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Effect of NMDA Antagonists on Rapid and Chronic Tolerance to Ethanol: Importance of Intoxicated Practice

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KHANNA, J. M., G. S. MORATO, A. CHAU, G. SHAH AND H. KALANT. *Effect of NMDA antagonists on rapid and chronic tolerance to ethanol: Importance of intoxicated practice.* PHARMACOL BIOCHEM BEHAV **48**(3) 755–763, 1994.—Recent studies from our laboratory have shown that NMDA antagonists ((+)MK-801 and ketamine) inhibit the development of both rapid and chronic tolerance to the motor-impairing (moving belt test) and hypothermic effects of ethanol. The present experiments were designed to determine a) the generality of this inhibition, by using a different test of motor function, the tilt-plane test, and b) the possible importance of the experimental paradigm (i.e., with and without intoxicated practice), for the effect of the NMDA antagonist on ethanol tolerance. Daily administration of ethanol 3.3 g/kg for 5 days produced the same degree of tolerance on this test, whether it was given as a single dose of 3.3 g/kg before the daily training session or as divided doses of 2.3 g/kg before and 1 g/kg immediately after the session. The inhibitory effect of a single dose of (+)MK-801 (0.25 mg/kg IP) on rapid tolerance did not last longer than 1 day. Therefore, daily administration of the NMDA antagonists was necessary to block development of chronic tolerance. Daily injection of (+)MK-801 (0.25 mg/kg IP) failed to block chronic tolerance, but inclusion of a second dose of (+)MK-801 daily, and progressive increase of this second dose during the chronic treatment period did block chronic tolerance. Unlike (+)MK-801, ketamine does not have motor-impairing effects of its own, and does not potentiate those of ethanol; it was, therefore, used in the remaining experiments. Groups of rats received ethanol (3.3 g/kg) or saline, either before a daily practice session on the tilt-plane or after it. Tolerance to the ethanol effect developed on both regimens, but more rapidly in the animals receiving ethanol before the practice sessions. Ketamine inhibited the development of tolerance in the before group, but had no effect on the tolerance development in the after group. These results suggest that ketamine will block only practice-learned tolerance and not tolerance acquired purely by pharmacological exposure, and posttrial administration of ketamine was ineffective in blocking tolerance. These experiments provide further evidence consistent with the hypothesis that NMDA antagonists block tolerance development through inhibition of learning.

Tolerance NMDA antagonists (+)MK-801 Ketamine Chronic Rapid

FUNCTIONAL tolerance to ethanol and other sedative hypnotic drugs has been produced by a variety of different methods of chronic administration, including liquid diet (17,30), pellet implantation (14), and vapor inhalation (10,13). The functional tolerance to ethanol observed in many of these studies can be attributed exclusively to repeated exposure to ethanol, without concern for learning or other nonpharmacological variables. However, there is considerable evidence that functional tolerance to ethanol is related to both pharmacological and nonpharmacological variables (12,16), including behavioral factors (4,5,23,24). While the role of behavioral factors in tolerance is not disputed, it is not yet clear whether there is a single mechanism of tolerance, which is merely modulated by the behavioral and environmental influences, or whether there are separate mechanisms for tolerance produced by pharmacological means and for environmentally condi-

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tioned or practice-learned tolerance (12,16). Unfortunately, many of these studies fail to distinguish between learning and pharmacological factors in tolerance, and it is, therefore, difficult to determine which factor is the dominant one.

Preliminary studies from our laboratory have shown that the NMDA antagonists (+)MK-801 and ketamine inhibit the development of both rapid and chronic tolerance to ethanol (18–22,36). In one of these studies (36), (+)MK-801 appeared to inhibit the tolerance equally in a learning and a purportedly nonlearning paradigm. However, it was recognized that the two paradigms may not have been adequately differentiated. The objective of the present work was, therefore, a) to describe the effect of the NMDA antagonists (+)MK-801 and ketamine on chronic tolerance to ethanol on a different performance test and b) to compare the effect of NMDA antagonists on practice-learned chronic tolerance vs. tolerance produced by pharmacological exposure in a minimum learning paradigm. Because there is a considerable body of evidence that NMDA receptors are involved in learning (8,25–27, 32,35), and acquisition of ethanol tolerance bears many similarities to learning (12,16), our prediction was that NMDA antagonists would affect only practice-learned tolerance and not tolerance acquired by pharmacological exposure in a minimum learning paradigm. Ketamine was preferred to (+)MK-801 for this part of the study because ketamine does not alter the acute motor impairment response to ethanol, whereas (+)MK-801 acutely enhances it (21,36). Both ketamine and (+)MK-801 also interfere with the hypothermic response to ethanol (21). Therefore, the present study was limited to the motor-impairment response to ethanol. To test the generalizability of our earlier findings, we used a different motor performance test, the tilt-plane test, instead of the more complex moving belt test used in the previous work (36).

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. Tap water was available at all times. The temperature of the vivarium room was maintained at 21 ± 1°C and lights were on from 0700 to 1900 h throughout the experiment. Each day, the animals were brought from the vivarium to the laboratory for injections and testing, and were returned to the vivarium after the last procedures of the day (testing or blood sampling).

Tilt-Plane Test

The tilting-plane test was used as a measure of motor impairment (1,11). The apparatus consists of a Lucite 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 30, 60, and 90 min after the injection of ethanol or saline. 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 drug effect. This generally occurred at about 30 min after injection in all ethanol groups, independently of pretreatment used. Blood samples (50 μl) for ethanol measurement were taken on test days from the rat's tail tip immediately after the last measurement of motor impairment. Blood ethanol was analyzed enzymatically as described previously (11).

