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On the biodisposition of ketamine and the pharmacology of its enantiomorphs in the rat



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# ON THE BIODISPOSITION OF KETAMINE AND THE PHARMACOLOGY OF ITS ENANTIOMORPHS IN THE RAT

by

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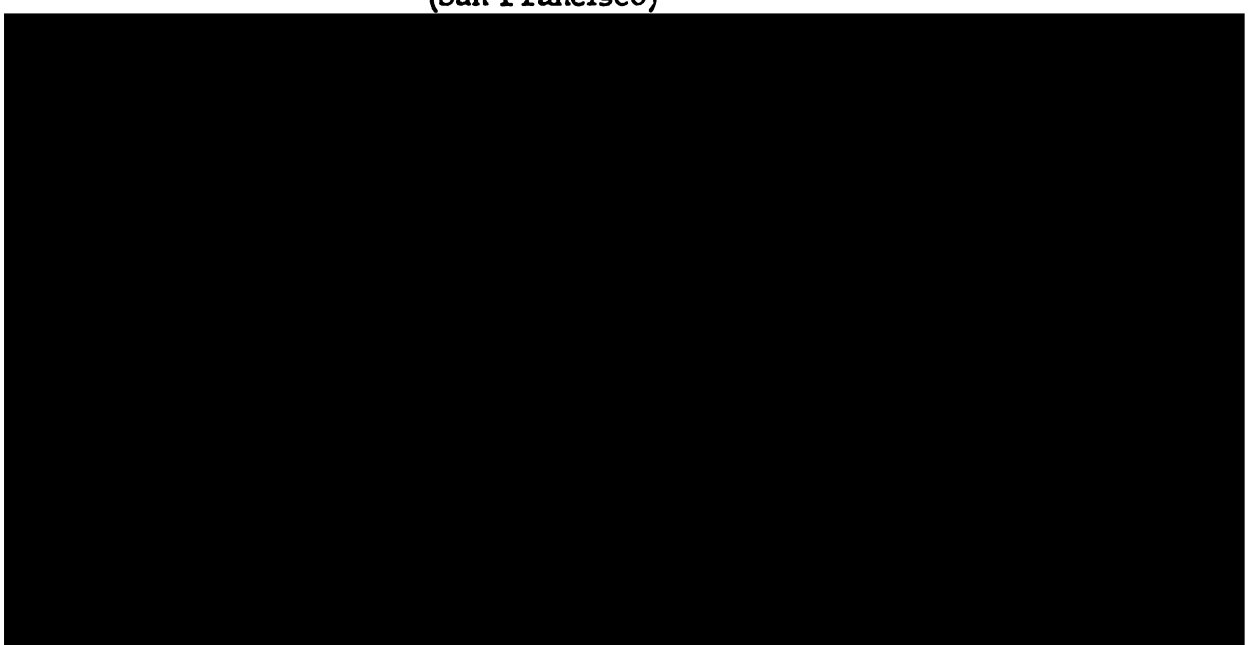

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# ABSTRACT

On The Bisdisposition of Ketamine and the Pharmacology of its Enantiomorphs in the Rat

Four pharmacologic actions of intravenous ketamine were studied in the rat. To elucidate the mechanism(s) terminating the pharmacologic effects, animals were pretreated with ketamine and agents anticipated to modify hepatic microsomal metabolism, including phenobarbital and SKF 525-A. SKF 525-A pretreatment markedly prolonged ataxia and stimulation of locomotor activity, in addition to significantly elevating brain and plasma levels subsequent to the initial 10 minutes following injection; thus hepatic metabolism appeared to play a prominent role in the termination of the posthypnotic effects of the drug. While significantly shortening the durations of the two posthypnotic events, phenobarbital and ketamine pretreatments also lowered the brain and plasma levels of ketamine. With all pretreatments, brain ketamine levels were almost identical at the cessation of hypnosis (25 μg/g of tissue) and ataxia (8-10 μg/g of tissue). No pretreatment altered either the duration of loss of righting reflex (hypnosis) or brain and plasma ketamine levels during the initial 10 minutes after injection. Approximately 70% of the injected drug was recovered from four tissues, skeletal muscle, gut, skin, and liver, at 10 minutes after injection; thus redistribution from brain to other tissues appeared to play a major role in the cessation of hypnosis. Ketamine pretreatment caused a 2-fold increase in the rate of its

in vitro hepatic microsomal metabolism. The same pretreatment also increased levels of cytochrome P-450 and NADPH-cytochrome c reductase in the liver, and the in vitro rates of hepatic microsomal metabolism of benzphetamine and 3,4-benzo(a)pyrene. Brain and plasma ketamine levels 30 minutes after injection were nearly identical in rats pretreated with ketamine and phenobarbital, although phenobarbital pretreatment resulted in a 4-fold increase in in vitro ketamine hepatic metabolism. The possible reasons for the lack of correlation between in vivo and in vitro results are discussed.

Hypnosis, ataxia, locomotor activity, and lethality, brain and plasma pharmacokinetic profiles, and the in vitro hepatic metabolism of racemic ketamine and its two optical isomers were studied in rats. At equimolar doses, the dextro isomer elicited periods of hypnosis and ataxia nearly twice those of the levo isomer. No significant differences were observed between the brain and plasma levels of either the individual isomers or the racemate following equimolar administration (30 mg/kg). Observed differences in the rates of the in vitro hepatic metabolism of the enantiomorphs did not appear to play an important role in the in vivo disposition of the isomers. From correlations between brain levels and pharmacological activities, differences in the potencies of the isomers appeared to have a pharmacodynamic rather than a pharmacokinetic basis. Following the administration of equihypnotic doses of the isomers, the posthypnotic stimulation of locomotor activity by the (+) enantiomorph was significantly less intense than that of

(-)-ketamine. In addition, the therapeutic index of the (+) isomer (3.50), expressed as the $LD_{50}$ over the 4 minute hypnotic dose, was greater than that of the racemate (2.73) or the levo isomer (1.93). These results suggest that the (+) isomer may provide anesthesia which is safer and has fewer posthypnotic side effects when administered at dosages equihypnotic to those of racemic ketamine or its (-) isomer.

# CHAPTER 1

## General Introduction

## Historical Perspective

The discovery of ether anesthesia in the middle of the nineteenth century stands, without reservation, as one of the significant medical discoveries of all time (77). Following the introduction of the gaseous and volatile anesthetics, the torturing pain, terror that surpassed all description, and death from traumatic shock, which had previously accompanied childbirth and surgery, were virtually eliminated (13) (149)(166). The excellent surgeon no longer had only seconds "to cut for the stone" or to amputate.

> "There were no shrieks, no violent strife,
> as usually occurred.
> There was merely gentle breathing,
> as in a peaceful sleep." (97)

It became immediately obvious, however, that the marvelous new gases were not without their faults (120). Within months following their introduction, deaths occurred during the administration of ether (8) and chloroform (110)(115). These early deaths, attributed to "congestion of the lungs" and "the circulation arrested at once, by a direct action on the heart", prompted medical scientists to search for better drugs and methods for their administration. Criteria, which are still applicable today, were set for the ideal anesthetic by those pioneer scientists. The perfect anesthetic would not be explosive as were, for example, ether ethylene, and cyclopropane. Unlike nitrous oxide, the ideal drug would provide skeletal muscle relaxation adequate for surgical procedures at dosage levels which would assure adequate patient respiratory function. The administration of the inexpensive, ideal drug would be simple. The onset of induction of anesthesia would be a rapid and pleasant experience as would be the termination of its effect. Very importantly,

the ideal anesthetic would be nontoxic. Unlike chloroform and ether, it would lack any cardiovascular and respiratory depressant properties.

Most of the research during the latter half of the nineteenth century was directed toward the discovery of both better anesthetics and new methods for their administration. Numerous techniques and devices, today considered routine, were developed. For example, Pravaz constructed the first hypodermic syringe in 1853, and, in 1880, Macewan obtained endotracheal anesthesia without resorting to tracheotomy. The gases, ethylene and cyclopropane were first used for general anesthesia during the late 1800's (92). Both were far from being ideal anesthetic agents. They were explosive and ethylene lacked the potency required to achieve adequate levels of surgical anesthesia. The very potent cyclopropane often caused marked respiratory depression.

The search for better anesthetics has continued into this century. Considerable research has been undertaken to elucidate the mechanism(s) of action of the anesthetics, which include such diverse compounds as simple olefins, barbiturates, halogenated hydrocarbons, various ethers, and even certain "inert" gases. In addition, studies have attempted to enumerate the multitude of factors influencing the duration and intensity of action of anesthetics in the body. Theories of the mechanism(s) of general anesthesia have attempted to explain how such diverse compounds as those mentioned above can produce the same overall effect. The demonstration that anesthetics selectively depress transmission through the multineural, polysynaptic pathways of the spinal cord, brain stem reticular formation, and cerebral cortex suggests a critical importance of synaptic transmission to anesthesia (81). Various theories,

ranging from the hypothesis that anesthetics block ionic channels at synaptic membranes (121) to the postulation that anesthesia results from the formation of gas hydrates or "icebergs" at crucial brain sites (127), have been proposed. None have been able to provide an adequate "structure activity" relationship common to the many structurally diverse anesthetic agents.

A major source of difficulty in the search for an unifying concept of anesthetic action has been the lack of information concerning anesthetic levels at their sites of action. The development of analytical methods for the detection of drugs and their metabolites in body tissues has progressed rapidly during the past thirty years (23). The often simple, highly selective, and very sensitive assays for drugs have greatly facilitated the understanding of the factors which influence the duration and intensity of action of anesthetics. They have also been essential in elucidating mechanisms of drug action, since the interpretation of studies with a therapeutic agent in vitro is open to question unless the concentration of the agent is also known in the living animal at its target site.

The gases of the nineteenth and early twentieth centuries have been largely replaced by the volatile halogenated hydrocarbons, e.g. halothane and methoxyflurane. The relative lack of respiratory irritation, flammability, and incidence of postanesthetic nausea and vomiting has made these compounds, especially halothane, the most popular anesthetic agents in the western world (54). The compounds are not without their limitations, however. Cardiovascular and respiratory depression have been observed with increased depth of anesthesia during the administration

of halothane and methoxyflurane (15)(137). Their adminstration requires the utilization of elaborate equipment and the constant monitoring of patient status. In addition, the high lipid solubility of these anesthetics often results in their accumulation in body fat depots, thus leading to a lengthy recovery from anesthesia following their administration.

The introduction of intravenous anesthetics has solved some of the problems associated with the inhalational agents. Attempts were made from time to time to induce surgical anesthesia by the administration of a variety of compounds, including the opiates, chloral hydrate, ether in saline, paraldehyde, and alcohol (93). All the preceding drugs were considered either unsafe or unsatisfactory for general anesthesia, and it was only with the introduction of the ultrashort acting barbiturates in the 1920's and 1930's that intravenous anesthesia became popular.

The prototype of the rapidly acting thiobarbiturates, thiopental, was given its first clinical trial at the Mayo Clinic in 1935 (102). The rapid onset of anestheisa following the intravenous administration of the highly lipid-soluble compound has been attributed to its rapid entry into highly perfused brain tissues (106)(111)(147). Subsequent to the loss of conciousness following intravenous thiopental, the ability to react in a reflex manner to external stimulii and, shortly thereafter, motor tone disappear. Excessive doses depress the vital medullary centers leading eventually to cardiovascular collapse and respiratory depression. This physiological response to thiopental is the same, with minor differences, as those for most conventional anesthetics.

Barbiturates, including thiopental, ultimately are metabolized by the liver to more polar compounds which are eliminated by renal excretion. Originally, the ultrashort pharmacologic action of thiopental was attributed to its rapid hepatic metabolism (53)(116). However, the evidence against metabolism as the important biodispositional factor in the termination of thiopental hypnosis was the observation that the plasma decay rate was only 10-15 percent/hour for several hours following injection of the thiobarbiturate. The hypothesis of rapid hepatic metabolism was soon discarded and for several years it was accepted that the evanescent effect of thiopental was due primarily to redistribution from brain to body fat (22). It soon became apparent, however, that the blood supply to body fat deposits was insufficient to transfer more than a small fraction of the total body thiopental there within the initial 5-10 minute period following drug injection, i.e. the period of hypnosis (130)(147). Subsequent studies suggested that the ultrashort action of thiopental resulted from the rapid redistribution of the drug from brain to well-perfused lean body tissues, e.g. skeletal muscle, and, eventually to body fat depots (72)(133).

The role of hepatic metabolism in the termination of the pharmacologic effects of thiopental deserves a final comment. Mark et al. (107) have shown an arterio-hepatic venous thiopental difference in humans which they have attributed to microsomal metabolism of the thiobarbiturate. However, these differences were determined at time points later than the,

> "...prompt awakening...due primarily to redistribution of thiopental from brain and other viscera to lean body mass and depot fat; at such early times the effect of biotransformation is negligible." (107)

In addition, by assuming that the liver metabolizes a constant fraction of thiopental per unit of time, Saidman and Eger (141) have suggested that hepatic metabolism will exert its greatest influence immediately following drug injection, i.e. when the liver receives the largest fraction of the injected thiopental. They concluded, nonetheless, that uptake in muscle played the dominant role in the early decline in the arterial thiopental concentration although the additional effects of metabolism and uptake in fat were important. Similarly, Sharma et al. (146) have demonstrated that the alteration of hepatic microsomal metabolism significantly influences postanesthetic depression following intravenous thiopental. Thus, at least two biodispositional phenomena, redistribution and hepatic metabolism, appear to play important roles in the termination of the pharmacologic effects of the ultrashort acting thiopental.

In addition to cardiovascular and respiratory depressant properties, several other factors, worthy of consideration, have detracted from the clinical utilization of thiopental as a general anesthetic. Post-operative increases in the sensitivity to somatic pain, i.e. hyperalgesia, have been reported following moderate doses of the thiobarbiturate (60). The sequelae of an intra-arterial injection of concentrated thiopental are well documented and may be quite serious (24). Following its repetitive administration, thiopental accumulates in body fat deposits, thus delaying complete recovery from the effects of the drug for an appreciable period of time. The accumulated thiobarbiturate also may augument the effects of other central depressant drugs.

The disadvantages of thiopental motivated the search for intravenous anesthetics with brief durations of action resulting from their rapid

elimination from the body, for example via metabolic pathways or excretory processes (59). New short acting barbiturates were evaluated (108) as well as the eugenols which are induction agents derived from the oil of cloves, and "minor tranquilizers" such as the benzodiazepine derivative, diazepam (59). Suffice it to say that each group of drugs had both distinct advantages and handicaps. In addition to a high incidence of postanesthetic nausea and vomiting, the marked cardiovascular depression and hypotension of the eugenol derivatives (20) offset their respiratory stimulating properties and rapid plasma hydrolysis (37). The delayed onset of action and respiratory depressant properties detracted from the analgetic properties of even low doses of diazepam. Thus the search for the ideal anesthetic agent continued.

## The Dissociative Anesthetics

The late 1950's ushered in a family of cyclohexylamines which produced a different state of anesthesia. This anesthetic state, characterized by catalepsy, catatonia, amnesia, and analgesia, was termed "dissociative anesthesia" (128). The term was originally defined on the basis of the dual electroencephalographic (EEG) effects of the cyclohexylamine derivatives. By depressing nervous activity of the thalamo-neocortical systems while simultaneously increasing that of the limbic system, the drugs caused a "functional dissociation" between the two systems (48). Other investigators interpreted the EEG effects, however, in terms of functional disorganization attributable to subclinical seizure activity in both the thalamus and limbic systems (89). Because of the latter interpretations of the EEG patterns, cataleptic

anesthesia was suggested as a more appropriate term to describe the anesthetic state evoked by the cyclohexylamines (168)(169). Clinically, dissociative anesthesia was described as a somnolent state in which the patient appeared not to be anesthesized, but rather "disconnected" from his surroundings (48).

Irrespective of a complete agreement on a precise terminology to describe the anesthetic state evoked by the cyclohexylamine derivatives, it became apparent that the compounds exhibited certain marked differences in pharmacological actions when compared to other more conventional anesthetics and to the thiobarbiturates.

Phencyclidine [1-(1-phenylcyclohexyl) piperidine] (fig. 1-1) was the first cyclohexylamine derivative to be studied as an anesthetic in the laboratory and the clinic (30)(78). Administered intravenously to man, the long acting drug produced an adequate anesthetic state in most subjects without significant cardiovascular and respiratory depression. However, its advantages were more than offset by a high incidence of posthypnotic emergence phenomena which included unmanageable mania and, in several instances, clonic convulsive movements (128). The duration of action and emergence excitement were too great to recommend use of the drug. It was discarded as a human anesthetic, although it is still used in veterinary medicine.

Structural modification of the cyclohexylamine nucleus led to the synthesis of cyclohexamine [1-(phenyl)-1-(ethylamino) cyclohexane] (fig. 1-1)(98). The shorter duration of action of cyclohexamine did not compensate for its high incidence of inadequate anesthesia and post-hypnotic agitation (43). Therefore, the drug never achieved extensive

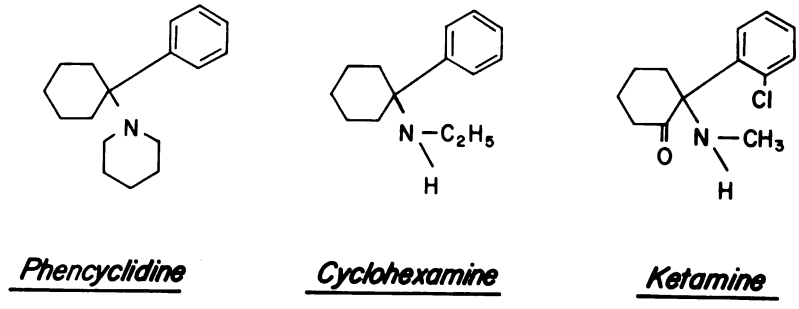



Figure 1-1. Structural formulas of the three dissociative anesthetic agents: phencyclidine, cyclohexamine, and ketamine.

clinical use in human beings. A similar fate befell the thiophene congener of the cyclohexylamines, CI-634 [2-(ethylamino)-2-(2-thienyl) cyclohexanone]. Its "intermediate duration of action" and inadequate anesthetic activity were very similar to cyclohexamine. However, because of the thiophene's rapid onset of action and wide safety margin between cataleptic and lethal doses, the drug was suggested to be useful for the immobilization of animals in the wild (32).

