Biotransformation and Disposition of Ketamine

TSUN CHANG AND ANTHONY J. GLAZKO

A great deal of information of the biotransformation and metabolic disposition of ketamine has been obtained in the Parke-Davis laboratories, but relatively little has been published [4, 6]. This chapter presents some basic information of the metabolic disposition of this drug in laboratory animals and in man, the background needed for a better understanding of the action of ketamine (CI-581; Ketalar*).

ANALYTICAL METHODS

Much of our earlier work with ketamine was based on use of the methyl orange procedure for assay of organic bases [3]. However, because of its lack of specificity and poor analytical sensitivity this technique was soon replaced by a fluorescent dye procedure using Xylene Red B [7]. Because of the low plasma levels of ketamine in human subjects and interference from other drugs, we finally resorted to the use of gas-liquid chromatography, which is a highly specific assay technique. For quantitative work, we originally used the *o*-trifluoromethyl analog of ketamine as an internal standard, and analyzed ketamine by chromatography without derivative formation [4]. More recently we have employed the *o*-bromo analog as an internal standard. We now recommend for quantitative work the use of a 3 percent OV-17 column at 195°C., preparation of the heptafluorobutyryl derivative, and

* Parke, Davis & Co., Detroit, Mich. 48232.

electron-capture detection [5]. The gas-liquid chromatography technique is capable of detecting several metabolites of ketamine in human plasma as well as the parent compound [10]. Thin-layer chromatography has also been useful for the detection of ketamine metabolites [4]. However, the most generally useful approach to the study of ketamine metabolism has been the radioisotope tracer technique, using the tritium-labeled drug [2] and oxygen-flask combustion techniques for liquid scintillation counting. Initial experiments in animals were carried out with the tritium attached to the ring carbon next to the cyclohexanone group; but oxidation at this position resulted in tritium being released and incorporated into body water. The labile tritium in the molecule was eliminated by treatment of the drug with strong alkali. The resulting product had a lower specific activity, but it was satisfactory for tracer studies in laboratory animals and in man.

PLASMA LEVELS

Ketamine is absorbed rapidly following peroral or parenteral administration to laboratory animals, with maximum levels appearing in blood plasma and in most tissues within 15 minutes. Rhesus monkeys receiving 25 mg. per kilogram intravenous doses of ketamine produced the plasma levels shown in Figure 1 (by colorimetric assay). The initial drop in plasma levels was very rapid (half-life, 10 minutes), due largely to the distribution of drug to the tissues. At time periods beyond 2 hours after administration, the ketamine levels fell more slowly (estimated half-life, about 2 hours). In a similar series of experiments two Rhesus monkeys were given tritium-labeled ketamine in 30 mg. per kilogram intravenous doses. The plasma levels estimated from total tritium activity are shown in Figure 2. In both animals the total radioactivity was significantly higher 15 minutes after administration than at 5 minutes. The 15-minute peak was followed by a first-order decrease in plasma levels, with an estimated half-life of 2.5 hours.

The apparent lag in the appearance of peak plasma levels of tritium was even more evident in dogs. Two animals receiving 20 mg. per kilogram intravenous doses of tritium-labeled ketamine showed peak plasma levels about one hour after administration, with lower

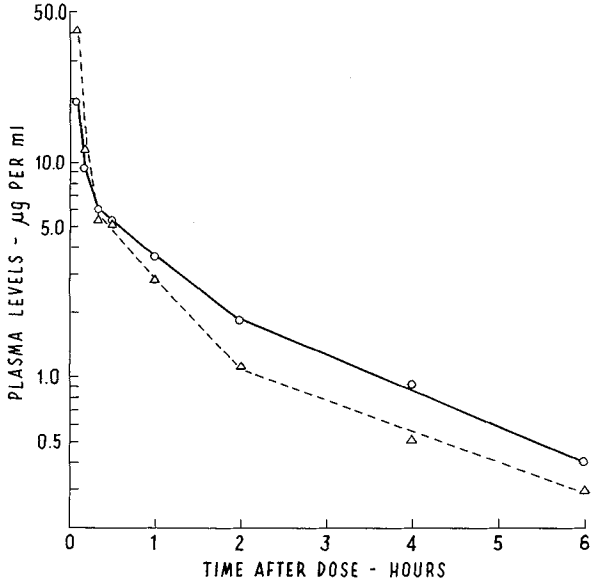

FIGURE 1. *Plasma levels of ketamine in two Rhesus monkeys following intravenous doses of 25 mg./kg. ketamine (methyl orange assay).*

levels present 15 and 30 minutes after administration, as shown in Figure 3. The relative proportion of unchanged drug to metabolites in each plasma specimen was estimated by thin-layer chromatography and measurement of recovered radioactivity. The proportion of ketamine decreased rapidly in the early time periods, whereas total metabolites reached a peak in one hour. This suggests that part of the ketamine is removed very rapidly from the blood by tissue elements and that, later, metabolites are returned slowly to the bloodstream. A similar pattern—rapid drug removal from the circulating blood followed by a slow return of metabolites—has been observed with diphenhydramine [8], where the effect is even more pronounced because of the tight binding of one of the metabolites to plasma albumin.