Experimental Procedure and Results

Experiment 1: Effect of single (3.3 g/kg) vs. divided dose (2.3 + 1.0 g/kg) on chronic ethanol tolerance development. Thirty-two rats were randomly divided into single and divided dose groups (16 per group). Each group was subdivided into equal control (S) and ethanol (E) groups. The single-dose E group received one dose of ethanol (3.3 g/kg IP) at zero time, whereas the divided-dose E group received 2.3 g/kg IP ethanol at zero time and 1.0 g/kg IP ethanol at 150 min. The saline groups received volumes of saline equal to those of the ethanol injections. On day 1, all rats were brought upstairs to the laboratory. Before zero time, and at 30, 60, and 90 min after ethanol or saline injections, the tilt-plane performance was measured. At 150 min, a second injection of ethanol or saline was given to the divided-dose groups only. The rats were then returned to their home cages. The day 1 procedure was repeated exactly on days 2, 3, and 4. On day 5, all rats received a challenge dose of ethanol (2.3 g/kg IP) followed by the same schedule of testing.

The results are shown in Fig. 1. A general linear model ANOVA (GLM-ANOVA) of maximum percent impairment values on day 5 showed no significant main effect of group, $F(1, 28) = 0.62$, $p > 0.4363$, and no significant group × treatment interaction, $F(1, 28) = 0.53$, $p > 0.4726$. However, there was a very significant main effect of treatment, $F(1, 28) = 19.24$, $p < 0.0001$. These results suggest that both E subgroups developed significant tolerance when compared to their respective S subgroups and the extent of this tolerance development was not different in single-dose and divided-dose groups.

Experiment 2: Effect of a single dose of (+)MK-801 on rapid tolerance to ethanol (Tilt-Plane Test). We have shown previously that rapid tolerance (two exposures to ethanol) is an excellent predictor of the features of chronic tolerance, and of the effects of various drug manipulations on it (19,21). Therefore, an initial experiment to test the effects of a single

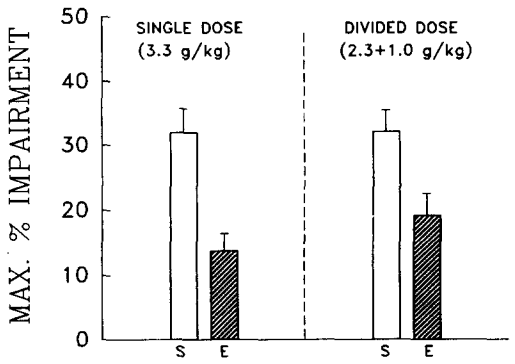

FIG. 1. Effect of daily administration of either single (3.3 g/kg) or divided (2.3 + 1.0 g/kg) dose of ethanol on tolerance development (Tilt-plane test). Tolerance to ethanol-induced motor impairment was assessed on day 5 with a challenge dose of ethanol given to all animals. S and E refer to saline and ethanol treatment groups. Results are presented as means ± SEM of eight animals per group.

dose of (+)MK-801 on rapid tolerance also served to examine the need for chronic dosage with the NMDA channel blocker in studying its effects on chronic tolerance.

Four groups of rats (n = 7 per group) were tested on the tilt-plane under the following treatments on day 1. At zero time, two groups received saline IP and two received 0.25 mg/kg of (+)MK-801. Thirty minutes later, one saline and one (+)MK-801 group were given ethanol (2.3 g/kg IP) and the other two groups received saline. Shortly before the first injections, and at 30, 60, and 90 min after the second injections, all groups were tested on the tilt-plane. At 120 min after the ethanol or second saline injections, all rats received additional ethanol (1.7 g/kg) or saline injections, respectively,. Thus, the total dose of ethanol in the S-E and MK-E groups was 4 g/kg. In previous work it had been found that a day 1 ethanol dose of 3-4 g/kg was required to produce rapid tolerance, but a single dose of this size was well beyond the linear part of the dose-response curve for the tilt-plane test. Therefore, the split-dosage schedule described here was necessary.

On day 2, all groups received an ethanol dose of 2.3 g/kg, were tested on the same schedule as for day 1, and were given a supplementary dose of ethanol (1.7 g/kg) at 120 min after the first injection. No (+)MK-801 or saline was used on day 2. On day 3, all groups were again tested after a single dose of ethanol (2.3 g/kg IP).

The results are shown in Fig. 2. On day 1, there was, as expected, a significant main effect of ethanol vs. saline, $F(1, 24) = 166.3, p < 0.001$. (+)MK-801 pretreatment produced a significant main effect, $F(1, 24) = 17.99, p < 0.0003$, indicative of some motor-impairing effect of the NMDA blocker, and there was a significant pretreatment × treatment interaction, $F(1, 24) = 6.80, p < 0.0154$, indicating potentiation of the ethanol effect by (+)MK-801. These findings are consistent with earlier observations of the acute effects of both substances (18,21,36).

