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BRIEF COMMUNICATION

Effect of D-Cycloserine on Rapid Tolerance to Ethanol

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KHANNA, J. M., H. KALANT, G. SHAH AND A. CHAU. *Effect of* D-*cycloserine on rapid tolerance to ethanol.* PHARMACOL BIOCHEM BEHAV **45**(4) 983–986, 1993.—We recently reported that the noncompetitive NMDA antagonists, (+)MK-801 and ketamine, block the development of rapid tolerance to ethanol. In the present article, we show that D-cycloserine (CS), an agonist at the glycine site of the NMDA receptor that enhances learning and memory, also enhances the development of rapid tolerance to ethanol. Rats were pretreated on day 1 with saline or CS, followed 30 min later by ethanol (2.3 g/kg, IP) or saline. At the end of motor impairment testing on the tilt-plane apparatus, a second injection of CS (3 mg/kg, IP, each time) or saline was given, followed 30 min later by ethanol or saline. Ethanol pretreatment alone (at this dose) did not result in rapid tolerance to ethanol on day 2. However, the group pretreated with CS and ethanol on day 1 showed significant tolerance on day 2 compared to other groups. Pretreatment with CS on day 1 did not affect the motor impairment response to the first exposure to ethanol whether this was on day 1 or day 2. In another experiment, administration of (+)MK-801 (0.25 mg/kg, IP) prior to CS abolished the rapid tolerance enhancement by CS. These findings are further evidence that the NMDA system, which requires activation by the glycine receptor, plays a major role in the development of at least some forms of ethanol tolerance.

NMDA antagonist (+)MK-801 D-Cycloserine Rapid tolerance Ethanol

THE results of our recent investigations suggested that NMDA receptors may play a role in the development of rapid and chronic tolerance to ethanol and rapid cross-tolerance between ethanol and other sedative-hypnotics. Tolerance and cross-tolerance to ethanol on day 2 were inhibited when NMDA antagonists [(+)MK-801 or ketamine] were administered prior to behavioral testing with ethanol on day 1 (7,9–11). Because NMDA receptors have been strongly implicated in learning and memory (2,12,14–16,18,20,21), and acquisition of ethanol tolerance bears many similarities to learning and memory (4,6), we concluded that the role of NMDA receptors in ethanol tolerance may be similar to their role in memory and learning. Results from other laboratories have also shown that (+)MK-801 and kynurenic acid attenuated the development of chronic tolerance to the analgesic effect of morphine (13,19).

It is possible that NMDA antagonists may have exerted their effects on tolerance development by some means other than interaction with the NMDA receptor complex. Such a possibility can be tested by producing changes opposite to that produced by NMDA antagonists, that is, by enhancing glutamatergic transmission. The NMDA receptor is known to be associated with a glycine coreceptor site that must be activated to permit the NMDA receptor site to respond to its agonist, glutamate (17). The objective of the present work, therefore, was to determine whether elevation of glutamatergic transmission with D-cycloserine (CS), an agonist at the glycine coreceptor, could accelerate the rate of tolerance development 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. Tapwater was available at all times. The temperature of the colony room

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was maintained at 21 ± 1°C and lights were on from 7:00 a.m.-7:00 p.m. throughout the experiment.

Test Procedures

Tilt-plane test. The tilting-plane test was used as a measure of motor impairment (1,3). The apparatus consists of a plane 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 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. Blood samples (50 μl) for ethanol measurement were taken on day 2 from the tail of the rat immediately after the last measurement of motor impairment. Blood ethanol was analysed by the enzymatic method described previously (5).

Experimental Procedure

Experiment 1: Effect of CS on development of rapid tolerance to ethanol. Rats were randomly divided into four groups. On day 1, two groups were injected with CS (3 mg/kg) while the other two groups received saline (S). This dose of CS has been shown to produce clear enhancement of learning on two different tasks (15). Thirty minutes later, rats from one of the CS and one of the S groups were given ethanol (E) (2.3 g/kg, IP) and the other two groups were again administered S. Before the first injection, and at successive 30-min intervals up to 90 min after E or S injection, the degree of motor impairment was assessed (tilt-plane test) in all animals. Immediately after the last measurement, a second IP injection of CS (3 mg/kg) or S was given because it was not known how long a period of CS action might be needed to modify tolerance acquisition. Preliminary work (in progress) suggests that only the CS dose preceding the E trial is required. At 120 min, another IP injection of E (0.7 g/kg) or S was given. This procedure of giving ethanol in two doses was employed because preliminary evidence suggested that a total dose of 3 g/kg might be just below the threshold for producing rapid tolerance but doses greater than 2.3 g/kg may not always fall in the linear part of the acute dose-response curve (8). Rats were then returned to their home cages. On day 2, an identical procedure was followed except that all animals received a challenge dose of 2.3 g/kg E and tolerance was assessed on the tilt-plane as described above. No CS or S pretreatment was given on day 2.

Experiment 2: Does (+)MK-801 block effect of CS on rapid tolerance to ethanol? For these studies, an experimental procedure similar to that described above was employed except that six groups of animals were used. Two of these groups received (+)MK-801 (0.25 mg/kg) and the other four groups received saline at zero time. Thirty minutes later, the two (+)MK-801 groups and two of the saline groups received CS (3 mg/kg) and the remaining two saline groups again received saline. At 60 min, rats from one of each pair of the treatment groups received ethanol (2.3 g/kg) and the others received saline. The rest of the day-1 and day-2 procedures were identical to those described above for Experiment 1.