The search continued for a more suitable congener of phencyclidine, a drug which would be a better anesthetic agent than phencyclidine and, yet, lack its postanesthetic effects. In 1961, ketamine [2-(o-chlorophenyl)-2-(methylamino) cyclohexanone] (fig. 1-1) was synthesized. Animal studies were begun in 1962 and clinical investigation was started in 1964. Unlike phencyclidine, ketamine did not cause convulsions, and its much shorter duration of action allowed for more accurate control of anesthesia by the use of supplemental doses (55). The fewer instances of emergence phenomena following ketamine's administration were of a much shorter duration than those observed following phencyclidine. Ketamine became commercially available in the spring of 1970 and is currently marketed by Parke, Davis & Company (Detroit, Michigan), the company which introduced the drug, and Bristol Laboratories (Syracuse, New York).

## Ketamine Pharmacology

Because it may be administered intravenously or intramuscularly without significant tissue irritation, ketamine is an extremely versatile agent. Following intravenous administration, the highly lipid soluble

drug (42) rapidly enters the brain to produce almost immediate anesthesia of short duration (41)(100). The time to onset and duration of anesthesia are more protracted following intramuscular ketamine.

Ketamine anesthesia is characterized by a trance-like, cataleptic state with generalized mild hypertonus (19)(47)(55)(94)(128), a tendency to keep the eyes open (19)(55)(91), and some degree of nystagmus (19)(96).

Pharyngeal and laryngeal reflexes have been reported to remain active when ketamine is used as a monoanesthetic (56)(90)(96). Despite the retention of these reflexes, however, tracheal soiling and aspiration have occurred following ketamine administration (129). In addition, ketamine stimulates marked salivation which must be blocked by an antisialagogue (96).

The rapid administration or overdosage of ketamine can cause apnea or marked respiratory depression (90)(91). However, the careful administration of ketamine rarely results in more than a transitory, slight respiratory depression (47)(114)(128)(163). Mild respiratory depression is rapidly followed by not only a normal respiratory volume, but also by normal response to carbon dioxide challenge (163). In any case, the respiratory depressant effects of ketamine are considerably less frequent and less intense than those observed following the administration of any other intravenous anesthetic agent with the possible exception of the eugenol derivatives (supra vide).

Ketamine generates an increase in cerebral spinal fluid (CSF) pressure, particularly in patients with abnormal flow dynamics and/or other intracranial pathology (145). Consequently, the drug

must be used with caution when a preexisting increase in CSF pressure is present.

A major difference between ketamine and the conventional hydrocarbon and barbiturate anesthetics is the cardiovascular stimulating action of this compound (59)(128)(160). This property of ketamine makes it especially valuable as a safe induction agent in patients for whom avoidance of cardiovascular depression is critical (9)(124). Increasing evidence indicates that the adrenergic nervous system must be intact for the cardiovascular stimulatory responses to occur (57)(142)(158)(159). Ketamine probably acts either directly by stimulating the central adrenergic centers (35)(36) or indirectly by inhibiting the neuronal reuptake of the catecholamines, especially norepinephrine (118)(157). It must be emphasized that the rapid injection of boluses of ketamine has been reported to depress the myocardium (158).

Notable analgesia accompanies the administration of ketamine (47)(55)(153)(163). In contrast to the thiobarbiturates (supra vide) ketamine provides satisfactory analgesia at subanesthetic doses (18)(140)(151).

The beneficial qualities of ketamine are being delineated and put to use for specific situations. Its ease of administration and rapid onset of action with minimal cardiorespiratory depression make it especially valuable for poor risk patients and children (9)(100)(124). Ketamine's broad therapeutic index (114) and apparent lack of chronic toxicity are indications for its use in clinical situations such as repeated burn dressings (14)(151) and recurrent radiotherapy for children (12)(49). It is particularly useful for cardiac catheterization

where myocardial depression and positive pressure ventilation may markedly change pressure and shunt abnormalities (96).

However, the incidence of postanesthetic phenomena observed following ketamine's clinical administration has deterred its wide acceptance for use in man (58)(62). Because of the desirable characteristics of ketamine anesthesia, various approaches have been employed in an attempt to minimize the purposeless movements, auditory and visual disturbances, and unpleasant dreams which follow the administration of the drug. One means employed to reduce the incidence and nature of the emergence phenomnea has been the elimination of external stimulation and hasty arousal of patients during the period or recovery from ketamine anesthesia (48)(100).

Narcotics, sedative-hypnotics, tranquilizers, anticholinergics, and combinations of these drugs, administered as premedications just prior to the end of anesthesia, also have been used to alleviate the emergence phenomena which occur following ketamine anesthesia (11)(17)(138)(140). Droperidol, pentazocine, and the opiate-hyoscine combinations appear to modify the emergence phenomena to a certain extent.

Admittedly, these clinical approaches have helped to diminish the postanesthetic effects of ketamine. Clearly, however, animal research, directed at both the precise definition of the specific mechanism(s) underlying ketamine's pharmacologic effects and the biodispositional events influencing the duration of action and response to the drug, affords the greatest opportunity for the elimination

of the adverse effects of ketamine anesthesia. Such an approach permits the rational development not only of specific pharmacologic antagonists of the adverse psychic effects of ketamine but also of new ketamine congeners with fewer undesirable properties.

Possibly because of the difficulties of extrapolation to man, few animal studies have been designed to elucidate the factors underlying ketamine's psychic effects. Nonetheless, posthypnotic central stimulatory and depressant actions, manifested by increases in spontaneous locomotor activity and ataxia, respectively, have been observed following the administration of hypnotic doses of ketamine to mice, rats, and dogs (31)(42)(114). If a relationship does exist between the animal and the human pharmacology of ketamine, the importance of further studies on the pharmacologic effects of ketamine in animals is certainly justified and cannot be overemphasized.

Similar logic applies to animal studies of the biodisposition of ketamine. Because a variety of factors are known to influence the biodisposition of drugs (69)(132), the judicious use of ketamine requires a complete knowledge of its biodisposition and an elucidation of the factors which may alter its absorption (e.g. from intramuscular injection sites), distribution, metabolism, and excretion.

Published information on the biodisposition of ketamine in laboratory animals and man was minimal until the introduction of a gas chromatographic method adequately sensitive and selective for the assay of ketamine, and its metabolites, in body tissues and fluids (26).

Because of ketamine's extreme lipid solubility, a "blood brain

barrier" to the drug is virtually nonexistent. Ketamine rapidly enters the brain and achieves maximal levels within 1 minute following its intravenous administration to rats (41). Brain and plasma levels of ketamine then rapidly decline during the next 10 minute period after intravenous administration (41)(42). Meanwhile, norketamine, the N-demethylated metabolite of ketamine, accumulates in brain tissue (41). However, it appears that the metabolite contributes only marginally to the hypnotic effect of ketamine (42).

A variety of mechanisms, ranging from hepatic metabolism to excretion via the kidneys and lungs, may operate to terminate the pharmacological actions of a drug (74). Pretreatment of rats with phenobarbital and SKF 525-A, agents anticipated to modify hepatic microsomal metabolism, had no effect on the duration of anesthesia, but markedly altered the duration of posthypnotic ataxia and locomotor stimulation following intravenous ketamine (42). Such studies suggested that at least two biodispositional events, one of which is hepatic metabolism, operate to terminate the pharmacologic effects of ketamine.

Ketamine is N-demethylated to norketamine [2-(o-chlorophenyl)-2-(amino) cyclohexanone], also commonly called metabolite I (fig. 1-2). Norketamine is further oxidized to [2-(o-chlorophenyl)-2-(amino) cyclohex-5-enone] which is commonly known as metabolite II. The N-demethylated metabolite has been reported to be the major metabolite in man (26)(34) and in the rat (34)(41). Two other potential intermediates, tentatively identified as [5-(hydroxyl)-

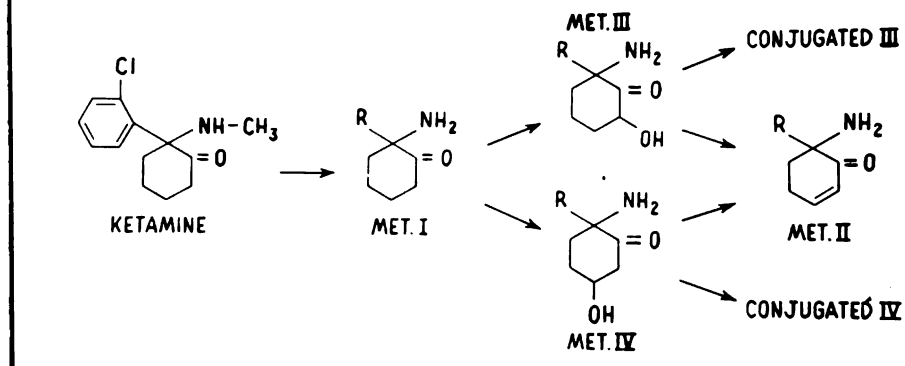



Figure 1-2. Proposed metabolic pathways for the biotransformation of ketamine. Taken from Chang and Glazko (34).

2-(o-chlorophenyl)-2-(amino) cyclohexanone] and [6-(hydroxyl)-2-(o-chlorophenyl)-2-(amino) cyclohexanone] have also been reported (34)(fig. 1-2). The stereochemistry of these hydroxylated intermediates is not known.

Certain species specific differences exist with respect to the overall patterns of metabolite disposition of ketamine. Norketamine is common to the rat, cat, monkey, and man (34)(41). [2-(o-chlorophenyl)-2-(amino) cyclohex-5-enone] does not appear to accumulate to the same extent in the rat as it does in primates. Such species differences in ketamine's metabolic disposition have received minimal attention to date, and it is unclear whether they result from qualitative differences in metabolic pathways or from variations in the rates of individual enzymatic reactions.

One final point deserves consideration. Up to this point the discussion of the pharmacology of ketamine has been concerned only with the biological activity and biodisposition of its racemic mixture. Although the ketamine enantiomers have been resolved (fig. 1-3), no studies of their pharmacology have been reported in the literature. Differences in the pharmacologic effects of the optical isomers of a variety of drugs, ranging from narcotic analgetics to drugs acting at the level of the autonomic nervous system, have been well established (1)(28)(33)(51)(52)(73)(152)(154)(155)(156). Therefore, because ketamine enantiomorphs are distinct molecular species, it is quite possible that pharmacologic and metabolic differences, both of a qualitative and quantitative nature, exist between the two ketamine optical isomers.

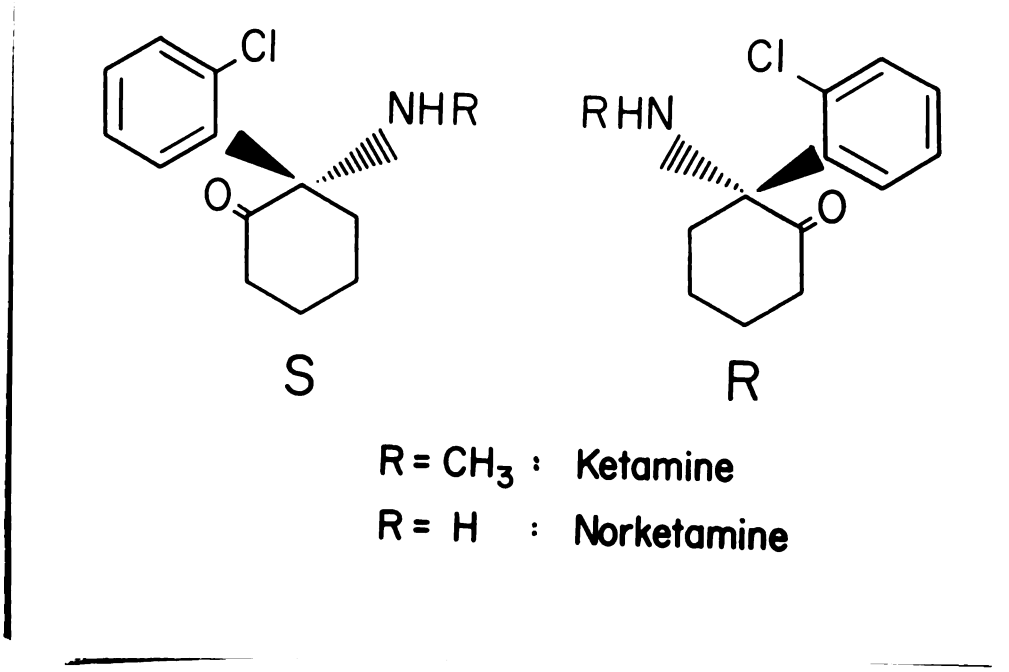



Figure 1-3. The enantiomorphs of ketamine and norketamine. Configuration about the asymmetric center is denoted by the S and R convention of Cahn et al. (27).

Proposed Research

Although the bulk of ketamine research has been clinically oriented, several studies have been directed toward a better understanding of the biodispositional events which influence the duration and intensity of the pharmacologic effects of ketamine in laboratory animals (41)(42). The purpose of the present research was to study further the influences of two biodispositional phenomena, hepatic metabolism and redistribution, on the pharmacological activity of ketamine. Information from such studies hopefully would be of value in the prediction of potential alterations of the anticipated pharmacologic effects of ketamine, e.g. alterations due to the presence of other drugs, to changes in "normal" physiological parameters, or to the development of pathologies. It was also hoped that the knowledge of the time course of the pharmacologic effects of ketamine, coupled with the information concerning the time dependence of its tissue distribution, might also contribute to the elucidation of the mechanism(s) underlying the pharmacologic effects of ketamine.

The second major thrust of this research was directed toward an evaluation of the pharmacologic activities of the two optical isomers of ketamine. Studies were designed to determine the existence of differences, if any, in the pharmacologic effects of the isomers. The ultimate goal of the isomer studies was to determine if one of the enantiomorphs, by virtue of greater safety or fewer undesirable pharmacological actions, might be of potentially greater clinical value than either its counterpart or racemic ketamine.

Rats were chosen for experimental study for several reasons. Obviously, they are easily handled, conveniently housed, and relatively inexpensive. In addition, much of the previous research on the biodisposition of ketamine was undertaken in rodents, thus providing a solid base for further study (34)(41)(42). Naturally, had our studies in rats yielded results of potential human clinical value, we assumed that extrapolation to man would pass "up" the biological ladder. That is, the isomers would be evaluated in various other species, including primates, prior to their clinical evaluation in man.

Organization of Research

The present research was divided into two parts. The first part, which is presented in Chapter 2, is concerned with studies on the biodisposition of ketamine in the rat. The tissue distribution of ketamine, and norketamine, was determined in rats pretreated with ketamine and two drugs anticipated to modify hepatic microsomal metabolism, phenobarbital and SKF 525-A. In addition, the effects of intravenous ketamine on the durations of three pharmacological actions of ketamine, hypnosis, ataxia, and locomotor activity, were examined. Finally, the influence of ketamine pretreatment on both its own *in vitro* hepatic microsomal metabolism and that of other compounds was studied in some detail.

The second part of this research, found in Chapter 3, is directed toward the examination of the pharmacologic actions and biodisposition of the ketamine enantiomorphs. The pharmacologic effects, acute toxicity, brain and plasma pharmacokinetic profiles,

and *in vitro* hepatic microsomal N-demethylation of the ketamine enantiomorphs was studied.

# CHAPTER 2

## Biodisposition of Ketamine in the Rat: Self-Induction of Metabolism

## INTRODUCTION

The ultimate and perhaps most intellectually attractive goal in pharmacology is the understanding of the molecular interactions of a drug with its "receptor site". It must be realized, however, that usually only a small portion of the total amount of drug in the body is at any time directly interacting with the receptors producing a pharmacological action. Yet it is the bulk of the drug, localized in parts of the body remote from the site of action that governs the kinetics of the movement of the drug through tissues of the body and its ultimate disappearance from the body. These kinetic factors, i.e. biodispositional events, thus underly the temporal course of the intensity of the pharmacologic effect and determine such practical matters as dosage schedules and even the field of useful clinical application of a drug.

The clinical advantages of ketamine over other anesthetic agents have been described (supra vide). In addition, it has been pointed out that the clinical usefulness of ketamine has been limited by the presence of undesirable emergence phenomena accompanying the administration of the drug. It is apparent, therefore, that the better that the biodispositional factors influencing ketamine's pharmacological actions are understood, the greater is the likelihood of understanding and perhaps reducing or eventually eliminating the adverse pharmacologic effects of the drug.

Three major mechanisms whereby drugs are ultimately removed from their sites of action, and, eventually, from the body are metabolism, storage, and excretion (74). Two biodispositional

phenomena, redistribution from brain to other body tissues and hepatic metabolism, have been suggested to be involved in the termination of ketamine's pharmacologic effects in the laboratory rat (42), in the cat (7), and in man (167). If redistribution and metabolism do play major roles in the cessation of the pharmacologic effects of ketamine, it is natural to question which body tissues serve as reservoirs or "sinks" for the drug and its primary N-demethylated metabolite, and to consider the effects of agents which modify hepatic drug metabolism on brain and plasma levels of ketamine and norketamine. These questions were approached by the determination of levels of ketamine and norketamine in various tissues at selected time intervals after intravenous administration and by examination of brain and plasma levels of ketamine in rats pretreated with drugs capable of modifying hepatic microsomal drug metabolism.

Central stimulation manifested by increased motor activity has been observed in rodents following the administration both of sub-hypnotic (31)(114) and hypnotic (42) doses of ketamine. Jiggle cages and subjective observation were used in these earlier studies. In the present study, electronic motility measurement was used to assess the intensity and duration of posthypnotic central stimulation following intravenous ketamine. In addition, the influence of hepatic function on the duration of agitation after ketamine administration was examined using drugs expected to modify hepatic drug metabolism.