In a study with tritium-labeled ketamine in human subjects [6], 1 mg. per kilogram intravenous doses produced peak plasma levels of 0.4 to 0.7 μg. per milliliter in 1 to 5 minutes, followed by a second peak of 0.6 to 0.7 μg. per milliliter in 1 to 2 hours after dosing. Thin-

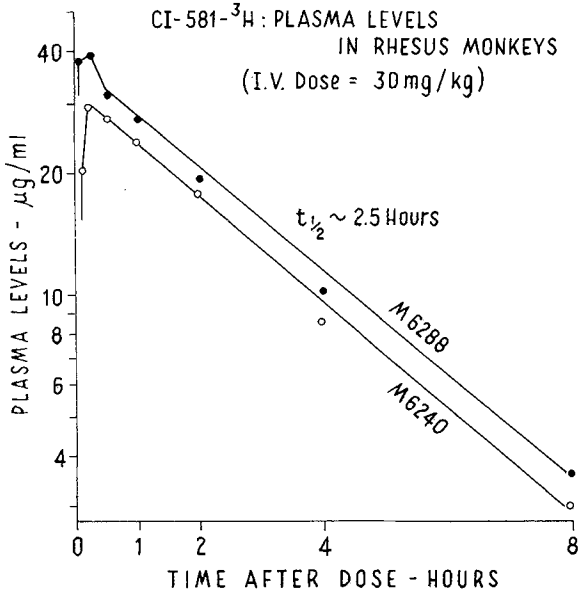

FIGURE 2. *Plasma levels of total tritium in two Rhesus monkeys following intravenous doses of 30 mg./kg. tritium-labeled ketamine.*

layer chromatography examination of the plasma revealed that the major component in the first peak was ketamine, while metabolites predominated in the second peak. Although the initial drop in plasma levels was rapid, this was followed by a slower drop, with half-lives extending to 9 hours or more during the first two days after administration. The tritium level in the red cells was nearly the same as in the plasma immediately after administration, and fell to 70 percent of the plasma levels in about 4 to 8 hours. The mean levels of tritium in the cerebrospinal fluid rose from 0.1 μg per milliliter 5 minutes after dosing to 0.20 to 0.23 μg. per milliliter in 1 to 2 hours.

In another study, in which unlabeled drug was employed [10], single intravenous doses of 2.2 mg. per kilogram produced peak ketamine levels of 1.0 to 1.8 μg. per milliliter 4 minutes after administration, with a mean half-life of about 18 minutes. Return of consciousness generally occurred when the ketamine plasma levels dropped below 1 μg. per milliliter.

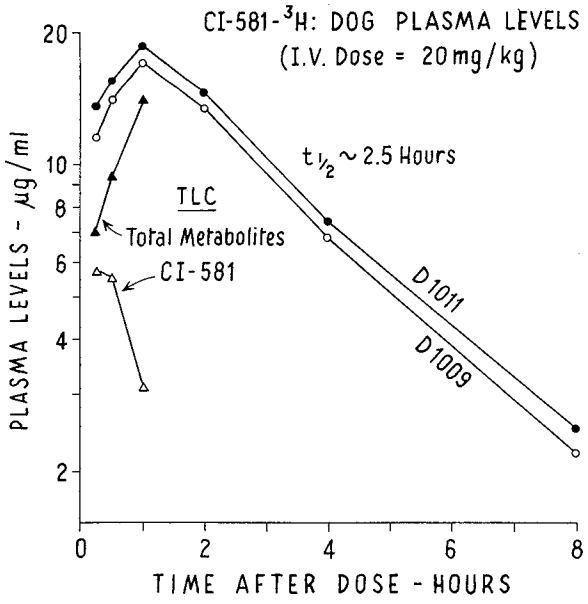

FIGURE 3. *Plasma levels of total tritium in two dogs following 20 mg./kg. intravenous doses tritium-labeled ketamine.* Triangles, *proportion of ketamine* (open) *to ketamine metabolites* (solid), *determined by thin-layer chromatography of pooled plasma specimens.*

TISSUE DISTRIBUTION

Ketamine appears to be distributed very rapidly into the body tissues, including the central nervous system. Rats receiving 50 mg. per kilogram peroral doses were sacrificed at different time periods up to two hours after administration, and representative tissue specimens were assayed by the colorimetric procedure. As shown in Figure 4, peak drug levels were found in most tissues 15 minutes after administration. The highest concentrations of unchanged drug occurred in body fat, liver, and kidneys, with lower concentrations in the lungs, brain, heart, and skeletal muscle, and lowest concentrations in the plasma. The half-life of ketamine in these tissues was about 15 to 20 minutes.

