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RAPID COMMUNICATION

Ketamine Retards Chronic but Not Acute Tolerance to Ethanol

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KHANNA, J. M., H. KALANT, J. WEINER, A. CHAU AND G. SHAH. *Ketamine retards chronic but not acute tolerance to ethanol.* PHARMACOL BIOCHEM BEHAV **42**(2) 347-350, 1992. – Motor impairment (tilt-plane test) was used to investigate whether the noncompetitive *N*-methyl-*D*-aspartate (NMDA) antagonist ketamine prevents the development of chronic and acute tolerance to ethanol. Rats were treated with ethanol or saline in the presence and absence of ketamine (separate groups) for 10 days and tested for ethanol tolerance in the absence of ketamine on the fifth and tenth days. In other studies, the effect of ketamine on acute tolerance to ethanol was examined. Rats that received ethanol daily without ketamine showed significant tolerance to ethanol on days 5 and 10, but those receiving ethanol plus ketamine daily showed significantly less tolerance to ethanol. Thus, ketamine interfered with the development of chronic tolerance just as it had been found previously to prevent rapid tolerance. In contrast, ketamine failed to block acute tolerance to ethanol. These results would suggest that the phenomena of acute tolerance and chronic tolerance have differences not previously reported.

NMDA antagonist Ketamine Ethanol Acute tolerance Chronic tolerance

TOLERANCE can develop and be measured within three different time frames that are characterized by different terms. The first and most commonly studied form of tolerance, designated *chronic tolerance*, is that which is seen to develop gradually and reach its maximum after several days or weeks of repeated administration of the drug. It usually includes both dispositional and functional components.

Acute tolerance is that which is seen during the course of a single drug exposure. This form of tolerance, originally described by Mellanby (13), was discounted by many investigators for a time on the grounds that it was based upon a distribution artifact arising from arterial-venous differences in drug concentration during the early period of drug absorption and distribution. However, the fact that such tolerance is seen even when the drug effect is related directly to the concentration in the brain (11) makes it clear that acute tolerance is indeed a true biological phenomenon.

A third form of tolerance, designated *rapid tolerance*, is that which is seen in response to a second dose of the drug given 8-24 h after the effect of the preceding dose has disappeared (2,4,7). This form of tolerance appears to be functional rather than dispositional, and its existence implies that some change produced by the first drug experience has outlasted the actual presence of the first dose of drug itself.

The nature of the relationship among these three time courses of tolerance, and the question of whether they rest upon the same or different mechanisms, is not clear. Recently, we compared rapid tolerance and cross-tolerance to ethanol and pentobarbital (7) with chronic tolerance and cross-tolerance to the same drugs (5,8). The similarity in results on rapid tolerance to those on chronic tolerance in two different tests, and in both directions, that is, lack of cross-tolerance to pentobarbital after ethanol pretreatment and clear evidence of cross-tolerance to ethanol after pentobarbital pretreatment, suggests that rapid tolerance may be an accurate predictor of chronic tolerance, although it does not permit any conclusion as to whether or not the two processes are identical. Moreover, no similar comparison of acute tolerance with the other two forms has yet been carried out.

There is a considerable body of evidence that the *N*-methyl-*D*-aspartate (NMDA) receptor is involved in learning and memory processes, and NMDA antagonists can block learning in animals (3,14,15,17). Since both rapid tolerance and chronic tolerance have been shown to be influenced by,

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or require the participation of, processes related to learning and memory (2), we hypothesized that the acquisition of ethanol tolerance should similarly be subject to interference by NMDA antagonists. Indeed, we found that the NMDA antagonists (+)MK-801 and ketamine blocked the development of rapid tolerance to ethanol and cross-tolerance to other sedative agents such as the benzodiazepines (9,10).

Whether NMDA antagonists also block the development of chronic and acute tolerance to ethanol is unknown. The present study,therefore, examines the effect of ketamine on the development of chronic and acute tolerance to ethanol on the tilt-plane test. Ketamine was preferred to (+)MK-801 because ketamine does not alter the acute motor-impairment response to ethanol, whereas (+)MK-801 acutely enhances it. Both (+)MK-801 and ketamine interfere with the hypothermic response to ethanol. Therefore, the present study was limited to ketamine and to the motor-impairment response to ethanol.

METHOD

Animals

Male Sprague-Dawley rats weighing 150–200 g were obtained from Charles River Laboratories (Montreal, Quebec). They were housed singly and fed a standard laboratory rat chow in a daily ration that was individually adjusted to maintain comparable body weights in the various groups. This was necessary because body weight affects performance in the tilt-plane test used in this work. Tapwater was available at all times. The temperature of the colony room was maintained at 21 ± 1°C and lights were on from 7 a.m.-7 p.m. throughout the experiment.

