Distribution in the Brain and Metabolism of Ketamine in the Rat after Intravenous Administration

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The anesthetic effects of ketamine and its distribution to CNS tissue were examined in rats following intravenous administration. Peak brain levels of ketamine were achieved less than a minute following injection. At all times studied, brain/plasma ratios of ketamine were 6.5:1. Study of regional brain levels indicated a preferential distribution of ketamine to the cerebral cortex 30 seconds and one minute after injection. The N-demethylated metabolite of ketamine also accumulated in the brain, reaching levels appreciably higher than that in plasma 10 minutes after administration. No evidence of the presence of the other metabolite, the cyclohexanone oxidation product, was found in either plasma or brain. Studies of biotransformation in vitro showed that brain tissue was incapable of metabolizing ketamine, while liver homogenates metabolized ketamine to the N-demethylated product exclusively. These observations on the disposition of ketamine and its metabolite are discussed in relation to the diverse actions of this dissociative anesthetic agent in the central nervous system. (Key words: Ketamine; Brain levels; Plasma levels; Metabolism; Sleep time.)

Increasing clinical experience with ketamine* monohydrochloride supports the contention that this compound produces an anesthetic state quite different from those produced by conventional hydrocarbons or barbiturates.† Ketamine produces numerous diverse central effects by mechanisms that are only partly understood.‡ § At the clinical level such actions may result in hallucinatory states, agitation, nystagmus, apnea, and extrapyramidal stimulation. Attempts to understand such actions may be facilitated by information relating to the disposition of ketamine and its metabolites, particularly their distribution to the brain. Disposition studies in man have necessarily been limited to the estimation of plasma and urinary concentrations of ketamine and its metabolites.¶ § Preliminary reports on tissue distribution and metabolism in laboratory animals have appeared,‖ but no information about ketamine distribution to CNS tissue is available. The availability of a sensitive gas–liquid chromatography procedure for the analysis of ketamine and its metabolites¶ has permitted this investigation relating plasma levels to distribution in the brain in the rat.

Methods

Disposition Studies

In all experiments male Sprague-Dawley rats (100–130 g) received injections into the tail vein of the appropriate concentrations of ketamine hydrochloride in saline solution in a total volume of 0.2 ml. The rats were observed for two hours following injection to assess duration of anesthesia and other pharmacologic effects. In other experiments, cardiac punctures in the left ventricle were performed at various times after iv injection for withdrawal of 1 to 3 ml of blood. Cardiac puncture was immediately followed by decapitation, removal of the brain, which was immediately placed on ice, and preparation of a 10 per cent homogenate of the whole brain in 0.9 per cent saline solution. Brains from other animals were dissected into...
four regions—cortex, midbrain, brainstem and cerebellum, and a 10 per cent homogenate of tissue from each region was prepared in 0.9 per cent saline solution. Heparinized blood was centrifuged for 15 minutes to obtain plasma, and the brain homogenate was centrifuged at 100,000 g for 30 minutes. Plasma and supernatant samples were extracted immediately or were refrigerated and extracted the following day and then assayed as described below for ketamine and its metabolites.

**Metabolism in Vitro**

Rats were decapitated and exsanguinated and the brains and portions of the livers were immediately removed, placed on ice, and cleaned of external blood. A 10 per cent homogenate was prepared in 0.9 per cent saline solution and used immediately. Tissue homogenates (0.5 ml) were placed in Erlenmeyer flasks containing 0.5 ml sodium phosphate buffer with nicotinamide (30.5 mg/ml), MgSO4 (6.2 mg/ml), and glucose-6-phosphate (10 mg/ml). 0.1 ml freshly prepared NADP (2 mg/ml), and 1.15 ml of 0.1 M sodium phosphate buffer, pH 7.35. To start the reaction 0.25 ml of ketamine hydrochloride (1 mg/ml) was added to each flask, mixed, and a 0.1-ml sample was transferred to 0.9 ml of 0.1-N HCl for assay of the initial ketamine concentration. The remaining reaction mixtures (2.4 ml) were incubated in a Dubnoff metabolic shaker for 30 min, after which 0.1 ml was transferred into 0.9 ml 0.1-N HCl for assay of ketamine and its metabolites.

**Extraction and Analysis of Ketamine and Metabolites**

From plasma or brain, 0.01-ml to 1.0-ml samples were extracted and derivatized for assay of ketamine base and its metabolites, using a slight modification of the procedure of Chang and Glazko.7 To simplify the procedure, the initial drying of the benzene layer with anhydrous sodium sulfate was omitted.

