The Repeated Administration of Ketamine Induces an Enhancement of Its Stimulant Action in Mice

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Received October 19, 1992 Accepted December 18, 1992

ABSTRACT—The modification of the stimulant and motor-incoordinate actions of ketamine induced by repeated administration was investigated by means of ambulatory activity and rota-rod performance in mice, respectively. Ketamine (12.5, 25 and 50 mg/kg, s.c.) increased the mouse's ambulation in a dose-dependent manner, and the repeated 5-times administration at 3- to 4-day intervals enhanced the increment effect. However, a disruption of the rota-rod performance by ketamine was not modified by the repeated treatment. These results suggest that a reverse tolerance to the stimulant action of ketamine is produced, and that a tolerance to its motor-incoordinate action may not cause the enhancement.

Keywords: Ambulatory activity, Ketamine, Reverse tolerance

Ketamine has not only hypnotic actions but also psycho-stimulant actions, including hallucination, restlessness and hyperexcitability, in humans (1). The stimulant actions of ketamine are manifested by ambulation (2, 3) and stereotypy (4) in rodents. Thus, the effects of ketamine on the rodent's behavior may be partially similar to those of psycho-stimulants such as amphetamines (5-7).

It is well known that repeated administration of amphetamines enhances the ambulation-increasing effect and/or the stereotypy-producing effect. These enhancement phenomena are called reverse tolerance or behavioral sensitization (5-7). Although the repeated treatment of ketamine produces a rapid tolerance to the hypnotic actions (8, 9), the modification of the stimulant actions by repeated administration has been scarcely investigated. In this study, we assessed whether the repeated ketamine administration in sub-anesthetic doses produced the tolerance or the reverse tolerance by means of ambulatory activity in mice. Furthermore, the effect of the repeated ketamine on rota-rod performance was investigated, because a motor-incoordinate effect of ketamine, i.e., ataxia, which was simultaneously observed at those doses (2, 3), may non-specifically modify the ambulation-increment.

Male ddY mice (Japan Laboratory Animals) at 7 weeks of age, weighing 26-35 g, were used. The animals were housed in groups of 10 in aluminum cages of 35 (D) × 25 (W) × 10 (H) cm with a wooden-flake floor mat, and they were given freely a solid diet (MF, Oriental Yeast) and tap water except during the times of experiment. The breeding room was controlled so that the light-dark cycle (light period; 6:00-18:00) and temperature (23±1℃) were almost constant.

The drug used in this study was racemic ketamine HCl (Ketalar Inj.®, Sankyo). The commercial preparation of ketamine was diluted by physiological saline (saline). The drug, and saline were administered subcutaneously, and the administration volume was fixed to 0.1 ml/10 g body weight.

The ambulatory activity of each mouse was measured by a tilting-type ambulometer (AMB-10, O'Hara & Co.) (10). Mice were individually placed in the Plexiglas activity cages and adapted to the cages for 30 min, and then the ambulatory activity was measured for 1.5 hr after the administration of ketamine (12.5, 25 and 50 mg/kg) or saline. These treatments were repeated for 5 times at 3- to 4-day intervals, following the experimental protocol in our laboratory to exclude accumulative effects of the drug (5, 6). In each drug test, 36-39 mice were used.

The rota-rod performance was assessed with a treadmill rod (Natume Co.), 8 cm in diameter, and the speed of 3 r.p.m. The trained mice that performed on the rod for more than 30 sec were used in the drug test. The performance time mounting on the rod was recorded (cut-off

time: 30 sec), and was scored to 6 criteria (0–4 sec: 0, 5–9 sec: 1, 10–14 sec: 2, 15–19 sec: 3, 20–24 sec: 4, 25–29 sec: 5, 30 sec: 6) (11). The rota-rod test was carried out at 10-min intervals for 60 min after ketamine (25 mg/kg) or saline administration. The 5-times treatments were repeated at 3- to 4-day intervals. In each drug test, 20 mice were used.

All measurements were carried out between 10:00 and 15:00.

The data in the ambulation and rota-rod tests were statistically compared by the paired Student's *t*-test and the Friedman analysis of variance, respectively. Differences were considered to be statistically significant when P values were less than 0.05.

As shown in Fig. 1, the administration of ketamine at 12.5, 25 and 50 mg/kg induced a dose-dependent increment in the mouse's ambulatory activity with ataxia, but no mice lost their righting reflex. Although saline administration did not produce any significant changes in the activity throughout the 5-times treatments, the repeated ketamine administration enhanced its ambulation-increasing action. Thus, the overall 1.5-hr activity counts in the 3rd and later administration of ketamine at 12.5 mg/kg and in the 2nd and later administration of ketamine at 25 and 50 mg/kg were significantly higher than the values in the 1st administration of the corresponding doses.

Table 1 indicates the results of the rota-rod test. Ketamine at 25 mg/kg completely inhibited the rota-rod performance for 20 min. The scores of the performance returned to the preadministration values at 40 min after the administration. The repeated ketamine treatment did not significantly modify the time-course change in the scores of the performance. Saline produced no significant changes in the rota-rod performance throughout the 5-times administrations (data not shown).

