

Interaction of Ketamine and Halothane in Rats

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The interaction of intramuscularly injected ketamine and its N-demethylated metabolite (metabolite I) with halothane was evaluated in rats. Five, 10, 20, or 50 mg/kg of ketamine alone or 20, 50, or 100 mg/kg of metabolite I alone produced less than 10 minutes of hypnosis. However, halothane anesthetic requirement (i.e., MAC) was depressed in a dose-dependent fashion as much as 56 per cent 1–2 hours and as much as 14 per cent 5–6 hours after injection of ketamine, 50 mg/kg, im. The reduction in MAC was correlated with brain levels of ketamine or metabolite I, suggesting a ketamine:metabolite I potency ratio of 3:1. The half-life of ketamine in plasma and brain was longer in the presence of halothane than when ketamine was given alone. It is concluded that ketamine is not a short-acting drug and that concomitant use with halothane would be expected to prolong further the duration of its action on the central nervous system. (Key words: Anesthetics, volatile, halothane; Anesthetics, intravenous, ketamine; Potency, anesthetic, halothane MAC; Interactions, ketamine-halothane.)

KETAMINE was introduced as a rapid, short-acting parenterally administered anesthetic. In conflict with the suggestion of its short action, several investigators have reported prolonged recovery.^{1–7} It is not known whether protracted recovery following ketamine administration is related to concomitant use of other drugs,^{8–10} formation of active ketamine metabolites, or persistence of ketamine itself.

Since no study has demonstrated the time course of the action of ketamine given in the presence of conventional inhalation anesthetics on the central nervous system, we sought to define the anesthetic properties of

ketamine alone and in the presence of halothane anesthesia by measuring the effect of ketamine-induced depression on the minimum alveolar concentration (MAC) of halothane. We also determined alterations in the halothane anesthetic requirement following administration of the principal metabolite of ketamine (namely, the N-demethylated metabolite of ketamine).^{11,12} By measuring brain and plasma levels of ketamine, metabolite I (N-demethylated ketamine), and metabolite II (cyclohexanone oxidation product of metabolite I),¹⁴ we were able to examine the relationships between drug levels and effects on halothane MAC.

Methods and Materials

Two hundred fifty-nine male Sprague-Dawley rats, weights 300–350 g, were divided into four groups. One group received ketamine alone while a second group received metabolite I only. The remaining two groups received the same doses of ketamine or metabolite I during halothane anesthesia.

KETAMINE ALONE

Twelve rats in each of four dose groups (total 48) received 5, 10, 20, or 50 mg/kg of ketamine (Ketalar) intramuscularly (im) in the hindlimb. We assessed the duration of 1) hypnosis (loss of the righting reflex); 2) "analgesia" (altered responsiveness to a painful mechanical stimulus applied to the tail¹⁵); 3) ataxia (abnormal gait). One, two, three, and four hours after each dose of ketamine, three rats were rapidly anesthetized with 100 per cent cyclopropane and blood was obtained by cardiac puncture. The blood was heparinized and centrifuged for 10 minutes to obtain plasma, which later was analyzed for ketamine and its metabolites. After cardiac puncture, the animals were decapitated. The cerebral hemispheres were transferred to ice and the superficial blood vessels removed. One part brain tissue was added to

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KETAMINE-HALOTHANE

Four groups of five rats (total 20) were anesthetized with halothane, tracheostomies performed, and anesthetic requirement (*i.e.*, MAC) determined as previously reported.¹³ Five, 10, 20, or 50 mg/kg of ketamine then were administered intramuscularly (one dose for each group of five rats) and MAC redetermined 1-2 hours (MAC 1-2), 3-4 hours (MAC 3-4) and 5-6 hours (MAC 5-6) after injection. These animals were sacrificed 6 hours after the ketamine injection.

Additionally, four groups of 20 rats (total 80) similarly were anesthetized with halothane and tracheostomized, and end-tidal halothane concentration was maintained at MAC as determined above. Ketamine, 5, 10, 20, or 50 mg/kg, was administered (one dose to each group of 20 rats), following which halothane concentrations were reduced parallel to the reductions determined previously in animals in which MAC values had been measured following identical doses. In each group of 20 animals five were sacrificed one hour, five two hours, five three hours, and five four hours after ketamine administration. Plasma and brain extract samples were obtained for ketamine, metabolite I, and metabolite II analyses as described earlier. The plasma and brain levels of metabolite I following im injection of ketamine were measured and the magnitudes of depression attributed to these levels of metabolite I determined from the percentage decreases in MAC 1-2 values after im administration of metabolite I alone. This despression in halothane anesthetic requirement resulting from the formation of metabolite I from ketamine then was subtracted from the measured depressions in MAC 1-2 following ketamine administration to ascertain the effects of ketamine itself.

