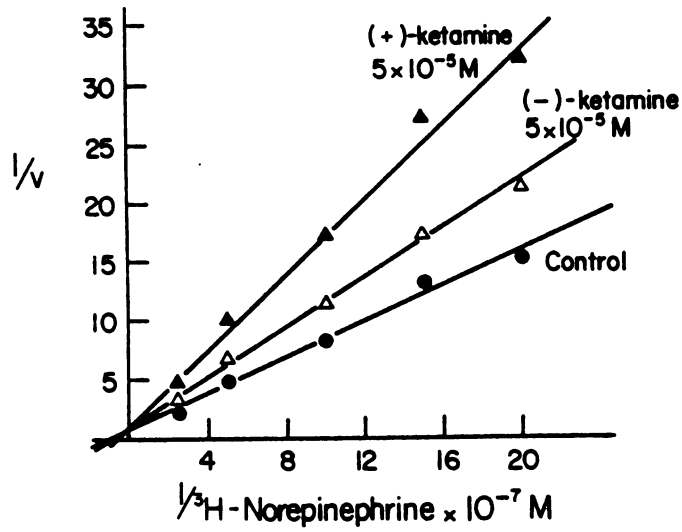



figure 3-17. Lineweaver-Burk plot of inhibition of $^{3}H$-norepinephrine uptake into synaptosomes of whole mouse brain by (+)- and (-)-ketamine. Synaptosomes were incubated (37°C) in duplicate for 5 minutes with $^{3}H$-norepinephrine concentrations varying from 5 nM to 40 nM in the presence or absence of 0.05 mM (+)- or (-)-ketamine. Points represent mean values of at least 4 experiments at each substrate concentration, differing by less than 6%. $1/V = (\text{moles/mg prot/min})^{-1} \times 10^{-12}$.

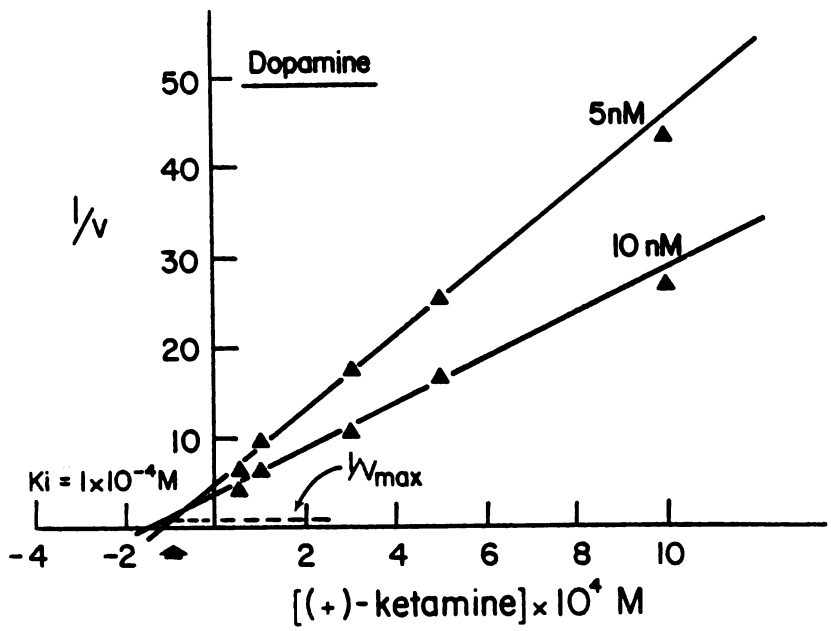



figure 3-18. Dixon plot of inhibition of $^{3}$H-dopamine uptake into synaptosomes of whole mouse brain by (+)-ketamine. Synaptosomes were incubated (37$^{o}$C) in duplicate for 5 minutes with 5 nM or 10 nM $^{3}$H-dopamine in the presence of varying concentrations of (+)-ketamine. The inhibitory constant ($k_i$) was obtained by direct observation from the plot. Points represent mean values of at least 4 experiments at each drug concentration, differing by less than 6%. 1/V = (moles/mg prot/min)$^{-1}$ x $10^{-12}$.

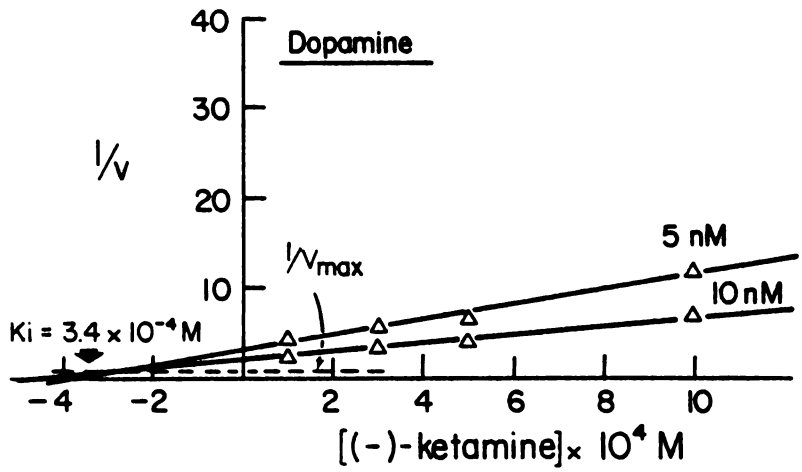



figure 3-19. Dixon plot of inhibition of $^{3}$H-dopamine uptake into synaptosomes of whole mouse brain by (-)-ketamine. Synaptosomes were incubated (37°C) in duplicate for 5 minutes with 5 nM or 10 nM $^{3}$H-dopamine in the presence of varying concentrations of (-)-ketamine. The inhibitory constant ($k_i$) was obtained by direct observation from the plot. Points represent mean values of at least 4 experiments at each drug concentration, differing by less than 6%. 1/V = (moles/ mg prot/min)$^{-1}$ x $10^{-12}$.