While the clinical use of ketamine does not ordinarily involve chronic administration, Cronin et al. (49) and Bennett and Bullimore (12)

have described repetitive ketamine anesthesia for radiotherapy in small children. The same authors also pointed out that children, receiving radiotherapy daily for a continuous period of 1-3 weeks, required increased doses of ketamine. The duration of satisfactory sedation of the children for the dose of ketamine which had previously proved to be satisfactory was shortened. Similarly, Bree et al. (21) reported that in monkeys "a tolerance to CI-581 developed following repeated anesthetization". To answer the question of whether or not the repetitive administration of ketamine would alter its pharmacologic and pharmacokinetic properties, we investigated the effects of ketamine pretreatment on the duration of its pharmacologic effects, brain and plasma levels of ketamine and its primary N-demethylated metabolite, and the rate of in vitro N-demethylation of ketamine in rats.

After it became apparent that ketamine pretreatment did alter the rate of the in vitro hepatic N-demethylation of ketamine, further studies were designed to assess the influences of ketamine pretreatment on the levels of hepatic microsomal cytochrome P-450 and NADPH-cytochrome c reductase activity. In addition, the rates of in vitro hepatic metabolism of benzphetamine and 3,4-benzo(a)pyrene were examined using microsomal fractions from ketamine pretreated rats.

## MATERIALS AND METHODS

### Intact Animal Experiments

Male, Sprague-Dawley rats (Simonsen Laboratories, Gilroy, Calif.), weighing 110-150 g, aged 33-36 days, and provided Berkeley Standard Diet

for Rats and water ad libitum, were used exlusively. Three rats per cage were housed in wire-meshed cages (24 X 18 X 18 cm) eight cm above indirect bedding (no additives; Blake, Moffit, and Towne, San Francisco) which was changed every other day. Environmental temperature was maintained at 21-23°C and the relative humidity at 55 ± 5%. The photoperiod was controlled to provide light from 0600 to 1800 hours; there was no twilight. To investigate the effects of i.v. ketamine in pretreated rats, animals were immobilized for a 30 sec caudal vein injection as previously described (41), and the duration of loss of righting reflex, duration of ataxia, and locomotor activity were examined. All studies were performed between 1000 and 1700 hours in order to minimize the possible influences of circadian rhythm (88)(134) on the results of the study.

The duration of loss of righting reflex was defined as the time interval between animal injection and the recovery of its righting reflex. The righting reflex was judged to be regained when the rat, which had been placed on its back immediately following drug injection, turned over on its front and repeatedly did so within 2 sec of being repositioned on its back.

The duration of ataxia was measured subjectively. Normal gait was defined as the ability of the animal to walk without crouching on its hindlimbs, to move without staggering or falling, and to balance on its hindlimbs without toppling. The ketamine injected animals were compared with uninjected rats and the observations of the animals were done in a "blind" fashion, i.e. the observer was unaware of the period of time that had passed between injection and cessation

of ataxia.

Locomotor activity was measured with an Electronic Motility Meter Fc 40 (Motron produckter, Stockholm, Sweden). Ketamine was injected and the rat was placed upon the motility meter immediately following the recovery of righting reflex. Activity was recorded for each of six consecutive 10-minute intervals and expressed as motility meter counts per 10-minute interval.

Quantitation of baseline meter activity is desirable when motility meter studies are undertaken. However, it is apparent, because of the possible influences on locomotor activity of hypnosis itself, that the validity of subtracting baseline activities of control rats, i.e. uninjected rats or those receiving caudal vein injections of vehicle (normal saline) alone, from those of rats receiving hypnotic doses of ketamine is questionable. Therefore, although the baseline motility meter activities for control rats (supra vide) were identical to those for animals pretreated with ketamine, SKF 525-A, and phenobarbital, they were not subtracted from those of pretreated rats following hypnotic doses of ketamine.

In Vivo Tissue Distribution

Rats receiving intravenous ketamine (30 mg/kg) were decapitated at various times after caudal vein injection. Heparin-treated blood samples, obtained by exsanguination following decapitation and representative tissue samples (whole brain, liver, skeletal muscle, kidney, heart, gut, and skin) were collected and homogenates (10% w/v) prepared from all tissues, except skin (5% w/v) in 0.1 N hydrochloric acid. Plasma samples, collected by centrifuging the blood samples

at 1000 X g for 10 minutes, and tissue supernatant samples, obtained by ultracentrifuging tissue homogenate samples at 100,000 X g for 60 minutes were then either extracted immediately or refrigerated and extracted the following day for chromatographic assay of ketamine and its metabolites as described later.

Preparation of Microsomal Fractions

To examine the effects of pretreatment with ketamine, phenobarbital, and 2-diethylaminoethyl 2,2-diphenylvalerate HCl (SKF 525-A) on the hepatic metabolism of ketamine, microsomes were prepared using a modification of the procedure described by Marietta et al. (105). Rats, previously starved for 14 hours, were decapitated and exsanguinated between 0900 and 1000 hours. Their livers were perfused immediately with 50 ml of ice cold 160 mM KCl-50 mM Tris-HCl, pH 7.40, excised, and placed on ice. All subsequent steps were carried out at 0-4 C. The pooled livers from 4 rats were minced with scissors and the tissue mince was homogenized with 2 volumes of the KCl buffer using 6 passes of the motor driven Teflon pestle of a Potter-Elvehjem homogenizer. Cell debris, nuclei, and mitochondria were removed by centrifugation at 10,000 X g (average) for 20 min. The mitochondrial supernatant was centrifuged for 60 minutes at 105,000 X g. The microsomal pellet was suspended in a volume of 160 mM KCl-50 mM Tris-HCl equal to that of the original mitochondrial supernatant and resedimented at 105,000 X g for 60 min. The washed microsomal pellet was resuspended in the KCl buffer, pH 7.40, to a final protein concentration of 8.0 mg of microsomal protein per ml as determined by the method of

Lowry et al. (101). To eliminate the possible influences of storage on hepatic microsomal metabolism and substrate-induced difference spectra (83), microsomes were freshly prepared for each study.

In Vitro Ketamine Metabolism

The in vitro microsomal N-demethylation of ketamine was estimated by incubating 0.3 ml of the prepared microsomal suspension with drug concentrations ranging from $5 \times 10^{-5}$ M to $1.5 \times 10^{-3}$ M in the case of the incubations with the microsomes from ketamine pretreated and control animals. Concentrations of ketamine used for incubations with the microsomes of phenobarbital pretreated rats ranged from $1 \times 10^{-4}$ M to $1.5 \times 10^{-3}$ M. The Erlenmeyer flasks also contained glucose-6-phosphate (12.5 mmol), $MgCl_2$ (25 mmol), glucose-6-phosphate dehydrogenase (2 I.E.U.), and nicotinamide adenine dinucleotide phosphate (1 mmol) adjusted to a final reaction volume of 2.5 ml with 160 mM KCl-50 mM Tris-HCl, pH 7.40. A Dubnoff metabolic incubator, shaking at a rate of 100 rpm, was used for incubations, all of which lasted for 15 minutes at 37°C in room air. The reaction was initiated by the addition of ketamine to the reaction vessel. Samples (0.1 ml) were taken at zero time and 15 minutes and transferred into 0.9 ml of 0.1 N hydrochloric acid for assay of the parent compound and its N-demethylated metabolite. Under these conditions, the rate of demethylation of ketamine was linear with time.

In Vitro Benzphetamine N-Demethylation

The N-demethylation of benzphetamine was determined by measuring the rate of formaldehyde formation (113)(122). In addition to containing

the same cofactors, at their respective concentrations, that been described for the in vitro metabolism of ketamine (supra vide), each 2.5 ml incubation also contained semicarbazide (.005 mmol) to trap the generated formaldehyde. Assay conditions were identical to those described in the section immediately above with the following exceptions. The 10 minute demethylation reaction was initiated by the addition of benzphetamine ($12.5 \times 10^{-3}$ mmol) and terminated by the addition of 0.1 ml of trichloroacetic acid (20%). After removal of the precipitated microsomal protein by centrifugation in an International Centrifuge at 1000 rpm for 15 minutes, 1.5 ml of the supernatant was mixed with 0.6 ml of double strength Nash Reagent (113) and incubated at 50°C for 45 minutes. After cooling to room temperature, the intensity of the yellow color was read at 415 nm using a Gilford 2000 Spectrophotometer. Corrections were made for formaldehyde produced by reactions not involving drug substrate by performing incubations in which only benzphetamine was omitted. Standard curves for the quantitation of formaldehyde present in the microsomal supernatant were prepared by carrying formaldehyde solutions, ranging in concentration from $1.3 \times 10^{-5}$ M, to $2.7 \times 10^{-4}$ M, through the Nash procedure (122).

3,4-Benzo(a)pyrene Hydroxylation

Hydroxylation of 3,4-benzo(a)pyrene was assayed using a modification of the method described by Nebert and Gelboin (123). Glucose-6-phosphate (5 mmol), $MgCl_2$ (3 mmol), nicotinamide adenine dinucleotide phosphate (1 mmol), glucose-6-phosphate dehydrogenase (0.7 I.E.U.), and microsomal protein (1.0 mg) were

added to the reaction vessel. The final reaction volume of 1.0 ml was buffered to a pH of 6.80 using 0.1 M sodium phosphate buffer. The assay was carried out at 37°C with a shaking rate of 80 rpm in a room darkened except for a yellow source. The reaction was initiated by the addition of 3,4-benzo(a)pyrene (0.24 µmol in 0.02 ml acetone) to the incubation mixture and stopped 10 minutes later by the addition of 1.0 ml acetone. Enzyme activities were determined in duplicate and compared to a blank to which acetone had been added prior to incubation.

After the addition of acetone, 3.25 ml of hexane were added and the mixture was reincubated with shaking at 37°C for 10 min. A 2.0 ml sample of the organic phase was extracted with 4.0 ml of 1 N sodium hydroxide. The concentration of the hydroxylated metabolite, 3-hydroxybenzo(a)pyrene, in the aqueous phase was determined using a SPF #2 Aminco-Bowman Spectrofluorometer. The excitation and emission wavelengths were 386 and 515 nm, respectively. The sensitivity and accuracy of the spectrofluorometer were checked using 3-hydroxybenzo(a)pyrene as a standard; the revovery of the 3-hydroxybenzo(a)pyrene ranged from 67 to 72%.

Measurement of Microsomal Spectra

Using a modification of the dithionite difference method of Omura and Sato (125), the cytochrome P-450 contents of hepatic microsomes, prepared from rats pretreated with phenobarbital and ketamine, were determined at room temperature (20-23°C) with a Shimadzu model MPS-50L recording spectrophotometer. All final microsomal suspensions had a protein concentration of 1 mg per ml and were

buffered to pH 7.70 with 0.1 M potassium phosphate buffer. The sample cuvette, which contained 2.0 ml of suspension, was gassed for 20 sec with carbon monoxide that had passed through 3 gassing towers each containing 200 ml of 0.05% sodium anthroquinone-2-sulphonate and 5% sodium dithionite in 0.1 N sodium hydroxide. After the addition of a few milligrams of solid sodium dithionite to both the sample and reference cuvettes, the difference spectrum was recorded for the wavelength interval encompassing 380 to 520 nm. The extinction due to cytochrome P-450 was taken to be the increment between 450 and 490 nm prior to the addition of dithionite subtracted from the difference after addition of the reducing agent. A molar extinction coefficient of 91 $cm^{-1}$ $mM^{-1}$ (113)(125) was employed for the determination of cytochrome P-450 content.

Spectral studies of the interaction of ketamine with hepatic microsomal cytochrome P-450 were accomplished using a modification of the methods described by Remmer et al. (135) and Imai and Sato (85). Solutions of ketamine were micropipetted into 1.0 ml of nonreduced microsomes contained in a Hellma self-masking spectrophotometric cell with a 4 mm wide light path. With each addition of ketamine to a sample cuvette, a corresponding volume of buffer was added to the reference cuvette. The microsomal suspension, prepared from the livers of untreated rats and containing 1.1 mg per ml of microsomal protein, was buffered with 160 mM KCl-50 mM Tris-HCl, pH 7.40. Difference spectra were recorded for the wavelength interval encompassing 350 to 480 nm using an Aminco DW-2-Dual-Wavelength Spectrophotometer. All spectra were recorded

at room temperature (20-23 C).

Determination of NADPH-Cytochrome C Reductase Activity

Cytochrome c reductase activity was determined essentially by the method of Masters et al. (109). 36 mmol of oxidized horse heart cytochrome c (Sigma Type III), 100 mmol of ehtylenediamine-tetraacetic acid (EDTA), and 30 µg of microsomal protein in 0.99 ml of 0.05 M potassium phosphate buffer, pH 7.70, were added to a 1.0 ml spectrophotometric cuvette. The reduction of cytochrome c was initiated by the addition of 0.1 ml of buffer containing 100 nmol of reduced nicotinamide adenine dinucleotide phosphate (NADPH). The reaction was followed at 500 nm and 25°C with a Gilford 2000 recording spectrophotometer and the rate of cytochrome c reduction was determined during the initial linear phase of the reaction. One unit of activity was defined as an absorbance change of 1.0 unit per minute at 500 nm and 25°C in a 1-cm light path. The unit of activity corresponded to a reduction of 47.6 nmol of cytochrome c per minute per ml of reaction mixture (109).

Assay of Ketamine and Norketamine

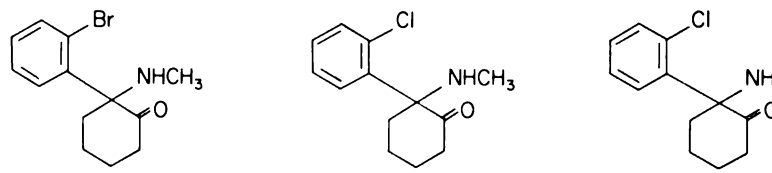
All samples were extracted and derivatized for assay of ketamine and norketamine bases using the modification by Cohen et al. (41) of the gas chromatographic procedure of Chang and Glazko (26). Because the procedure has been extensively detailed (40)(41), only our modifications of the Cohen procedure that were used for this particular study will be described:

A. Although the stock standard solutions prepared for this work were identical to those described by Cohen (40),

our working standard solutions of ketamine, norketamine, and the brominated internal standard [2-(o-bromophenyl)-2-(methylamino) cyclohexanone] (fig. 2-1) for the gas chromatographic assay contained 1.0, 0.5, and 5.0 ug of free base per ml of 0.1 N hydrochloric acid, respectively.

B. Gas chromatographic apparatus and conditions were similar with the following exception. The flow rate of the carrier gas, Argon 95%/Methane 5%, was adjusted to 24-25 ml/min, thus giving better resolution of the drug peaks. Therefore, new retention times were 50 sec for norketamine, 93 sec for ketamine, and 129 sec for the internal standard. Samples were injected every 15-18 min.

C. The extraction procedure for the present studies was modified in the following manner. Following the addition of heptafluorobutyric anhydride (0.1 ml) and pyridine (0.1 ml) to the benzene extract contained in the glass stoppered conical centrifuge tubes, the stoppered tubes were vigorously hand shaken for 5 sec and, then, immediately vortexed. The tubes were allowed to stand at room temperature for 30 min, instead of 60 min as described by Cohen (40)(41), and were vortexed every 10 minutes during that period. The modification, which allowed a more rapid extraction and derivitization of samples containing ketamine and norketamine, did not influence the derivatization of ketamine or norketamine as evidenced by no change in the ratio of their peak heights to that of the internal standard. Recoveries from known standard solutions of ketamine




*Internal Standard* *Ketamine* *Norketamine*

Figure 2-1. Structural formulas of the brominated internal standard for the ketamine and norketamine gas chromatographic assay, ketamine, and norketamine.

and norketamine, using the 30 min procedure were identical to those following the 60 minute derivatization.

Preparation of Tissue Standard Curves

Standard curves were prepared for each tissue that was examined in this study. To prepare the curves for the representative tissues, male, Sprague-Dawley rats (110-150 g) were decapitated and exsanguinated. Homogenates (10% w/v) in 0.1 N hydrochloric acid were prepared from whole brain, liver, skeletal muscle (gastrocnemius, biceps femoris, and gracilius), heart, kidney, and gut. The working solutions of the tissue standards, which were used to prepare standard curves, contained 1.0, 0.5, and 5.0 ug/ml of the ketamine, norketamine, and internal standard free bases, respectively. A similar procedure was used for skin, but, because of the viscosity of the 10% homogenate, a 5% w/v homogenate was prepared. Tissue supernatants were obtained by ultracentrifugation of the homogenates at 100,000 X g for 60 minutes. Aliquots of the supernatants, ranging from 0.05 to 0.60 ml, were extracted and standard curves were prepared for ketamine and norketamine. Standard curves for whole blood and plasma were prepared as described by Cohen et al. (40)(41). Recovery of ketamine and norketamine from plasma and the tissue homogenates ranged from 96-99%. Duplicate assays of samples varied less than 3%. As were Cohen's (40), our standard curves were linear for concentrations of ketamine ranging from 25 to 300 ng per ml. Norketamine standard curves were linear from 12.5 to 150 ng per ml. In subsequent gas chromatographic analyses, plasma and tissue supernatant samples

were diluted so that levels of ketamine and norketamine could be calculated from the linear segments of their respective standard curves.

Pretreatment Schedules

Animals pretreated with phenobarbital were injected intraperitoneally (i.p.) twice daily for 3 days with drug (35 mg/kg/ injection) with the final injection given 18 hours prior to ensuing experimentation. Likewise, ketamine was administered i.p. twice daily for 3 days at a dose of 40 mg/kg/ injection with one final injection also 18 hours preceding further experimental manipulation. 2-Diethylaminoethyl 2,2-diphenylvalerate HCl (SKF 525-A) was injected i.p. at a dose of 25 mg/kg 60 minutes prior to subsequent experimentation . Control animals were concurrently injected with equal volumes of 0.9% NaCl, the vehicle for the three drugs used to pretreat the rats.

