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Effect Of NMDA Antagonists On Rapid Tolerance To Ethanol Under Two Different Testing Paradigms

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KHANNA J. M., G. SHAH AND A. CHAU. *Effect of NMDA antagonists on rapid tolerance to ethanol under two different testing paradigms.* PHARMACOL BIOCHEM BEHAV **57**(4) 693–697, 1997.—We have recently reported that pretreatment with NMDA receptor antagonists [(+)MK-801 and ketamine] inhibited the development of rapid tolerance to ethanol hypothermia and motor-impairment on day 2 in animals receiving ethanol on day 1, compared to the control group pretreated with saline. In these studies rats were tested at 30, 60, 90 and 120 min after ethanol on both day 1 and 2. In the present report we compared the development of rapid tolerance under 2 different conditions: (1) in groups of rats that were tested on the tilt-plane at all test times (Testing or Intoxicated Practice group), (2) in groups of rats that were not tested on the tilt-plane but were handled at all test times on day 1 (dummy testing). Rats were pretreated with ethanol or saline on day 1 and tested with ethanol on day 2 in all the above studies. Both testing (intoxicated practice) and dummy testing of animals on day 1 after pretreatment with ethanol produced rapid tolerance to ethanol on day 2. However, (+)MK-801 or ketamine pretreatment, which blocked rapid tolerance in the intoxicated practice testing paradigm, failed to block rapid tolerance in the dummy testing paradigm. Similar results were obtained for rapid tolerance and for the effect of ketamine in the hypothermia experiment. These findings suggest that NMDA antagonists block rapid tolerance in the intoxicated testing paradigm but not in the dummy testing paradigm. However, whether the two types of rapid tolerance tested in the present experiments are indeed different or interrelated remains to be further investigated. © 1997 Elsevier Science Inc.

Tolerance Rapid NMDA antagonists Testing Dummy testing

REPEATED administration of ethanol and other drugs has been shown to produce decreasing effects at the same dosage, i.e. tolerance. This term is usually synonymous with chronic tolerance if no other temporal parameters have been specified. The temporal characterization of the development and loss of chronic tolerance has been extensively characterized (7,8). However, an even single experience of ethanol-induced hypothermia has been shown to reduce significantly the hypothermic effects of ethanol in the mouse (4). Similarly, in the rat a single experience of ethanol-induced impairment in the tilt-plane or moving belt test resulted in a decrease of the motor-impairment in a subsequent test under ethanol (2,9). One of the most interesting aspects of this type of tolerance, usually referred to as rapid tolerance, is that the magnitude of the observed tolerance is often not significantly different from that observed following chronic ethanol treatment.

Intoxicated practice has been shown to enhance the development of chronic tolerance (3,11,12). Britrán and Kalant (2) did show that different amounts of intoxicated practice on day 1 produced different degrees of rapid tolerance on day 2, but the paradigm did not distinguish between effects of actual practice and possible effects of associated stimuli i.e. between operant and associative learning. NMDA blockers were previously shown to prevent both rapid and chronic tolerance, (10,13) when intoxicated practice was given, but not without intoxicated practice. These experiments again failed to distinguish between operant and associative components of the practice situation. The present experiments were therefore designed to answer both these questions. Both hypothermia and motor-impairment (tilt-plane test) were used to examine the effect of the NMDA antagonist (i.e. ketamine) on tolerance development under different treatment paradigms.

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Since (+)MK-801 produces hyperthermic effects of its own and can interfere with the hypothermic response to ethanol, the present study with (+)MK-801 was restricted to the motor-impairment response (tilt-plane test) to ethanol.

MATERIALS AND METHODS

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 which 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 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 tilt-plane test was used as a measure of motor-impairment (1,5). The apparatus consists of a Lucite plane which is hinged at one end, around which it can be inclined at a fixed angular velocity (approximately 4s) 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. The degree of post-drug ataxia was assessed as the percentage change in sliding angle, compared to the same animal's pre-drug value. Maximum impairment, regardless of the time of its occurrence, was employed as the measure of ethanol effect. This generally occurred at 30 min after injection in all 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 (6).

Hypothermia Test

A thermistor probe was inserted 5 cm into the rectum and left until a stable temperature recording was obtained (approximately 30 s) on a Yellow Springs Instrument electrical thermometer. This was done prior to and at successive 30-min intervals after the intraperitoneal test injection until the temperature began to return to normal. This generally occurred about 120 min after injection of ethanol.

Statistical Methods

Results in the various experiments were analysed by one-, two- or three-way ANOVA as required, using the GLM-ANOVA program in the NCSS statistical package for PCs. NCSS is a trademark of Dr. Jerry L. Hintze; 329 North 1000 East, Kaysville, Utah 84037.