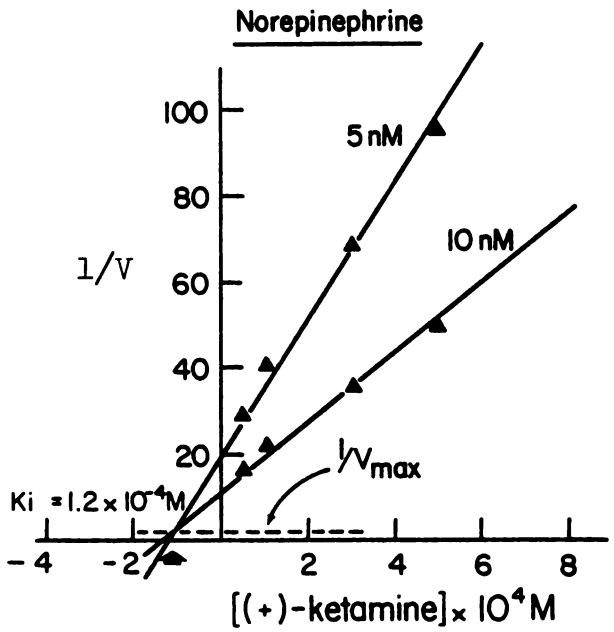



figure 3-20. Dixon plot of inhibition of $^{3}$H-norepinephrine uptake into synaptosomes of whole mouse brain by (+)-ketamine. Synaptosomes were incubated (37°C) in duplicate for 5 minutes with 5 nM or 10 nM $^{3}$H-norepinephrine in the presence of varying concentrations of (+)-ketamine. The inhibitory constant ($k_i$) was obtained by direct observation from the plot. Points represent mean values of at least 4 experiments at each drug concentration, differing by less than 6%. 1/V = (moles/mg prot/min)$^{-1}$ x $10^{-12}$.

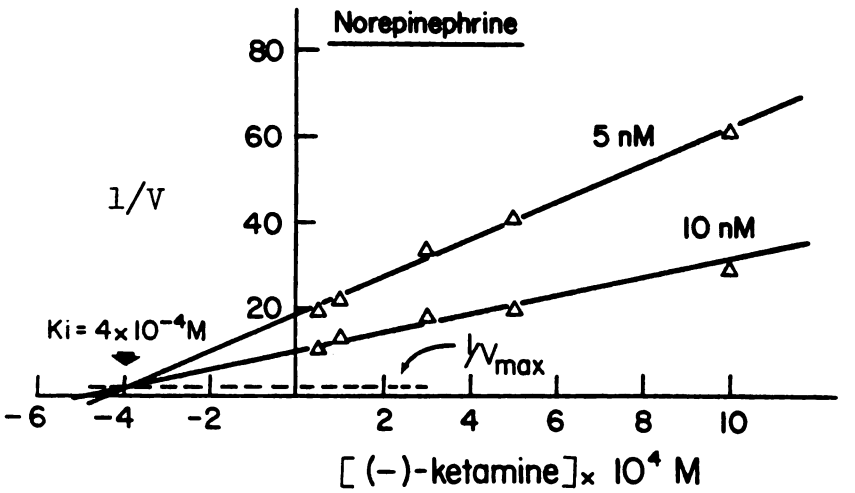



figure 3-21. Dixon plot of inhibition of $^{3}$H-norepinephrine uptake into synaptosomes of whole mouse brain by (-)-ketamine. Synaptosomes were incubated (37°C) in duplicate for 5 minutes with 5 nM or 10 nM $^{3}$H-norepinephrine in the presence of varying concentrations of (-)-ketamine. The inhibitory constant ($k_i$) was obtained by direct observation from the plot. Points represent mean values of at least 4 experiments at each drug concentration, differing by less than 6%. 1/V = (moles/mg prot/min)$^{-1}$ x $10^{-12}$.

for $^{3}$H-dopamine reuptake while the respective $k_i$ constants for (+)- and (-)-ketamine in the $^{3}$H-norepinephrine studies were 0.12 and 0.40 mM.

Effects of (+)-Ketamine and (-)-Ketamine on Uptake of $^{3}$H-Serotonin in Mouse Synaptosomes

Differential effects were also observed in the interaction of the ketamine enantiomers with the serotonergic reuptake process. However, in contrast with the relative inhibitory potencies noted in catecholamine uptake, the (-)-enantiomer exhibited the greater potency in this system. Inspection of $IC_{50}$ values in Table 3-8 shows that (-)-ketamine had twice the potency of (+)-ketamine as an inhibitor of $^{3}$H-serotonin transport into mouse synaptosomes. Furthermore, both isomers displayed greater potency for interfering with serotonin uptake relative to their activities on dopamine and norepinephrine reuptake.

