The Development of an Acute Tolerance to Ketamine

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A rapidly developing tolerance to the hypnotic effect of ketamine in rats and mice has been investigated. On the basis of plasma and brain concentrations of ketamine at the time the animals wakened, it seemed likely that a central nervous system tolerance to the drug explained the shortened sleeping times noted.

THE rapid development of tolerance to central nervous system (CNS) depression by a number of drugs has been reported. Drugs with which this effect has been associated include, among others, ethyl alcohol,[1] pentobarbital,[2] thiopental,[3,4] glutethimide,[5] and diazepam.[6] Two of the effects noted were a shortened sleeping time following repetition of the same dose of the drug, and an increase in the blood level required to produce an observed effect. Mirsky's group,[1] in 1941, described this latter type of acute tolerance to ethanol in the rabbit and in man, and Brodie and colleagues,[3] a decade later, showed that in the human, thiopental plasma levels on waking were higher after a large dose of the drug than after a smaller one. An acutely developing tolerance to the effect of thiopental on the rate of cerebral O_2 consumption has been described.[7]

For the past several years, a new short-acting IV anesthetic agent, ketamine, has been in clinical use. Animal experiments showed that, in monkeys, repeated injections to a total of 6 did not result in a change in sleeping time.[8] Extensive clinical experience seemed to indicate that repeated injections of the drug required little change in dosage and were not followed by any change in observed anesthetic time.

While we were investigating this drug for possible enzyme inductive effects, a sharp decrease in the sleeping time of rats consequent to the 2nd injection of the drug was observed. The abrupt onset suggested a rapidly developed tolerance. We have investigated this possibility.

MATERIALS AND METHODS

Animal Experiments.—Male rats weighing between 190 and 250 gm, obtained from a local supplier,† were housed in air-conditioned quarters and fed *ad libitum* on standard laboratory feed. Groups of rats were given IP injections of ketamine (90 mg/kg), and their sleeping times measured from loss to regaining of the righting reflex. One group received only the initial injection, and from these brain and plasma samples were obtained at the time of recovery of the righting reflex. The samples were analyzed for ketamine concentration. Other groups were allowed to recover and then further injections of ketamine (90 mg/kg IP) were given at 5, 6, 24, 48, and 72 hours following the 1st injection. Brain and plasma samples were obtained at the time of recovery of the righting reflex at each time interval. All the animals used in a sequence of experiments came from the same shipment lot.

Another set of experiments on male rats used a dose of 80 mg/kg IP of ketamine. These animals were tested only for sleeping time at 24-hour intervals to a total of 5 exposures.

Other sleeping time studies used male mice of 20 to 30 gm weight given ketamine (100 mg/kg IP). Sleeping time was recorded after the 1st, 2nd, 3rd, and 4th injections, administered at 24-hour intervals.

Chemical Analyses. — Ketamine concentrations in plasma were estimated as previously described.[9] Brain samples were assayed by making a 25 percent homogenate

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TABLE

Sleeping Time, Brain and Plasma Levels of Ketamine and Brain to Plasma Concentration Ratio of Ketamine at Recovery of Righting Reflex in Male Rats Given Ketamine (90 mg/kg IP) at the Indicated Times

Injection number	Time of injection, hours	n	Sleeping time ± SEM, min	n	Brain concentrations of ketamine on recovery of righting reflex ± SEM, μg/g	n	Plasma concentrations of ketamine on recovery of righting reflex ± SEM, μg/ml	Brain/ plasma ratio of ketamine
1	0	47	23.7 ± 1	18	12.04 ± 0.79	17	2.84 ± 0.25	4.25
2	24	39	17.4 ± 1*	24	13.73 ± 0.78†	24	3.68 ± 0.27*	3.74
3	48	18	14.1 ± 1*	12	14.15 ± 1.3†	13	4.3 ± 0.63*	3.3
4	72	5	16.2 ± 2.9*	5	15.34 ± 3.36†	5	3.59 ± 0.35*	4.27

*Differs from 1st injection $p<0.05$.

†Differs from 1st injection $p<0.1$.