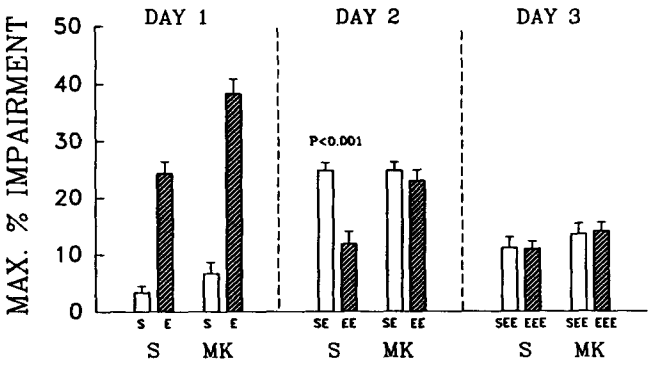

FIG. 2. Effect of a single dose of (+)MK-801 on the development of rapid tolerance to ethanol in the tilt-plane test. The lower of the two lines of letters below the horizontal axis indicates the pretreatments received on day 1 only: S = saline, MK = (+)MK-801. No pretreatments were given on days 2 and 3; the letters S and MK serve only to differentiate the groups on the basis of their day 1 pretreatments. The upper line of letters indicates the treatments on the different days. On day 1, S = saline and E = ethanol, 2.3 g/kg IP. On day 2, SE and EE indicate that all groups received ethanol despite their different treatments on day 1. On day 3, SEE and EEE indicate that all groups again received ethanol. All ethanol-treated animals on days 1 and 2 received a supplementary dose of ethanol (1.7 g/kg) after completion of the tests. Rapid tolerance to ethanol was assessed on days 2 and 3. See text for further details. Results are presented as means ± SEM of seven animals per group.

On day 2, the group (S-EE) that had received saline and ethanol on day 1 now showed significant tolerance to ethanol, when compared to its own day 1 score, $t(12) = 4.10, p < 0.01$, and to the day 2 ethanol effect in the group that had received only saline (S-S) on day 1, $t(12) = 4.964, p < 0.001$. In contrast, the day 1 MK-E group did not show tolerance to ethanol on day 2, compared to the MK-S group, $t(12) = 0.747, p > 0.50$. An ANOVA for the day 2 results of all four groups showed a significant main effect of ethanol, $F(1, 24) = 16.66, p < 0.0004$, and a significant interaction of ethanol × (+)MK-801, $F(1, 24) = 9.24, p < 0.0056$, indicating that the MK-E group had not developed rapid tolerance. Moreover, its response to ethanol on day 2 was identical to that of the S-E group on day 1, $t(12) = 0.199, p > 0.80$, indicating that the previous day's exposure to (+)MK-801 had no lasting effect on the acute response to ethanol. On day 3, all groups showed tolerance relative to their day 1 scores, and relative to the day 2 scores for the S-E and the two MK groups, $F(1, 36) = 63.52, p < 0.0001$.

These results indicate that the ability of (+)MK-801 to inhibit tolerance development was limited to the day on which the (+)MK-801 was administered. When the same groups were tested the next day under ethanol in the absence of (+)MK-801, the alcohol experience was able to produce rapid tolerance that could be demonstrated on day 3. These findings are consistent with the known short half life of (+)MK-801 [87 ± 8 min; see (29)] and indicate that a single dose did not produce any long-lasting interference with the ability to develop tolerance. Similarly, ketamine has a half life in both plasma and brain of about 20 min (34). Therefore, it was concluded that daily administration of (+)MK-801 or ketamine would be necessary to examine the effects of NMDA channel blockade on the development of chronic tolerance to ethanol in the following experiments.

Experiment 3: Effect of chronic (+)MK-801 on ethanol tolerance (Tilt-Plane Test). Rats were randomly divided into four groups. On day 1, the treatments and procedures were identical to those used in Experiment 1. The day 1 procedure was repeated exactly the same way for days 2, 3, and 4. On day 5, an identical test procedure was followed except that all animals received an ethanol challenge dose (2.3 g/kg IP) and no (+)MK-801 or saline pretreatment was given.

The results of this experiment are shown in Fig. 3. Baseline scores (preinjection) in all groups averaged about 40.0 ± 2.0° over the course of the experiment. ANOVA showed a significant effect of days, $F(4, 110) = 4.91, p < 0.0011$, but no significant differences among groups, $F(3, 110) = 1.73, p > 0.1647$, and no significant groups × days interaction, $F(12, 110) = 0.59, p > 0.8491$. On day 1, (+)MK-801 enhanced the motor-impairment response to ethanol, and the response of this group (MK-E) did not change on days 2, 3, and 4. The saline-ethanol group (S-E) showed the expected motor-impairment response on day 1, and this was significantly reduced by day 2. This response did not change over days 3 and 4. There was no significant change in the response of either (S-S) or (MK-S) groups over days 1 to 4. Comparison of maximum percent impairment by a challenge dose of ethanol on day 5 showed similar reductions in maximum percent impairment in MK-E and S-E rats. There was also no difference between S-S and MK-S groups. A two-way general linear model (GLM) ANOVA for maximum percent impairment values for the day 5 results showed that chronic pretreatment with (+)MK-801 did not block chronic tolerance development to ethanol-induced motor impairment because there was no significant effect of (+)MK-801 pretreatment, $F(1, 22) =$