EXPERIMENTAL PROCEDURE

Experiment 1: Effect of Ketamine on Rapid Tolerance Development to Ethanol in Testing vs. Dummy Testing Paradigms

Tilt-plane test. On day 1, 64 rats were randomly divided into eight groups. Four groups were assigned for normal testing procedure and the remaining four groups for the dummy-testing procedure. Two groups from each testing category received ketamine (1 mg/kg IP) whereas the remaining groups were given saline. Thirty min later, one of the ketamine and one of the saline groups in each procedure were injected with ethanol (2.3 g/kg IP) and the other groups received saline. For Testing groups, motor-impairment was assessed (tilt-plane test) prior to the injection and at 30, 60 and 90 min after the respective ethanol or saline injection. For Dummy Testing, rats were only placed on the tilt-plane prior to injection and at 30, 60 and 90 min after injection, without tilting the plane. At 120 min all ethanol rats were given an extra dose of ethanol (1.7 g/kg) and the saline rats were given saline injections. Then they were returned to their home cages. On day 2, a challenge dose of ethanol (2.3 g/kg) was given to all rats to assess rapid tolerance; no pretreatment or post-test injections were given.

Hypothermia Test. On day 1, 58 rats were randomly divided into eight groups. Four groups were assigned to the normal testing procedure ($n = 6$ or 7 each group) and the remaining four groups to the dummy testing procedure ($n = 8$ each group). Two groups from each testing category were injected with ketamine (1 mg/kg, IP) and the other two groups were given saline. Thirty min. later, one ketamine and one saline group were injected with ethanol (2.0 g/kg, IP) whereas the remaining groups received saline. For normal testing groups the hypothermic response was measured prior to injections and after successive 30 min. intervals up to 120 min. after ethanol or saline injections. All rats in the dummy-testing groups were only picked up from their cages for handling at each of the test times, but the temperature probe was not inserted and no measurement were taken except for the initial measurement before the injection. At 120 min. all ethanol rats were given a second dose of ethanol (2.0 g/kg, IP) and the other rats received saline, after which they were returned to their home cages. On day 2, all rats were challenged with ethanol (2.0 g/kg) to assess rapid tolerance; no pretreatment or post-test injections were given.

Experiment 2: Effect of (+)MK-801 on Development of Rapid Tolerance to Ethanol in Testing vs. Dummy Testing. (Tilt-plane test)

The experimental procedure for this study was identical to that described in experiment 1 above with ketamine, except that on day 1 rats in the corresponding groups were injected with (+)MK-801 (0.25 mg/kg) instead of ketamine. On day 2, a challenge dose of ethanol (2.3 g/kg) was given to all rats and no pretreatment or posttest injections were given.

RESULTS

Experiment 1: Effect of Ketamine on Rapid Tolerance to Ethanol in Testing (T) vs. Dummy Testing (DT) Methods

Tilt-plane test. The results of this experiment are shown in Fig. 1. On day 1, rats injected with ethanol showed the expected motor-impairment response and administration of ketamine did not significantly affect the magnitude of this response. A GLM-ANOVA of maximum percent impairment values showed that the main effect of group (T vs. DT) was not significant ($F(1, 55) = 0.42$, $p > 0.5208$) but there was a significant main effect of ketamine (S vs. K) pretreatment ($F(1, 55) = 5.50$, $p < 0.0226$) and of ethanol (SE vs. EE) treatment ($F(1, 55) = 22.56$, $p < 0.0001$). Thus rats pretreated with saline (S) on day 1 and injected with ethanol on both days (EE) showed a significantly lower motor-impairment re-

sponse to ethanol on day 2 in both the T group, and the DT group than the rats that received saline (SE) on day 1 did. The pretreatment (S vs. K) × treatment (SE vs. EE) interaction was also significant ($F(1, 55) = 4.02$, $p < 0.0499$). These results suggested that ketamine (K) blocked rapid tolerance to the motor-impairing effect of ethanol only in the T group and not in the DT group.

Hypothermia Test. The results of this experiment are shown in Fig. 2. On day 1, rats injected with ethanol showed their expected hypothermic response and administration of ketamine did not significantly affect the magnitude of this response. A GLM-ANOVA of ΔT_{max}°C values on day 2 showed a significant main effect of (T vs. DT) group ($F(1, 50) = 11.61$, $p < 0.0013$) but a non-significant main effect of (K vs. S) pretreatment ($F(1, 50) = 0.38$, $p > 0.5421$). The main effect of treatment (SE vs. EE was significant ($F(1, 50) = 28.07$, $p < 0.0001$), and there was a significant group (T vs. DT) × treatment (SE vs. EE) interaction ($F(1, 50) = 4.56$, $p < 0.0377$). There was a significant triple interaction for group (T vs. DT) × pretreatment (S vs. K) × treatment (SE vs. EE) ($F(1, 50) = 4.56$, $p < 0.0377$), which required a further breakdown analysis for only the T and DT groups.