**Table 1. Duration of Ketamine Effects as a Function of Dose**

<table>
<thead>
<tr>
<th>Ketamine Hydrochloride, mg/kg</th>
<th>Number of Rats</th>
<th>Number of Deaths</th>
<th>Time to Recovery of Righting Reflex (min)</th>
<th>Time to End of Ataxia (min)</th>
<th>Time to End of Agitation (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4</td>
<td>0</td>
<td>—</td>
<td>5.13 ± 0.31</td>
<td>12.5 ± 1.26</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0</td>
<td>3.94 ± 0.41</td>
<td>12.00 ± 0.71</td>
<td>23.8 ± 1.38</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>0</td>
<td>7.50 ± 0.35</td>
<td>30.00 ± 1.68</td>
<td>40.5 ± 1.32</td>
</tr>
<tr>
<td>40</td>
<td>5</td>
<td>1</td>
<td>13.00 ± 1.22</td>
<td>39.50 ± 1.55</td>
<td>54.5 ± 3.33</td>
</tr>
<tr>
<td>60</td>
<td>5</td>
<td>2</td>
<td>32.67 ± 5.91</td>
<td>63.3 ± 14.83</td>
<td>69.3 ± 9.26</td>
</tr>
<tr>
<td>80</td>
<td>4</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Rats were injected with ketamine via tail vein at the doses indicated. At doses greater than 10 mg/kg, loss of the righting reflex occurred during injection (15 sec). Times to recovery of the righting reflex and to cessation of ataxia and agitation were estimated from the end of injection time. Data represent means ± SE.
and the tubes were left at room temperature after derivatization and stirred every 10 min for an hour. Analyses were carried out using a Varian aerograph series 1200 gas chromatograph with an electron capture detector of titanium tritide foil. Column preparation and gas chromatographic conditions were similar to those reported by Chang and Glazko. Standard curves based on ratios of peak heights of standards to height of internal standard (2-amino-2-[0-bromophenyl]-2-methylamino-cyclohexanone) for ketamine, metabolite I, and metabolite II were found to be linear over the range of 0.01 to 1.0 μg for ketamine and metabolite II and 0.002 to 0.2 μg for metabolite I. It should be noted that this technique is approximately ten times more sensitive than that reported by Chang and Glazko. Recovery of standard solutions of ketamine and its metabolites from plasma and brain homogenates was 95-98 per cent. Duplicate assays agreed, with a variation of ±4 per cent, and standard curves were prepared for each series of analyses.

A typical separation of ketamine and its N-demethylated metabolite (metabolite I) following extraction from rat brain tissue is shown in figure 1. Retention times were 1 min 5 sec for metabolite I, 2 min for ketamine, and 2 min 45 sec for internal standard. Standard solutions of metabolite II added to brain homogenate had a retention time of 1 min 35 sec.

Results

Table 1 shows the times of recovery of the righting reflex, disappearance of ataxia, and cessation of agitation as a function of ketamine dosage following intravenous injection. Signs of increased extrapyramidal movement were evident at doses of more than 10 mg/kg during the period of loss of the righting reflex. Corneal reflexes remained intact at doses as large as 40 mg/kg but were lost at the higher dose used. After regaining the righting reflex the rats manifested marked salivation and were agitated but ataxic. They frequently moved backwards in circular movements and appeared to have impaired balance. Agitation, as judged by increased motility, continued after the disappearance of ataxia. By this mode of administration, a dose of about 60 mg/kg was found to be close to the LD₅₀. Since ketamine hydrochloride at 20 mg/kg provided a satisfactory period of loss of the righting reflex (7-8 minutes), this dose was chosen for subsequent studies of brain distribution.

Following iv administration, ketamine entered the brain very rapidly, to achieve maximal levels within a minute (fig. 2). Brain levels then declined, paralleling the decrease in plasma concentration, and recovery of the righting reflex occurred when brain ketamine levels were approximately 30 μg/g of tissue. Despite the rapid decrease of brain ketamine levels, CNS tissue retained the compound preferentially, as evidence by brain:plasma ratios of approximately 6.5:1 at all times of measurement.

The N-demethylated metabolite (I) was detectable in plasma at 30 seconds and reached a plateau of 2.25 μg/ml between 5 and 10 minutes (fig. 3). Metabolite I was first detectable in brain tissue at 1 minute and continued to increase in concentration of the CNS during the 10 minutes of the experiment. As with the parent compound, the rat brain appeared to retain metabolite I, the brain:plasma ratio reaching 2.5:1 at 10 minutes. Metabolite II, the cyclohexanone oxidation product, was not detectable in plasma or brain tissue during the time intervals studied in these experiments.