Test Procedures

Tilt-plane test. The tilt-plane test was used as a measure of motor impairment (1,5). The apparatus consists of a plane that is hinged at one end, around which it can be inclined at a fixed angular velocity through a range of 55° above the horizontal axis. The animal is placed on the slightly roughened surface of the plane, which is then tilted until the animal slides from the starting position. The test measure is the angle at which the animal begins to slide. The sliding angle was measured before and at 30, 60, and 90 min after the injection of ethanol. The degree of postdrug ataxia was assessed as the percentage change in sliding angle compared to the same animal's predrug value. Maximum impairment, regardless of the time of its occurrence, was employed as the measure of ethanol effect. This generally occurred about 30 min after injection.

Experimental Procedures

Effect of chronic ketamine and ethanol treatment on ethanol tolerance on the tilt-plane test.

Procedure. On day 1, rats were brought upstairs to the laboratory and randomly divided into four groups. Two groups received IP saline and the other two groups were injected with ketamine (1 mg/kg) at zero time. After 30 min, one of the saline and one of the ketamine groups received 2.3 g/kg IP ethanol and the remaining two groups were injected with saline. Before the injections and at 30, 60, and 90 min after ethanol or saline injections the tilt-plane performance was measured. At 150 min, all rats received their respective additional ketamine (1 mg/kg) or saline injections to ensure continued effective concentration of the antagonist for several hours after the test. Rats were then returned to their home cages.

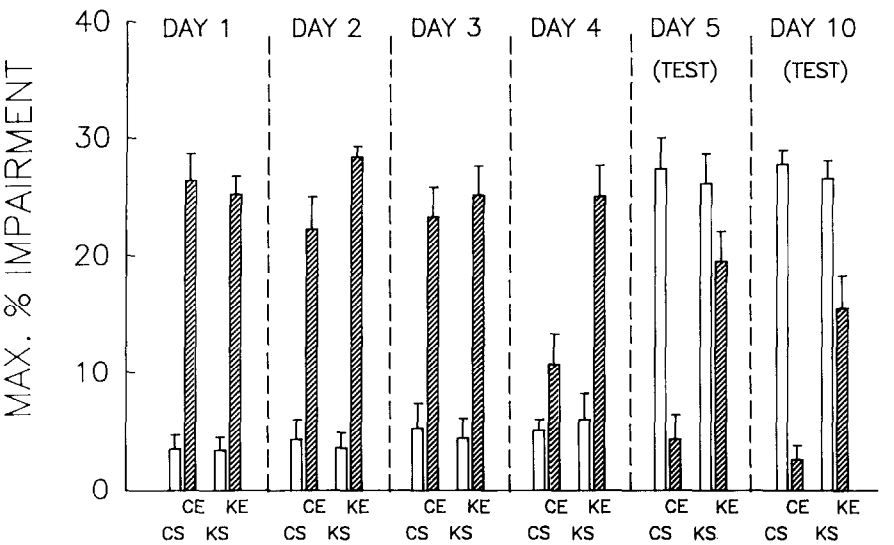

FIG. 1. Effect of ketamine (K) on the development of chronic tolerance to ethanol (tilt-plane test). Two groups received ketamine (K) with ethanol or saline and another two control groups (C) received saline, with ethanol or saline, on day 1. This procedure was repeated on days 2, 3, and 4 and on days 6, 7, 8, and 9. Doses of ketamine and ethanol were increased at intervals (see the Methods section). CS and KS represent groups treated chronically with saline and ketamine in the absence of ethanol (plain bars), whereas CE and KE represent groups treated with saline and ketamine in the presence of ethanol (cross-hatched bars). Chronic tolerance to ethanol-induced motor impairment was assessed on days 5 and 10, when all groups received a challenge dose of ethanol. No ketamine pretreatment was given on test days. Values shown are means ± SEM. n = 7 animals per group.

The day 1 procedure was repeated exactly on day 2. On days 3 and 4, an identical test procedure was followed except both the first and second ketamine doses were increased by 0.5 mg each time (i.e., total ketamine dose was 3 mg/kg on day 3 and 4 mg/kg on day 4) and an additional dose of ethanol (1 g/kg) or saline was given at 180 min. On day 5, all animals received a challenge dose of ethanol (2.3 g/kg, IP) followed by the same schedule of testing. No ketamine or saline pretreatment was given on the test day, but ethanol, ketamine, or saline posttreatments remained unchanged. Animals were then returned to their home cages. On days 6–9, animals were given in their home cages a single dose of ketamine (4 mg/kg) followed 30 min later by a single dose of ethanol (3.3 g/kg). On day 10, all rats received only a challenge dose of ethanol (2.3 g/kg, IP), followed by the same schedule of testing, to test for ethanol tolerance again. Blood samples (50 μl) for ethanol measurement were taken from the rat's tail tip immediately after the last measurement of motor impairment on test days 5 and 10. Blood ethanol was analyzed by the enzymatic method described previously (6).