A similar experiment was carried out in rats using a peroral dose

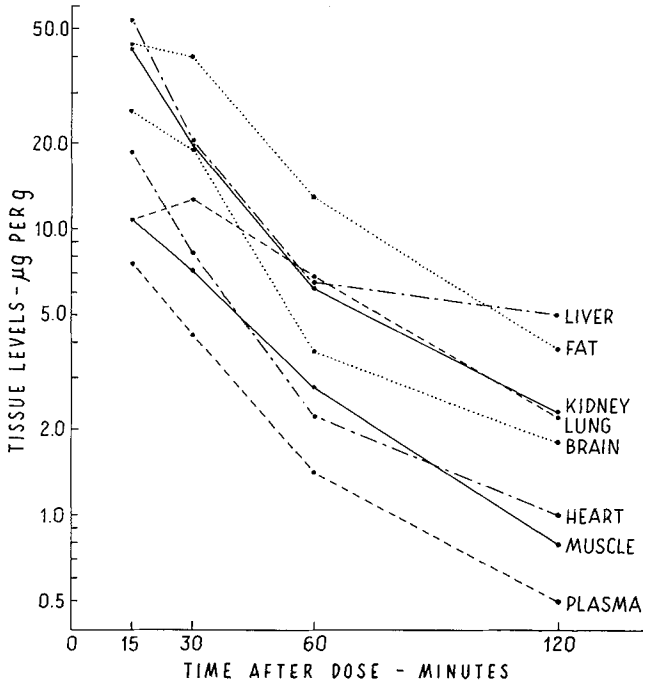

FIGURE 4. *Tissue levels of ketamine in rats at different time periods following a 50 mg./kg. peroral dose (methyl orange assay).*

of 40 mg. per kilogram of the tritium-labeled drug. Observations were extended over a 70-hour period after administration, with the results shown in Figure 5. An initial sharp drop in plasma levels occurred in the first few hours after administration, followed by a gradual lengthening of half-life to several hours in the 2- to 8-hour postdose period, and to 24 hours or more in later time periods. This appears to have resulted from the presence of drug metabolites which are not detected by the colorimetric assay procedure. The most interesting differences between the radiometric and colorimetric assays were found in the brain, lungs, and body fat. Where early high levels of unchanged drug were detected by colorimetric assay, relatively low levels were found by radiometric assay in the later time periods.

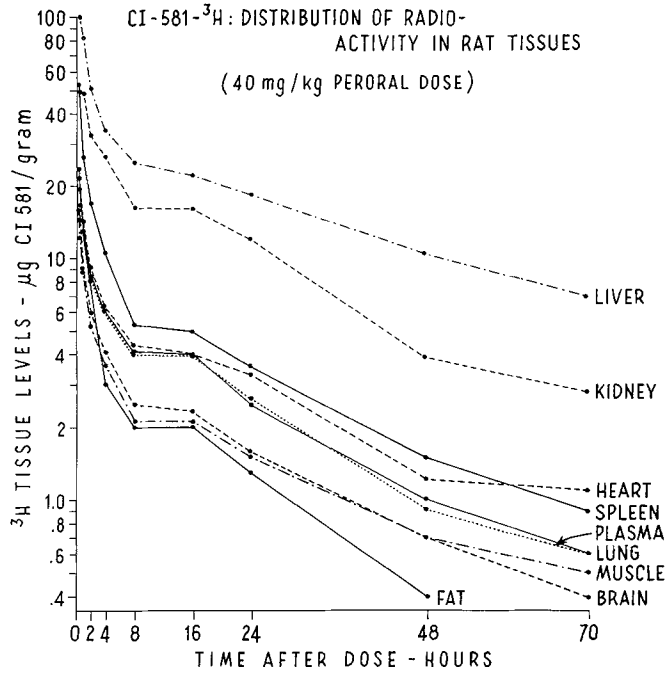

FIGURE 5. *Tissue levels of total tritium in male rats (250 gm.) at different time periods following a 40 mg./kg. peroral dose of tritium-ketamine.*

EXCRETION

Balance studies with tritium-labeled ketamine in laboratory animals provided good information on the routes of excretion and on species differences in excretion patterns. Albino rats were given 54 mg. per kilogram doses of tritium-labeled ketamine by different routes of administration, and complete collections of urine and feces were made over five-day periods. Assays for total tritium by liquid scintillation counting (expressed as percent of dose) are shown in Table 1. Urinary excretion accounted for 56 to 75 percent of dose given by intramuscular, intraperitoneal, and peroral routes. Fecal excretion accounted for 23 to 29 percent of dose, indicating that biliary excretion could be involved in the metabolic disposition of this drug.

TABLE 1. *Excretion of tritium-labeled ketamine metabolites in rat urine and feces (dose, 54 mg. per kilogram). Data expressed as % of dose.*

Time After Dose (hrs.)	Peroral Route[a]		Intramuscular Route[b]		Intraperitoneal Route[a]	
	Urine (%)	Feces (%)	Urine (%)	Feces (%)	Urine (%)	Feces (%)
0–24	65	17	41	15	47	17
24–48	10	6	8	10	8	6
48–72			4	2	3	2
72–96	0	0	2	1	1	1
96–120			1	1	0	0
120–168	. . . .	. . . .	0	0	0	0
(0–120)	75	23	56	29	59	26
Total	98		85		85	

[a] Mean value for 5 animals.
[b] Mean value for 4 animals.