(w/v) of brain in KCl 1.15 percent. To 6 ml of the homogenate was added 3 ml of 20 percent metaphosphoric acid. This was shaken on a vortex mixer, then centrifuged. Supernatant (5 ml) was placed in a reaction vessel containing 15 ml of chloroform and 20 μg of pheniramine maleate as an internal standard. NaOH (10 N) 0.5 ml was added to make the mixture strongly alkaline. From this point, treatment was as described earlier[9] for the assay of ketamine in plasma. Standard curves were constructed for each day's determinations from the results of known concentrations of ketamine in brain homogenate subjected to the same procedure. The peak height ratio of ketamine to internal standard related linearly to ketamine concentrations.

RESULTS

The results of repeated injections of ketamine into rats at 24-hour intervals for 4 successive doses are summarized in the table. Sleeping time decreased abruptly (26%) between the 1st and 2nd doses. A further but lesser decrease occurred between the 2nd and 3rd exposures. The decrease in sleeping time was accompanied by an increase in the brain and plasma ketamine levels at the time of wakening. Differences between initial values of each parameter and subsequent values at each exposure were significant, as indicated in the table. The brain/plasma ratio at wakening showed an initial decrease, but returned to its original value on the 4th exposure.

Figures 1 and 2 illustrate the changes in sleeping time and brain and plasma ketamine levels on waking between the 1st exposure and a repeated injection at 5 and at 6 hours. When a 2nd injection of ketamine was given 5 hours after the 1st, the animals slept a significantly longer time following

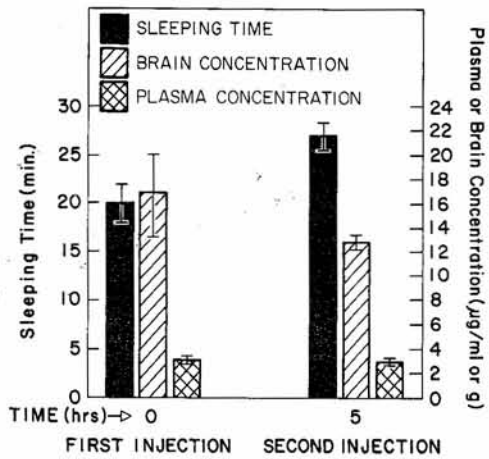

FIG 1. Sleeping time, waking brain and plasma concentration of ketamine in rats following the 1st and 2nd injections of ketamine (90 mg/kg IP) 5 hours apart.

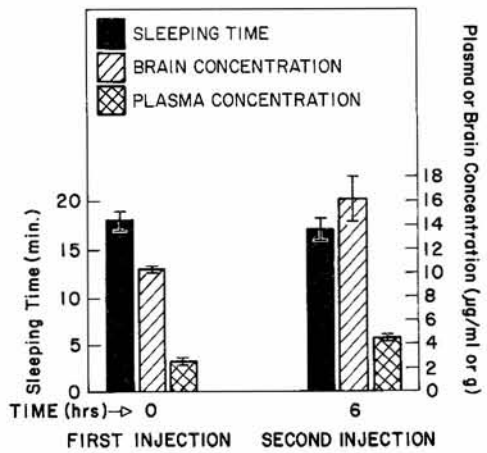

FIG 2. Sleeping time, waking brain and plasma concentration of ketamine in rats following the 1st and 2nd injections of ketamine (90 mg/kg IP) 6 hours apart.

the 2nd injection ($p<0.025$). The waking brain and plasma levels after the 2nd exposure were not different than after the 1st ($p<0.2$, $p<0.3$). When the 2nd injection was given 6 hours after the 1st, sleeping time was now decreased slightly. The difference between overall means was not significant, but between values where a paired *t*-test was possible, the difference was highly significant ($p<0.025$). Waking brain and plasma levels were significantly higher than after the 1st injection ($p<0.01$).

Figure 3 illustrates the effect of consecutive injections of ketamine, 80 mg/kg IP, on the sleeping time of rats. The decline in sleeping time noted, particularly between the 1st and 2nd injections, is similar to that noted when a dose of 90 mg/kg IP was given.