The present experiment clearly demonstrated that the repeated administration of ketamine produced a reverse tolerance to its ambulation-increasing effect in mice, like

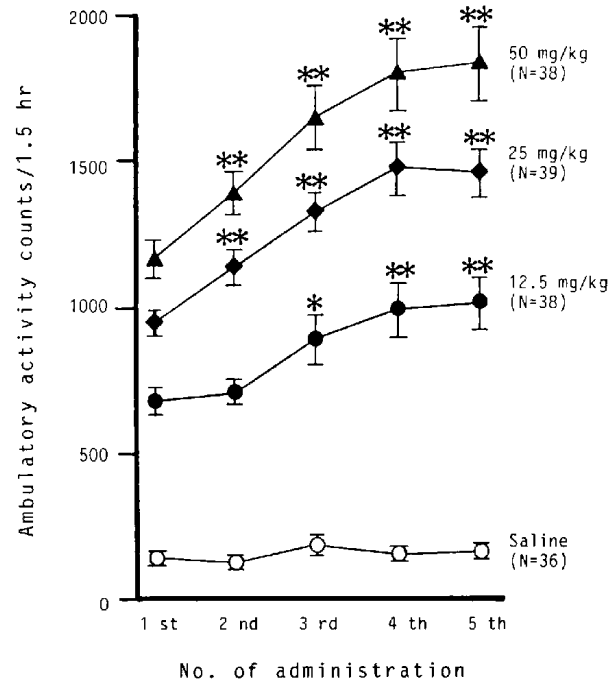

Fig. 1. Changes in the 1.5-hr overall ambulatory activity counts after repeated administration of ketamine (12.5, 25 and 50 mg/kg, s.c.) for 5 times at 3- to 4-day intervals in mice. Each point indicates a mean value±S.E.M. *, **: Significantly different from the value in the 1st administration within each group ($P<0.05$, $P<0.01$).

that of amphetamines (5–7). Ketamine, unlike the psycho-stimulants, induced an ataxia with the ambulation-increment. Therefore, it is suspected that the ataxia obstructs the ambulation, and that an enhancement of the ambulation-increasing effect of ketamine non-specifically results from a development of a tolerance to the ataxia-inducing action in the repeated administration. However, the results of the rota-rod test, suitable for the detection of the motor incoordination including ataxia (11), indicated that the tolerance to the disruption of the rota-rod per-

Table 1. Changes in scores of rota-rod performance after repeated administration of ketamine (25 mg/kg, s.c.) for 5 times at 3- to 4-day intervals in mice

Number of administration	Scores of rota-rod performance						
	0	10	20	30	40	50	60 (min)
1st	6 (6–6)	0 (0–0)	0 (0–0)	4.5 (1–6)	6 (6–6)	6 (5–6)	6 (6–6)
2nd	6 (6–6)	0 (0–0)	0 (0–0)	6 (5–6)	6 (6–6)	6 (6–6)	6 (6–6)
3rd	6 (6–6)	0 (0–0)	0 (0–0)	5.5 (2–6)	6 (6–6)	6 (6–6)	6 (6–6)
4th	6 (6–6)	0 (0–0)	0 (0–0)	3 (0–6)	6 (6–6)	6 (6–6)	6 (6–6)
5th	6 (6–6)	0 (0–0)	0 (0–0)	5 (1–6)	6 (4–6)	6 (6–6)	6 (6–6)

Each value represents the median and interquartile range. Twenty mice were used.

formance was not produced after the repeated ketamine administration. An accumulation of ketamine and its metabolites by the intermittent treatment may not also be candidates for the enhancement of the ambulation-increasing effect, because there is no evidence of the accumulation of ketamine and its metabolites in rat brain even after 10 daily administration of ketamine at an anesthetic dose (9).

It was reported that the presynaptic dopamine neurons in the nucleus accumbens mediate the ambulation-increment induced by ketamine in mice (2). We also reported that ketamine increases the ambulatory activity by facilitating a dopamine release from a newly synthesized pool at the presynaptic level in mouse brain (3). Thus, it is suggested that ketamine shares partially common mechanisms of action with amphetamines (12). The central dopaminergic system is considered to play an important role in the production of reverse tolerance to amphetamines (6, 7). It is probable that a hyperactivation in the dopaminergic system produces the reverse tolerance to the ambulation-increasing effect of ketamine.

Recently, it has been postulated that changes in presynaptic dopamine neurons and concomitant enhancement of the dopamine release, but not changes in the sensitivity of postsynaptic dopamine receptors, are involved in the production of the reverse tolerance to amphetamines (7). It is not clear whether such a mechanism contributes to the production of the ketamine-induced reverse tolerance as demonstrated in our study. Phencyclidine (13) and MK-801 (14), which are similar to ketamine in behavioral and NMDA antagonistic properties (4), are reported to produce the reverse tolerance to their ambulation-increasing effect after the repeated administration, but not a cross-sensitization to amphetamines. Ketamine-sensitized mice also do not display the cross-sensitization to methamphetamine (Y. Uchihashi, unpublished data). These results suggest that the reverse tolerance to ketamine is produced through a different dopaminergic mechanism from that of amphetamines, probably via an agonistic action on sigma receptors and/or an antagonistic action on NMDA receptors (13, 14).

Livingston and Waterman (9) demonstrated that the tolerance to the hypnotic action of ketamine is associated with an increased hepatic metabolism in rats. In addition to such a mechanism, the enhancement of the stimulant action of this drug may contribute to a rapid development of tolerance to the hypnotic action (8, 9).

The characteristics of the reverse tolerance to amphetamines in experimental animals are considered to be intimately correlated to the psychotoxic effects of the drugs in humans, i.e., amphetamine psychosis (6, 7). However, it is not proved whether the psychotoxic effects of ketamine in humans are enhanced after its repeated administration (15). Therefore, further investigations, including case studies in humans, are required.

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