Rectal temperatures were maintained at $37 \pm 1^\circ\text{C}$ with a heating pad. End-tidal or "alveolar" gas samples were analyzed for halothane and carbon dioxide using infrared analyzers.

METABOLITE I-HALOTHANE

Three groups of 25 rats (total 75) were prepared as in the ketamine-halothane study

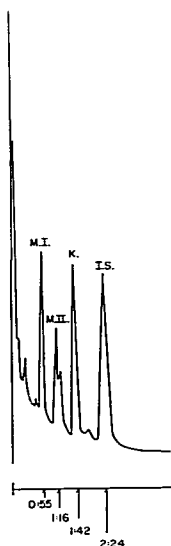


FIG. 1. Representative gas-liquid chromatogram of metabolite I (M.I.), metabolite II (M.II), ketamine (K.), and internal standard (I.S.) following extraction from rat brain tissue. Retention times (minutes:seconds) are indicated under the respective peaks.

nine parts physiologic saline solution, homogenized, and centrifuged at $100,000 \times g$ for 60 minutes. The supernatant was analyzed for ketamine, metabolite I, and metabolite II.

METABOLITE I ALONE

Groups of 12 rats (total 36) received, intramuscularly, 20, 50, or 100 mg/kg metabolite I (dissolved in 0.1 N HCl and diluted to the appropriate concentration with saline solution). The pharmacologic effects evaluated included hypnosis, analgesia, and ataxia. Three animals in each group were sacrificed at one-hour intervals and plasma and brain samples obtained as described above.

TABLE 1. Duration of Pharmacologic Effects after Intramuscular Administration of Ketamine or Its Principal Metabolite in the Rat*

	Dose (mg/kg)	Number of Animals	Hypnosis (Min)	Analgesia (Min)	Ataxia (Min)
Ketamine	5	12	0	2 ± 1	8 ± 1
	10	12	0	10 ± 2	21 ± 1
	20	12	1 ± 1	28 ± 5	38 ± 2
	50	12	9 ± 2	54 ± 7	72 ± 5
Metabolite I	20	12	0	5 ± 3	10 ± 3
	50	12	0	9 ± 3	29 ± 3
	100	12	1 ± 1	26 ± 7	69 ± 9

* Values are means ± SE.

except that each group received 20, 50, or 100 mg/kg of metabolite I im. At each dose, MAC changes were determined in five animals while the remaining 20 rats were maintained at comparable halothane levels. As in the previous study, equal numbers of these 20 rats were sacrificed one, two, three, and four hours after injection of metabolite I. Plasma and brain extract samples were obtained as described above for analysis of metabolites I and II.

EXTRACTION AND ANALYSIS OF KETAMINE, METABOLITE I AND METABOLITE II

Plasma (0.01–1.0 ml samples) and brain (0.1–2.0 ml samples) levels of ketamine and its two principal metabolites were assayed by gas-liquid chromatography (GLC) using an electron capture detector (tritium foil) with a modified version of the procedure of Chang and Glazko.^{12,14} The carrier gas was 95 per cent argon/5 per cent methane at a flow rate of 20 ml/min. The column temperature was maintained at 192 C. A GLC record demonstrating the separation pattern of ketamine, metabolite I, and metabolite II extracted from rat brain tissue following ketamine, 50 mg/kg, im, is shown in figure 1. Standard curves based on peak height ratios of standards to internal standard (2-amino-2 [O-bromophenyl]-2-methyl-amino-cyclohexanone were linear from 0.01 to 1.0 µg for ketamine and metabolite II and from 0.002 to 0.5 µg for metabolite I.

The plasma and brain levels of ketamine and its two principal metabolites were plotted against time on semilogarithmic paper

and a linear regression obtained by the method of least squares. Similar log dose-response curves also were plotted for ketamine and metabolite I. Finally, the relationship between drug levels in the plasma or brain and the effect on halothane MAC also were described with linear regression analyses.