Additional studies were carried out with normal dogs and monkeys, and with animals in which bile fistulas had been established by surgical techniques. Following intravenous administration of tritium-labeled ketamine in doses of 20 to 30 mg. per kilogram of body weight, complete collections of urine, bile, and feces were made and measured for radioactivity. The results are shown in Table 2. With two normal dogs 69 to 78 percent of the dose was recovered in the urine and 7 to 8 percent in the feces. Bile-fistula animals excreted about the same amount of tritium in the urine, but 12 to 15 percent of the dose was recovered in the bile over a two-day period, with greatly reduced amounts of tritium in the feces. The Rhesus monkeys excreted a larger proportion of tritium in the urine (91 to 93 percent), and only small amounts in the feces. However, in one bile-fistula monkey, over 8 percent of the dose was recovered in the bile. These observations suggest that significant amounts of drug metabolites may be excreted in the bile, and that reabsorption from the intestinal tract may occur.

Balance studies were also carried out with normal human subjects who received 1 mg. per kilogram intravenous doses of tritium-labeled

TABLE 2. *Excretion of tritium-labeled ketamine metabolites in dogs and monkeys (intravenous dose, 20 to 30 mg. per kilogram).*

Species	Collection Period (hrs.)	Dose Recovered (%)			
		Urine	Bile	Feces	Total
Dog	0–96	78.0	. . . .	7.0	85.0
Dog	0–96	69.0	. . . .	8.0	77.0
BF dog[a]	0–48	71.2	12.2	2.5	85.9
BF dog[a]	0–48	76.9	15.5	1.0	93.4
Monkey	0–72	91.0	. . . .	0.0	91.0
Monkey	0–72	93.0	. . . .	2.0	95.0
BF monkey[a]	0–96	90.6	8.6	0.2	99.4

[a] Bile-fistula preparations.

ketamine [6]. The mean five-day recovery of tritium in urine represented 91 percent of the dose, with only 3 percent in the feces. Excretion in man thus appears to be more like that in the Rhesus monkey than in the dog or rat. The peak excretion rate occurred within 4 hours after administration, and the major portion of the tritium was excreted in the urine within 24 hours. However, small amounts of tritium continued to appear in the urine for several days.

PLACENTAL TRANSFER

Ketamine appears to be transferred readily across the placenta in Rhesus monkeys and dogs, based on chromatographic evidence of unchanged drug in fetal tissues. In one monkey a dose of ketamine of 25 mg. per kilogram was administered intravenously 50 minutes prior to delivery of the fetus, followed by a booster dose (15 mg. per kilogram) 25 minutes after the initial dose. Maternal body weight was 8.23 kilograms. The presence of unchanged ketamine in the placenta and in the fetal liver and brain was established by thin-layer and gas-liquid chromatography procedures. Assay data obtained with the methyl orange procedure are shown in Table 3. They indicate rapid distribution of ketamine to fetal tissues, including brain.

TABLE 3. *Ketamine concentrations in fetal tissues of a newborn Rhesus monkey (methyl orange assay).*

Fetal Tissue	Ketamine Concentration (μg./gm.)	Fetal Tissue	Ketamine Concentration (μg./gm.)
Liver	18.5	Heart	9.2
Spleen	21.2	Brain	10.2
Kidneys	10.7	Carcass	8.3
Lungs	9.3	Plasma	4.7
GI tract	22.5	Placenta	16.4

Ketamine was also administered to one pregnant dog near term, using a single intravenous dose of 20 mg. per kilogram followed 5 minutes later by continuous intravenous infusion of 2 mg. per kilogram per minute. Maternal plasma levels by the colorimetric assay procedure ranged from 16 μg. per milliliter 20 minutes after administration to 30 μg. per milliliter in 65 minutes. The first pup was delivered 75 minutes after the first dose, with a total of five pups being delivered at 4-minute intervals. During this period the maternal plasma levels of ketamine ranged from 32 to 35 μg. per milliliter. Parallel fetal plasma levels ranged from 23 to 28 μg. per milliliter, or about 75 percent of the corresponding maternal levels. Drug concentrations in the fetal livers ranged from 64 to 122 μg. per milliliter, indicating rapid distribution of drug into the fetal tissues.

Placental transfer of ketamine in human subjects was established in collaboration with Dr. Brian Little at Cleveland Metropolitan General Hospital, with assays being performed in the Parke-Davis Laboratories [10]. The expectant mothers were given either (1) a priming dose of 2.2 mg. per kilogram followed by continuous infusion at 0.11 mg. per kilogram per minute, or (2) a priming dose of 1.5 mg. per kilogram and continuous infusion at 0.08 mg. per kilogram per minute, just prior to parturition. The mean maternal plasma levels at delivery were 2.1 μg. per milliliter in the *a* series and 1.6 μg. per milliliter in the *b* series. The fetal cord blood levels averaged about 60 percent of the maternal levels in both series.

NATURE OF METABOLITES

In our early work with ketamine [4], human urine was made alkaline and extracted directly with chloroform. The extract was then examined by gas-liquid chromatography, with the results shown in Figure 6. Two major metabolites were recognized, together with a small amount of unchanged drug. Application of the gas-liquid chromatography procedure to 72-hour (unhydrolyzed) urine specimens accounted for 22 to 24 percent of the dose.