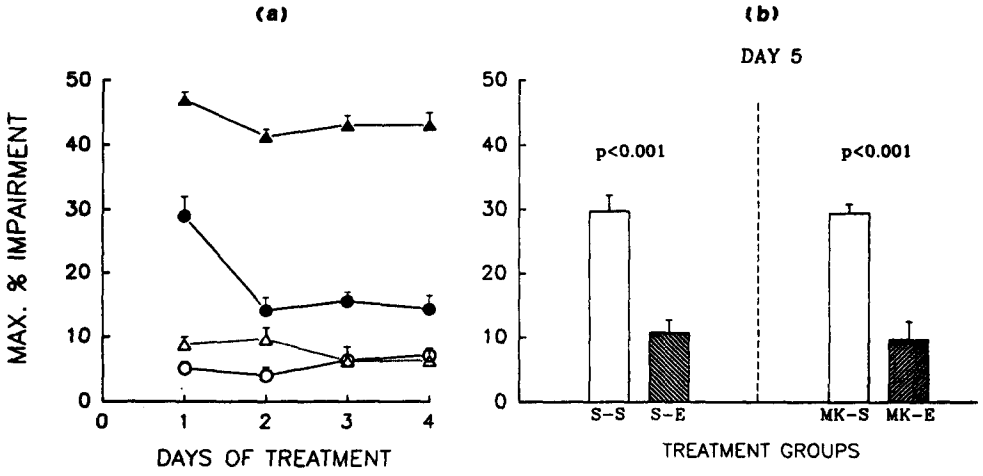

FIG. 3. Effect of chronic (+)MK-801 treatment on ethanol tolerance (tilt-plane test). Two groups received (+)MK-801 [with ethanol (MK-E; ▲) or saline (MK-S; △)] and another two groups received saline [with ethanol (S-E; ●) or saline (S-S; ○)] on day 1 to day 4 (see the Method section for details). Tolerance to ethanol-induced motor impairment was assessed on day 5 with a challenge dose of ethanol given to all animals. (a) Effect of daily (+)MK-801 and ethanol treatment up to day 4 and (b) effect of chronic (+)MK-801 treatment on ethanol-induced motor impairment on day 5. Results are presented as mean ± SEM of six to seven animals.

0.10, $p > 0.757$, nor a significant pretreatment × treatment interaction: $F(1, 22) = 0.03$, $p > 0.863$. Treatment effect (ethanol vs. saline), however, was significant, $F(1, 22) = 71.99$, $p < 0.0001$.

Experiment 4: Effect of increasing doses of (+)MK-801 on ethanol tolerance (Tilt-Plane Test). The lack of effect of (+)MK-801, despite previous demonstrations that it blocked chronic tolerance to ethanol on the moving belt test (36), raised the possibility that the dosage in Experiment 3 was insufficient. Another experiment was, therefore, performed, using a second dose of (+)MK-801 to extend the duration of its action. In addition, to allow for the possibility that tolerance might develop to (+)MK-801 itself (2), the size of the second dose was progressively increased during the chronic treatment period.

Rats were randomly divided into four groups ($n = 7$). On day 1, at zero time two groups received IP saline and the other two groups were injected with (+)MK-801 (0.25 mg/kg). After 30 min, one of the saline and one of the (+)MK-801 groups received 2.3 g/kg IP ethanol and the remaining two groups received saline. Before the zero time injections and at 30, 60, and 90 min after the ethanol or second saline injections the tilt-plane performance was measured. At 120 min after initial ethanol or saline injections all rats received additional (+)MK-801 (0.1 mg/kg) or saline injections and at 150 min their respective ethanol (1 g/kg) or saline injections. Rats were then returned to their home cages. The day 1 procedure was repeated exactly the same way on days 2 and 3 except that the second dose of (+)MK-801 was increased to 0.2 and 0.25 mg/kg on the second and third day, respectively. On day 4, all animals received a challenge dose of ethanol (2.3 g/kg IP) followed by the same schedule of testing, but no (+)MK-801 or saline pretreatment was given on the test day. The total daily dose of (+)MK-801 (0.50 mg/kg) on this day was given at 150 min and ethanol posttreatment dose of 1 g/kg was given at 180 min. The animals were then returned to their home cages. On days 5 to 8, animals were given in their home cages a single dose of (+)MK-801 (0.50 mg/kg) followed 30 min later by a single dose of ethanol (3.3 g/kg). On day 9, all rats received only a challenge dose of ethanol (2.3 g/kg) followed by the same schedule of testing, to test for ethanol tolerance again. Blood ethanol samples were taken at the end of motor-impairment tests on days 4 and 9.

The results of this experiment are shown in Fig. 4. The S-E group showed the expected motor-impairment response on day 1, but this was reduced on day 2 and the reduction was significant by day 3. There was no significant change in the responses to saline in either the S-S or the MK-S groups over days 1 to 3. (+)MK-801 significantly enhanced motor-impairment response to ethanol (MK-E group) on day 1, and this response did not change on days 2 and 3. On days 4 and 9, when all these groups were challenged with ethanol, there was a significant development of ethanol tolerance in the control groups but not in the MK-E group.

A two-way ANOVA of the results on day 4 showed significant main effects of pretreatment, $F(1, 24) = 16.97$, $p < 0.0004$, and of ethanol treatment, $F(1, 24) = 19.99$, $p < 0.0002$, as well as a significant pretreatment × treatment interaction, $F(1, 24) = 12.42$, $p < 0.0017$. Day 9 ANOVA results did not show a significant (+)MK-801 pretreatment effect, $F(1, 22) = 2.45$, $p > 0.1321$, but the effect of ethanol treatment was significant, $F(1, 22) = 17.35$, $p < 0.0004$. (+)MK-801 pretreatment still blocked the development of chronic tolerance to ethanol, because there was a significant pretreatment × treatment interaction, $F(1, 22) = 11.41$, $p < 0.0027$.