Results

Only the 50 mg/kg dose of ketamine gave significant hypnosis, while analgesia and ataxia followed all doses of ketamine and metabolite I, the duration of each pharmacologic effect being related to dose (table 1). The onset of analgesia was not as rapid as the onset of hypnosis.

Brain ketamine concentrations (fig. 3) were approximately four times those in plasma (fig. 2) in both halothane-anesthetized and un-anesthetized rats at all intervals studied. The half-life of ketamine in plasma and brain was prolonged significantly ($P < 0.05$) by halothane anesthesia.

Both ketamine and metabolite I depressed MAC (figs. 4 and 5). Although MAC returned towards control levels with time, significant depressions remained 5–6 hours after injection of 50 mg/kg ketamine or 100 mg/kg metabolite I. Correlation coefficients of 0.966 and 0.938 were ascertained by linear regression analyses of the depression in halothane MAC 1–2 as a function of the logarithms of the doses of ketamine and metabolite I, respectively. The log dose-response curves for ketamine and metabolite I are essentially parallel (slopes of 0.055 and 0.048 Δ MAC/mg

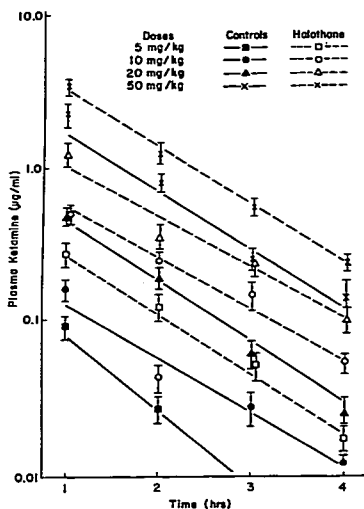


FIG. 2. Changes in plasma levels of ketamine with time in unanesthetized (control) and anesthetized (halothane) animals following im injection of ketamine. Values are means; vertical bars represent ± 1 SE.

drug/kg body weight, respectively) and suggest a potency ratio for ketamine:metabolite I of approximately 3:1.

Ketamine and metabolite I in the plasma correlated with reductions in halothane MAC 1-2 (fig. 6). Similarly, brain levels of ketamine and metabolite I correlated with MAC 1-2 (fig. 7). The amount of metabolite I formed accounted for about 20-25 per cent of the measured decrease in halothane MAC 1-2 following im injection of im ketamine. For example, MAC 1-2 was found to be decreased by 40 per cent following administration of ketamine, 20 mg/kg, im (fig. 4). By extrapolation, the amount of metabolite I measured in the plasma ($0.98 \pm .21$ $\mu\text{g/ml}$) would have resulted in about a 10 per cent reduction in MAC 1-2 (fig. 6). Thus, the percentage depression in MAC 1-2 corresponding to plasma ketamine alone was esti-

mated to be 30 per cent (fig. 6). Similarly, the amount of metabolite I found in the brain ($1.33 \pm .17$ $\mu\text{g/g}$) would have accounted for a 7 per cent reduction in MAC 1-2 (fig. 7). Hence, for this particular dose of ketamine, metabolite I levels in the plasma and brain would account for 25 and 18 per cent, respectively, of the total decrease in halothane MAC 1-2.

Although metabolite II was detected in the brain following im injection of metabolite I, significant brain levels of metabolite II were found only after administration of 20 or 50 mg/kg, im (table 2). In addition, there was no correlation between decreases in halothane MAC and levels of metabolite II following either ketamine or metabolite I.

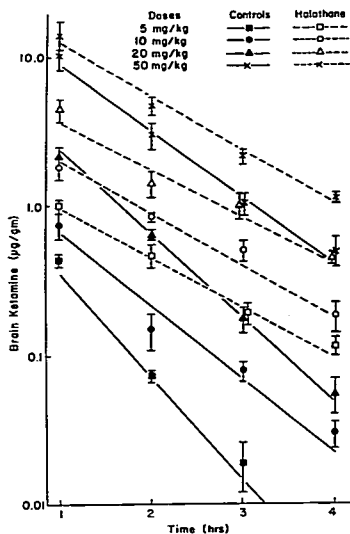


FIG. 3. Changes in brain levels of ketamine with time in unanesthetized (control) and anesthetized (halothane) animals following im injection of ketamine. Values are means; vertical bars represent ± 1 SE.