The two-way ANOVA for the T group showed no significant main effect of pretreatment (S vs. K) ($F(1, 22) = 0.43$, $p > 0.517$) but the main effect of treatment (SE vs. EE) was significant ($F(1, 22) = 7.65$, $p < 0.0113$) and pretreatment (S vs. K) × treatment (SE vs. EE) interaction was significant ($F(1, 22) = 7.65$, $p < 0.0113$). These results suggested that, for the T group, rapid tolerance to ethanol developed in saline pretreated rats and ketamine pretreatment blocked it. The ANOVA for the DT group showed no significant main effect of pretreatment (S vs. K) ($F(1, 28) = 0.10$, $p > 0.7599$). The main effect of treatment (SE vs. EE) was significant ($F(1, 28) = 23.39$, $p < 0.0001$), but the pretreatment (S vs. K) × treatment (SE vs. EE) interaction was not significant ($F(1, 28) = 0.52$, $p > 0.4774$). These results suggested that for the DT group, rapid tolerance to ethanol developed to a similar extent in both saline and ketamine pretreated rats.

Experiment 2: Effect of (+)MK-801 (MK) on Rapid Tolerance Development to Ethanol in Testing (T) vs. Dummy Testing (DT)

The results of this experiment are shown in Fig. 3. The day 1 maximum percent impairment values for the T group were subjected to a GLM-ANOVA. There was a significant main effect of S vs. MK pretreatment ($F(1, 28) = 9.94$, $p < 0.0038$) and S vs. E treatment ($F(1, 28) = 237.27$, $p < 0.0001$). There was also a significant pretreatment (S vs. MK) × treatment (S vs. E) interaction ($F(1, 28) = 6.91$, $p < 0.0138$). The post-hoc Duncan's multiple range test showed MK pretreated groups were significantly ($p < 0.05$) different from S pretreated groups. These results suggested that on day 1, MK pretreatment significantly enhanced the motor-impairment response due to ethanol in the T group of rats.

A GLM-ANOVA for maximum percent impairment values for the day 2 results showed significant main effects of T vs. DT groups ($F(1, 56) = 5.33$, $p < 0.0247$) and S vs. MK pretreatment ($F(1, 56) = 4.46$, $p < 0.0392$). There was also a significant group (T vs. DT) × pretreatment (S vs. MK) interaction ($F(1, 56) = 7.76$, $p < 0.0073$) suggesting that (+)MK-801 pretreatment did not block rapid tolerance development in the DT group. The main effect of treatment (SE vs. EE) was significant ($F(1, 56) = 27.70$, $p < 0.0001$). The lack of a significant group (T vs. DT) × treatment (SE vs. EE) interaction ($F(1, 56) = 0.13$, $p < 0.717$) suggests that day 1 ethanol treatment had a similar effect on day 2 response of both T and DT groups. The pretreatment (S vs. MK) × treatment interaction was significant ($F(1, 56) = 9.42$, $p < 0.0003$) and there was also a significant triple interaction of group (T vs. DT) × pretreatment (S vs. MK) × treatment (SE vs. EE) ($F(1, 56) = 14.78$, $p < 0.0003$) which suggested that (+)MK-801 blocked rapid tolerance to the motor-impairing effects of ethanol only in the T group and not in the DT group.

There was no significant difference in blood ethanol levels taken on day 2 after the last measurement of temperature or motor impairment in any of the above experiments (data not shown).

DISCUSSION

The results of this study show that rapid tolerance to ethanol on day 2 occurred under both Testing and Dummy Testing paradigms. This would suggest that the behavioral experience of the tilt plane or the actual testing of the hypothermic response is not critical to the development of rapid tolerance. In other studies (Khanna et al, submitted for publication) we found that rapid tolerance to ethanol on day 2 did not occur if animals were left alone in their cages after their respective ethanol or saline injections on day 1, i.e. some kind of experience on day 1, either intoxicated practice or dummy testing (i.e. associative learning), is required for the production of rapid tolerance. Although there appeared to be greater tolerance in the testing than in the dummy testing group (Fig. 3), the difference between these two groups was not statistically significant. Similarly, there was no significant difference between testing and dummy testing groups in the other two experiments (Figs. 1 & 2).