Chemicals

All reagents were of analytical grade. Sources of materials used for the gas chromatographic assay have been enumerated by Cohen (40). 3,4-Benzo(a)pyrene, trisma HCl, NADP, EDTA, cytochrome c, NADPH, acetylacetone, and glucose-6-phosphate dehydrogenase were obtained from Sigma Chemical Company (St. Louis, Missouri). Glucose-6-phosphate was purchased from CalBiochem (San Diego, California). Ammonium acetate, sodium and potassium phosphates, magnesium, sodium, and potassium chlorides, acetone, hexane, and formaldehyde were obtained from Mallinckrodt Chemical Works (St. Louis, Missouri). Sodium dithionite was

purchased from Eastman Organic Chemicals (Rochester, New York). 3-Hydroxybenzo(a)pyrene was obtained from Dr. H. V. Gelboin of the National Cancer Institute. Ketamine hydrochloride crystals and solution (Ketalar) were supplied by Parke, Davis & Company (Detroit, Michigan) and SKF 525-A was a gift from Smith Kline and French Laboratories (Philadelphia, Pennsylvania).

Statistical Analyses

Differences between the means of the pharmacologic effects and tissue levels of ketamine and norketamine were evaluated using Student's t test for unpaired data (170). P values less than 0.05 were judged to be significant. Kinetic data was analyzed from a weighted regression line plotted using the FORTRAN program of Cleland (38)(39).

## RESULTS

### Ketamine Distribution in Selected Tissues

After intravenous ketamine (30 mg/kg), peak levels were achieved in brain within 1 minute and declined rapidly during the initial 10 minutes after injection (table 2-1; fig. 2-2). Simultaneously, levels of injected ketamine in skeletal muscle, liver, gut, and skin were rising to the plateau levels eventually achieved 10 to 20 minutes after drug administration (fig. 2-2). Subsequently, levels of drug in all tissues declined slowly, paralleling the plasma decay of ketamine.

Data from table 2-1 show that less than 1% of the ketamine administered i.v. remained in the plasma at 10 minutes after injection, and at that time nearly 70% of the parent drug was

TABLE 2-1

Percentage Recovery of Ketamine from Selected Tissues

Ketamine (30 mg/kg) was injected into the caudal veins of rats subsequently decapitated at the indicated times after injection to collect tissues for assay. Data express ketamine and nor ketamine (metabolite I) recovered from each tissue as percentages of the total amount of ketamine administered. Calculations are based on the mean drug concentrations shown in fig. 2-2, and the tissue weights per 100 g of rat cited in the text.

| | 1 Min[a] | 5 Min | 10 Min | 20 Min | 30 Min | 60 Min |
|---|---|---|---|---|---|---|
| Ketamine percent recovery (tissue) | | | | | | |
| Muscle | 33.0 | 37.1 | 37.8 | 26.6 | 16.2 | 11.4 |
| Skin | 6.0 | 8.3 | 12.1 | 16.2 | 12.5 | 6.2 |
| Liver | 4.3 | 7.2 | 7.7 | 2.9 | 2.4 | 1.7 |
| Gut | 8.1 | 10.8 | 10.9 | 10.1 | 6.2 | |
| Kidneys | 4.9 | 3.3 | 2.8 | 1.5 | 1.3 | 0.7 |
| Heart | 1.1 | 0.6 | 0.5 | 0.4 | 0.2 | 0.1 |
| Brain | 4.0 | 2.0 | 0.7 | 0.3 | 0.3 | 0.1 |
| Plasma | 2.2 | 1.3 | 0.8 | 0.5 | 0.4 | 0.3 |
| Total percent accounted for | 63.6 | 70.6 | 73.3 | 58.5 | 39.5 | 20.5 |
| Metabolite I[b] percent recovery (tissue) | | | | | | |
| Muscle | 0.5 | 3.6 | 5.6 | 7.5 | 8.4 | 8.4 |
| Skin | 0 | 1.2 | 1.8 | 3.3 | 4.0 | 6.7 |
| Liver | 0.6 | 4.6 | 6.7 | 7.3 | 8.9 | 6.0 |
| Gut | 0.5 | 1.6 | 3.1 | 4.8 | 5.6 | |
| Kidneys | 0 | 0.4 | 0.7 | 1.0 | 1.4 | 1.4 |
| Heart | 0 | 0.2 | 0.3 | 0.2 | 0.2 | 0.2 |
| Brain | 0 | 0.2 | 0.3 | 0.2 | 0.2 | 0.2 |
| Plasma | 0.1 | 0.4 | 0.7 | 0.8 | 1.0 | 1.1 |
| Total percent accounted for | 1.7 | 12.2 | 19.2 | 25.1 | 29.7 | 24.0 |

[a] Time after injection.

[b] Calculated as ketamine (moles) metabolized to metabolite I.

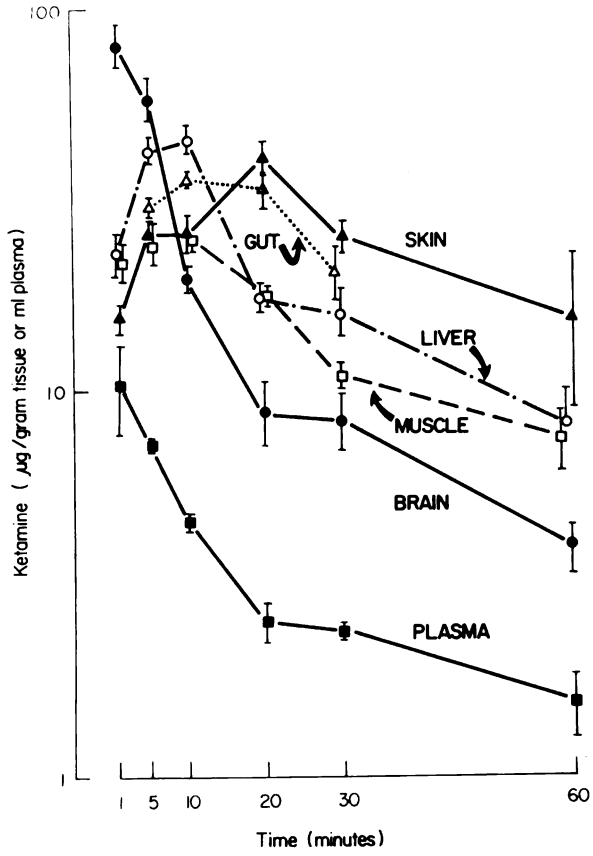



Figure 2-2. Distribution of ketamine after its caudal vein administration (30 mg/kg) to rats. At the indicated times, animals were decapitated for gas chromatographic assay of ketamine levels in selected tissues. Values are means for five animals with vertical bars representing $\pm$ S. E. M.

localized within skeletal muscle, skin, gut, and liver[1]. Such tissues appear to serve as major reservoirs for ketamine. Estimation of percent recovery of ketamine 10 minutes subsequent to intravenous administration shows that 73% of the injected drug was recovered as the parent molecule, while nearly 20% of the dose was recovered as metabolite I (norketamine). In addition, although not presented, determinations of ketamine and metabolite I (norketamine) levels in "whole rat homogenates" prepared from animals killed 10 minutes after intravenous ketamine administration (30 mg/kg) disclosed 74% of the injected drug recovered as ketamine and 24% recovered as the N-demethylated metabolite, norketamine.

Pharmacologic Actions of Intravenous Ketamine in Pretreated Rats

The durations of three pharmacologic effects of intravenous ketamine (30 mg/kg) were determined in animals pretreated with ketamine, phenobarbital, and SKF 525-A. No pretreatment had any significant effect on the duration of loss of righting reflex (hypnosis) after injection (table 2-2). However, SKF 525-A pretreatment caused marked prolongation of the durations of ataxia ($p < .01$) as well as the duration of posthypnotic locomotor activity (fig. 2-3). In control experiments it was determined that SKF 525-A itself had no significant influence on locomotor activity. Phenobarbital and ketamine pretreatments resulted in significant decreases in the durations of ataxia and locomotor activity observed following ketamine administration (table 2-2; fig. 2-4). It should be noted

[1] In this and subsequent calculations, the following data from our laboratory and Goldstein and Aronow (72) are used: for 100 g of rat, plasma 5 g, muscle 45 g, kidneys 2 g, liver 5 g, skin 12 g, brain 1 g, heart 1, gut 9 g.

TABLE 2-2

Effects of Ketamine HCl, Phenobarbital, and SKF 525-A Pretreatments on Pharmacologic Effects of I.V. Ketamine

Ketamine hydrochloride (30 mg/kg) was injected into the caudal vein of pretreated rats and durations of effects were measured from the end of injection as detailed under "Materials and Methods". Values for durations of loss of righting reflex and ataxia represent means ±S.E.M. of four animals.

| Pretreatment | Duration of Loss of Righting Reflex (minutes) | Duration of Ataxia (minutes) |
|---|---|---|
| Control | 8.0 ± 1.7 | 27.2 ± 5.0 |
| SKF 525-A | 9.9 ± 2.1 | 58.1 ± 7.5[a] |
| Ketamine | 8.1 ± 2.0 | 18.1 ± 3.0[a] |
| Phenobarbital | 7.9 ± 1.8 | 19.3 ± 2.5[a] |

a P value $<.01$ by Student's unpaired t test

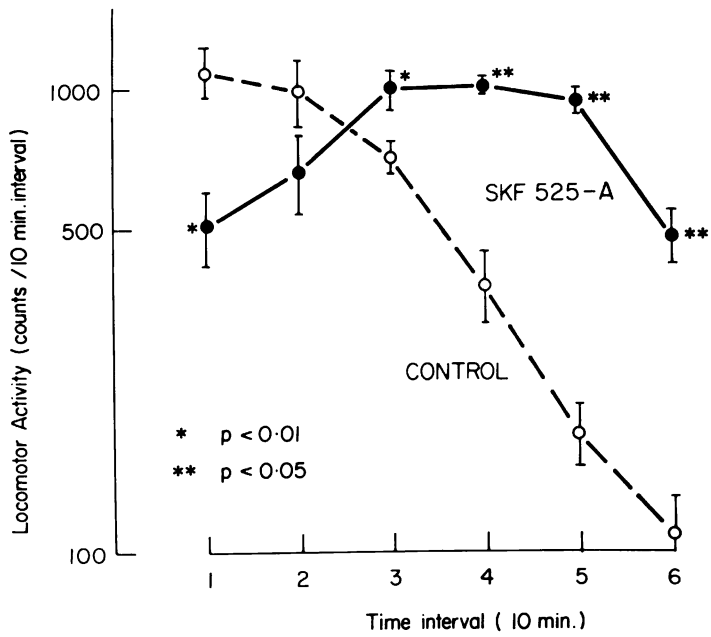



Figure 2-3. Locomotor activity after caudal vein injection of ketamine (30 mg/kg) in rats pretreated with SKF 525-A. Motility meter activity was recorded for individual rats during each of six consecutive 10-minute intervals subsequent to cessation of ketamine hypnosis and is expressed as counts per 10-minute interval. Values represent means (±S. E. M.) of five experiments.

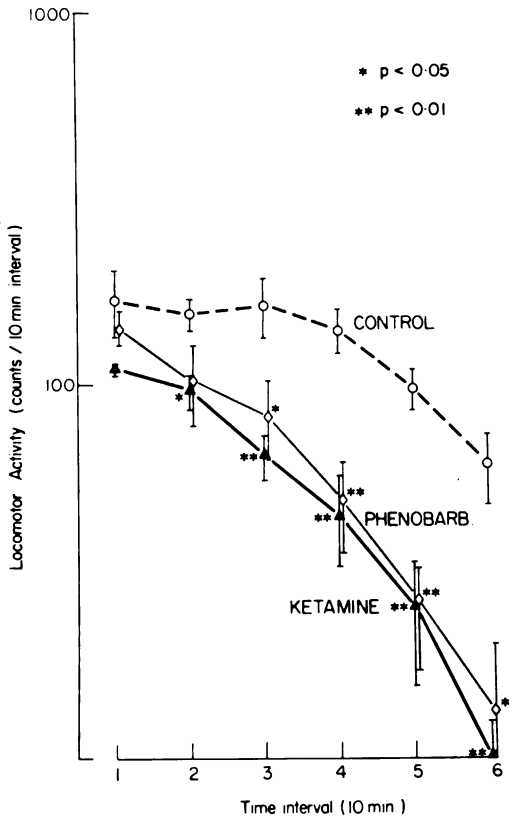



Figure 2-4. Locomotor activity after caudal vein administration of ketamine (30 mg/kg) to rats pretreated with ketamine and phenobarbital. Motility meter activity was recorded for individual rats during each of six consecutive 10-minute intervals subsequent to cessation of ketamine hypnosis and is expressed as counts per 10-minute interval. Values represent means (± S. E. M.) of five experiments.

that the various pretreatments had the same effects when ketamine was administered at a dosage of 20 mg/kg i.v. (table 2-3).

Ataxia observed after intravenous ketamine was considered to be of central rather than peripheral origin. Interestingly, ketamine has been reported to prolong the neuromuscular blockade observed following the administration os succinylcholine and d-tubocurarine to the cat (50), decamethonium to the rabbit (16), and d-tubocurarine to man (63). Administered by itself, however, ketamine has been observed to lack neuromuscular blocking properties at anesthetic dosage levels (16)(50) and, in fact, has been reported to facillitate muscle twitch in the rabbit (16). Our examination of the rat phrenic nerve-diaphragm preparation showed that levels of ketamine and norketamine that occur in skeletal muscle in vivo had no effects on neuromuscular transmission.

Posthypnotic locomotor activity observed following ketamine administration appeared to be specific properties of the drug. The intravenous administration of doses of thiopental, equihypnotic to those of ketamine, caused a minor decrement, rather than an increase, in locomotor activity compared to control animals.

Plasma and Brain Levels of Ketamine and Norketamine in Rats Pretreated with Ketamine and SKF 525-A

After intravenous ketamine (30 mg/kg) to rats, plasma levels of the drug declined rapidly ($T_{\frac{1}{2}}$ approximately 10 minutes) and neither pretreatment schedule significantly altered the plasma levels of ketamine during the initial 10 minute period after injection (fig 2-5). At subsequent time points, SKF 525-A pretreatment significantly elevated plasma ketamine levels up to and including

TABLE 2-3

Effects of Ketamine HCl, Phenobarbital, and SKF 525-A Pretreatments on Pharmacologic Effects of I.V. Ketamine (20 mg/kg)

Ketamine hydrochloride (20 mg/kg) was injected into the caudal vein of pretreated rats and durations of effects were measured from the end of injection as detailed under "Materials and Methods". Values for durations of loss of righting reflex and ataxia represent means ±S.E.M. of four animals.

| Pretreatment | Duration of Loss of Righting Reflex (minutes) | Duration of Ataxia (minutes) |
|---|---|---|
| Control | 6.2 ± 0.5 | 22.2 ± 1.2 |
| SKF 525-A | 5.4 ± 0.8 | 42.7 ± 4.4[a] |
| Ketamine | 5.4 ± 0.5 | 16.9 ± 1.1[a] |
| Phenobarbital | 6.4 ± 0.7 | 16.4 ± 1.8[a] |

a P value $< .05$ by Student's unpaired t test

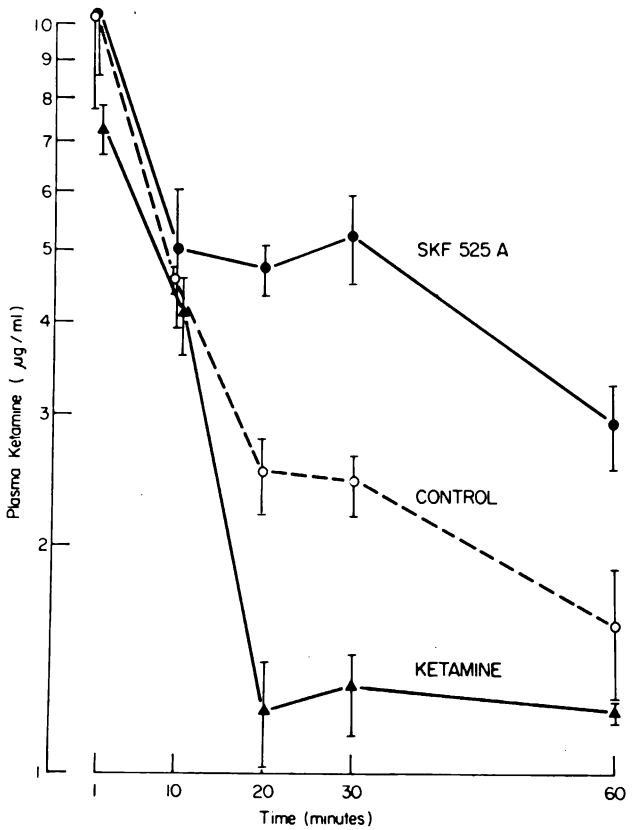



Figure 2-5. Plasma levels of ketamine after caudal vein administration of ketamine (30 mg/kg) to rats. Animals pretreated with ketamine and SKF 525-A were decapitated at the indicated times after i.v. injection for gas chromatographic assay of plasma ketamine levels. Values are means of four experiments and vertical bars represent ±S.E.M.

60 minutes. By contrast, ketamine pretreatment lowered plasma levels of injected ketamine although the effect was not statistically significant at the 60 minute time point.