Effect of ketamine on acute tolerance development to ethanol. The day before the experiment, three practice runs were given to each rat on the tilt-plane. Only those with a sliding angle of 40° or higher were used for the acute tolerance experiment. On the experimental day, rats were brought to the laboratory in the morning. Their initial performance on the tilt-plane was recorded. Rats with equal performance were paired together. In each pair of rats, one received IP ketamine (1 mg/kg) and the other received saline. Thirty minutes later, each rat was injected IP with one of the four test doses of ethanol, that is, 2.35, 2.6, 2.9, and 3.2 g/kg. Rats receiving the lower doses of ethanol (2.35 and 2.6 g/kg) were tested on the tilt-plane at 30 min after injection and every 10 min thereafter. Similarly, rats that received the higher doses of ethanol (2.9 and 3.2 g/kg) were tested at 60 min thereafter. Their performance was recorded until they reached a standard criterion of 35° angle. A tail blood sample was collected and the time was noted as well. This experiment was repeated with a higher dose of ketamine (4 mg/kg, IP).

RESULTS

Effects of Chronic Ketamine Treatment on Ethanol Tolerance (Tilt-Plane Test)

The results of this experiment are shown in Fig. 1. The saline-ethanol group (CE) showed the expected motor-impairment response on day 1, which did not change over days 2 and 3 but was significantly reduced on days 4–10; indeed, by day 10 the effect of ethanol was no greater than that of saline. There was no significant change in either saline control (CS) or ketamine control (KS) group responses to saline over days 1–4. When these two groups were exposed to ethanol alone, on days 5 and 10, they showed the same effect as the CE and ketamine ethanol (KE) groups did on day 1. Ketamine did not affect the motor-impairment response to ethanol on day 1, and the response of this group (KE) did not change on days 2, 3, and 4. The effect of ethanol alone, on days 5 and 10, did show some decrease in group KE, but it was still markedly greater than in group CE. A two-way analysis of variance (ANOVA) for maximum percent impairment values for the day 5 results showed that chronic pretreatment with ketamine retarded the development of chronic tolerance to ethanol-induced motor impairment because there was a significant effect of pretreatment, $F(1, 24) = 7.78$, $p < 0.010$, treatment, $F(1, 24) = 35.73$, $p < 0.0001$, and a significant pretreatment × treatment interaction, $F(1, 24) = 10.94$, $p < 0.003$. When maximum percent impairment data for day 10 groups were subjected to a two-way ANOVA, it again showed a significant effect of pretreatment, $F(1, 24) = 10.27$, $p < 0.0038$, treatment, $F(1, 24) = 98.63$, $p < 0.0001$, and a significant pretreatment × treatment interaction, $F(1, 24) = 14.95$, $p < 0.0007$, that suggested impaired development of tolerance.

Effect of Ketamine on Development of Acute Tolerance to Ethanol

The time to reach the standard criterion of recovery was positively correlated with the dose of ethanol, but this relationship was not altered by pretreatment with ketamine. Thus, in the saline pretreatment group the recovery times after the 2.35-, 2.6-, 2.9- and 3.2-g/kg doses were 43.8 ± 3.2, 45.0 ± 4.2, 70.0 ± 3.8, and 71.4 ± 2.6 min, respectively, and in the ketamine group the corresponding recovery times were 47.5 ± 4, 47.5 ± 4.5, 70.0 ± 1.9, and 72.9 ± 6.8 min, respectively.

The log dose-response curve for blood levels at the time of recovery to criterion in ketamine- and saline-pretreated groups is shown in Fig. 2(a). There is clear evidence of acute tolerance