Metabolite I was identified initially as the synthetic N-demethylated derivative of ketamine from its gas-liquid chromatography retention time and comparison with the synthetic preparation [4]. It was later isolated from monkey urine and identified chemically. *Metabolite II* was also isolated from monkey urine by extraction with carbon tetra-

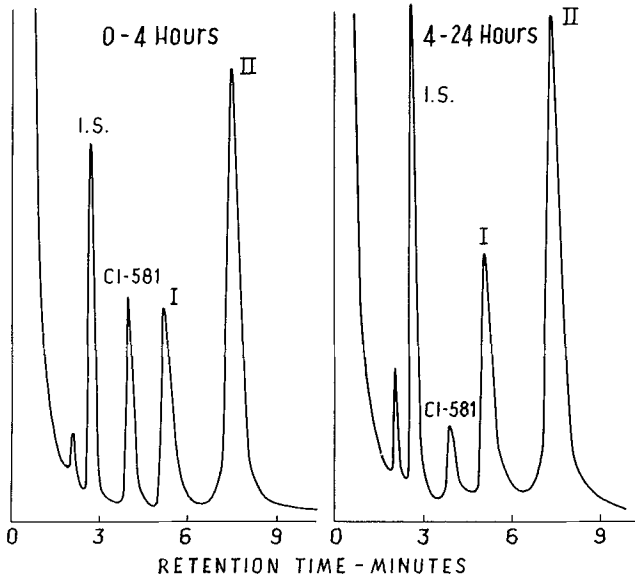

FIGURE 6. *Gas-liquid chromatography (1% ECNSS at 170° C.) of chloroform extracts of human urine (unhydrolyzed), from a subject receiving a 500-mg. intravenous dose of ketamine.* (I.S., *internal standard* [o-*trifluoromethyl analog of ketamine*]; CI-581, *ketamine;* I, II, *ketamine metabolites.*) (*Urine collection periods indicated in Figure.*)

chloride and by countercurrent extraction with 1,2-dichloroethane and pH 3.7 citrate buffer (0.2M), to separate it from ketamine and from metabolite I. This metabolite proved to have a most unexpected chemical structure: a double bond introduced into the cyclohexane ring (a cyclohex*ene*one derivative of metabolite I) [4]. Thus, metabolite II represents an oxidation product resulting from the removal of two hydrogen atoms from adjacent carbons in the cyclohexane ring. The chemical structure of metabolite II was eventually confirmed by synthesis of this compound and comparison of chromatographic properties. Metabolite I exhibited some ketaminelike activity in animal tests, being about one-tenth as active as ketamine; metabolite II showed considerably lower activity.

Gas-liquid chromatography examination of rat urine, cat urine, and human urine yielded the retention times shown in Figure 7. Cat urine

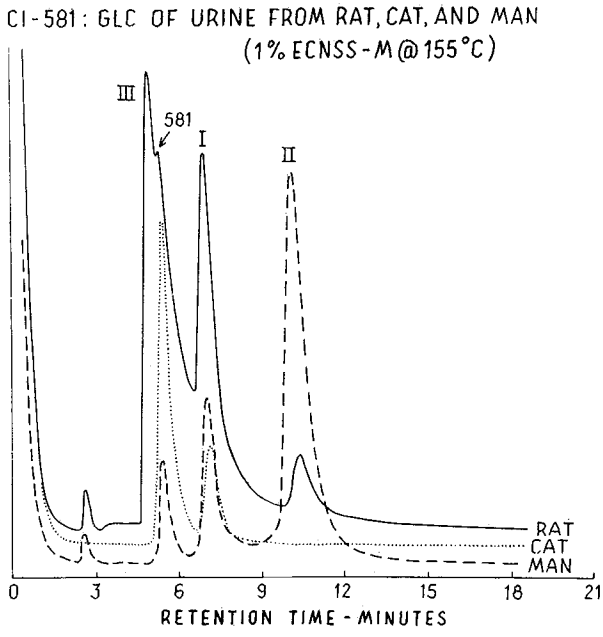

FIGURE 7. *Gas-liquid chromatography of chloroform extracts of unhydrolyzed rat, cat, and human urine after administration of ketamine.* (CI-581, *unchanged ketamine;* I, II, III, *ketamine metabolites.*)

did not contain any significant amount of metabolite II, although free ketamine and metabolite I were present. Rat urine contained metabolite II, with considerable amounts of metabolite I and free ketamine. The ketamine peak in rat urine appeared as a shoulder on a broader peak because of the presence of a third metabolite (III). This metabolite was also detected in human urine and monkey urine following acid hydrolysis. Metabolites I and II have also been identified in human material and fetal plasma [10].

Thin-layer chromatography analysis of unhydrolyzed monkey urine using tritium-labeled ketamine produced the chromatographic pattern shown in Figure 8. Metabolites I and II ran together at high Rf (13 percent of total tritium); a large polar fraction (70 percent) was seen at low Rf, consisting mainly of metabolites conjugated with glucuronic