Reciprocal plots of various concentrations of each ketamine enantiomorph indicated the inhibition in each case to be competitive in nature. The effect of 50 $\mu$M of (-)- and (+)-ketamine on the synaptosomal uptake of $^{3}$H-serotonin caused an increase in the value of the Michaelis constant ($2.0 \times 10^{-7}$ M and $3.9 \times 10^{-7}$ M, respectively) from control ($8.9 \times 10^{-7}$M), while $V_{max}$ (3.1 pmol/mg/min) remained the same (figure 3-22).

In kinetic studies, the inhibitory constant ($k_i$) of the (+)-enantiomer was determined to be twice that of the (-)-enantiomer. The $k_i$ values, evaluated by Dixon plots, were 0.02 mM and 0.04 mM for (-)- and (+)-ketamine respectively

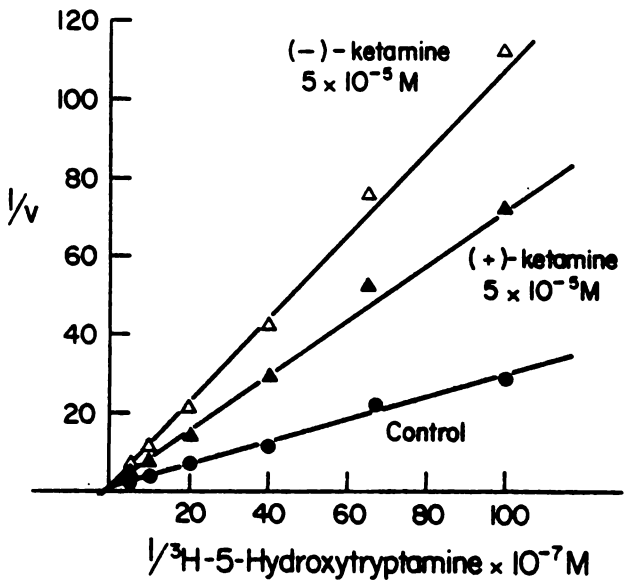



figure 3-22. Lineweaver-Burk plot of inhibition of $^{3}$H-serotonin uptake into synaptosomes of whole mouse brain by (+)- and (-)-ketamine. Synaptosomes were incubated (37°C) in duplicate for 5 minutes with $^{3}$H-serotonin concentrations varying from 1 nM to 20 nM in the presence or absence of 0.05 mM (+)- or (-)-ketamine. Points represent mean values of at least 4 experiments at each substrate concentration, differing by less than 6%. 1/V = (moles/mg prot/min)$^{-1}$ x $10^{-12}$.

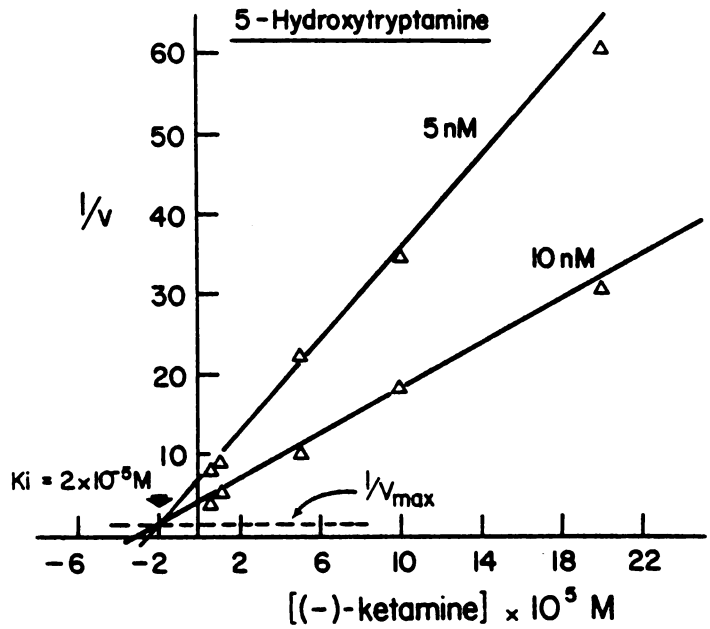



figure 3-23. Dixon plot of inhibition of $^{3}$H-serotonin uptake into synaptosomes of whole mouse brain by (-)-ketamine. Synaptosomes were incubated (37°C) in duplicate for 5 minutes with 5 nM or 10 nM $^{3}$H-serotonin in the presence of varying concentrations of (-)-ketamine. The inhibitory constant ($k_i$) was obtained by direct observation from the plot. Points represent mean values of at least 4 experiments at each drug concentration, differing by less than 6%. 1/V = (moles/mg prot/min)$^{-1}$ x $10^{-12}$.