Experiment 5: Effect of chronic ketamine on tolerance to ethanol in intoxicated and nonintoxicated practice groups. Experiment 4 showed that the higher dosage of (+)MK-801 did, indeed, prevent chronic tolerance to ethanol on the tilt-plane test. The purpose of Experiment 5 was to see whether the role of the NMDA receptor was primarily in a learning component of the tolerance process. However, as noted in the Introduction, (+)MK-801 itself has a motor-impairing effect

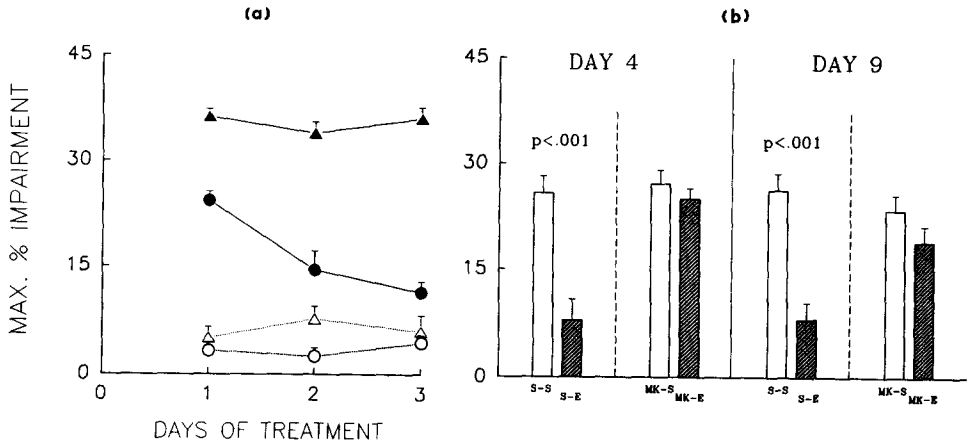

FIG. 4. Effect of increasing doses of (+)MK-801 on ethanol tolerance (tilt-plane test). Two groups received (+)MK-801 [with ethanol (MK-E; ▲) or saline (MK-S; △)] and another two groups received saline [with ethanol (S-E; ●) or saline (S-S; ○)] on day 1 to day 3 (see Experimental Procedure section for details). Tolerance to ethanol-induced motor impairment was assessed on day 4 and day 9 with a challenge dose of ethanol given to all animals. (a) Effect of daily increasing doses of (+)MK-801 (0.35 mg/kg, 0.45 mg/kg, 0.50 mg/kg on days 1, 2, and 3, respectively) and ethanol treatment and (b) effect of chronic (+)MK-801 treatment on ethanol-induced motor impairment on day 4 and day 9. Results are presented as means ± SEM of six to seven animals.

that is synergistic with that of ethanol, whereas ketamine (also an NMDA channel blocker) does not. Therefore, to simplify the interpretation, ketamine was used in this experiment in place of (+)MK-801.

Forty-eight rats were randomly divided into six groups (n = 8 each). These groups were assigned as follows: two control groups, two intoxicated practice groups (P), and two nonintoxicated practice groups (NP). At zero time on day 1, one of the control groups and one of the P groups were injected with ketamine (1.5 mg/kg IP) and all other groups received saline IP After 30 min, P groups received ethanol (2.3 g/kg IP) and all other groups were given IP saline. Before the zero time injections and at 30, 60, and 90 min after ethanol or saline injections, the tilt-plane performance was measured. At 120 min a second injection of ketamine (1.5 g/kg) was given to ketamine-receiving groups and one of the NP groups received the combined ketamine dose (3.0 mg/kg IP), whereas all other groups received IP saline. At 150 min, a second injection of ethanol (1.0 g/kg) was given to P groups, while the NP groups were given the combined ethanol dose (3.3 g/kg). Control groups again received IP saline. Rats were then returned to their home cages.

The day 1 procedure was repeated on days 2, 3, and 4, except that both the first and second ketamine doses were increased by 0.5 mg each time (i.e., total ketamine dose was 4, 5, and 6 mg/kg on days 2, 3, and 4, respectively). 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. On days 6–11, animals were treated in exactly the same way as for days 1–4 without further increase in ketamine (3 + 3 mg/kg) or ethanol (2.3 + 1 g/kg) doses. On days 5 and 12 all rats were challenged with ethanol alone (2.3 g/kg IP).

The results of this experiment are shown in Fig. 5. The two P groups showed their expected motor-impairment response on day 1, and ketamine pretreatment had no effect on this response. The P group receiving saline and ethanol (SP) had progressively lower motor-impairment scores over days 2, 3, and 4, approaching a theoretical minimum plateau of about 10%. A GLM ANOVA showed significant main effects of group [KP vs. SP; $F(1, 140) = 36.81, p < 0.0001$], and days, $F(10, 140) = 9.24, p < 0.0001$. There was only a marginally significant group × days interaction, $F(10, 140) = 1.81$, $p > 0.065$. This was due mainly to the effect of the latter plateau in the SP group, because a separate ANOVA of days 1–4 showed a highly significant interaction. The other P group, receiving ketamine and ethanol (KP), also showed a gradual decrease over days 1–11; though the slope of the linear regression for this group was low (−1.005), it was highly significant ($p < 0.0001$).

A GLM-ANOVA for maximum percent impairment data on day 5 showed a significant group (control, P, or NP) effect, $F(2, 42) = 9.12, p < 0.0005$, but a nonsignificant pretreatment (ketamine, saline) effect, $F(1, 42) = 3.27, p > 0.0777$. There was a significant group × pretreatment interaction, $F(2, 42) = 4.25, p < 0.0209$, suggesting that P and NP groups responded differently to different pretreatments. Further breakdown comparison of only saline-pretreated groups showed significant difference among groups, $F(2, 21) = 24.83, p < 0.0001$. A post hoc Newman-Keul's range test showed that the saline control group had significantly greater maximum percent impairment than the P group ($p < 0.05$), but it was not different from the NP group. A GLM-ANOVA for ketamine-pretreated groups showed there was no difference among control, P, or NP groups, $F(2, 21) = 0.31, p > 0.7351$; this fact suggests that ketamine blocked tolerance in the P group.