Figure 4 shows the distribution of ketamine with time in four regions of the rat brain: cerebellum, brainstem, midbrain, and cerebral cortex. At 30 seconds and at 1 minute after injection, the levels of ketamine were significantly higher in the cerebral cortex than in the other regions of the brain (P < 0.05, Student’s t test for paired data). No marked change in regional brain distribution was apparent at later times, and the declines in regional levels of ketamine were similar to that observed for the whole brain (fig. 2). Metabolite I was found in all regions of the brain, but with no significant difference in concentration among regions (approximately 4 and 6 μg/g tissue at 5 and 10 minutes, respectively).

Table 2 shows the results of experiments evaluating ketamine metabolism by liver and brain tissue in vitro. Liver homogenates metabolized ketamine at a rate of approximately 25 μg/g tissue/min, the only detectable prod-
Fig. 2. Plasma and brain levels of ketamine following iv administration. Ketamine hydrochloride, 20 mg/kg, was injected into the tail vein of each rat. Samples of plasma and whole brain were assayed for ketamine at appropriate times. Points represent mean values from four animals ± standard errors.

Fig. 3. Plasma and brain levels of metabolite I following ketamine administration. Ketamine hydrochloride, 20 mg/kg, was injected into the tail vein of each rat. Samples of plasma and whole brain were assayed for metabolite I at appropriate times. Points represent mean values from four animals ± standard errors.
TABLE 2. Metabolism of Ketamine by Rat Brain and Liver Homogenates

<table>
<thead>
<tr>
<th>Number of</th>
<th>P</th>
<th>Ketamine (μg/ml Medium)</th>
<th>Metabolite I (μg/ml Medium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiments</td>
<td></td>
<td>1*</td>
<td>10*</td>
</tr>
<tr>
<td>Saline control</td>
<td>4</td>
<td>&gt;0.5</td>
<td>94.5 ± 2.83</td>
</tr>
<tr>
<td>Brain</td>
<td>4</td>
<td>&gt;0.1</td>
<td>97.4 ± 0.57</td>
</tr>
<tr>
<td>Liver</td>
<td>4</td>
<td>&lt;0.05</td>
<td>95.5 ± 2.47</td>
</tr>
</tbody>
</table>

Ketamine was added to rat liver and brain homogenates and incubated for 30 minutes at 37°C (see Methods). Samples were taken at zero time (t₀) and 30 minutes (t₁₀) and analyzed by gas chromatography for ketamine and metabolites. Data represent means ± SE derived from four animals. Student's t-test was used for calculation of P values.

Discussion

The gas-liquid chromatographic procedure for estimation of ketamine and its metabolites in plasma appears suitable for determinations in other tissues, including brain. In these studies of the cerebral distribution of ketamine, rats were used as experimental animals, and it is of interest that diverse CNS effects which resulted in behavioral manifestations analogous to those seen in man were apparent. Extrapyramidal effects were observed during the period of loss of the righting reflex. While ataxia and agitation were apparent for prolonged periods following recovery of the righting reflex, these effects also occurred quite rapidly in animals given ketamine in doses that did not result in loss of the righting reflex.

Given intravenously, ketamine produced loss of the righting reflex at doses lower than that necessary to produce the same effect when given intraperitoneally to rats. The earliest plasma level estimated (30 seconds) represented a total plasma ketamine content of 40 μg/100 g of rat (assuming a plasma volume of 3 ml), or approximately 2 per cent of the injected dose. This suggests extremely rapid distribution of ketamine to tissue compartments, and this may well be the case in man. For example, a similar calculation based on reported plasma levels in man 2 minutes after intravenous administration suggests that at this time the total plasma content represents only 5 to 6 per cent of the injected dose. In this respect, ketamine appears to be similar, in terms of tissue distribution, to short-acting barbiturates such as thiopental.