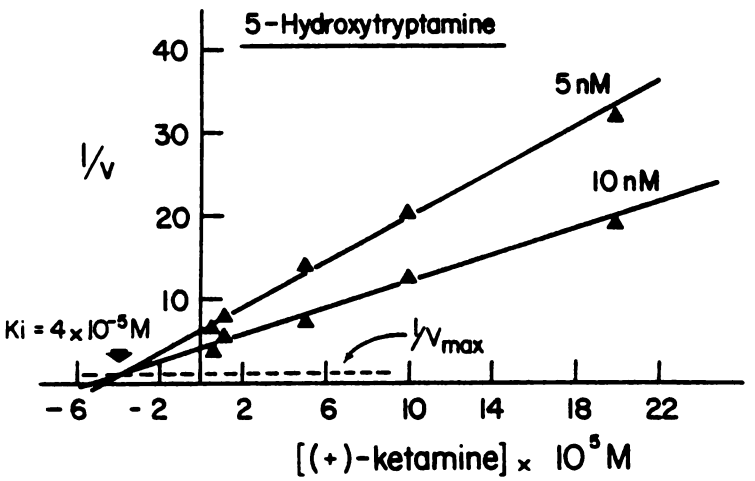



figure 3-24. Dixon plot of inhibition of $^{3}$H-serotonin uptake into synaptosomes of whole mouse brain by (+)-ketamine. Synaptosomes were incubated (37°C) in duplicate for 5 minutes with 5 nM or 10 nM $^{3}$H-serotonin in the presence of varying concentrations of (+)-ketamine. The inhibitory constant ($k_i$) was obtained by direct observation from the plot. Points represent mean values of at least 4 experiments at each drug concentration, differing by less than 6%. 1/V = (moles/mg prot/min)$^{-1}$ x $10^{-12}$.

(figures 3-23 and 3-24).

$^{3}$H-Phencyclidine Binding Assays

The effects of phencyclidine, racemic ketamine and the ketamine optical isomers on specific $^{3}$H-phencyclidine binding in brain homogenates were examined by use of the method described by Zukin and Zukin (1979). The initial binding assays were performed by studying specific $^{3}$H-phencyclidine binding in the presence of various concentrations of phencyclidine and racemic ketamine in rat brain homogenates. In these experiments phencyclidine and (±)-ketamine inhibited $^{3}$H-phencyclidine binding in a concentration dependent manner and the $IC_{50}$ values (± S.E.M.) estimated from log-probit plots were (3.2 ± .3) x $10^{-7}$ M and (2.1 ± .2) x $10^{-5}$ M for phencyclidine and racemic ketamine respectively. In similar experiments, only in mouse brain homogenates, the ability of phencyclidine, the ketamine enantiomers and racemic ketamine to displace $^{3}$H-phencyclidine binding was compared. The effects of various concentrations of phencyclidine and the ketamine isomers are shown in figure 3-25. $IC_{50}$ values (± S.E.M.) determined from log-probit plots were (2.7 ± .3) x $10^{-7}$ M for phencyclidine and (2.9 ± .6) x $10^{-5}$ M and (2.7 ± .2) x $10^{-5}$ M for (+)- and (-)-ketamine respectively. Racemic ketamine exhibited an $IC_{50}$ of (2.5 ± .3) x $10^{-5}$ M in this assay.

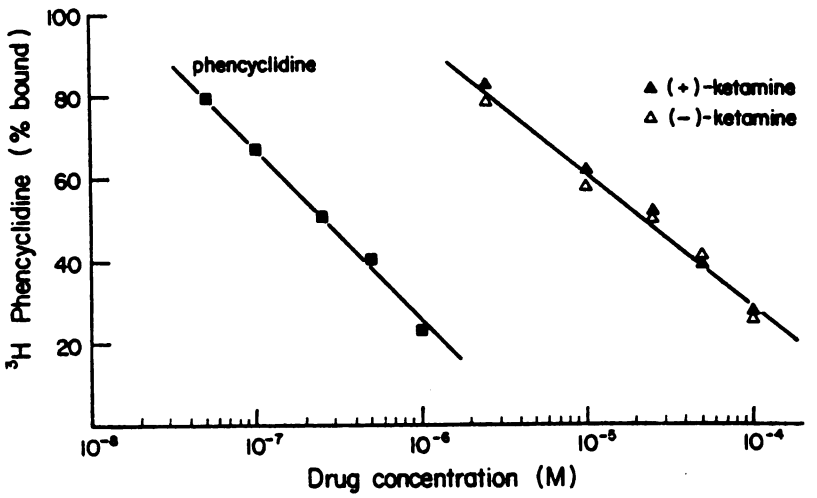



figure 3-25. Displacement of $^3$H-phencyclidine binding in mouse brain homogenates by phencyclidine and the ketamine enantiomers. $^3$H-phencyclidine (7.0 nM) was incubated at 4°C for 45 minutes with brain homogenates in the presence of the indicated concentrations of drugs. Each determination was done in triplicate and repeated at least twice. Non-specific binding obtained in the presence of 0.1 mM cold phencyclidine was subtracted from all experimental points.

# CHAPTER FOUR: DISCUSSION

DISCUSSION

The present results demonstrate that the reported differences in hypnotic and locomotor stimulation potencies of the optical isomers of ketamine in the Sprague-Dawley rat (Marietta et al, 1977) also occur in the ICR mouse. In addition, the more potent isomer, (+)-ketamine, for these two effects was also shown to have the greatest analgesic potency. However, estimation of potency ratios reveals that (+)-ketamine is approximately 3 x more potent in terms of analgesia and only 1.5 x more potent in the induction of hypnosis than the (-)-isomer, while the duration of hypnosis was 3 x longer with (+)-ketamine than with (-)-ketamine following intravenous administration of equimolar doses. Marked analgesia could be detected at dose levels well below those causing loss of the righting reflex and this was most evident in the case of (+)-ketamine. As an analgesic agent, this isomer showed the additional advantage of causing less locomotor stimulation than (-)-ketamine when used at an equivalent analgesic dose.