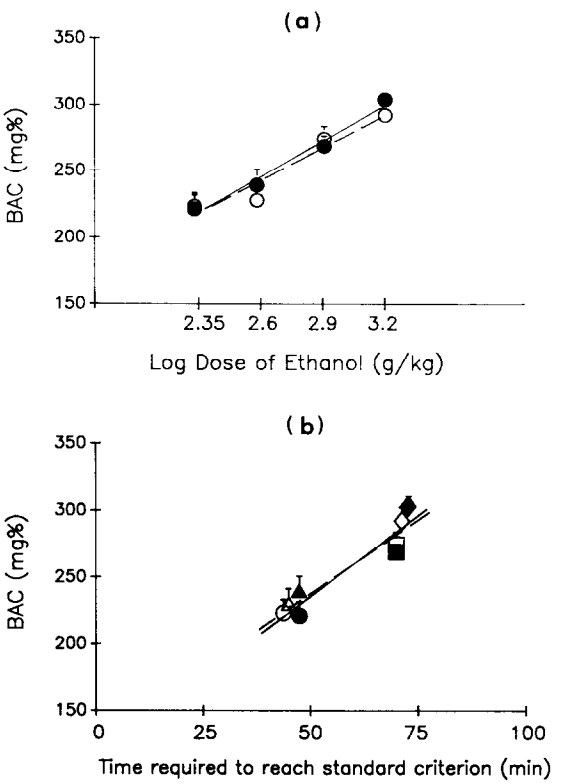

FIG. 2. (a) Log dose-response curve for blood ethanol levels on recovery. (●), Ketamine-pretreated rats; (○), saline-pretreated rats. Values shown are means ± SEM. $n = 8$ animals per group except for the highest dose, where $n = 7$. (b) Time required to reach standard criterion (min) on the tilt-plane test and blood ethanol levels on recovery in ketamine-pretreated and control rats. The four test doses of ethanol, that is, 2.35, 2.6, 2.9, and 3.2 g/kg, are represented by circles, triangles, squares, and diamonds, respectively. (●), Ketamine-pretreated rats; (○), saline-pretreated rats. Values shown are means ± SEM. $n = 8$ animals per group except for the highest dose, where $n = 7$.

development in the form of a progressive dose-dependent increase of blood ethanol level on recovery. However, there is no significant difference between the two pretreatments with respect to blood alcohol levels on recovery.

Figure 2(b) shows the blood levels of ethanol at recovery vs. the time required to reach recovery. There is again clear evidence of acute tolerance in the form of significantly higher blood ethanol levels on recovery in the groups that recovered at later times. However, blood ethanol levels were not significantly different in the ketamine- compared to the saline-pretreated groups.

Similar results were found when a higher dose of ketamine (4 mg/kg) was employed (data not shown).

DISCUSSION

Recently, we reported that NMDA antagonists [(+)MK-801 and ketamine] prevent the development of rapid tolerance to ethanol, that is, tolerance that is seen in response to a second dose of ethanol given 24 h after the effect of the first dose of ethanol has disappeared (10). In other studies, NMDA antagonists also blocked rapid cross-tolerance from ethanol to chlordiazepoxide and vice versa (9). Although the nature of the relationship between rapid and chronic tolerance and cross-tolerance is not clear, the similarity in results on rapid tolerance to those reported in models of chronic tolerance (5,7,8) suggests that NMDA antagonists would inhibit chronic tolerance in a similar manner. It was, therefore, not surprising to find that the NMDA antagonist did impair the development of chronic tolerance to ethanol.

The present results show that ethanol yields a reliable dose–response curve for time required to reach recovery on the tilt-plane test and that there is no difference between the ethanol dose–response curves in animals pretreated with ketamine or saline. Evidence for acute (within session) tolerance is seen (Fig. 2) in that the ethanol level in the blood at the time of recovery was higher the larger the dose given and hence the longer the time elapsed between ethanol administration and recovery. These results are consistent with those reported in the literature for ethanol and other drugs. Maynert and Klingman (12) reported significant increases in plasma concentration of various hypnosedative drugs at the time of disappearance of ataxia as the initial dose was increased. Similarly, LeBlanc et al. (11) showed a progressive shift toward higher brain levels of alcohol for the same degree of motor impairment with increasing time after alcohol administration.

Since there was no significant difference between ketamine and control groups with respect to blood levels of ethanol at recovery after different ethanol doses, when motor impairment was measured, it appears that ketamine does not affect acute tolerance development. A possible objection to this conclusion might arise from the fact that the animals were retested repeatedly to determine the point of recovery. Conceivably, the retesting might have provided enough stimulus to the development of acute tolerance to overcome the inhibitory effect of ketamine. This seems improbable, because a ketamine effect was seen in the earlier studies on rapid tolerance (9), which also involved repeated tests. However, experiments are now being conducted to test this question specifically. Although it would have been ideal to use brain ethanol concentration at recovery, it seems unlikely that pharmacokinetic factors, that is, arterial-venous differences in ethanol concentration during the early period of absorption and distribution, are of importance in the findings described here. The venous blood alcohol measurements used here to demonstrate acute tolerance reflected brain alcohol levels since blood alcohol measurements on recovery were carried out at a time (30 min postinjection) long after the attainment of equilibrium between blood and brain levels (16).

In conclusion, the present results indicate that the NMDA type of glutamate receptor is involved in chronic and rapid tolerance but not in acute tolerance to ethanol. These observations, however, do not permit any conclusion as to the nature of its involvement or to the possible relation between acute and chronic tolerance.

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