In the rat, rapid distribution to the brain occurred, brain levels of ketamine at 30 seconds representing about 150 μg/100 g of rat, or approximately 7.5 per cent of the injected dose. This rapid entry of ketamine into the brain, along with the rapid onset of its anesthetic effect, suggests an almost complete absence of a blood-brain barrier to this agent. At every time of measurement, the brain: plasma level ratio was 6.5:1, indicating a preferential accumulation and retention of ketamine by the brain. The rate of entry and accumulation of drugs in cerebral tissue is classically considered to be a function of blood flow to the brain and tissue solubility at physiologic pH. The increases in cerebral blood flow in dogs and man that occur almost immediately after administration of ketamine may contribute to the rapid achievement of high brain levels. Although ketamine would exist largely in the ionized form at physiologic pH, it is possible that it has properties similar to those of thiopental in terms of high lipid solubility. Since little is known of the precise mechanisms of drug accumulation in the CNS, it is also possible that active transport plays some role with regard to the high brain levels of ketamine. The maintenance of high brain:plasma ratios may reflect the solubility.
and possibly also tissue binding of this agent. Preliminary reports noting that tissue concentrations of ketamine may exceed plasma levels in laboratory animals have appeared, but no precise information about this aspect of disposition of the drug has been published.

Study of the regional CNS distribution of ketamine (fig. 4) indicated that the cerebral cortex achieved higher levels than other regions in the early periods after intravenous administration. While this may be a function of differences in regional blood flows and solubility, it may have pertinence regarding possible sites of action of ketamine, which have been suggested to involve higher centers. The \( \alpha \)-demethylated product of ketamine (metabolite I) appears to be a major metabolite in man, and was formed quite rapidly in the rat (fig. 3). Metabolite I also accumulated in CNS tissue of the rat to give brain levels greater than those of plasma. In fact, rapid distribution of metabolite I into body tissues may account for the plateauing of the plasma levels of this metabolic product. Rat liver homogenates were capable of \( \alpha \)-dealkylating ketamine to metabolite I at a rapid rate (table 2). No evidence of further metabolism to the oxidation product (metabolite II) was found in either in-vivo or in-vitro experiments, which supports the suggestion that, in the rat, metabolite I is the major breakdown product of ketamine. While one must extrapolate from in-vitro experiments to the intact animal with caution, it is of interest that the rate of hepatic degradation in the rat could account for almost 50 per cent of the administered dose of ketamine (20 mg/kg) during the time when the righting reflex was lost. This suggests that, at least in this species, metabolic transformation of ketamine may play a significant role in the termination of certain of the drug's CNS actions. If this is the case, ketamine is quite different from thiopental in this respect. In man, plasma ketamine levels decrease rapidly after an iv injection of 2.2 mg/kg, consciousness returning with plasma concentrations in the range 0.7 to 1.0 \( \mu \)g/ml. Since metabolite I rapidly appears in the plasma in man, it is reasonable to suggest that metabolic degradation may also be significant here with respect to duration of action. We are unaware of any clinical evidence that might suggest unusual responses to ketamine in situations of gross hepatic impairment, but some degree of caution is suggested until it becomes apparent that accumulation is not a problem in man.
Homogenates of rat brain did not metabolize ketamine, and we tentatively conclude that the accumulation of the x-demethylated product in the CNS follows extracerebral degradation of the parent compound. The relatively high, prolonged brain levels of metabolite I raise the possible that certain of the diverse CNS effects observed following administration of ketamine could be caused by this compound. This is probably not the case with respect to ataxia and agitation. These effects occurred rapidly in animals that did not lose their righting reflexes, 1 or 2 minutes after injection and prior to the establishment of significant plasma levels of metabolite I. However, it cannot be ruled out that both actions in the CNS, perhaps more delayed in onset, could be caused by metabolic products of ketamine.

Appreciation is extended to Charles Pudwill for development of the gas chromatographic assay, and to Parke-Davis for generously supplying ketamine hydrochloride.

References

Circulation

PERFUSION PRESSURE AND CEREBRAL BLOOD FLOW In the normal subject, autoregulation maintains cerebral blood flow (CBF) constant until the arterial blood pressure falls to 50–55 mm Hg. Below this pressure, CBF falls sharply and results in the characteristic EEG findings. The arterial blood pressure below which CBF becomes inadequate during the nonpulsatile flow of cardiopulmonary bypass (CPB) is still uncertain, and studies of the effect of elevated central venous pressure (CVP) on CBF have produced conflicting results. According to the authors, a mean pressure of 50 mm Hg is necessary to provide adequate CBF during CPB. They correlated 257 recordings of arterial pressure and 191 recordings of CVP with quantitative EEG findings during open-heart operations on 34 patients. A decrease in mean arterial blood pressure to below 50 mm Hg was invariably associated with EEG slowing. There was no correlation between venous pressure and EEG pattern. (Juneja, L., Flynn, R. E., and Berger, R. L.: The Arterial, Venous Pressures and the Electroencephalogram during Open Heart Surgery. Acta Neurol. Scand. 48: 163, 1972.)