FIG. 4. Changes in halothane MAC at various times after im injection of ketamine. Values are means; vertical bars represent ± 1 SE.

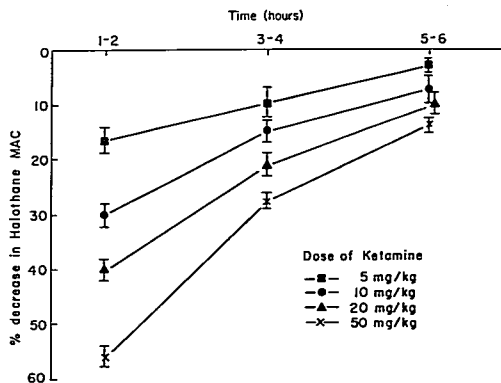
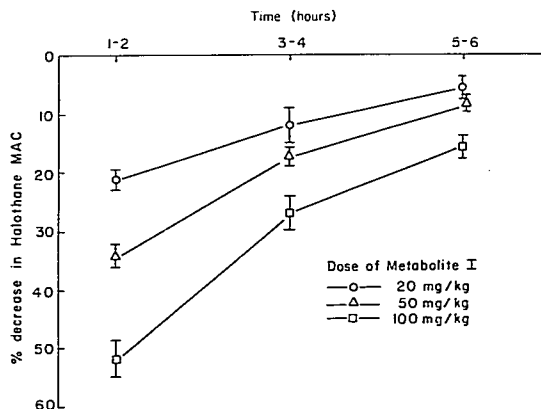


FIG. 5. Changes in halothane MAC at various times after im injection of metabolite I. Values are means; vertical bars represent ± 1 SE.



Discussion

Ketamine and its principal metabolite both depressed halothane anesthetic requirement in a dose-dependent fashion. More importantly, significant depressions in halothane MAC were found at times when identical doses of ketamine or metabolite I administered alone had no observable pharmacologic

effect. We have also correlated these depressions in halothane MAC with brain levels of ketamine and thereby, this represents the first report correlating alterations in anesthetic requirement with brain levels of a fixed agent.

These data do not support the suggestion that ketamine is a "short-acting" agent. Doses of ketamine producing less than 10

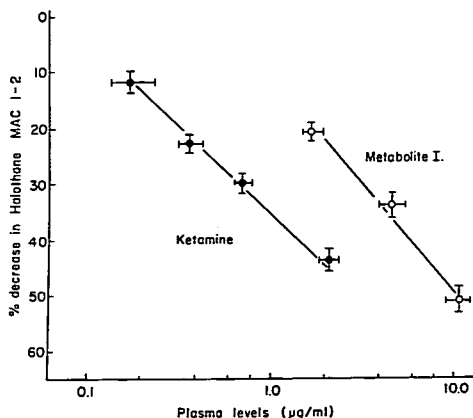


FIG. 6. Correlations between plasma levels of ketamine or metabolite I and halothane MAC 1-2 after im injection of ketamine or metabolite I, respectively. The effect of metabolite I formation has been subtracted from the measured decrease in MAC 1-2 after im injection of ketamine in order to demonstrate the effect of ketamine alone. Values are means \pm 1 SE.

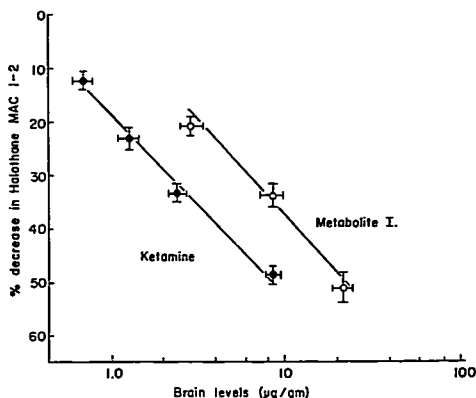


FIG. 7. Correlations between brain levels of ketamine or metabolite I and halothane MAC 1-2 after im injection of ketamine or metabolite I, respectively. The effect of metabolite I formation has been subtracted from the measured decrease in MAC 1-2 after im injection of ketamine in order to demonstrate the effect of ketamine alone. Values are means \pm 1 SE.