A GLM-ANOVA of maximum percent impairment values for day 12 showed a significant group effect, $F(2, 42) = 11.96, p < 0.0001$, that was due to the production of tolerance in both P and NP groups relative to the controls. The effect of pretreatment (ketamine vs. saline) was significant, $F(1, 42) = 6.59, p < 0.0139$, and a significant group × pretreatment interaction was seen, $F(2, 42) = 6.12, p < 0.0047$. To analyze the group difference further, separate

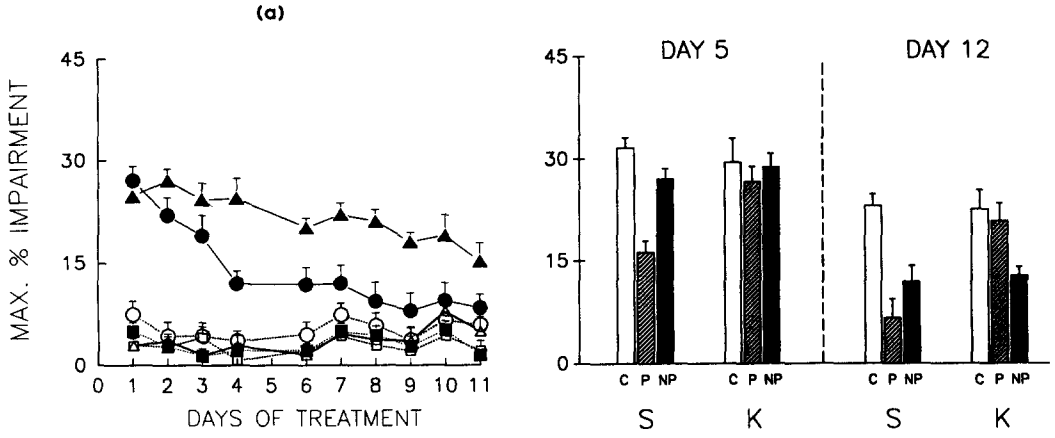

FIG. 5. Effect of chronic ketamine treatment on tolerance to ethanol in intoxicated and nonintoxicated practice groups. On days 1–4 and days 5–11, two control groups received saline (S/C; ○) or ketamine (K/C; △) followed by saline. Two intoxicated practice groups received saline (S/P; ●) or ketamine (K/P; △) followed by ethanol. Two nonintoxicated practice groups received saline (S/NP; □) or ketamine (K/NP; ■) followed by ethanol (combined ketamine and ethanol doses given after practice). Total ketamine doses given were 3, 4, 5, and 6 mg/kg on days 1, 2, 3, and 4, respectively. This dose was maintained for the remainder of the experiment. Tolerance to ethanol-induced motor impairment was assessed on day 5 and day 12 (see Experimental Procedure section for details). (a) Effect of daily treatment with ketamine and ethanol from day 1 to day 11 and (b) effect of chronic ketamine treatment on ethanol-induced motor impairment on day 5 and day 12 for control, intoxicated practice, and nonintoxicated practice groups. Results are presented as means ± SEM of eight animals.

analyses were carried out for each pretreatment. Comparison of only saline-pretreated groups showed significant differences among groups, $F(2, 21) = 13.13$, $p < 0.0002$. A post hoc Newman-Keul's range test showed that the saline control group had significantly greater maximum percent impairment than both P and NP groups receiving ethanol ($p < 0.05$ in each case). Similarly, GLM-ANOVA for ketamine groups showed significant group effect, $F(2, 21) = 5.04$, $p < 0.0163$. The post hoc Newman-Keul's range test showed that ketamine control group had significantly greater maximum percent impairment than the NP group ($p < 0.05$) but was not different from the P group. These analyses indicate that ketamine pretreatment blocked chronic tolerance in the P group but not in the NP group.

Blood ethanol levels in Experiment 5 taken at the end of motor-impairment measurement on days 5 and 12 are shown in Fig. 6. There was no significant difference on either day in ethanol levels among the various groups. Similarly, there was no difference in blood ethanol levels among various groups in Experiments 2 and 3 (data not shown). It is, therefore, unlikely that changes in ethanol pharmacokinetics played a significant role in the findings described.

Experiment 6: Effect of chronic ketamine administration, either before or after behavioral testing with ethanol, on tolerance to ethanol. In all the studies described above, (+)MK-801 or ketamine was administered before ethanol. The purpose of this experiment was to examine whether administration of ketamine after behavioral testing with ethanol can alter the tolerance to ethanol. On day 1, 32 rats were divided into two groups, Before and After ($n = 16$ each). Each group was further subdivided into saline (S) and ketamine (K) groups ($n = 8$ each). On day 1 at zero time, Before groups received S or K (3 mg/kg). Thirty minutes later all four subgroups received ethanol (3.3 g/kg). Before any injections, and 30, 60, and 90 min after ethanol, all groups were tested on the tilt-plane. At 150 min after first injections, the After groups were injected with S or K (3 mg/kg). The rats were returned to their home cages. On days 2, 3, and 4, the day 1 procedure was repeated the same way except that the dose of ketamine was increased by 1 mg daily to 4, 5, and 6 mg/kg, respectively. On day 5, all rats were subjected to ethanol tolerance test with a dose of 2.3 g/kg IP and their tilt-plane performance was measured. There was no S or K injection given to any group on the test day.