Certain of the pharmacological differences displayed by the ketamine enantiomers in rodents have since been revealed at the clinical level according to a recent preliminary study of the individual ketamine enantiomorphs in surgical patients (White et al, 1980). This study disclosed differences in anesthetic potencies, intraoperative effects, analgesia, physical side effects, incidences and types of postanesthetic emergence phenomena, and anesthetic

preferences between the optical isomers of ketamine. (+)-Ketamine exhibited greater potency as an anesthetic and analgesic agent than (-)-ketamine while spontaneous motor activity, hallucinations, delerium and unpleasant dreams were more common following (-)-ketamine than (+)-ketamine. Finally, patients found (+)-ketamine more acceptable as an anesthetic than either the racemate or (-)-ketamine.

Enantiomeric differences in pharmacological activity have been associated with various stereoselective factors including transport to active sites (Ames et al, 1977), metabolism (Jenner and Testa, 1973) and interactions with receptors (Jacquet et al, 1977; Mohler and Okada, 1977). The differences in pharmacological potencies between the two optical ketamine isomers observed in this study do not appear to be due to marked differences in biodisposition. The brain levels of the less potent (-)-isomer were equal to (or, at 10 minutes, greater than) those of (+)-ketamine. Similarly, except for one time point (10 minutes), no major differences were detected in brain levels of norketamine of the isomers while a very small amount of metabolite II (hydroxylated metabolites) was found in the brain in the case of the weaker isomer at later time periods. The relative contribution of norketamine or other metabolites derived from ketamine to the pharmacological effects of the ketamine enantiomers is yet to be determined. The biodisposition data, nevertheless, suggest the observed potency differences of the ketamine enantiomorphs reside, in large part, in

pharmacodynamic factors involving stereochemical properties of the two enantiomers.

No significant differences were detected in the plasma concentrations of (+)- and (-)-ketamine following subcutaneous administration. However, the plasma level of norketamine derived from the (-)-isomer was significantly lower than that of (+)-norketamine at all time intervals. This appeared to be compensated by an increase in the plasma levels of the subsequent hydroxylated products (metabolite II) of the (-)-isomer compared to the same metabolite of (+)-ketamine. These observations support the suggestion of stereoselective metabolism of the ketamine isomers, at least in the ICR mouse. Since plasma (and brain) levels of the parent isomers were equivalent, stereoselectivity of metabolism does not appear to occur at the level of N-demethylation but rather during the process of ring oxidation with conversion of normetabolites to the hydroxylated derivatives. Substrate stereoselective metabolism has been described for a number of enantiomers including amphetamine (Gunne and Gallard, 1967; Dring et al, 1970), barbiturates (Palmer et al, 1970) and psychotomimetics (Gal et al, 1976) which also involves hepatic microsomal oxidation reactions.

In spite of the differential metabolism of (-)-norketamine signified by significantly lower amounts of this metabolite in the plasma compared to levels measured for (+)-norketamine, the brain concentration of (-)-norketamine remained equal to or greater than that of its enantiomer.

This preferential accumulation of the N-demethylated metabolite of (-)-ketamine in the brain has also been reported in the rat (Marietta et al, 1977) and may be indicative of stereoselective active transport of (-)-norketamine into the CNS or preferential binding of (-)-norketamine to brain tissue sites. Alternatively, it is possible differences may exist between the optical isomers with regard to brain metabolism, although in vitro brain metabolism of racemic ketamine was not observed at the level of N-demethylation in rat brain (Cohen and Trevor, 1974). For example, (+)-norketamine could be selectively converted to undetected metabolites in the brain, subsequently lowering its brain to plasma ratio. Another possibility could be differential stimulation of blood flow to the brain following (-)-ketamine administration, but such stimulation should have been reflected in increased transport of the parent compound to the brain over the same time period and this was not observed. The concentrations which drugs achieve in their distribution between tissues and plasma are also affected by binding to plasma protein, in that high protein binding in the plasma leads to low tissue to plasma concentration ratios. In this regard, (+)-norketamine could be preferentially binding to plasma protein, resulting in lower brain to plasma concentration than that observed for (-)-norketamine. Protein plasma binding has also been shown to decrease the rate of hepatic extraction of some drugs from the plasma and this could be reflected in a decreased

rate of metabolism observed for (+)-norketamine.

The narcotic antagonist, naloxone, blocked the anti-nociceptive activity of racemic ketamine while atropine pre-treatment had no significant affect on ketamine analgesia. Naloxone can antagonize various drug effects on the CNS including general anesthesia (Finck et al, 1977), ethanol dependence (Blum et al, 1977) and analgesia caused by nitrous oxide (Berkowitz et al, 1976). It is possible that certain of these antagonist effects are due to nonspecific, analeptic actions of naloxone. In the case of the analgesic effects of ketamine the present data do not support a nonspecific action of naloxone since the animals given naloxone plus saline exhibited the same writhing response as saline control. In considering possible mechanisms for analgesia, ketamine could act to potentiate or release endogenous peptides with resulting analgesic effects as has been suggested for nitrous oxide analgesia (Berkowitz et al, 1976). Alternatively, ketamine may interact directly with narcotic analgesic receptors. This possibility was examined by studying the effects of ketamine on narcotic antagonist binding. Both of the individual ketamine enantiomers displayed competitive binding activities in displacing $^{3}$H-naloxone from specific binding sites. Stereoselective binding occurred in the opiate binding experiments with (+)-ketamine exhibiting twice the affinity of (-)-ketamine for interaction with opiate receptors. However, the inhibitory concentrations required for the ketamine agents to inhibit $^{3}$H-naloxone were much greater

than that displayed by the potent opiate, levorphanol, and even greater than that measured for its non-analgesic enantiomer, dextrorphan. Since the analgesic potencies of the opiates are correlated with their ability to compete with $^{3}$H-naloxone binding to the opiate receptors in vitro (Pert and Snyder, 1973), it appears direct interaction with the opiate receptor is unlikely to be the basis for the analgesic effect of ketamine or for the differential analgesic actions of its enantiomers. This does not preclude the possibility that ketamine in some manner potentiates the effect of or releases endogenous endorphins at opiate analgesic receptor sites.