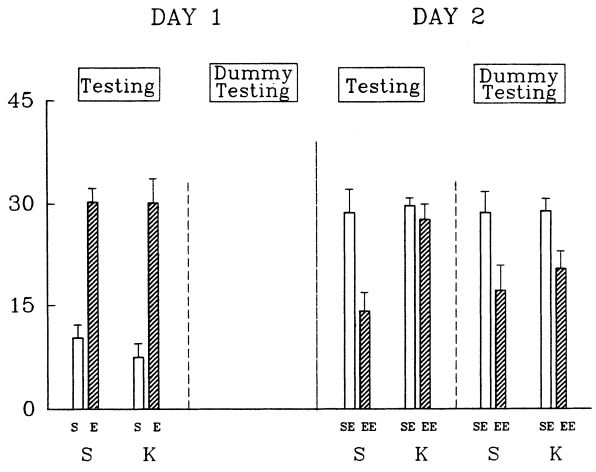

FIG. 1. Effect of ketamine on the rapid tolerance to ethanol-induced motor impairment under Testing and Dummy Testing paradigms. Four groups received ketamine (K; 1 mg/kg) and another 4 groups received saline (S) on day 1. Thirty min later, rats from 2 of the K and 2 of the S groups were given ethanol (E; 2.3 g/kg IP) and the other 2 groups from each received S. Half of the rats from each treatment group were tested at successive 30-min intervals up to 90 min on the tilt-plane test (Testing) while the other half from each treatment group was handled at the corresponding times but was not tested on the tilt-plane (Dummy Testing). On day 2, all groups were injected IP with E (2.3 g/kg). Thus, group EE received E on both day 1 and the test day, whereas the SE group received S on day 1 and E on the test day. Animals in the control group were given S while those in the drug group were given K on day 1 only. Results shown are means ± SEM of 7–8 animals per group.

The most interesting aspect of this work is that pretreatment with the NMDA antagonists (+)MK-801 and ketamine, which was shown earlier (10,13) to block rapid tolerance in the intoxicated testing paradigm, failed to block rapid tolerance in the dummy testing paradigm. These findings would suggest that the mechanism of tolerance produced in the intoxicated practice paradigm is different from that of the dummy testing procedure, i.e. there is specificity in the tolerance induced by intoxicated practice vs. simple handling of the animals. However, this explanation does not appear to completely explain the findings. That is, if these were completely different or noninterrelated mechanisms, the NMDA antagonists should only block the intoxicated practice tolerance, leaving the nonintoxicated practice tolerance intact. Specifically, NMDA antagonists should block only tolerance from intoxicated practice in the EE-K group, but there should be tolerance remaining in the EE-K group from the nonintoxicated (dummy experience) tolerance. This did not appear to be the case. NMDA antagonists completely blocked tolerance in the EE-K group. Therefore, the results suggest that there is an interaction between the two types of tolerance and that when there is intoxicated practice it nullifies the effect of nonintoxicated practice. This possibility would suggest that the two types of rapid tolerance tested in the present experiment are indeed different, but interrelated.

Rapid tolerance in the testing paradigm is mainly learned intoxicated practice tolerance (operant model tolerance). The day 1 tilt-plane tests at 30, 60 and 90 min are very strong stimuli to the development of ethanol tolerance. Since NMDA receptors have been strongly implicated in memory and learning, it is not surprising that NMDA antagonists would block this learned tolerance. Although the handling process (rats being picked up 3 times after IP ethanol) is a cue to the rat, the difference in the ability of NMDA antagonists to block these two forms of rapid ethanol tolerance may lie in the different contribution of learning to each form.

Ketamine has a half life in both plasma and brain of about 20 min. Similarly, (+)MK-801 half life is 87 ± 8 min (10). Given the short half-life of the NMDA antagonists, it could be argued that more frequent or higher doses of NMDA antagonists might also block dummy tolerance. Although we cannot rule out this possibility, the major point of this work is that doses of NMDA antagonists that do block intoxicated practice tolerance do not block dummy practice tolerance. Moreover, this seems unlikely because although the half life for (+)MK-801 is relatively short, its half life within the NMDA receptor channel may be considerably longer due to the high affinity of the ion channel binding site for (+)MK-801 and its slow dissociation from this site (13). These findings would indicate that a single dose of the NMDA antagonists would be expected to be effective at critical times in the protocol (30-120 minutes after injection) and the simultaneous presence of the NMDA antagonist and ethanol in plasma does not seem to be necessary in order for the NMDA antagonist to affect tolerance.