On days 1, 2, 3, and 4 all groups showed their expected motor-impairment responses due to ethanol and there was no significant difference among the various groups (data not shown). A GLM-ANOVA for maximum percent impairment

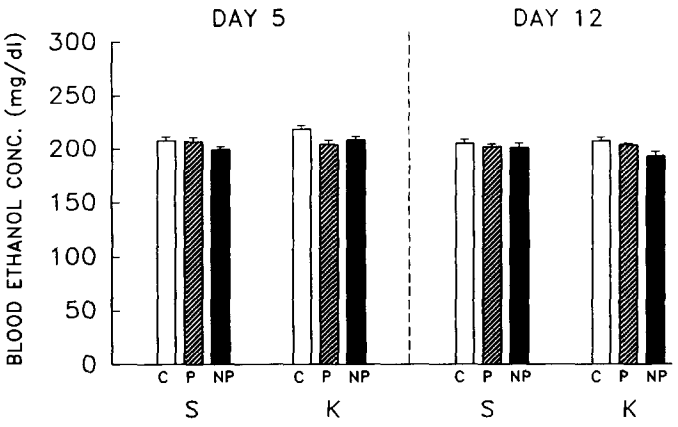

FIG. 6. Blood ethanol levels on day 5 and day 12 in various groups in Experiment 3. S and K refer to saline and ketamine treatment groups and C, P, and NP refer to control, intoxicated practice, and nonintoxicated practice groups. Results are shown as means ± SEM of eight animals per group.

on day 5 (Fig. 7) showed no significant main effects of group (before vs. after), $F(1, 28) = 1.66$, $p > 0.2077$, or chronic treatment (ketamine vs. saline), $F(1, 28) = 3.13$, $p > 0.0878$, and no significant group × treatment interaction, $F(1, 28) = 2.27$, $p > 0.1428$. The absence of significant main effects reflected the fact that daily ethanol treatment produced tolerance to ethanol in all groups except the group receiving ketamine before ethanol. Separate analyses were, therefore, carried out for ketamine and saline (before vs. after) groups. Comparison of only the ketamine-treated groups showed a significant difference, $F(1, 14) = 4.63$, $p < 0.0493$, suggesting blockade of ethanol tolerance development when ketamine was administered before, but not after, ethanol. Comparison of only the saline control groups showed that both groups had developed tolerance to ethanol and they were not significantly different, $F(1, 14) = 0.02$, $p > 0.8880$.

DISCUSSION

In agreement with recent work (21), (+)MK-801 but not ketamine administration produced some motor incoordination on its own and also significantly enhanced the effects of ethanol. The reason for the dissimilarity between (+)MK-801 and ketamine is not clear, because both are blockers of the cation channel linked to the NMDA receptor and bind to the same site in the channel (9). It may be related to the relative doses of the NMDA antagonists used or to some differential effects on other neurochemical systems. Recent studies have shown that a variety of NMDA antagonists enhanced in a dose-dependent manner the duration of ethanol and pentobarbital anesthesia and reduced the MAC of volatile anesthetics (6,7). Large doses of ketamine are known to produce anesthesia and subanesthetic doses do produce behavioral effects (15,28). Therefore, it is possible that larger doses of ketamine than those used here might have also enhanced the motor incoordination effect of ethanol.

Recently, we reported that ketamine retarded chronic tolerance to ethanol, when ketamine doses were increased daily (19). Similar results were found in this study, when the (+)MK-801 doses were increased. However, chronic tolerance to ethanol was not blocked if the (+)MK-801 dose was not increased (Experiment 3). The fact that increasing doses of the NMDA antagonists are required to effectively block chronic tolerance would suggest either that tolerance develops to the effect of the antagonists themselves, or that higher doses of the antagonists are required to inhibit tolerance than those used in Experiment 3. It is not possible to comment on the development of tolerance to (+)MK-801 in Experiment 4 because doses of (+)MK-801 were increased daily. However, in Experiment 3, in which a fixed dose of (+)MK-801 was used daily, no evidence of tolerance to (+)MK-801 was seen. Lack of tolerance to (+)MK-801 was also reported by Wu et al. (36) using the moving-belt test. The inability to see tolerance in both of these studies may be due to the ceiling effect, and systematic dose–response studies are required to address this issue. In a recent study, Beart and Lodge (2) reported that chronic treatment with (+)MK-801 (0.5 mg/kg twice daily for 7 days) resulted in reduced responses to NMDA and reduced density of NMDA receptors compared with control animals.

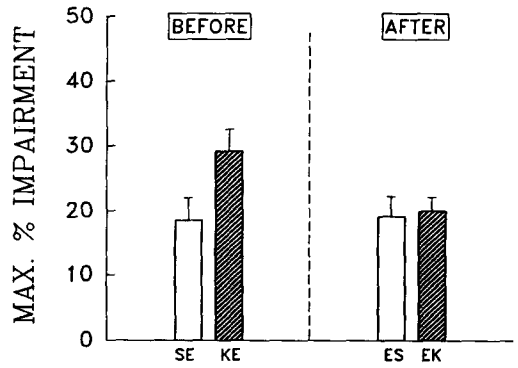

FIG. 7. Effect of chronic ketamine administration, either before or after behavioral testing with ethanol, on tolerance to ethanol (Tilt-plane test). Tolerance to ethanol-induced motor impairment was assessed on day 5 with a challenge dose of ethanol given to all animals. SE and KE refers to groups that received saline and ketamine before behavioral testing with ethanol, whereas ES and EK refers to groups that received saline and ketamine after behavioral testing with ethanol. Results are presented as means ± SEM of eight animals per group.