minutes of hypnosis resulted in analgesia and ataxia lasting an hour or longer. Furthermore, we have demonstrated that subanesthetic doses of ketamine resulted in depression of halothane MAC for as long as 6 hours. The effects of ketamine in depressing the central nervous system, as reflected by decreases in anesthetic requirement, were of even longer

duration than any of the apparent pharmacologic effects and occurred at relatively low levels of the drug. For example, ketamine levels in the rat brain were approximately 30 µg/g when the rat regained the righting reflex and 13 µg/g at the termination of the analgetic period following im injection of ketamine (unpublished data), yet signifi-

cant dose-related depressions of halothane anesthetic requirement were found at brain levels ranging from 0.1 to 10 $\mu\text{g/g}$ (fig. 7).

The prolonged action of ketamine is due in part to persistence of ketamine resulting from its high lipid solubility,¹⁵ and in part to the conversion of ketamine to metabolite I, which itself possesses weak anesthetic properties. We estimate that approximately 75–80 per cent of the prolonged depression of anesthetic requirement following ketamine administration is attributable to ketamine itself and that the remaining 20–25 per cent is related to the formation of metabolite I. Our estimates of the relative potency of metabolite I indicate a potency approximately three times that reported by previous investigators.^{11,16} Those investigators suggested a potency ratio of 10:1 between ketamine and metabolite I from comparisons of the durations of loss of the righting reflex. We estimate a potency ratio of 3:1 from comparison of brain levels producing 50 per cent depression of MAC (fig. 7). This also agrees with the 3:1 ratio of the ketamine-to-metabolite I dose required to decrease MAC by 50 per cent. The parallelism of the log dose-response curves for ketamine and metabolite I is consistent with similar mechanisms of action in their effects on halothane MAC.

Metabolite II was found in the plasma and brain following administration of the larger doses of ketamine and all doses of metabolite I, although metabolite II was not found by others following intravenous administration of equipotent doses of ketamine.^{12,15} We have no explanation for this apparent discrepancy. However, the levels of metabolite II we detected were significantly lower than the levels of either ketamine or metabolite I. Since no measurable brain level of metabolite II was found after ketamine, 5 or 10 mg/kg, im, and since the plasma and brain levels of metabolite II were relatively constant with time after ketamine, 20 or 50 mg/kg, im, it would seem unlikely that metabolite II contributes significantly to the ketamine-induced depression of the halothane anesthetic requirement. Metabolite II has

TABLE 2. Maximum Metabolite II in Plasma and Brain after IM Ketamine or Metabolite I during Halothane Anesthesia*

	Dose (mg/kg)	Plasma ($\mu\text{g/ml}$)	Brain ($\mu\text{g/g}$)
Ketamine	5	0.12 \pm 0.03	0
	10	0.22 \pm 0.11	0
	20	0.66 \pm 0.08	0.38 \pm 0.16
	50	1.73 \pm 0.33	1.94 \pm 0.34
Metabolite I	20	0.46 \pm 0.15	0.96 \pm 0.13
	50	2.53 \pm 0.43	3.59 \pm 0.43
	100	4.66 \pm 0.84	7.88 \pm 0.51

* Values are means \pm 1 SE.

been reported to have 0.01 the anesthetic activity of ketamine.¹⁶

This study suggests that halothane alters the action of ketamine and metabolite I by influencing their biodisposition. This is evidenced by prolongation of the plasma half-life, and thereby the brain half-life, of ketamine. We have not determined the specific changes in biodisposition that produce this result, but alterations in cardiac output and hence ketamine's uptake, distribution and/or redistribution, as well as a reduction in its metabolism,¹⁷ are likely possibilities. For example, a decrease in blood flow at the site of intramuscular injection of thiopental has been shown to slow the uptake and reduce the peak brain level, while elevating the levels in the brain, 60–140 minutes after administration.¹⁸

In summary, demonstrated correlations between brain levels of ketamine or metabolite I and reductions in halothane MAC suggest that these two agents are jointly responsible for the lethargy seen clinically after recovery from the anesthetic effects of ketamine. The concomitant administration of a potent inhalational anesthetic (e.g., halothane) would be expected to prolong further the post-hypnotic lethargy associated with ketamine.

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