A similar pattern was observed for brain levels of ketamine (fig. 2-6). Levels of the drug were maximal within 1 minute after intravenous injection and declined rapidly during the initial 10 minutes ($T_{\frac{1}{2}}$ approximately 8 minutes). Although neither pretreatment schedule significantly modified the levels of ketamine during the first 10-minute interval, SKF 525-A pretreatment significantly increased brain ketamine levels at all time points examined after ten minutes. While ketamine pretreatment decreased the brain levels of ketamine at 20, 30, and 60 minutes, only the 30 minute ketamine brain level was significantly decreased ($p < .05$). Estimation of brain levels of ketamine at time points of recovery of righting reflex indicated no differences between control animals and those pretreated with ketamine or SKF 525-A. In all groups of animals, brain levels of ketamine were nearly identical (approximately 9 μg/g of tissue) at the point of cessation of ataxia.

The plasma norketamine pharmacokinetic patterns of rats pretreated with SKF 525-A differed statistically from those of control animals only at the 10-minute time point following the intravenous administration of ketamine (30 mg/kg)(fig. 2-7). Norketamine levels in ketamine pretreated rats were similar to those of control animals for the initial 20-minute interval following i.v. ketamine (30 mg/kg). Subsequently, the levels of norketamine

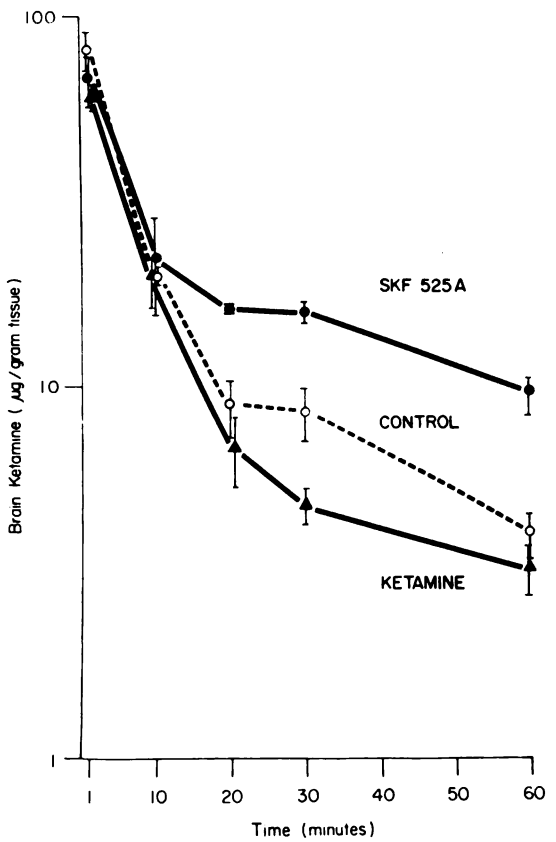



Figure 2-6. Brain levels of ketamine after caudal vein administration of ketamine (30 mg/kg) to rats. Animals pretreated with ketamine and SKF 525-A were decapitated at the indicated times after i.v. injection for gas chromatographic assay of brain ketamine levels. Values are means of four experiments and vertical bars represent ±S.E.M.

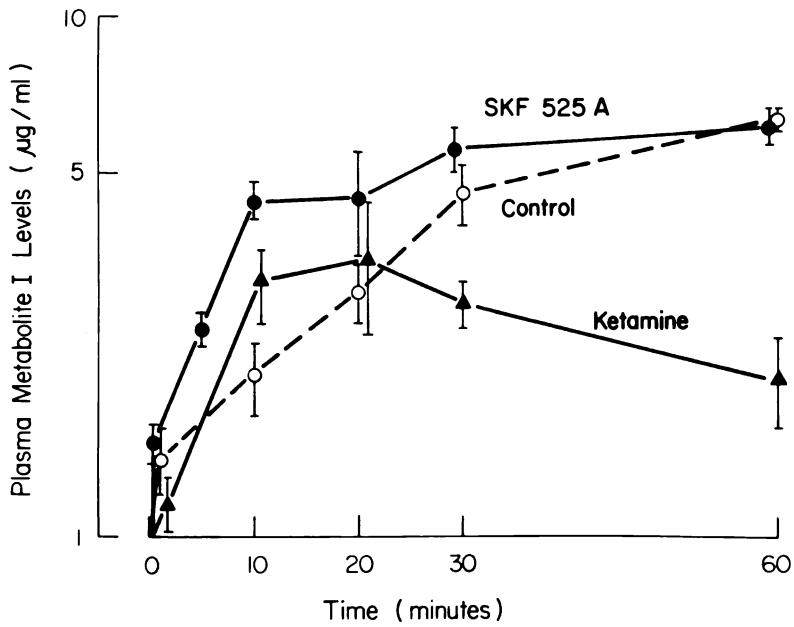



Figure 2-7. Plasma levels of norketamine after caudal vein administration of ketamine (30 mg/kg) to rats. Animals pretreated with ketamine and SKF 525-A were decapitated at the indicated times after i.v. injection for gas chromatographic assay of plasma norketamine levels. Values are means of four experiments and vertical bars represent ±S.E.M.

in the plasma of ketamine pretreated rats were statistically lower ($p<.05$) than those of control animals.

Brain norketamine pharmacokinetic patterns for the animals pretreated with ketamine and SKF 525-A were similar to those observed for plasma (fig. 2-8). Peak norketamine levels of 6-8 µg/g were achieved in the brains of SKF 525-A and control rats 20 minutes following the intravenous ketamine (30 mg/kg) and remained elevated throughout the 60-minute time period chosen for study. Brain norketamine levels in ketamine pretreated rats peaked during the initial 10 to 20 minute interval following intravenous ketamine. The levels of norketamine in ketamine pretreated rats thereafter declined to levels approximately 30-50% those in control animals (fig. 2-8).

In Vitro Metabolism of Ketamine

The in vitro N-demethylation of ketamine by rat hepatic microsomes was investigated in rats pretreated with phenobarbital and ketamine. Kinetic data presented in table 2-4 disclose that ketamine pretreatment doubled the maximal rate (Vmax) of ketamine N-demethylation ($p<.05$). Phenobarbital pretreatment caused more than a 4-fold increase in the maximal rate of ketamine metabolism ($p<.05$). SKF 525-A pretreatment has been shown to decrease the rate of N-demethylation of ketamine by more than 30% (42).

Pretreatment with ketamine had no influence on the apparent Michaelis constant (Km) for the N-demethylation of ketamine (table 2-4). However, the apparent Km for the rate of ketamine N-demethylation in phenobarbital pretreated animals was significantly

TABLE 2-4

Kinetic Analyses of the Hepatic Microsomal N-Demethylation of Ketamine in Phenobarbital and Ketamine Pretreated Rats

The hepatic microsomal metabolism of ketamine was estimated and analyzed as detailed under "Materials and Methods". Each pretreatment group consisted of 6 rats which were randomly assigned to subgroups consisting of 2 animals. Kinetic constants were calculated from duplicate samples of the microsomes prepared from the pooled livers of each subgroup.

| Pretreatment | Km ± S.E.M. (mM) | Vmax ± S.E.M. (nmol N-demethylated/mg of protein/hr) |
|---|---|---|
| Control | .127 ± .010 | 207 ± 16 |
| Ketamine | .149 ± .015 | 435 ± 33[a] |
| Phenobarbital | .218 ± .028[a] | 894 ± 45[a] |

a P value $< .05$

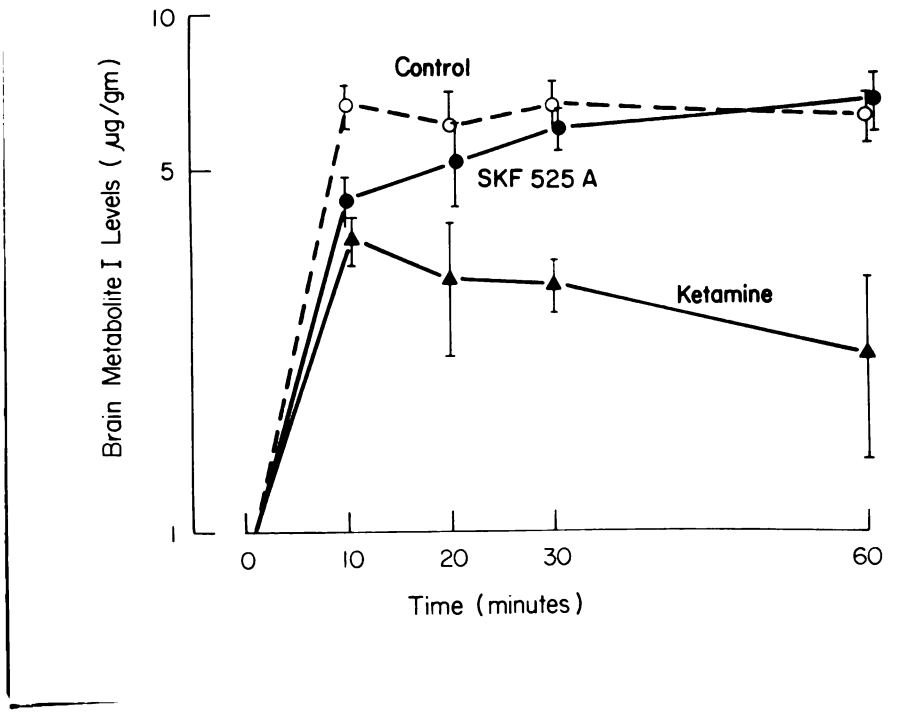



Figure 2-8. Brain levels of norketamine after caudal vein administration of ketamine (30 mg/kg) to rats. Animals pretreated with ketamine and SKF 525-A were decapitated at the indicated times after i.v. injection for gas chromatographic assay of brain norketamine levels. Values are means of four experiments and vertical bars represent ±S.E.M.

greater ($p < .01$) than that for control animals (table 2-4).

Effects of Ketamine and Phenobarbital Pretreatments on Cytochrome P-450 Levels and NADPH-Cytochrome C Reductase Activity in Rats

Phenobarbital and ketamine pretreatments both significantly elevated the levels of hepatic microsomal cytochrome P-450 (table 2-5). The phenobarbital pretreatment tripled the level of P-450 ($p < .01$), whereas ketamine pretreatment caused a 50% increase in the microsomal content of the hemoprotein ($p < .05$).

Similarly, as shown in table 2-5, both pretreatments significantly increased NADPH-cytochrome c reductase activity of the liver microsomes ($p < .05$). As was the case for cytochrome P-450, the phenobarbital pretreatment enhanced the cytochrome c reductase activity to a greater extent than did the ketamine pretreatment. However, the differences in the cytochrome c reductase activity were not as marked as those observed for the levels of P-450.

In Vitro Benzphetamine N-Demethylation and 3,4-Benzo(a)pyrene Hydroxylation in Ketamine and Phenobarbital Pretreated Rats

Both ketamine and phenobarbital pretreatments significantly increased the rate of benzphetamine N-demethylation (table 2-5). The more than 4-fold enhancement of benzphetamine N-demethylation by phenobarbital pretreatment paralleled the effect the identical pretreatment had on the hepatic microsomal N-demethylation of ketamine (table 2-4). However, ketamine pretreatment caused a nearly 3-fold increase in the rate of benzphetamine N-demethylation, an increase which was greater than the 2-fold increase that the pretreatment with ketamine exerted over its own rate of *in vitro* hepatic N-demethylation (tables 2-4 and 2-5).

TABLE 2-5

Effect of Ketamine and Phenobarbital Pretreatments on Hepatic Microsomal Cytochrome P-450 Levels, NADPH-Cytochrome C Reductase Activity, Benzphetamine N-demethylation, and 3,4-Benzo(a)pyrene Hydroxylation

Cytochrome P-450 levels, NADPH-cytochrome c reductase activity, benzphetamine N-demethylation, and 3,4-benzo(a)pyrene hydroxylation were determined using hepatic microsomes prepared from pooled livers of phenobarbital and ketamine pretreated rats as detailed under "Materials and Methods". Values represent duplicate assays as described in the legend of figure 2-4.

| | Control ($\bar{X} \pm$ S.E.M.) | Ketamine ($\bar{X} \pm$ S.E.M.) | Phenobarbital ($\bar{X} \pm$ S.E.M.) |
|---|---|---|---|
| CYTOCHROME P-450 (nmol/mg protein) | 0.38 ± .03 | 0.57 ± .04[b] | 1.13 ± .03[a] |
| CYTOCHROME C REDUCTASE (nmol cytochrome c reduced/mg protein/min) | 50.23 ± 4.23 | 70.27 ± 3.65[b] | 86.19 ± 12.08[b] |
| BENZPHETAMINE N-DEMETHYLATION (nmol HCHO formed/ mg protein/min) | 2.23 ± 0.16 | 6.03 ± 0.59[a] | 9.45 ± 0.85[a] |
| 3,4-BENZO(a)PYRENE HYDROXYLATION (nmol/mg protein/min) | .054 ± .006 | .119 ± .003[a] | .197 ± .018[a] |

a P value $<$ .01 by Student's unpaired t test

b P value $<$ .05 by Student's unpaired t test

3,4-Benzo(a)pyrene hydroxylation was significantly increased ($p < .01$) by both ketamine and phenobarbital pretreatments (table 2-5). The extents to which the two pretreatments increased the rate of 3,4-benzo(a)pyrene hydroxylation (table 2-5) paralleled those to which they enhanced ketamine N-demethylation (table 2-4).

Interaction of Ketamine with Washed Microsomes

Figure 2-9 discloses that the addition of ketamine (0.146 mM) to washed, nonreduced hepatic microsomes from untreated rats produced a characteristic type I shift in the absorbance spectrum ($\lambda$max, 385 nm; $\lambda$min, 420 nm).

Effect of Phenobarbital and Ketamine Pretreatments on Brain and Plasma Levels of Ketamine and Norketamine

Both pretreatments significantly decreased ($p < .05$) the plasma levels of ketamine at the 30- and 40-minute time points following the intravenous administration of the drug (30 mg/kg)(fig. 2-10). Similarly, brain levels of ketamine were lower in the ketamine and phenobarbital pretreated rats (fig. 2-11). The plasma and brain ketamine levels in rats pretreated with ketamine were interposed between those of control and phenobarbital pretreated animals (figs. 2-10 and 2-11). However, at the times chosen for study, there were no statistical differences in either the brain or plasma ketamine levels in the rats pretreated with phenobarbital and ketamine.

Brain and plasma levels of norketamine in rats were significantly less ($p < .05$) than those of control animals (figs. 2-12 and 2-13). The norketamine levels of ketamine pretreated animals fell between those of control and phenobarbital pretreated rats. Decreased

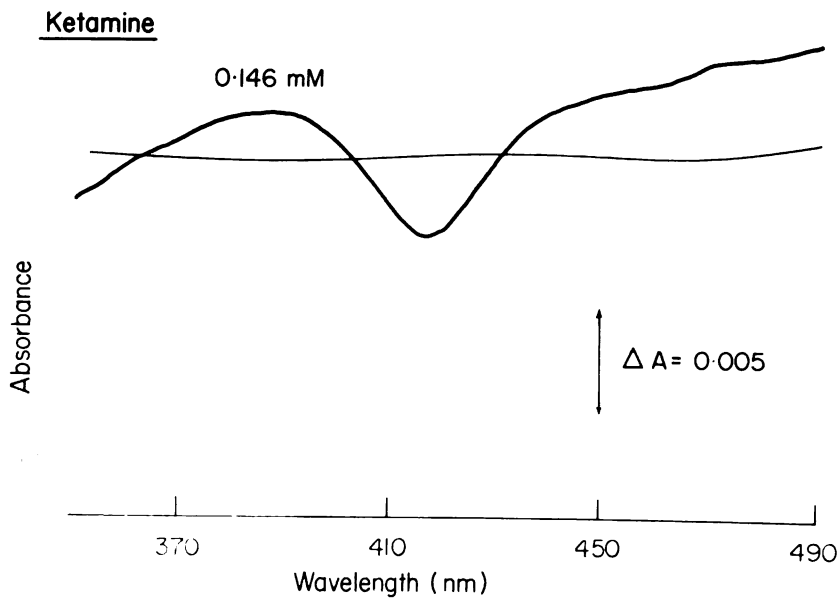



Figure 2-9. Type I cytochrome P-450 binding spectrum for ketamine (0.146 mM) using microsomes from untreated rats as detailed under "Materials and Methods".

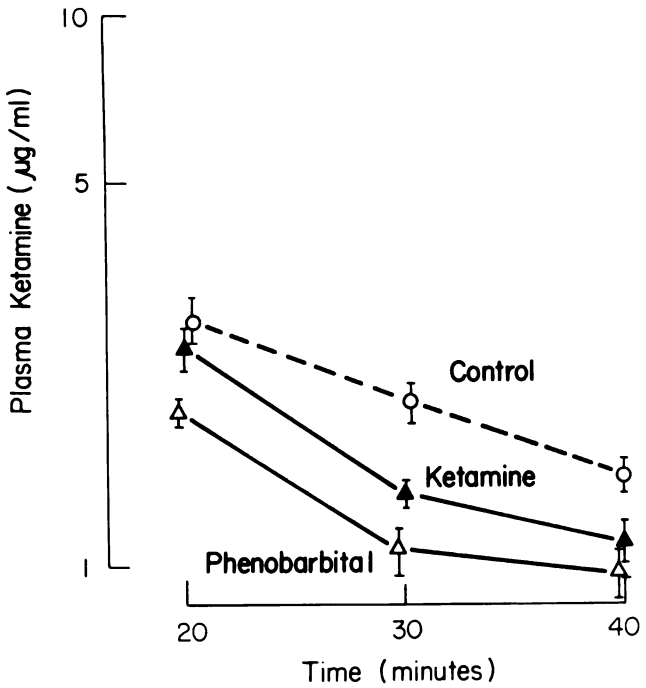



Figure 2-10. Plasma levels of ketamine following the caudal vein injection of ketamine (30 mg/kg) to phenobarbital and ketamine pretreated rats. Animals were decapitated at the indicated times after i.v. injection for gas chromatographic assay of plasma ketamine levels. Values represent means (±S.E.M) of five experiments.