The important finding of these experiments is that the inhibitory effect of ketamine on tolerance was evident if the antagonist was administered prior to behavioral testing under ethanol, and not if it was administered before ethanol without behavioral testing. Similarly, administration of ketamine after behavioral testing with ethanol did not block the development of tolerance. These results, taken together, suggest that NMDA antagonists block the acquisition of tolerance by preventing the retention of learning that occurs during practice under ethanol, but have no effect if consolidation of the learning has already taken place before the NMDA antagonist is given. These results are in full agreement with several studies in the literature, in which the NMDA antagonists have been shown to impair various forms of learning only if the NMDA antagonist is administered prior to training (8,25–27,32,35).

The present findings could also be interpreted as evidence for state-dependent learning, in that a (+)MK-801 or ketamine cue that was present during chronic ethanol treatment was not present on ethanol-only test days. Thus, the absence of ethanol tolerance on the test days might be attributable to absence of a state-linked cue that had been linked to a state-dependent learned tolerance. Arguing against this interpretation, however, is the fact that during the first four treatment days in Experiment 5 (Fig. 5a), when the respective state cues were constant, the ketamine-ethanol group did not develop tolerance, whereas the saline-ethanol group did. Only after experiencing ethanol alone, on day 5, did the ketamine-ethanol group show some slight degree of tolerance during the subsequent treatment days. The same lack of tolerance development during the treatment period, when (+)MK-801 state cues were constant, was also noted by Wu et al. (36). A similar argument against state-dependent learning applies to the effect of ketamine on rapid tolerance to ethanol (20). In studies by others, NPC 12626 (a competitive antagonist of NMDA receptors) prevented the induction of LTP and disrupted spontaneous alternation and inhibitory avoidance performance when administered before but not after training (33). Moreover, NPC was effective when administered before training as well as before testing.

These results may appear at odds with those of a recent study from this laboratory (36) in which it was found that (+)MK-801 impaired the development of tolerance to ethanol in both the practice and nonpractice groups. However, there are three main differences between the two studies. First, Wu et al. (36) employed (+)MK-801, whereas ketamine was used in the practice and nonpractice portion of the present study.

Ketamine in the doses used here does not produce motor impairment, whereas (+)MK-801 produces motor incoordination of its own as well as potentiating the effects of ethanol on motor incoordination. Second, the earlier study employed a highly learned task, the moving-belt test, whereas the present one used the tilt-plane test for which animals require no prior training. Either the difference in the tests used for measuring tolerance, or greater intoxication in the nonpractice group due to the combined effects of ethanol and (+)MK-801, may have resulted in a different outcome despite the absence of opportunity for learning in the test itself. Third, the dose of (+)MK-801 used by Wu et al. (36) might have been relatively greater than the dose of ketamine used in this study. If so, it may have been enough to affect both types of tolerance while the lower dose of ketamine was able to differentiate between them. Studies with different NMDA antagonists, different doses, and different test systems are required to clarify this issue. In any event, the results of the present study are consistent with various findings pertaining to (+)MK-801 and other NMDA antagonists on learning.

Animals were tested for tolerance and the inhibitory effect of ketamine on day 5 and day 12. The practice group developed tolerance on day 5, whereas no tolerance was seen in the nonpractice group at this time. Tolerance did not increase further in the practice group but did in the nonpractice group, so that the two groups were equally tolerant on day 12. It could be argued that the tolerance seen in the nonpractice group on day 12 is the result of test practice under the influence of ethanol (without ketamine) on day 5. Although this possibility cannot be ruled out, previous studies in this laboratory with the moving-belt test have shown that the administration of test doses of similar magnitude every 4th day (or less frequently) did not result in tolerance (23,24). Therefore, it seems unlikely that the tolerance seen in the nonpractice group on day 12 is the result of practice session on test day 5. If the tolerance on day 12 in the nonpractice group had been due to a nonketamine session on day 5, then there should also have been tolerance on day 12 in the practice group receiving chronic ketamine because they also had a ketamine-free session on day 5. Therefore, it seems likely that tolerance in the practice and nonpractice groups resulted from different processes, and only the former was affected by ketamine.

The results with ethanol in the present study differ from those obtained with morphine by Ben-Eliyahu et al. (3), who used a model of morphine tolerance (i.e., sustained release preparation containing (+)MK-801 and morphine) in which the involvement of learning is minimized. These authors reported a block of morphine tolerance by (+)MK-801 for up to 12 days. Their findings suggest that (+)MK-801 can prevent the development of nonassociative morphine tolerance. In other studies, LY 274614 (a competitive NMDA receptor antagonist) was reported to reverse the development of morphine tolerance without reducing the analgesic response (31). The authors postulated that activation of an NMDA receptor may be required for both the initiation as well as the maintenance of morphine tolerance.

(+)MK-801 or ketamine administration did not significantly affect blood ethanol levels in any of the experiments conducted. It is, therefore, unlikely that changes in the pharmacokinetics of ethanol are playing a significant role in the findings described here. In conclusion, the present results suggest that NMDA antagonists block chronic tolerance by preventing some adaptation that occurs during intoxicated practice. Additional studies are required to confirm this interpretation.

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