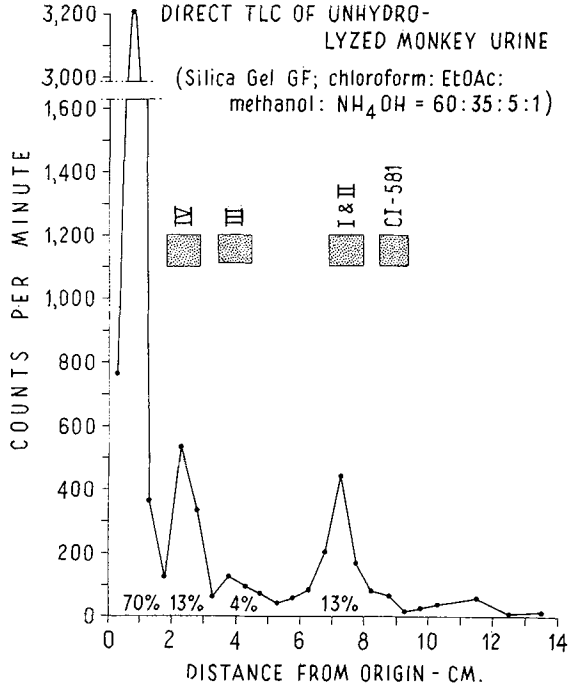

FIGURE 8. *Direct thin-layer chromatography of unhydrolyzed urine from a Rhesus monkey after administration of tritium-ketamine (solvent system A). Percentage figures represent fraction of total tritium found under each peak.*

acid; metabolite III was observed at intermediate Rf (4 percent). In addition a new peak (metabolite IV, 13 percent) was seen between that of the low Rf component and metabolite III. Examination of the same monkey urine by thin-layer chromatography after acid hydrolysis produced the results shown in Figure 9. Metabolite III showed a large increase in peak height (to 69 percent) caused by release of additional metabolite III from the previously conjugated form. Although the low Rf polar fraction was greatly reduced by this treatment (to 31 percent), some of the compounds in that fraction retained their polar characteristics after acid treatment, representing unidentified metabolites.

Metabolite III was isolated from monkey urine by countercurrent extraction in chloroform and pH 4.8 acetate buffer. Structural work indicated that metabolite III was a primary amine resulting from

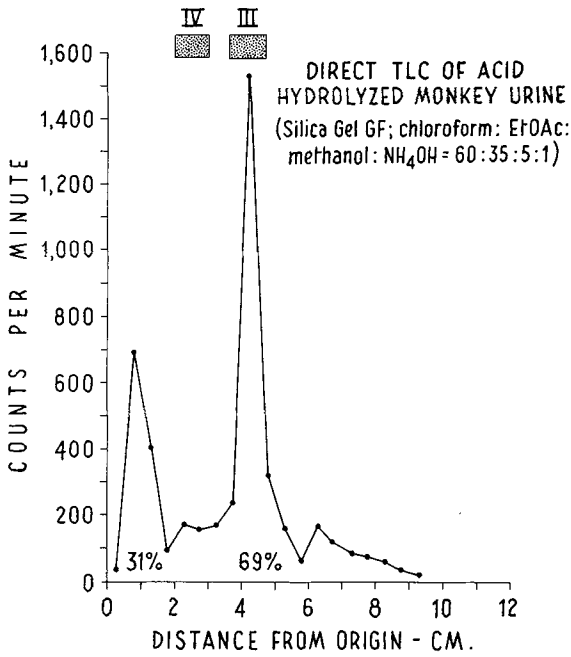

FIGURE 9. *Thin-layer chromatography of acid-hydrolyzed urine from a Rhesus monkey after administration of tritium ketamine. Urine pre-extracted with chloroform before acid hydrolysis to remove unconjugated metabolites (same urine specimen as in Fig. 8).*

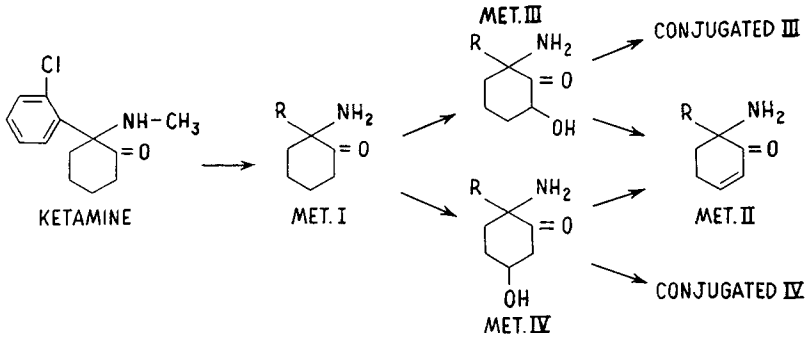

FIGURE 10. *Biotransformation pathway for ketamine includes N-dealkylation (metabolite I), hydroxylation of the cyclohexanone ring (metabolites III and IV), conjugation with glucuronic acid, and dehydration of the hydroxylated metabolites to form the cyclohexene derivative (metabolite II).*

N-dealkylation of ketamine that was hydroxylated in the cyclohexanone ring, on the carbon next to the carbonyl group. *Metabolite IV* was also isolated from monkey urine, using thin-layer chromatography to separate it from other metabolites. Exposure of metabolite IV to alkali resulted in the formation of metabolite II, a reaction characteristic of β-hydroxy carbonyl compounds. The final product showed functional groups identical with those found in metabolite III. The compound thus appears to be a 5-hydroxycyclohexanone derivative of metabolite I. Both metabolites III and IV appeared to be conjugated with glucuronic acid, yielding the aglycones when hydrolyzed with β-glucuronidase. Attempts to synthesize metabolites III and IV have been unsuccessful, and the possibility of ketaminelike activity in these metabolites has not been examined. A tentative scheme summarizing the biotransformation of ketamine is shown in Figure 10.