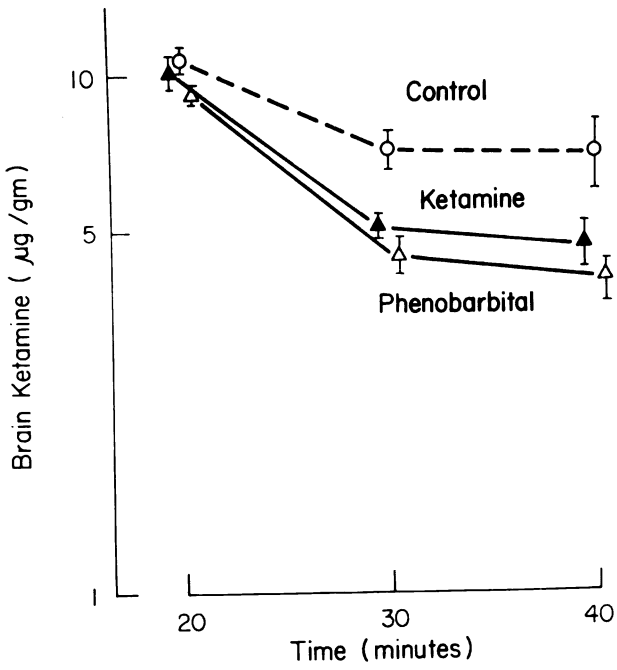



Figure 2-11. Brain levels of ketamine following the caudal vein injection of ketamine (30 mg/kg) to phenobarbital and ketamine pretreated rats. Animals were decapitated at the indicated times after i.v. injection for gas chromatographic assay of brain ketamine levels. Values represent means (±S.E.M) of five experiments.

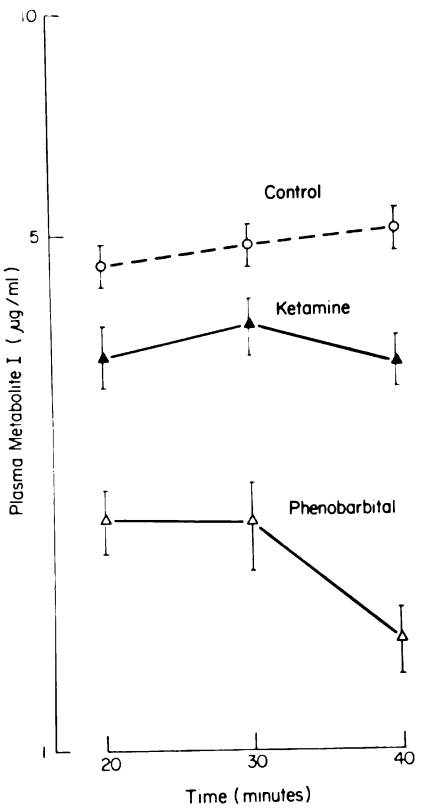



Figure 2-12. Plasma levels of norketamine following the caudal vein injection of ketamine (30 mg/kg) to phenobarbital and ketamine pretreated rats. Animals were decapitated at the indicated times after i.v. injection for gas chromatographic assay of plasma norketamine levels. Values represent means (±S.E.M) of five experiments.

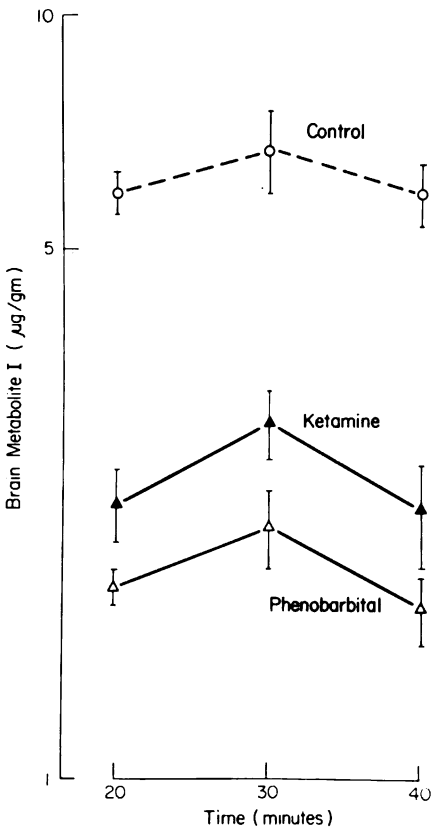



Figure 2-13. Brain levels of norketamine following the caudal vein injection of ketamine (30 mg/kg) to phenobarbital and ketamine pretreated rats. Animals were decapitated at the indicated times after i.v. injection for gas chromatographic assay of brain norketamine levels. Values represent means (±S.E.M) of five experiments.

plasma levels of norketamine (fig. 2-12), along with decreased levels of ketamine (fig. 2-10), suggest that ketamine and phenobarbital pretreatments may augument the metabolism of ketamine both at the N-demethylation step and at a level of the further metabolic conversion of norketamine.

## DISCUSSION

Three pharmacologic actions of intravenous ketamine (30 mg/kg) were studied in rats, namely the duration of loss of righting reflex (hypnosis) and two posthypnotic events, ataxia and locomotor activity. Pretreatment of rats with ketamine and drugs anticipated to modify hepatic microsomal drug metabolism (6)(44)(45)(46) had no effect on the duration of loss of righting reflex (tables 2-2 and 2-3), but markedly altered the durations of the posthypnotic drug effects (tables 2-2 and 2-3; figs. 2-3 and 2-4). Moreover, ketamine and SKF 525-A pretreatments influenced brain and plasma levels of intravenous ketamine only subsequent to the initial 10 minutes following intravenous injection (figs. 2-5 and 2-6). Irrespective of pretreatment schedule, brain levels of ketamine were similar in all animals at the cessation of hypnosis as well as ataxia (fig. 2-6). Subsequent to the initial 20 minutes following intravenous ketamine administration (30 mg/kg), brain and plasma norketamine levels in ketamine pretreated rats were significantly lower than those of control and SKF 525-A pretreated animals (figs. 2-7 and 2-8). Simultaneously, brain and plasma norketamine pharmacokinetic patterns of control and SKF 525-A pretreated rats were nearly identical (fig. 2-7 and 2-8). From correlations between brain

levels of norketamine and the duration and intensity of the post-hypnotic pharmacologic effects of intravenous ketamine (30 mg/kg) in control and SKF 525-A pretreated animals, therefore, the contribution of norketamine to the posthypnotic effects of ketamine appears minimal or, possibly, masked by the more intense pharmacologic activity of the parent compound.

The intact animal data coupled with those on brain and plasma levels observed following intravenous ketamine (30 mg/kg) suggest that two separate biodispositional events are involved in the termination of the pharmacologic actions of the drug. Hepatic metabolism appears to play a prominent role in the termination of the post-hypnotic effects of ketamine. However, because agents anticipated to modify hepatic drug metabolism had no influence on the duration of loss of righting reflex (hypnosis) observed following intravenous ketamine, other biodispositional phenomena apparently play important roles in the termination of hypnosis.

Because of its lipid solubility, rapid entry into brain tissue, and relatively short duration of action, ketamine has been compared with short acting thiobarbiturates (42)(59). It is conceivable, therefore, that redistribution events operate to terminate ketamine hypnosis as in the case of the thiobarbiturate, thiopental (72)(133). Peak levels of ketamine occur in brain, heart, kidney, and plasma within 1 minute after intravenous ketamine (30 mg/kg) and, subsequently, fall rapidly during the initial 10 to 20 minutes after injection (table 2-1; fig. 2-2). Simultaneously, levels of ketamine in gut, liver, skin, and skeletal muscle rise to plateau levels

within 5 to 20 minutes following injection. Ten minutes after intravenous administration, nearly 70% of the parent drug is localized within the gut, liver, skin, and skeletal muscle (table 2-1). Thus, it appears likely that these four tissues serve as reservoirs for both injected ketamine and ketamine which has redistributed from brain into plasma.

Numerous factors have been shown to alter the biodisposition and pharmacologic actions of a variety of therapeutic agents. Age, sex, and state of health (66)(70), as well as environmental influences, including bedding materials, ambient temperature, cage crowding, and painful stimulii (162), have been shown to influence the responses of laboratory animals and man to drugs. One of the most important factors contributing to alterations in drug action and biodisposition is the administration of drugs which either stimulate or depress hepatic drug microsomal metabolism (46)(64)(95)(103)(104)(136). Several investigators have suggested that the repetitive administration of ketamine leads to the development of tolerance to further administrations of the drug (12)(21)(49). Tolerance to drugs, e.g. barbiturates and opiates, can arise from cellular adaptation to the agents, from increased elimination of the drugs, e.g. via increased hepatic metabolism, or from a combination of cellular and metabolic events (75). To our knowledge, only one study concerning the influences of repetitive ketamine administration on hepatic metabolism has been reported in the literature. Chang and Glazko (34) reported ketamine administration (50 mg/kg i.v. at 16- to 24-hour intervals for three doses) to lack stimulatory

effects on the hepatic metabolism of N-methylaniline. Their results suggested that ketamine pretreatment does not enhance the activity of microsomal enzymes responsible for N-demethylation in the rat when estimated 42 hours after the final intravenous ketamine injection. However, our results indicate that this is not the case (tables 2-2 and 2-3; figs. 2-5 and 2-6). Investigation of the in vitro microsomal metabolism of ketamine revealed more than a 2-fold increase in ketamine N-demethylation in rats pretreated with ketamine (table 2-4).

Remmer (136) has listed several general properties that chemical compounds must have if they are to display a potential inducing capacity. Ketamine fulfills the requirements of a high lipid to water partition (42) and a type I cytochrome P-450 binding spectrum (fig. 2-9). Furthermore, in addition to increasing levels of hepatic microsomal cytochrome P-450 (table 2-5), ketamine pretreatment also stimulates the hepatic microsomal metabolism of two substrates traditionally used for the study of in vitro hepatic metabolism, 3,4-benzo(a)pyrene and benzphetamine (table 2-5). Ketamine's relatively short plasma half-life of approximately 2 hours (34) detracts from its potential as an inducing agent. Its situation is analogous to that of the short acting barbiturate, hexobarbital. Although much more lipid soluble than phenobarbital, hexobarbital is a much weaker inducing agent in male rats (136). The probable reason for the differences in ability to induce hepatic metabolism can be attributed to the fact that hexobarbital is rapidly metabolized in the rat. It has a half-life of about 1 hour, whereas the half-life of phenobarbital is nearly

11 hours. Similar correlations between plasma half-life and "stimulatory potencies" have been reported for other barbiturates (161).

Inducers of drug metabolism have been classified according to their effects on various components of the electron transport system associated with the liver microsomes (68). Phenobarbital-like inducers cause increases in NADPH-cytochrome c reductase, cytochrome P-450, and the rate of metabolism of a large number of drugs including type I cytochrome P-450 binding substrates such as aminopyrine and benzphetamine (46)(61)(126). By contrast, the polycyclic hydrocarbons, such as 3-methylcholanthrene and 3,4-benzo(a)pyrene, increase neither NADPH-cytochrome c reductase nor the metabolism of type I compounds (46)(164). Apparently, 3-methylcholanthrene-like inducers exert their effect by stimulating the synthesis of an unusual hemoprotein, variously called P-446 (84), P-448 (3)(5)(71)(117), and $P_1$-450 (150).

The polycyclic hydrocarbons, in addition to increasing the maximal rates of metabolism (Vmax) of various substrates, also markedly alter the apparent Michaelis constants (Km) of hepatic microsomal metabolism of drugs in rodents (2)(3)(4). These marked changes in Km and Vmax, coupled with the shift towards shorter wavelengths in the 450 nm absorption maximum of the CO difference spectrum (*supra vide*), suggest a qualitative, as well as a quantitative difference in the nature of the hemoprotein induced by 3-methylcholanthrene-like drugs (2). Phenobarbital, on the other hand, often significantly increases Vmax of the metabolism

of type I substrates such as ketamine without markedly influencing the apparent Km of their hepatic metabolism (76)(82)(139). However, treatment with the long acting barbiturate has been shown to increase the Michaelis constant of the hepatic metabolism of the type II substrate, aniline (79).

Results from this study suggest that ketamine is a phenobarbital-like inducing agent in rats. Ketamine pretreatment enhanced NADPH-cytochrome c reductase activity (table 2-5) and significantly increased both its own rate of in vitro hepatic microsomal metabolism and that of other type I substrates (tables 2-4 and 2-5). In addition, ketamine pretreatment did not significantly influence the Km of its own hepatic N-demethylation. The "inducing potential" of ketamine, however, is limited by its relatively short plasma half-life (supra vide).

Significantly, although the pretreatment of rats with phenobarbital resulted in more than a 4-fold increase in the in vitro metabolism of ketamine (table 2-4), comparison of phenobarbital and ketamine pretreatments shows no significant differences in their influence on the durations of pharmacologic actions following intravenous ketamine injection (table 2-2; fig. 2-4). Furthermore, 30 minutes after the i.v. administration of ketamine to pretreated rats, brain and plasma levels of ketamine and its nor-metabolite were similar for both pretreatments (fig. 2-10, 2-11, 2-12, and 2-13). One explanation for the similar in vivo effects of ketamine and phenobarbital pretreatments, even though their in vitro effects were markedly different, lies in the conditions selected for the assay

of ketamine N-demethylation *in vitro* following the two pretreatments. Ketamine substrate concentrations chosen for the assay both obeyed requirements for classical Michaelis-Menten saturation (hyperbolic) kinetics and, also, were within the range of sensitivity of the gas chromatographic method used to detect levels of ketamine and norketamine. The *in vitro* levels of ketamine, however, were probably 5 to 150 times greater than those observed in the liver *in vivo*. This apparent lack between *in vivo* and *in vitro* results will be discussed in greater detail in the Discussion section of Chapter 3. However, the observations on the effects of ketamine and phenobarbital pretreatments do raise the question of the validity of predicting *in vivo* metabolic events by extrapolating directly from *in vitro* studies without careful consideration of the many factors affecting drug metabolism (67).

# CHAPTER 3

## The Pharmacology of the Ketamine Enantiomorphs in the Rat

## INTRODUCTION

Due to its rapid onset of action, wide margin of safety, and lack of cardiorespiratory depression, unequalled by any other currently available general anesthetic, ketamine has been shown to be a particularly useful anesthetic in certain clinical situations (9)(47). Nevertheless, as has been pointed out, the high incidence of postanesthetic emergence phenomena, including hallucinations, vocalization, and purposeless movements observed following the clinical administration of ketamine (58)(96), has deterred its wide acceptance. Ketamine bears a chiral center at C-2 (fig. 1-3). Although numerous studies of the animal and human pharmacology have been reported in the literature (supra vide), there have been no published studies on the pharmacologic actions of the two enantiomorphs of ketamine. Since marked variations in the pharmacological actions and biodisposition of optical isomers have been well documented (28)(86), we undertook the following studies to determine if one of the ketamine enantiomorphs had distinctive properties.

Initial experiments were designed to assess differences, if any, in the durations and intensities of three pharmacologic effects (loss of righting reflex, ataxia, and increased locomotor activity) observed following the caudal vein administration to rats of equimolar doses of the two enantiomorphs. In addition, the relative durations and intensities of the posthypnotic effects (ataxia and locomotor activity) of the isomers were compared following their intravenous administrations at equihypnotic doses. Since initial studies

indicated differences in the pharmacologic potencies of the two isomers, we questioned whether it was possible that such differences in biological activity could be due to differences in the biodisposition of of the two enantiomorphs, e.g. tissue distribution (25) or rate of hepatic metabolism (80). We approached these questions by determining brain and plasma levels of the enantiomorphs and their nor-metabolites at selected time intervals following their i.v. equimolar administration, and by studying their in vitro hepatic microsomal metabolism.

Finally experiments were conducted to determine the relative acute toxicities of the two ketamine isomers. $LD_{50}$ values and therapeutic indices, expressed as the ratio of the $LD_{50}$ to that dose of the isomer eliciting a 4 minute period of hypnosis (loss of righting reflex), have been calculated for each isomer.

## MATERIALS AND METHODS

### Intact Animal Experiments

The intact animal experiments were performed exactly as described in Chapter 2 under "Materials and Methods". To determine lethality, rats, receiving 30 second caudal vein injections of ketamine or its enantiomorphs, were observed for a period of 30 minutes. However, all animals that died, did so within 3 to 5 minutes as judged by cardiac standstill.

### In Vivo Brain and Plasma Distribution

Brain and plasma levels of unmetabolized parent amine and metabolically formed norketamine (fig. 1-3) following intravenous administration of (+)-, (-)-, (±)-ketamine and (±)-norketamine

were determined by gas chromatography as described previously (40)(41). Recoveries of (+)-, (-)-, and (±)-ketamine from 0.1 N hydrochloric acid, plasma, and brain homogenate supernatant were determined using the methodology described both by Cohen (40) and under "Materials and Methods" in Chapter 2. As shown in figures 3-1, 3-2, and 3-3, recoveries of (+)-, (-)-, and (±)-ketamine from 0.1 N hydrochloric acid, plasma, and brain homogenate supernatant were identical. The recovery of ketamine and its two enantiomorphs from plasma and brain ranged from 97 to 102% of the recovery of the compounds from 0.1 N hydrochloric acid. Similarly, the recovery of (±)-norketamine from brain homogenate supernatant and plasma has been shown to range from 96 to 99% of that from 0.1 N hydrochloric acid (40)(41). The norketamine enantiomorphs were not available for recovery studies. Additionally, lack of analytical procedures precluded the possiblilty of determining the enantiomorphic compositions of brain and plasma ketamine and norketamine following administration of the racemic drug.

Preparation of Microsomal Fractions

To examine the hepatic metabolism of the ketamine enantiomorphs, microsomes were prepared from the livers of untreated rats using methodology identical to that described under "Materials and Methods" in Chapter 2.