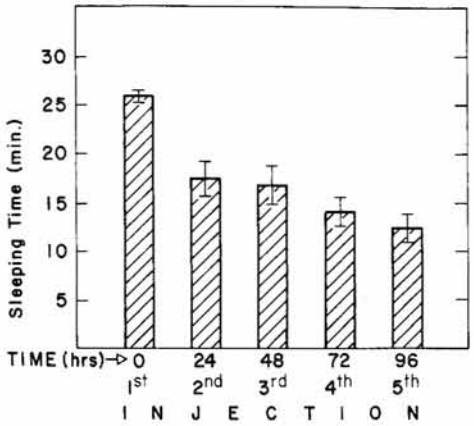

FIG 3. Sleeping time of rats following 5 successive doses of ketamine (80 mg/kg IP) at 24-hour intervals.

Mice respond to repeated injections of ketamine in a manner paralleling the response of rats (fig 4). Sleeping time decreases sharply between the 1st and 2nd doses and also between the 2nd and 3rd doses. The sleeping time after the 4th dose, however, is not significantly different than that after the 2nd dose.

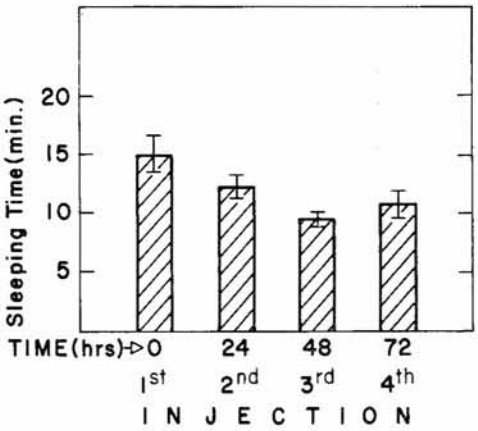

FIG 4. Sleeping time of mice following 4 successive doses of ketamine (100 mg/kg IP) at 24-hour intervals.

DISCUSSION

The development of an acute tolerance to ketamine, evident in the present work, and to other CNS depressants reported elsewhere by others,[1-7] could be explained in various ways: by an increase in ability to biotransform the agent; by an alteration in the blood-brain barrier to the drug; or by an increase in resistance of CNS cells to the effect of the drug. An alteration in ability to biotransform the drug seems unlikely, since the onset of induction of drug-metabolizing enzymes is a relatively slow process compared with the rapid onset of the acute tolerance witnessed here. Early work done at the Parke-Davis Company* also showed that repeated parenteral doses of ketamine HCl administered to rats did not increase the *N*-demethylating capacity of the hepatic microsomal enzymes. Further, if a more rapid metabolism were the cause, there is little reason to expect a change in waking plasma and brain concentrations of the drug. A change in the blood-brain barrier is possible, but does not seem likely in view of the very rapid onset of anesthesia in all species given the drug IV.

The table does show some variation in the brain/plasma ratio between control and previously exposed animals. The deviations do not explain the higher waking brain concentrations of ketamine in the latter animals, and the ratio returns to that of the control animals at a time when the animals are still sleeping a shorter time and waking with higher brain concentrations. A change in blood-brain barrier would not be expected to alter the waking brain concentrations of the drug. By contrast, if the neurons of the CNS became rapidly resistant to the effect of the drug, a higher plasma and brain level on waking would be expected. This explanation would seem to fit best the data presented here.

Rapidly developing accommodation to the CNS effects of many sedatives and hypnotics has been reported. In Mirsky's investigation of the effect of ethanol in the rabbit and in man,[1] the effect of the drug in both

*Unpublished data.

species (coma in the rabbit, drunkenness in man) disappeared while blood levels of ethanol were considerably above those noted at the onset of the effect. In both species, repeated administration of the drug showed that higher levels were required to produce the same effect at each subsequent exposure. In the rabbit, the repetition could be carried to several exposures, each resulting in a further rise of blood ethanol at which symptoms disappeared.