SPECIES DIFFERENCES IN BIOTRANSFORMATION

Some marked differences in the biotransformation of ketamine by different species of animals are evident from the data shown in Figure 7. Two different thin-layer chromatography systems were used to examine urine, plasma, and tissue specimens from animals receiving tri-

tium-labeled ketamine. Sections (0.5 cm.) of the coating were scraped off and radioactivity was determined by liquid scintillation counting to determine the relative proportion of each metabolite present. *System A* made use of the silica gel GF_{254}* (250 μ) with a solvent consisting of chloroform:ethylacetate:methanol:ammonia (60:35:5:1). This separated metabolites III and IV, but metabolites I and II appeared as a single peak. These metabolites were separated in *system B,* using plates coated with aluminum oxide HF_{254} (Brinkman, 250 μ) and a solvent consisting of chloroform:cyclohexane:diethylamine (60:40:2). However, this system did not separate metabolites III and IV. For effective separation of all metabolites, system B was used first; the region corresponding to III+IV was scraped off, eluted with methanol, and rechromatographed in system A. Thus, unchanged ketamine and all four recognized metabolites can be determined individually.

In 24-hour urine specimens from rats, dogs, and monkeys receiving tritium-ketamine, approximately two-thirds of the excretion products were present as polar conjugates. Chromatographic examination of the nonconjugated metabolites revealed traces of unchanged ketamine (1 to 5 percent), some metabolite III and metabolite IV (5 to 15 percent), and some I+II (7 to 16 percent). Acid hydrolysis of monkey urine resulted in a major increase in metabolite III (52 percent of total tritium), and a decrease in the low Rf fraction (21 percent). Thin-layer chromatography analysis of monkey bile also indicated the presence of conjugated forms of metabolites III and IV. Current evidence indicates that these metabolites may undergo enzymatic hydrolysis in the intestinal tract and be reabsorbed.

Thin-layer chromatography analysis of monkey plasma produced the results shown in Table 4. Using intravenous doses of tritium-ketamine (30 mg. per kilogram), plasma samples taken 5 minutes after administration contained mainly unchanged drug, with small amounts of metabolites I+II, III, and IV also present. In the 15- to 30-minute period after administration, the proportion of ketamine decreased to 47 to 59 percent, with a marked increase in metabolite III and a smaller increase in metabolite IV. Table 5 presents data on the metab-

* Brinkman Inst., Inc., Westbury, N.Y. 11590.

TABLE 4. *Unconjugated metabolites in monkey plasma (intravenous dose, 30 mg./kg. of tritium-labeled ketamine).*

Time After Dose (min.)	Relative Concentrations			
	Ketamine (%)	Metabolite III (%)	Metabolite IV (%)	Metabolite I + II (%)
5	80	8	3	9
15	59	20	9	12
30	47	30	13	10

olites present in rat plasma, liver, and brain, at different time periods after administration of tritium-ketamine. Unchanged drug was found in the brain and liver 15 minutes after administration, with considerable amounts of metabolite I+II also present; metabolite III increased in later time periods.

A similar chromatography study was conducted on pooled plasma specimens from three human subjects receiving 1 mg. per kilogram intravenous doses of tritium-labeled ketamine [6]. The results, shown in Table 6, indicate that unchanged ketamine was the predominant

TABLE 5. *Tritium-labeled ketamine metabolites in rat tissues (thin-layer chromatography).*

Tissue	Time After Dose (min.)	Ketamine (%)	Metabolite III (%)	Metabolite I + II (%)
Plasma	30	0	75	25
	60	0	74	26
Liver	15	49	6	45
	60	0	23	67
Brain	15	51	10	39
	30	0	71	29
	60	0	79	21

TABLE 6. *Unconjugated metabolites in human plasma (intravenous dose, 1 mg./kg. of tritium-labeled ketamine).*

Time After Dose (min.)	Relative Concentrations		
	Ketamine (%)	Metabolite I (%)	Metabolite II (%)
1	100	0	0
5	100	0	0
15	81	19	0
30	71	29	0
60	25	16	59
120	12	13	73

component in the first few minutes after administration. Metabolite I appeared within 15 minutes and reached a peak in about 30 minutes after administration; metabolite II appeared within one hour. The acid-labile conjugates in urine accounted for one-third of the excreted tritium in the first two to four hours, and increased to two-thirds in later time periods. Some unchanged ketamine was found in urine in the first four hours after administration with large amounts of metabolite II and smaller amounts of metabolites III and IV. After this time period no significant amounts of ketamine could be detected in the urine, although large amounts of metabolite II and smaller amounts of metabolites III and IV were present. The identity of these metabolites was established by isolation and chemical characterization, and by gas-liquid chromatography of the metabolites in comparison with known reference standards. Approximately 20 percent of the total tritium in human urine could not be extracted with ethyl acetate after acid hydrolysis, presumably representing unknown metabolites.