In Vitro Enantiomorph Metabolism

The *in vitro* microsomal N-demethylations of racemic ketamine and its two optical isomers were estimated using the same procedure described for the *in vitro* metabolism of ketamine under "Materials

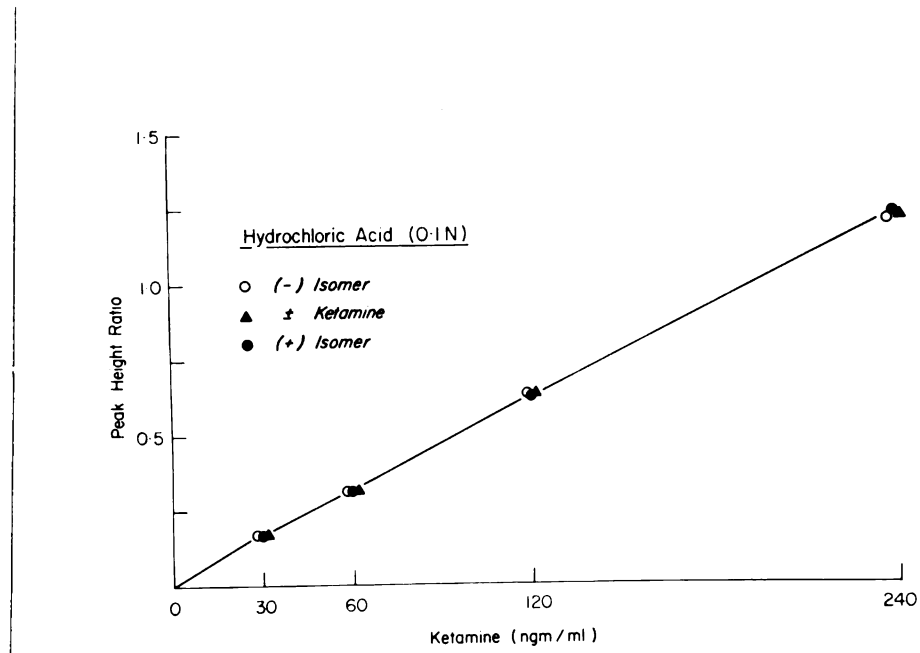



Figure 3-1. Standard curves for (+)-, (±)- and (-)-ketamine extracted from 0.1 N hydrochloric acid as detailed under "Materials and Methods". Each point represents the mean of duplicate assays run on three consecutive days. Because of their relatively small values, standard errors were unable to be depicted on the illustration.

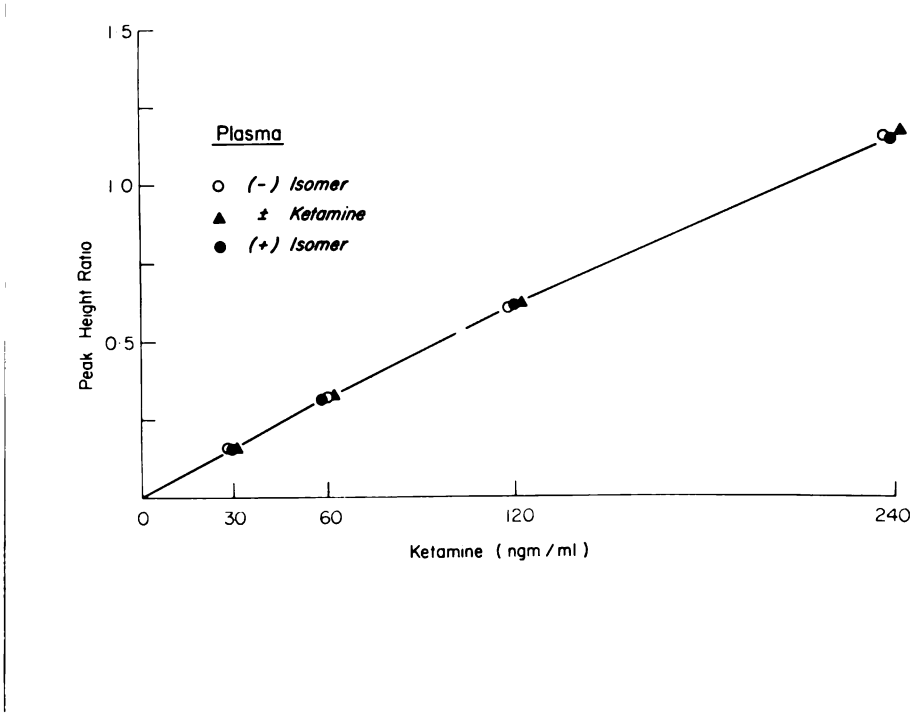



Figure 3-2. Standard curves for (+)-, (±)- and (-)-ketamine extracted from separated rat plasma as detailed under "Materials and Methods". Each point represents the mean of duplicate assays run on three consecutive days. Because of their relatively small values, standard errors were unable to be depicted on the illustration.

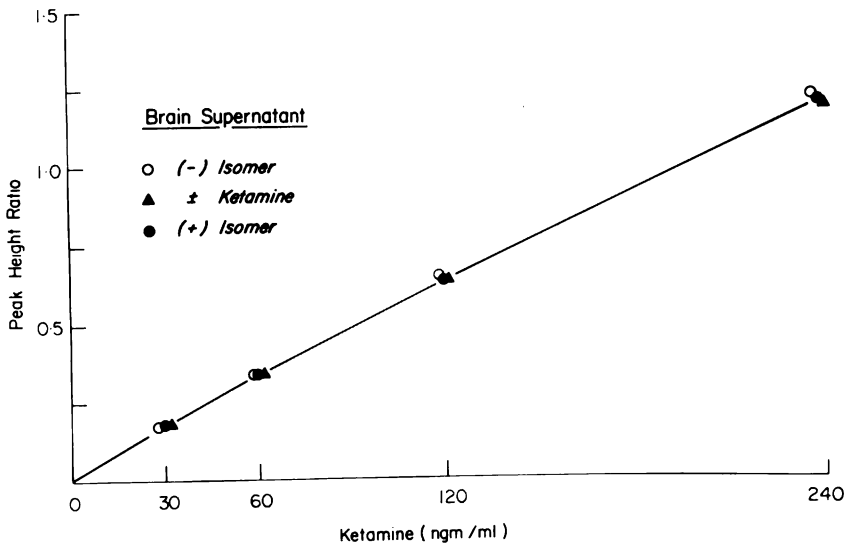



Figure 3-3. Standard curves for (+)-, (±)- and (-)-ketamine extracted from brain supernatant as detailed under "Materials and Methods". Each point represents the mean of duplicate assays run on three consecutive days. Because of their relatively small values, standard errors were unable to be depicted on the illustration.

and Methods" in Chapter 2. Concentrations of (+)-, (-)-, and (±)-ketamine in the Erlenmeyer reaction flasks ranged from $5 \times 10^{-5}$ M to $1.5 \times 10^{-3}$ M.

Chemicals

Crystalline ketamine HCl was a gift from Parke, Davis & Company (Detroit, Michigan). The ketamine enantiomorphs used in these studies were very generously supplied by Messrs. Wm. F. Minor and T. W. Hudyma of Bristol Laboratories (Syracuse, New York)[2]. They had $[\alpha]_D^{25}$ = +92.48 (c 2.00, water) and $[\alpha]_D^{25}$ = -91.88 (c 2.00, water). Both compounds melted at 259-261 C (decomp). The absence of a labile functionality about the chiral center suggests that racemization of the enantiomorphs is highly unlikely.

Statistical Analyses

$LD_{50}$ values were calculated using the methodology of Litchfield and Wilcoxon (99). Student's t test for unpaired data was used to evaluate differences between means of the pharmacologic effects and tissue levels of the isomeric and racemic ketamines and their N-demethylated metabolites. P values less than .05 were judged significant. Kinetic data was analyzed from a weighted regression line plotted using the FORTRAN program of Cleland (38)(39).

## RESULTS

### The Pharmacologic Actions of the Ketamine Enantiomorphs

Dose-dependent increases in the durations of loss of righting reflex and ataxia occurred following the caudal vein administration

[2] United Kingdom Patent Specification No. 1,330,878. Bristol Meyers Corp.: Ketamine Resolution, 1973. Copies may be obtained from the Patent Office, 25 Southhampton Bldgs, London, WC2A 1AY.

of equimolar doses, ranging from 10 to 40 mg/kg, of racemic and isomeric ketamine to rats (tables 3-1 and 3-2). With respect to these two central depressant effects, the (+) isomer was twice as potent as (-)-ketamine. Significant lethality occurred following the administration of the 40 mg/kg doses of (+)-ketamine and (±)-ketamine, thus invalidating the results for the two drugs at that dosage. The levo-isomer was not significantly lethal at the 40 mg/kg dosage.

Results obtained for posthypnotic stimulation of locomotor activity, following the i.v. administration of equimolar doses of racemic and isomeric ketamine to rats, were not statistically different. Nevertheless, a pattern emerged suggesting the locomotor activity of (-)-ketamine to be more intense than that of the dextro isomer. Subsequent intravenous administration of equihypnotic dosages of (+)-ketamine (12.5 mg/kg), (±)-ketamine (20 mg/kg), and (-)-ketamine (30 mg/kg) confirmed, both in terms of magnitude (table 3-3) and duration (fig. 3-4), that the locomotor activity of (+)-ketamine was significantly less intense ($p < .05$) than that of the (-) enantiomorph. Equihypnotic doses of the two isomers were also equi-ataxic.

Brain and Plasma Levels of the Ketamine Optical Isomers and their N-Demethylated Metabolites

Identical brain pharmacokinetic patterns were observed for ketamine and its two enantiomorphs during the initial 30 minute interval following their i.v. administration (30 mg/kg) to rats (fig. 3-5). These data, coupled with those from table 3-1 showed that the brain levels of (+)-ketamine, (±)-ketamine, and (-)-ketamine were 17, 27,

TABLE 3-1

The Duration of Loss of Righting Reflex Following i.v. Administration of the Ketamine Enantiomers

The duration of loss of righting reflex was determined following the caudal vein administration (10-40 mg free base/kg) of the three compounds as detailed under "Materials and Methods". Values represent means (±S.E.M.) of five experiments.

| Dosage (mg/kg) | (-)-Ketamine (minutes) | (±)-Ketamine (minutes) | (+)-Ketamine (minutes) |
|---|---|---|---|
| 10 | 1.76 ± 0.30[a] | 3.10 ± 0.26 | 4.10 ± 0.38[a] |
| 20 | 3.84 ± 0.24[a] | 5.80 ± 0.64 | 7.80 ± 0.42[a] |
| 30 | 5.02 ± 0.20[a] | 7.50 ± 0.55 | 11.90 ± 1.48[a] |
| 40 | 8.72 ± 0.72 | ——— | ——— |

a P value < .05 by Student's unpaired t test

TABLE 3-2

The Duration of Ataxia Following i.v. Administration of the Ketamine Enantiomorphs

The duration of ataxia was determined following the caudal vein administration (10-40 mg free base/kg/kg) of the three compounds as detailed under "Materials and Methods". Values represent means (±S.E.M.) of five experiments.

| Dosage (mg/kg) | (-)-Ketamine (minutes) | (±)-Ketamine (minutes) | (+)-Ketamine (minutes) |
|---|---|---|---|
| 10 | 9.46 ± 0.65[a] | 13.65 ± 0.31 | 19.28 ± 0.46[a] |
| 20 | 16.50 ± 0.59[a] | 23.13 ± 0.73 | 30.74 ± 1.81[a] |
| 30 | 23.24 ± 0.70[a] | 29.30 ± 1.75 | 41.05 ± 1.65[a] |
| 40 | 35.36 ± 3.14 | — | — |

a P value $< .01$ by Student's unpaired t test

TABLE 3-3

Stimulation of Locomotor Activity by Equihypnotic Doses of the Ketamine Optical Isomers

Following the caudal vein administration of the ketamine enantiomorphs, locomotor activity was measured as detailed under "Materials and Methods". Doses of each isomer eliciting 5 min of hypnosis (loss of righting reflex) are given in the "Results" section. Values represent the means (±S.E.M.) for 6 studies.

| | (-)-Ketamine | (±)-Ketamine | (+)-Ketamine |
|---|---|---|---|
| Duration of Loss of Righting Reflex (minutes) | 4.93 ± 0.34 | 5.27 ± 0.46 | 5.22 ± 0.40 |
| Locomotor Activity (counts/hour) | 5133 ± 700 | 4326 ± 516 | 3027 ± 382[a] |

a Differs from (-)-Ketamine; P value $<.05$ by Student's unpaired t test

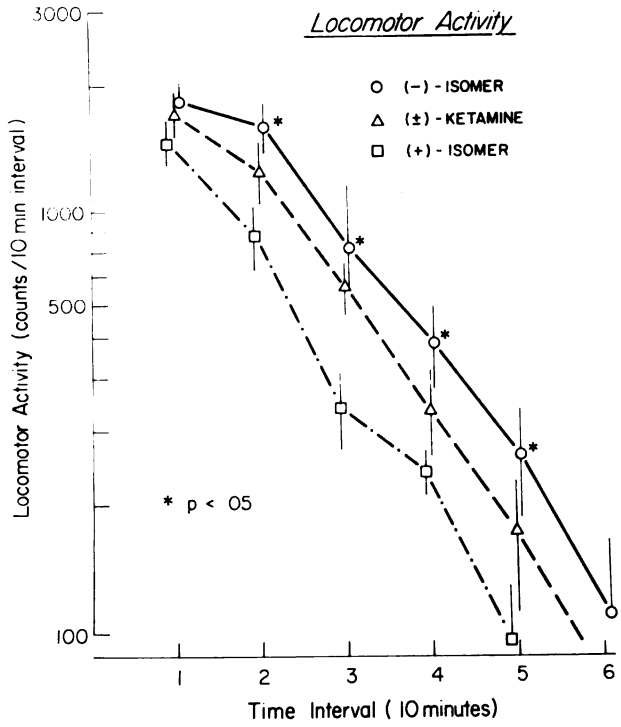



Figure 3-4. Locomotor activity following the equihypnotic, caudal vein administration of ketamine and its enantiomorphs (see text for doses). Motility meter activity was recorded for individual rats during each of six consecutive 10-minute intervals subsequent to cessation of ketamine hypnosis and is expressed as counts per 10-minute interval. Values represent means (±S. E. M.) of five experiments.

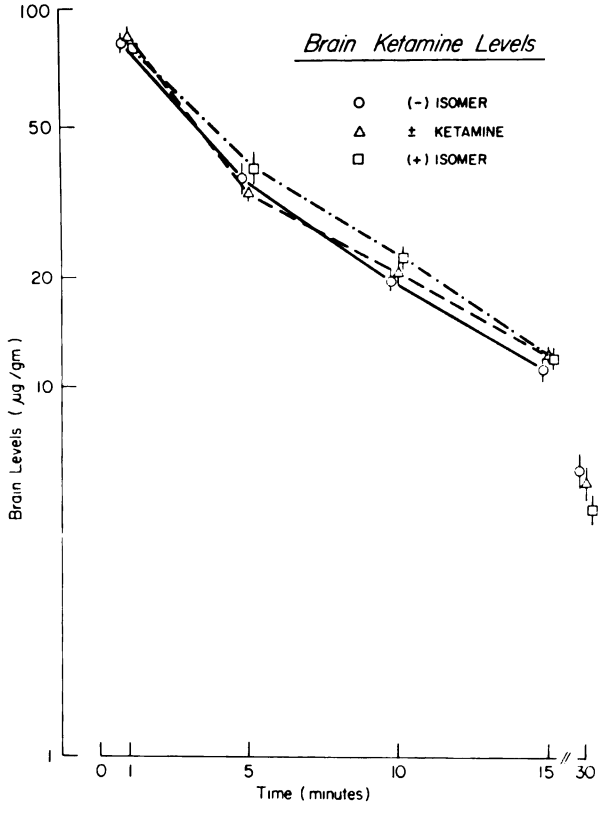



Figure 3-5. Brain levels of ketamine and its enatiomorphs following caudal vein administration (30 mg/kg) to rats. Animals were decapitated at the indicated times following i.v. injection for gas chromatographic assay of brain isomeric and racemic ketamine levels. Values are means of five experiments and vertical bars represent ±S. E. M.

and 40 µg/g, respectively, at the time points of recovery of righting reflex.

Plasma levels of the three ketamine compounds also were identical following i.v. drug administration (30 mg/kg)(fig. 3-6). Similarly, although the plasma levels of the primary N-demethylated metabolites (norketamines) of (-)-ketamine and (+)-ketamine were statistically different at the 5 minute time point, the plasma pharmacokinetic patterns of the nor-metabolites of the two isomers were otherwise identical (fig. 3-7). However, marked differences in the brain levels of the N-demethylated metabolites occurred following the i.v. administration (30 mg/kg) of the parent isomers (fig 3-8). The nor-metabolite of (-)-ketamine atttained brain levels two to three times greater than the nor-metabolite of (+)-ketamine. Levels of norketamine following racemic ketamine were interposed between those of the two isomers.