The work presented in this paper demonstrates a similar response. As shown in the table and in figures 3 and 4, subsequent exposures to ketamine in both rats and mice resulted in progressively shorter sleeping times to at least 3 administrations. A similar response to repeated injections of thiopental was noted in the male rat by Singh.[10] These observations all indicate that the limit of tolerance is not reached after 1 or even 2 moderate doses of the drugs used. Larger doses appear to elicit a greater response. This conclusion was also indicated in earlier work by Brodie[3] and by Dundee's group.[4]

Barnett and Fiore[6] showed the development of a tolerance to diazepam in cats by testing the linguomandibular reflex. Their experiments indicated the tolerance was due to a metabolite of diazepam, which presumably had a good affinity for the same CNS receptors as diazepam, but had a much weaker agonist effect and therefore acted as a partial antagonist to diazepam.

In the experiments here, a similar explanation might pertain. The biotransformation products of ketamine are presumed to be pharmacologically inactive, and they are also excreted in both urine and feces. The original investigative work by the Parke-Davis Company* showed that about 74 percent of an IP dose in the rat appeared in the urine and feces by 24 hours (as measured by radioactivity). It therefore seems unlikely that competition for receptor sites by an inactive metabolite would explain the shortened sleeping time observed in this work, following subsequent doses of ketamine at 24-hour intervals. Such an explanation might be postulated for the tolerance which developed by 6 hours.

The time required to demonstrate a tolerance in this work was 6 hours. Animals reinjected at 5 hours slept longer than after their 1st exposure (fig 2). It is possible that there was a residual effect from the 1st dose still present by 5 hours which could account for the increased sleeping time. The data presented here do not suggest any definite mechanism. At 6 hours after an initial dose, a 2nd dose of ketamine resulted in a shortened sleeping time, apparently by a mechanism of CNS tolerance to the drug, since the animals were waking with higher brain and plasma levels of ketamine (fig 3). Singh[10] reported that a minimum of 4 hours was required for female rats to develop tolerance to thiopental, a time quite similar to that observed here.

This work demonstrates a rapidly developing tolerance to the CNS-depressant effect of ketamine. The degree of the tolerance is of modest dimension, but was demonstrated in rats at 2 different dosage levels and in a different species (mice). The tolerance to ketamine resembles the tolerance to a number of other CNS depressants and seems to represent an unidentified adaptation of CNS neurons which permits them to accommodate to the presence of a depressant drug.

*Unpublished data.

REFERENCES

1. Mirsky JA, Piker P, Rosenbaum M, et al: Adaptation of the central nervous system to varying concentrations of alcohol in the blood. Q J Stud Alcohol 2:35-45, 1941

2. Aston R: Acute tolerance indices for pentobarbital in male and female rats. J Pharmacol Exp Ther 152:350-353, 1966

3. Brodie BB, Mark LC, Lief PA, et al: Acute tolerance to thiopental. J Pharmacol Exp Ther 102:215-218, 1951

4. Dundee JW, Price HL, Dripps RD: Acute tolerance to thiopentone in man. Br J Anaesth 28: 344-352, 1956

5. Curry SH, Norris H: Acute tolerance to a sedative in man. Br J Pharmacol 38:450P-451P, 1970

6. Barnett A, Fiore JW: Acute tolerance to diazepam in cats and its possible relationship to diazepam metabolism. Eur J Pharmacol 13:239-243, 1971

7. Altenburg BM, Michenfelder JD, Theye RA: Acute tolerance to thiopental in canine cerebral oxygen consumption studies. Anesthesiology 31: 443-448, 1969

8. McCarthy DA, Chen G, Kaump DH, et al: General anesthetic and other pharmacological properties of 2-(o-chlorophenyl)-2-methylamino cyclohexanone HCl (CI 581). J New Drugs 5:21-33, 1965

9. Lo JN, Cumming JF: Interaction between sedative premedicants and ketamine in man and in isolated, perfused rat livers. Anesthesiology 43: 307-312, 1975

10. Singh JM: Clinical signs and development of tolerance to thiopental. Arch Int Pharmacodyn 187:199-208, 1970