The effects of the ketamine enantiomorphs on the cholinergic system were examined at the level of the muscarinic receptor and acetylcholinesterase activity. Ketamine analgesia was not significantly affected by prior administration of atropine which suggests muscarinic action does not play an important role in the antinociceptive activity of ketamine. At peripheral sites of action ketamine appears to possess weak anticholinergic properties (Kloog et al, 1977; Mahmoodi et al, 1980). In addition, behavioral studies in laboratory animals indicate ketamine shares some of the pharmacological actions of antimuscarinic agents including stereotyped behavior (Scheel-Kruger, 1970), rotational behavior (Kelly and Miller, 1975) and increased locomotor activity (Payne and Andersson, 1967). In the muscarinic binding experiments the ketamine enantiomers showed no

difference in their ability to compete for $^{3}$H-QNB binding sites while displaying inhibitory concentration values 4 to 5 orders of magnitude more than those typically measured for muscarinic antagonists in displacing $^{3}$H-QNB binding (Birdsall and Hulme, 1976). A comparison of *in vivo* concentrations of the ketamine isomers with their *in vitro* $IC_{50}$ values indicates minimal binding by the enantiomers would occur at a low subanesthetic dose (7 mg/kg) while the concentration of ketamine in the brain at recovery of the righting reflex ($9 \times 10^{-5}$ M) is near the $IC_{50}$ values of the ketamine enantiomers for inhibition of muscarinic binding and, as such, measurable binding might occur at that time period (Table 3-5). Although it is clear that the affinities of the ketamine agents for muscarinic receptors are within the range of concentrations which can be achieved in the brain, it is not evident whether ketamine is acting as an agonist or antagonist at these central muscarinic sites. Binding studies by themselves indicate only the degree to which a compound competes with a labeled ligand for particular receptor sites. In the case of phencyclidine, however, evidence from quantum mechanical techniques (Maayani et al, 1973) and more recent regional binding studies in rat brain (Aronstam et al, 1980) indicate the binding of phencyclidine to central receptors exhibits properties more characteristic of muscarinic antagonists than agonists.

Examination of the interaction of the ketamine enantiomers with acetylcholinesterase revealed stereoselective inhibition

of acetylcholinesterase activity in the presence of the enantiomorphs with (-)-ketamine showing the greater potency in this regard. The mixed nature of inhibition exhibited by both ketamine isomers in these experiments is not unlike that displayed by a number of cholinergic ligands (Kato et al, 1970; Kitz et al, 1970). It has been proposed that acetylcholinesterase possesses non-catalytic anionic sites which modulate the enzyme activity (Kitz et al,1970; Roufogalis and Quist, 1972). The non-competitive component of ketamine inhibition may be a reflection of an interaction of the charged nitrogen group ($pK_a$ = 7.5) of the ketamine molecule with these peripheral anionic sites.

The inhibitory concentrations necessary for the ketamine compounds to reduce acetylcholinesterase activity indicate these agents are very weak acetylcholinesterase inhibitors and only at the peak brain levels achieved during anesthesia would there be a measurable reduction of acetylcholinesterase action. However, previous biochemical studies have been unable to demonstrate a change in acetylcholine levels throughout the duration of ketamine anesthesia although a slight reduction in acetylcholine turnover has been measured in some brain regions (Ngai et al, 1978). Both isomers displayed a greater potency in binding to muscarinic receptors relative to acetylcholinesterase inhibition, thus any muscarinic action resulting from reduced acetylcholinesterase activity might be counteracted by an antagonist action by ketamine at muscarinic receptors. In this regard, weak anticholinergic

action by ketamine observed in smooth muscle and intact ganglia preparations, indicates the ability of ketamine to inhibit acetylcholinesterase does not predominate at peripheral sites of action. In conclusion, the relative weak anticholinesterase activities of the ketamine enantiomers are unlikely to be the basis for their differential effects and probably contribute little, if any, to the pharmacological actions of ketamine in general.

It is possible, however, that an antimuscarinic action of ketamine might be related to the behavioral responses observed following ketamine administration. In laboratory animals, muscarinic antagonists not only elicit motor behavior similar to that caused by ketamine but have been shown to potentiate amphetamine induced motor activity (Campbell et al, 1969; Fibiger et al, 1970), an action which has been attributed to the modification of a cholinergic inhibitory influence on amphetamine's activation of the dopaminergic system (Thornburg and Moore, 1973). Of relevance is the observation that both antimuscarinic agents and drugs which increase dopaminergic activity have the potential to elicit psychotomimetic behavior in humans (Friedhoff and Alpert, 1973). The interaction of ketamine with the cholinergic system and its ability to inhibit reuptake of dopamine may underlie certain of the psychic emergence reactions seen with this agent.