In Vitro Hepatic Metabolism of the Ketamine Enantiomorphs

The highly lipid-soluble N-demethylated metabolite of ketamine rapidly enters the brain (42). Therefore, it appeared possible that the plasma norketamine levels, which failed to reflect differences in the brain levels of the primary metabolites of the individual isomers, might also obscure differences in the in vivo hepatic metabolism of the individual isomers. For that reason, the hepatic metabolism of the isomers was examined in vitro using liver microsomal preparations. Results presented in table 3-4 show that the maximal velocity (Vmax) and apparent Michaelis constant (Km) calculated for the hepatic microsomal N-demethylation of (-)-ketamine were

TABLE 3-4

Kinetic Analysis of the Hepatic Microsomal N-Demethylation of the Ketamine Enantiomorphs

The hepatic microsomal N-demethylation of the ketamine isomers was estimated and analyzed as detailed under "Materials and Methods". Kinetic constants were calculated from triplicate samples of the pooled livers of four animals.

| | Km ± S.E.M. (mM) | Vmax ± S.E.M. (nmol N-demethylated/mg of protein/hr) |
|---|---|---|
| (-)-Ketamine | .158 ± 0.25 | 291.9 ± 11.9 |
| (±)-Ketamine | .177 ± 0.20 | 330.0 ± 10.1 |
| (+)-Ketamine | .256 ± 0.56 | 467.8 ± 30.3[a] |

a P value $<.05$

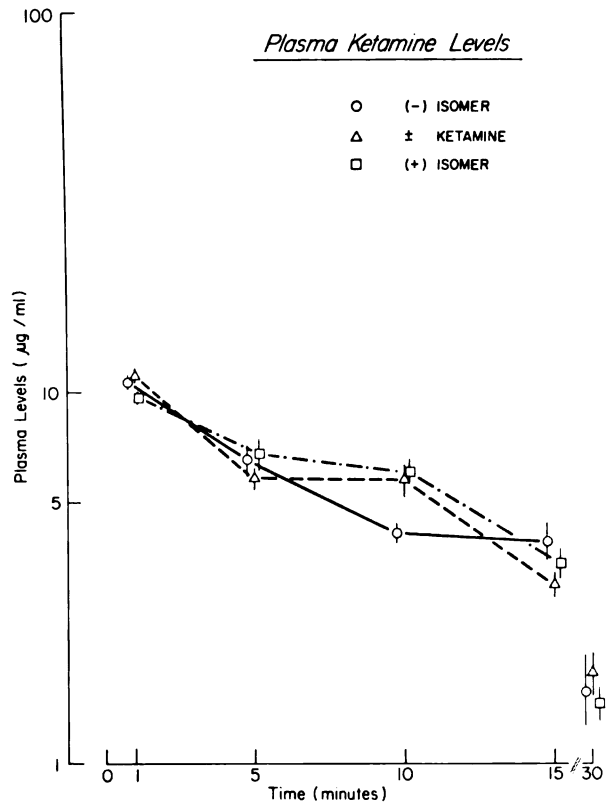



Figure 3-6. Plasma levels of ketamine and its enatiomorphs following caudal vein administration (30 mg/kg) to rats. Animals were decapitated at the indicated times following i.v. injection for gas chromatographic assay of plasma isomeric and racemic ketamine levels. Values are means of five experiments and vertical bars represent ±S.E.M.

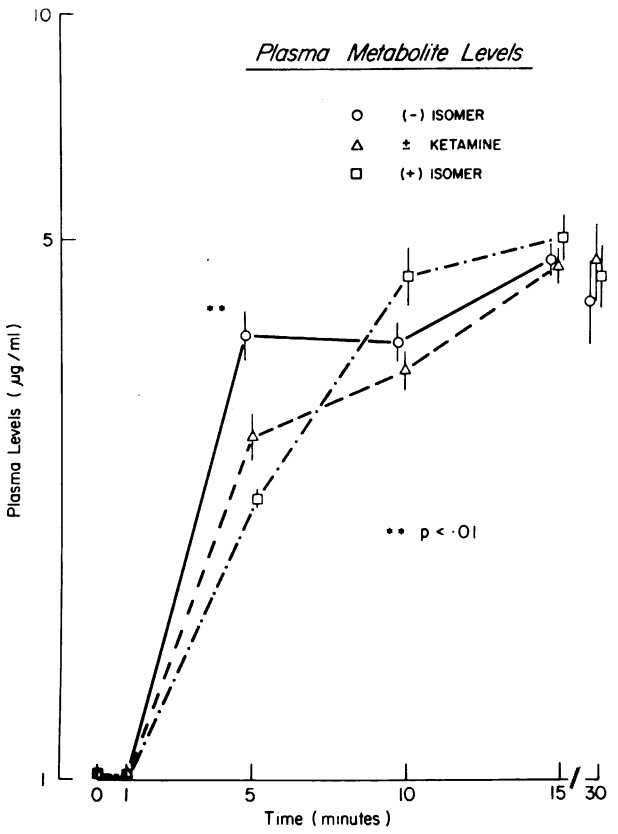



Figure 3-7. Plasma levels of the N-demethylated metabolites of ketamine and its two enantiomorphs following i.v. administration (30 mg/kg) of the parent molecules to rats. Animals were decapitated at the indicated times following tail vein injection for gas chromatographic assay of plasma metabolite levels. Values represent means of five experiments and vertical bars indicate $\pm$S.E.M.

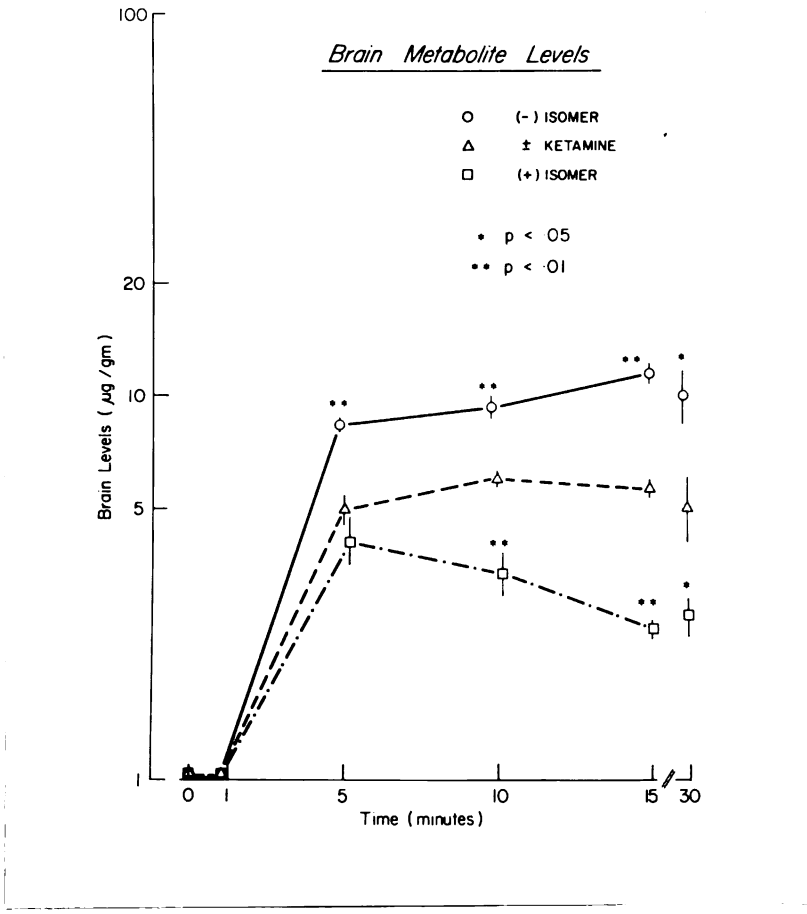



Figure 3-8. Brain levels of the N-demethylated metabolites of ketamine and its two enantiomorphs following i.v. administration (30 mg/kg) of the parent drugs to rats. Animals were decapitated at the indicated times after tail vein injection for gas chromatographic assay of brain metabolite levels. Values represent means of five experiments and vertical bars indicate $\pm$S.E.M.

approximately 60% of those of the (+) isomer. The higher apparent rate of *in vitro* metabolism of (+)-ketamine was not reflected in brain or plasma levels of the isomer or its nor-metabolite (*supra vide*). In addition, determination of the levels of both the ketamine isomers and their nor-metabolites in "whole rat homogenates", prepared from animals killed 10 minutes after intravenous isomer injection (30 mg/kg), disclosed identical levels of the parent drugs and their N-demethylated metabolites. The lack of a correlation between the *in vivo* and *in vitro* results are discussed below.

Acute Toxicity of the Ketamine Enantiomorphs

Dose-dependent increases in lethality were observed following intravenous administration of equimolar amounts (10 mg/kg to 60 mg/kg) of ketamine and its isomers to rats (table 3-5). Identical results were obtained for (+)-ketamine and racemic ketamine. However, the (-) isomer was slightly less toxic than the (+) isomer. The "therapeutic indices" of the three compounds were derived by dividing their respective $LD_{50}$ values calculated from the data in table 3-5 (99) with that dose of each drug eliciting a 4 minute period of hypnosis. As shown in table 3-6, the "therapeutic index" of (+)-ketamine is nearly twice that of the levo isomer. A similar difference was obtained by calculating therapeutic indices for the isomers and racemate using doses of the three drugs which evoked 7 minutes of hypnosis.

DISCUSSION

The importance of stereochemical influences on variations

TABLE 3-5

The Lethality of the Ketamine Enantiomorphs

Following the caudal vein administration of the isomers, all aminals were observed for a 30 minute period. However, all animals that died did so within 5 minutes following the 30 second i.v. injection. Values represent animals dying/animals tested.

| Dose | (-)-Ketamine | (±)-Ketamine | (+)-Ketamine |
|---|---|---|---|
| (mg free base/kg) | | | |
| 10 | 0/5 | 0/5 | 0/5 |
| 20 | 0/5 | 0/5 | 0/5 |
| 30 | 0/5 | 1/5 | 1/5 |
| 35 | 1/5 | 2/5 | 2/5 |
| 40 | 4/9 | 7/9 | 7/9 |
| 50 | 4/5 | 5/5 | 5/5 |
| 60 | 5/5 | — | — |

TABLE 3-6

Therapeutic Indices of the Ketamine Enantiomorphs

Therapeutic indices of the three isomers were derived by dividing their respective $LD_{50}$ values, calculated from the data in Table 3-5 using the methodology of Litch-Field and Wilcoxon (99), by the doses of the isomers eliciting a 4 minute period of hypnosis. 95% confidence limits are presented for the $LD_{50}$ values.

| | (-)-Ketamine | (±)-Ketamine | (+)-Ketamine |
|---|---|---|---|
| $LD_{50}$ (mg/kg) | 41.5 | 35.0 | 35.0 |
| (C.L. 95%) | (36.5-47.1) | (31.2-39.2) | (31.2-39.2) |
| Hypnosis (4 min) | 21.5 | 12.8 | 10.0 |
| Therapeutic Index | 1.93 | 2.73 | 3.50 |

in the pharmacological actions and biodisposition of drugs has been well documented (28)(86). Simply considering drugs which exert their primary actions on the central nervous system discloses marked differences in activity between the optical isomers of narcotic-analgetics (10)(131), amphetamine (119)(144), hallucinogens (148), hexobarbital (165), and even certain antipsychotic drugs (143).

The present study discloses significant differences in the pharmacologic effects of the two enantiomorphs of ketamine in the rat. Following intravenous injections of equimolar amounts of the individual isomers, (+)-ketamine evoked periods of loss of righting reflex and ataxia twice the duration of those observed following the administration of the levo drug (tables 3-1 and 3-2). Potency variations between optical isomers have been attributed to differences in drug-receptor (pharmacodynamic) interactions (10)(131) and, also, to differences in agonist concentrations at target areas (25). Since the brain and plasma pharmacokinetic profiles of the ketamine enantiomorphs are identical following their intravenous administration (30 mg/kg)(figs. 3-5 and 3-6), the differences in their central depressant effects appear to be pharmacodynamic in nature. Interestingly, the observed stereoselectivity of the general anesthetic effect of the ketamine isomers, molecules which possess identical physico-chemical properties (e.g. $pK_A$ value, lipid solubility) in the absence of a chiral interaction, contrasts with the rather nonspecific general structural requirements for the volatile and gaseous anesthetics.

Following the intravenous administration of the ketamine isomers to rats at equihypnotic doses, (-)-ketamine increased posthypnotic

locomotor activity to a greater extent than the (+) isomer (table 3-3; fig. 3-4). Following i.v. (-)-ketamine, the brain levels of nor-ketamine, the primary metabolite of the compound with greater posthypnotic stimulatory properties, were nearly 3 times those following (+)-ketamine (30 mg/kg)(fig. 3-8). Whether or not the differences in locomotor activities caused by the two enantiomorphs is a consequence of metabolite accumulation in the brain is a question that certainly will deserve further consideration.

Based on our in vitro studies the differences in the brain levels of the nor-metabolites of the two isomers apparently can not be attributed to differences in their rates of hepatic N-demethylation. To the contrary, the maximal rate of in vitro hepatic microsomal N-demethylation of the (-) isomer is only 60% that of the dextro isomer (table 3-4). In addition, plasma levels of the isomers and their N-demethylated metabolites (figs. 3-6 and 3-7) suggest little difference, if any, between the in vivo rates of metabolism of the isomers. In discussing in vivo aspects of drug metabolism, Gillette (67) has pointed out that differences between in vivo and in vitro rates of drug metabolism, or inhibition of drug metabolism by certain agents, may be attributed to the observation "that the free concentration of most drugs in the body is considerably below the concentration required to half-saturate the enzyme (Km)". Following its intravenous administration (30 mg/kg) to rats, ketamine achieves maximal in vivo levels of 30 μg (125 nmol) per gram of wet liver tissue (fig. 2-2). Since each gram of liver contains 20-30 mg of microsomal protein (112), levels of 4-6 nmol of ketamine per mg

microsomal protein are achieved in vivo. However, substrate concentrations used to study the in vitro hepatic metabolism of ketamine and its isomers ranged from $5 \times 10^{-5}$ M to $1.5 \times 10^{-3}$ M. Therefore, because each ml of in vitro microsomal incubate contained 1.5 mg microsomal protein, levels of the isomers and racemate, which ranged from 33-1000 nmol of ketamine per mg microsomal protein in vitro, were 5 to 150 times the maximal levels attained in vivo. Interestingly, at the lowest substrate concentrations used in vitro, $5 \times 10^{-5}$ M, which was approximately one-third Km, the rates of N-demethylation of (+)-ketamine, (±)-ketamine, and (-)-ketamine were 96.0, 94.8, and 98.5 nmol/mg of protein/hr, respectively. Significant differences in the in vitro rates of N-demethylation of the isomers and racemate were observed only at substrate concentrations greater than $1 \times 10^{-4}$ M.

Jonsson (87) has suggested that L-amphetamine, possibly due to its greater affinity for hepatic microsomal enzymes, may inhibit the p-hydroxylation of the D-isomer in rats. Although our results suggest that the rates of in vivo metabolism of the ketamine isomers are similar (supra vide), it is interesting to note that (-)-ketamine not only has a greater apparent affinity than (+)-ketamine for the microsomal enzyme system in vitro, but is also N-demethylated in vitro more slowly than the (+) isomer (table 3-4). These results suggest that the differences between the in vitro rates of N-demethylation of racemic and dextro ketamine (table 3-4) may result from the inhibition of the hepatic metabolism of (+)-ketamine by the levo

isomer present in the racemic mixture.

"Therapeutic indices" calculated for the isomers and racemate in the rat show that the index for (+)-ketamine is twice that for the levo isomer which, in turn, is less than that of the racemate (table 3-6). Thus, the (+) isomer appears not only to be a "safer" compound than both the racemate and the levo enantiomorph, but also appears to have significantly less posthypnotic stimulation as measured by locomotor activity than (-)-ketamine (table 3-3; fig. 3-4). Further studies with the ketamine enantiomorphs, therefore, appear warranted both in the rat and other laboratory animals. Hopefully, such studies will establish whether one isomer offers a potential advantage over the other and racemic ketamine in terms of enhancement of the desirable properties with diminution of the undesirable actions of ketamine anesthesia.

# SUMMARY

The first part of this study was designed to facilitate a better understanding of the biodispositional events which influence the duration and intensity of the pharmacologic effects of ketamine in the rat. Two biodispositional phenomena, redistribution events and hepatic metabolism, were shown to play prominent roles in the termination of the central pharmacologic actions of intravenous ketamine (30 mg/kg). Hepatic metabolism played a major role in the termination of the two posthypnotic events--ataxia and locomotor activity--observed in this study, while redistribution of ketamine from brain to other tissues, primarily skeletal muscle, gut, skin, and liver, terminated hypnosis. Pretreatment of rats with ketamine increased both the in vitro hepatic microsomal metabolism of ketamine and the in vivo rate of plasma decay of ketamine following its injection intravenously. Ketamine pretreatment also increased levels of cytochrome P-450 and NADPH-cytochrome c reductase in the liver and the in vitro rates of hepatic microsomal metabolism of benzphetamine and 3,4-benzo(a)pyrene. In addition, ketamine pretreatment shortened the durations of the two post-hypnotic effects of intravenous ketamine observed in this study, but did not influence the duration of hypnosis after drug administration. Brain and plasma ketamine levels 30 minutes after injection were nearly identical in rats pretreated with ketamine and phenobarbital, although phenobarbital pretreatment resulted in a 4-fold increase in in vitro ketamine hepatic metabolism. The lack of correlation between the in vitro and in vivo effects of ketamine and phenobarbital

pretreatments was probably attributable to differences between levels of ketamine occurring in the livers of pretreated rats *in vivo* and those used *in vitro* to study the hepatic metabolism of ketamine with microsomes from ketamine and phenobarbital pretreated rats.

The second part of this study was directed toward an evaluation of the pharmacologic activities of the two optical isomers of ketamine. At equimolar doses, the dextro isomer elicited periods of hypnosis and ataxia nearly twice those of the levo isomer. Since the brain and plasma levels of the isomers and racemate were identical following equimolar administration (30 mg/kg) of the three compounds, observed differences in the pharmacological activities of the ketamine enantiomorphs appeared to have a pharmacodynamic rather than a pharmacokinetic basis. *In vitro* differences in the rates of hepatic microsomal metabolism of the individual isomers did not appear to play an important role in the disposition of the optical isomers *in vivo*. At equihypnotic doses, (-)-ketamine increased posthypnotic locomotor activity to a greater extent than the (+) isomer. Following i.v. (-)-ketamine the brain levels of norketamine, the primary metabolite of the compound with greater posthypnotic stimulatory properties, was nearly 3 times those following (+)-ketamine. Whether or not the differences in locomotor activities caused by the two enantiomorphs is a consequence of metabolite accumulation in the brain is a question which deserves further consideration. Acute toxicity testing showed that (+)-ketamine has a higher therapeutic index (3.50) than that of the racemate (2.73) or the levo isomer (1.93).

These results suggested that the (+) isomer may provide anesthesia which is safer and has fewer posthypnotic side effects when administered at dosages equihypnotic to those of racemic ketamine or its levo isomer.

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