ENZYMES AND KETAMINE BIOTRANSFORMATION

In some of our earliest experiments with ketamine [4], rat liver homogenates fortified with magnesium chloride, nicotinamide, and nicotinamide-adenine dinucleotide phosphate were incubated with

ketamine for two hours at 37°C. Gas-liquid chromatography analysis of the reaction products indicated that metabolite I was produced by N-dealkylation of ketamine. When synthetic metabolite I was incubated with rat liver homogenates, metabolite III was identified as an intermediate oxidation product; but metabolite II was not formed. Using liver homogenates from a Rhesus monkey, ketamine was also converted to metabolite I by dealkylation. However, incubation of metabolite I with monkey liver homogenates resulted in the appearance of metabolite II. The relatively small amount of metabolite II in rat urine and the accumulation of large amounts of metabolite III, as indicated in Figure 7, appear to result from a species difference in the enzymatic mechanism responsible for the further oxidation of metabolite III to form metabolite II. The presence of large amounts of metabolite II in human urine indicates that the biotransformation of ketamine in man may be more closely related to that of the monkey than the rat.

Experiments were also carried out to determine whether ketamine could induce the formation of enzymes responsible for the biotransformation of ketamine. Rats were administered ketamine (50 mg. per kilogram) intravenously at 16- to 24-hour intervals for three doses, and were sacrificed about 42 hours after the last dose. The liver microsome fraction, fortified with enzyme cofactors, was incubated with N-methylaniline for 15 minutes, and the extent of demethylation was assayed by the Bratton-Marshall procedure. The demethylation rate was not significantly different from that observed in control animals. Also, there was no significant increase in liver weight relative to body weight. These observations indicate that ketamine probably does not enhance the activity of microsomal enzymes responsible for N-dealkylation in the rat.

PROTEIN BINDING

The protein-binding characteristics of ketamine and its metabolites were explored in some detail. Our initial studies involved the use of Sephadex G25 columns* [1]. The results indicated that ketamine itself

* Pharmacia Laboratories Inc., Piscataway, N.J. 08854.

was not bound to bovine serum albumin to any appreciable extent. In later work we used the equilibrium dialysis technique of Goldstein [9] with 4 percent human serum albumin solutions. No significant binding was found with ketamine or metabolites I or II (synthetic); metabolites III and IV were not available for study. Bilirubin (40 μg. per milliliter) in 4 percent human serum albumin was also dialyzed against various concentrations of ketamine, metabolite I, and metabolite II (4 to 48 μg. per milliliter). There was no indication that bilirubin was released from its binding sites on the protein in the presence of these agents.

ACKNOWLEDGMENTS

We wish to thank the following individuals in the Chemical Pharmacology Section at Parke-Davis for their assistance with various phases of the work: C. E. Blackburn, P. Borondy, W. A. Dill, J. Lewis, D. Mourer, L. Mulligan, L. Peterson, and A. Savory. We are also indebted to R. Bonczak and T. Reutner for help with animal work. Metabolites were obtained from Dr. R. F. Parcell.

REFERENCES

1. Barlow, C. F., Firemark, H., and Roth, L. J. Drug-plasma binding measured by Sephadex. *J. Pharm. Pharmacol.* 14:550, 1962.
2. Blackburn, C. E., and Ober, R. E. Tritium labelling of 2-(*o*-chlorophenyl)-2-(methylamino)cyclohexanone (ketamine; Cl-581) by acid catalyzed exchange: An example of intramolecular competition for tritium between substituted aromatic and cyclohexane rings. *J. Labelled Compds.* 3:38, 1967.
3. Brodie, B. B., Udenfriend, S., and Dill, W. A. The estimation of basic organic compounds in biological material: V. Estimation by salt formation with methyl orange. *J. Biol. Chem.* 168:335, 1947.
4. Chang, T., Dill, W. A., and Glazko, A. J. Metabolic disposition of 2-(*o*-chlorophenyl)-2-methylaminocyclohexanone hydrochloride in laboratory animals and in man. *Fed. Proc.* 24:268, 1965.
5. Chang, T., and Glazko, A. J. A gas chromatographic assay for ketamine in human plasma. *Anesthesiology* 36:401, 1972.

6. Chang, T., Savory, A., Albin, M., Goulet, R., and Glazko, A. J. Metabolic disposition of tritium-labelled ketamine (Ketalar; CI-581) in normal human subjects. *Clin. Res.* 18:597, 1970.
7. Dill, W. A., Chucot, L., Chang, T., and Glazko, A. J. Determination of ketamine in blood plasma. *Anesthesiology* 34:73, 1971.
8. Drach, J. C., Howell, J. P., Borondy, P. E., and Glazko, A. J. Species differences in the metabolism of diphenhydramine (Benadryl). *Proc. Soc. Exp. Biol. Med.* 135:849, 1970.
9. Goldstein, A. The interactions of drugs and plasma proteins. *J. Pharmacol. Exp. Ther.* 95:102, 1949.
10. Little, B., Chang, T., Chucot, L., Dill, W. A., Enrile, L., Glazko, A. J., Jassani, M., Kretchmer, H., and Sweet, A. Y. A study of ketamine as an obstetrical anesthetic agent. *Am. J. Obstet. Gynecol.* 113:247, 1972.