Experiments to examine the effects of the ketamine enantiomorphs on the uptake of dopamine, norepinephrine and serotonin into mouse synaptosomes disclosed that the

biogenic amine reuptake processes were sensitive to the steric features of the enantiomers, evidenced by differences in $IC_{50}$ values and inhibitory constants of the isomers. (+)-Ketamine displayed greater potency in inhibiting dopamine and norepinephrine uptake into synaptosomes while serotonin uptake was more sensitive to (-)-ketamine. Stereoselective differences on inhibition of catecholamine and serotonin reuptake have also been noted for the enantiomers of amphetamine (Coyle and Snyder, 1969; Harris and Baldessarini, 1973; Holmes and Rutledge, 1976) with (+)-amphetamine, the more active central stimulant, showing the greater potency in this regard. A comparison of the inhibitory concentrations of ketamine on dopamine and norepinephrine uptake with those of amphetamine and its analogues (Harris and Baldessarini, 1973; Tseng et al, 1976) and tricyclics (Koide and Uyemura, 1980), agents whose activities have been associated with inhibition of catecholamine uptake, reveals the ketamine isomers to be 2 to 3 orders of magnitude less potent in this regard. Relative to these compounds, the ketamine agents would probably be ranked as very weak inhibitors of catecholamine uptake, at best. However, if the concentrations of ketamine achieved *in vivo* are related to the inhibitory concentrations measured *in vitro*, it appears that enough ketamine is potentially available for some inhibition of dopamine and norepinephrine uptake to occur. For example, during the approximate time interval (5 to 15 minutes) of locomotor stimulation and analgesic action at an analgesic

dose of ketamine, the accompanying brain concentrations (Table 3-5) would result in 30-40% and 10-20% inhibition of catecholamine uptake by (+)- and (-)-ketamine respectively. At a dose (2-3 x) necessary for (-)-ketamine to cause locomotor stimulation or analgesia equivalent to (+)-ketamine, the (-)-isomer would display the same approximate degree of inhibition of catecholamine uptake as (+)-ketamine, assuming a concomitant increase (2-3 x) in brain levels.

Both noradrenergic and dopaminergic mechanisms have been associated with drug induced motor behavior (Schildkraut and Kety, 1967; Snyder et al, 1970) and may contribute, in part, to the behavioral activity elicited by ketamine. In addition, as mentioned previously, the disinhibition of the effects of acetylcholine by an antimuscarinic action of ketamine may magnify an effect of ketamine on dopaminergic activation. The noradrenergic system is also thought to play a role in analgesia (Balagura and Ralph, 1973) and weak analgesic action has been observed with amphetamine and other sympathomimetic amines in humans and laboratory animals (Innes and Nickerson, 1975). If noradrenergic activity is influencing the analgesic effect of ketamine then it may contribute to the greater analgesic action of (+)-ketamine, the more potent isomer in inhibition of norepinephrine reuptake.

Both ketamine enantiomers preferentially inhibited serotonin accumulation in synaptosomes relative to their action on catecholamine uptake and this was most evident with (-)-ketamine. The $IC_{50}$ values and inhibitory constants

for serotonin uptake determined *in vitro* are comparable with *in vivo* brain levels following a subanesthetic dose of ketamine and below the brain levels measured during hypnosis. Serotonin has been associated with hallucinogenic behavior and also is considered to play a role in pain perception and sleep (Costa et al, 1974). It is of interest that (-)-ketamine, a more potent inhibitor of serotonin uptake than (+)-ketamine, is less potent than (+)-ketamine in its hypnotic and analgesic activity yet has been shown to exhibit more psychotomimetic behavior in humans than the (+)-isomer at equivalent therapeutic doses. This suggests the possibility that interaction with serotonin reuptake may not be the important factor in mediating ketamine analgesia or anesthesia but may play a role in psychotomimetic actions of ketamine.

Certain of the psychotomimetic emergence reactions produced by ketamine resemble those seen after administration of LSD and other LSD-like agents (Freedman, 1968). In addition, the EEG activity observed with ketamine is similar to that induced by LSD and other indoleamine hallucinogenic agents (Kayami and Iwoma, 1972; Mori et al, 1972; Winters et al, 1972), characterized by significant increased discharges in the hippocampus, a portion of the limbic system associated with emotional behavior. Additional evidence for selective hippocampal activation following ketamine administration was demonstrated recently in rat brain (Nelson et al, 1980). Ketamine was shown to cause a significant increase in glucose

utilization in the hippocampus, indicative of increased functional activity, while discrete regions of the brain associated with sensory systems (medial geniculate and inferior colliculus) showed decreased uptake of glucose during ketamine action. Similar changes in glucose metabolism in selective regions of the rat brain were observed following a behaviorally active dose of phencyclidine (Meibach, 1979).

Although ketamine and LSD may share some similarities in behavioral action and EEG activity, a common neurochemical action is less obvious. It has been proposed that the hallucinogenic actions of LSD and other hallucinogenic indoleamines entail a suppression of raphe neuronal activity by stimulation of serotonin receptors at presynaptic sites located in the midbrain raphe nuclei thus freeing synaptic target cells (e.g. in hippocampus) from the inhibitory activity of serotonin (Haigler and Aghajanian, 1977). However, Aghajanian and coworkers have shown phencyclidine has no effect on midbrain raphe nuclei activity (Aghajanian et al, 1970) suggesting phencyclidine is not acting at the same presynaptic sites as LSD; there are no reports of ketamine action in this regard.