Recently, we reported that ketamine retarded chronic tolerance to ethanol only if ketamine was administered prior to behavioral testing under ethanol, and not if it was administered before ethanol without behavioral testing, although chronic tolerance to ethanol developed on both regimens (10). These results suggested that ketamine will block only practice learned tolerance and not tolerance acquired by pharmacological exposure. These results are in agreement with studies of Szabo et al. (13) who reported that NMDA re-

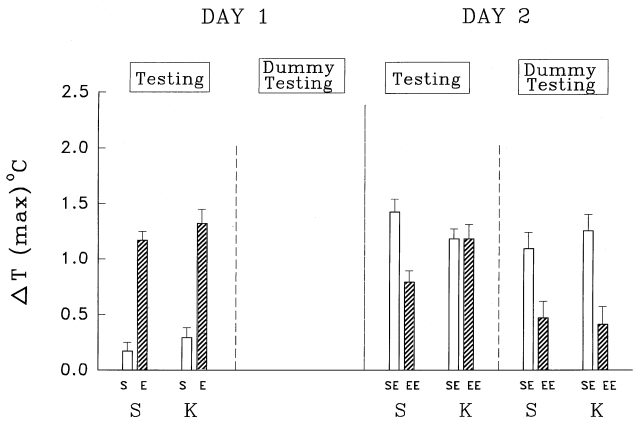

FIG. 2. Effect of ketamine on the rapid tolerance to ethanol-induced hypothermia under Testing and Dummy Testing paradigms. Four groups received ketamine (K; 1 mg/kg) and another 4 groups received saline (S) on day 1. Thirty min later, rats from 2 of the K and 2 of the S groups were given ethanol (E; 2.0 g/kg IP) and the other 2 groups from each received S. Half of the rats from each treatment group were tested for hypothermia at successive 30-min intervals up to 120 min (Testing) while the other half from each treatment group was handled at the corresponding times but was not tested for hypothermia (Dummy Testing). On day 2, all groups were injected IP with E (2.0 g/kg). Group EE received E on both day 1 and the test day, whereas the SE group received S on day 1 and E on the test day. Animals in the control group were given S while those in the drug group were given K on day 1 only. Results shown are means ± SEM of 6–8 animals per group.

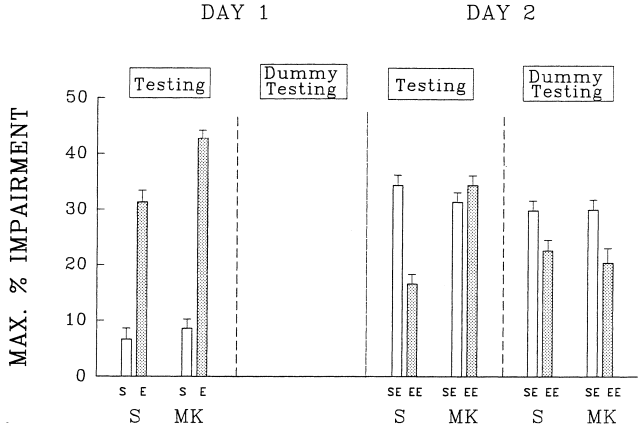

FIG. 3. Effect of (+)MK-801 on the rapid tolerance to ethanol-induced motor impairment under Testing and Dummy Testing paradigms. Four groups received (+)MK-801 (MK; 0.25 mg/kg) and another 4 groups received saline (S) on day 1. Thirty min later, rats from 2 of the MK and 2 of the S groups were given ethanol (E; 2.3 g/kg IP) and the other 2 groups from each received S. Half of the rats from each treatment group were tested at successive 30-min intervals up to 90 min on the tilt-plane test (Testing) while the other half from each treatment group was handled at the corresponding times but was not tested on the tilt-plane (Dummy Testing). On day 2, all groups were injected IP with E (2.3 g/kg). Group EE received E on day 1 and the test day whereas the SE group received S on day 1 and E on the test day. Animals in the control group were given S while those in the drug group were given MK on day 1 only. Results shown are means ± SEM of 8 animals per group.

ceptor antagonist, dizocilpine blocked environmental dependent tolerance but had no effect on the development of environment independent tolerance.

The present findings with the testing and dummy testing paradigms could be interpreted as evidence for rapid learned and pharmacological tolerance respectively since NMDA antagonists selectively blocked rapid tolerance in the testing paradigm but failed to block it in the dummy testing paradigm. If other manipulations which are known to selectively block chronic learned and pharmacological tolerance affect testing and dummy testing rapid tolerance in a similar manner, rapid tolerance might prove to be a useful and rapid tool to examine both learned and pharmacological tolerance.

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