However, a drug which does share some similarity in neurochemical properties with ketamine (and phencyclidine) and has been shown to be a potent hallucinogen in humans is paramethoxyamphetamine (Shulgin et al, 1969). This amphetamine derivative differs from the parent compound not only in its hallucinogenic properties but also in its

preferential inhibition of serotonin relative to its effect on catecholamine uptake (Tseng et al, 1976). Harris and coworkers compared the effects of a variety of hallucinogenic and non-hallucinogenic derivatives of amphetamine and tryptamine, including paramethoxyamphetamine and LSD, on schedule-controlled behavior and correlated behavioral effects with their reported potencies in producing subjective effects in humans (Harris et al, 1978). In this report the observation was made that the most consistent neurochemical correlate among the hallucinogenic agents was stimulation of the serotonergic system. In this respect, it is possible that certain of the hallucinogenic behaviors of ketamine may be due to its interaction with the serotonergic system. Additional evidence of a serotonergic role in ketamine action is the report that, at subanesthetic concentrations, ketamine selectively inhibited dorsal root potentials eminating from the Raphe Magnus Nucleus which are thought to be serotonergically mediated (Larson, in press). In the same study, a similar action but of longer duration was exhibited by phencyclidine.

In the phencyclidine binding experiments, the inhibitory concentrations of phencyclidine necessary to reduce $^{3}$H-phencyclidine binding by 50% in rat and mouse brain homogenates were similar to those determined in rat brain by previous laboratories (Vincent et al, 1979; Zukin and Zukin, 1979). There was no significant difference in the $IC_{50}$ values of the ketamine enantiomers in displacing $^{3}$H-phencyclidine while

the $IC_{50}$ values for phencyclidine were 2 orders of magnitude less than that required by the ketamine agents to reduce the radioactive binding. Recently, Maayani and Weinstein demonstrated that $^{3}$H-phencyclidine binds to the filters used in the binding assays with characteristics similar to the "specific" binding found in brain tissue (Maayani and Weinstein, 1980). Moreover, if the brain tissue is boiled, phencyclidine binding properties remain essentially the same, suggesting phencyclidine binding sites are not true receptors but merely a reflection of saturable binding to non-specific sites on filters or tissue. Maayani and Weinstein point out that the relative low apparent affinity of $^{3}$H-phencyclidine ($\sim 10^{-7}$) for binding sites and consequent high dissociation rate of bound $^{3}$H-phencyclidine may preclude the use of the rapid filtration method with this ligand. Given these observations, if the ketamine agents were competing for artefactual binding sites then, of course, analysis of the present data is meaningless; but if, in fact, the ketamine compounds were displacing $^{3}$H-phencyclidine from specific phencyclidine receptors in brain tissue then these data reveal no differences in the ability of the ketamine enantiomers to bind to these receptors. In the latter case, this would suggest differential effects of the enantiomorphs of ketamine do not reside in stereoselective binding to phencyclidine receptors. The status of these binding studies awaits further clarification and the evidence for the existence of phencyclidine receptors may depend on the availability of a specific ligand with

higher affinity and/or use of methods other than rapid filtration.

In summary, the ketamine enantiomers exhibited differential effects in their pharmacological actions and in their interaction and effects on various components of several brain neurotransmitter systems. (+)-Ketamine displayed greater potency than (-)-ketamine in causing analgesia, anesthesia and locomotor stimulation, however the (-)-enantiomer elicited greater locomotor activity than the (+)-enantiomer following equianalgesic doses. At the neurochemical level, the (+)-isomer exhibited a greater potency in inhibiting norepinephrine and dopamine uptake into brain synaptosomes and for displacing $^{3}$H-naloxone binding in brain homogenates than (-)-ketamine. Serotonin uptake and acetylcholinesterase activity in brain were more sensitive to inhibition by (-)-ketamine while the ketamine enantiomorphs reduced $^{3}$H-QNB and $^{3}$H-phencyclidine binding with equal potency. Relative to their other neurochemical actions, both isomers displayed the greatest potency in their interaction with the serotonergic system and in reducing $^{3}$H-phencyclidine binding. Both the fact that stereospecificity of action was demonstrated by the ketamine enantiomers and the observation that no differences in biodisposition of the enantiomers and their metabolites were found in the brain provide support for the notion that interaction at specific sites of action or receptors underlie

the observed pharmacological differences of the ketamine enantiomorphs.

Albeit the ketamine agents exhibited weak activities at the neurochemical level, on the basis of inhibitory concentrations measured in vitro, but one must not discount the importance of physico-chemical properties of a drug in predicting its pharmacological activity. Ketamine is a highly lipid soluble compound. Based on its partitioning characteristics in organic solvents ketamine is 5 to 10 times more soluble than the generally acknowledged extremely lipid soluble agent, thiopental (Mayer et al, 1959; Cohen, 1973), and like thiopental enters the brain readily. One also can not ignore the possibility that it may be a particular combination of "weak" neurochemical events which contributes to a pharmacological action. For example, the model proposed by Friedhoff and Alpert hypothesizes that psychotic symptoms are the result of an imbalance between dopaminergic and cholinergic systems in brain regions involving emotional expression whereby disruption of this balance either by increasing dopaminergic activity or decreasing cholinergic action exacerbates or elicits psychotomimetic behavior (Friedhoff and Alpert, 1973). A relative weak dopaminergic activation and anticholinergic action by ketamine, when combined, could potentiate such an imbalance. Ketamine and its congeners have a myriad of pharmacological actions and to sort through and correlate these activities with underlying events is exceedingly difficult. An understanding

of how these agents act may well await the identification and characterization of specific receptors to the arylcyclohexylamine class of neuropharmacological agents.

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