RESULTS

Experiment 1

The baseline (preinjection) sliding angles of the four groups were closely matched: 39.3 ± 0.7, 39.3 ± 0.7, 40.0 ± 0.5, and 39.6 ± 0.7°, $F(3, 24) = 0.27, p > 0.847$, n.s. The maximum effect occurred at 30 min post-E in all groups. On day 1, animals receiving ethanol showed the expected degree of motor impairment (Fig. 1), which was not affected by CS pretreatment. Analysis of maximum percent impairment scores by general linear model analysis of variance (GLM-ANOVA) showed a significant main effect of ethanol treatment, $F(1, 24) = 117.33, p < 0.0001$ but no significant effect of pretreatment, $F(1, 24) = 0.13, p > 0.726$, n.s., nor pretreatment × treatment interaction, $F(1, 24) = 0.01, p > 0.938$, n.s. The results of the rapid tolerance test on day 2 showed significantly lower motor impairing effects of E in rats treated with E on day 1 (EE) than in those that had received S (SE) when both had been pretreated with CS on day 1, $t(12) = 3.79, p < 0.005$. Maximum percent impairment, however, was almost identical when SE vs. EE were compared for S-pretreated (control) animals, $t(12) = 0.07, p > 0.95$. A GLM ANOVA showed significant pretreatment × treatment interaction, $F(1, 24) = 5.56, p < 0.0268$, suggesting that CS pretreatment enhanced rapid tolerance to ethanol. There was no significant difference in blood ethanol levels taken on day 2 at the end of motor impairment measurement in CS-pretreated groups compared to control groups. Blood ethanol levels in the four groups were: CS (SE), 215 ± 4; CS (EE), 215 ± 5; control (SE), 217 ± 5; control (EE), 210 ± 5 mg/100 ml; $n = 7$ animals per group.

Experiment 2

The results of this experiment are shown in Fig. 2. On day 1, (+)MK-801 produced a small degree of motor impairment,

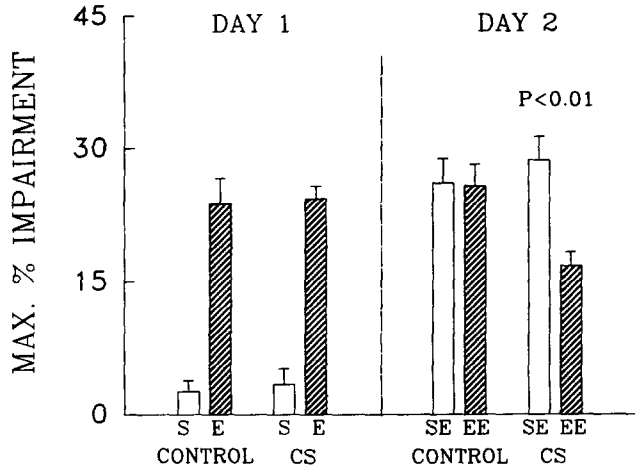

FIG. 1. Effect of D-cycloserine (CS) on the development of rapid tolerance to ethanol (E) (tilt-plane test). Two groups received CS [with ethanol and saline (S)] and another two groups received saline (with ethanol and saline) on day 1. Rapid tolerance to ethanol was assessed on day 2, when all groups received a challenge dose of ethanol. Group EE received ethanol on day 1 and the test day whereas the SE group received saline on day 1 and ethanol on the test day. Animals in the control groups were given vehicle (saline), while those in the drug group were given CS only on day 1 (see the Method section for details). Results shown are means ± SEM; $n = 7$ animals per group).

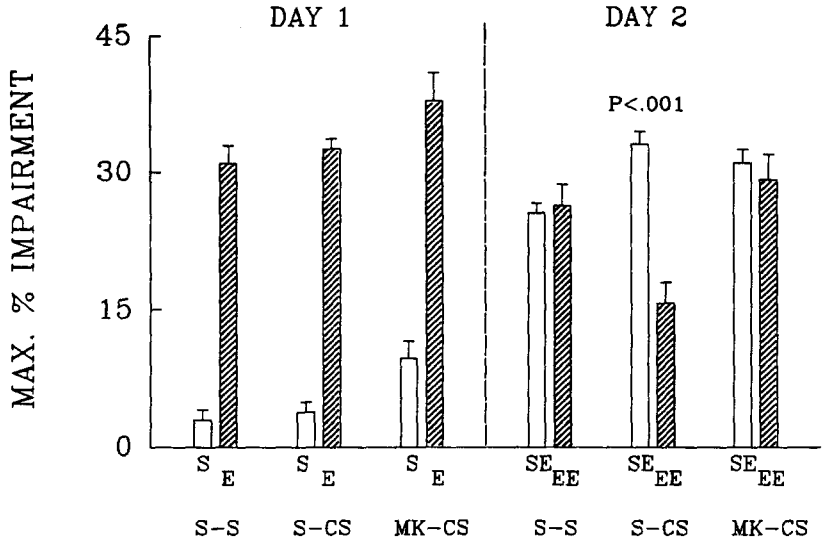

FIG. 2. Effect of (+)MK-801 and D-cycloserine (CS) treatment on rapid tolerance development to ethanol (E). Two groups received CS [with ethanol and saline (S)], two groups received saline (with ethanol and saline), and another two groups received (+)MK-801 and CS (with ethanol and saline) on day 1. (+)MK-801 was given 30 min prior to CS and the four other groups were administered vehicle (saline) at this time. Ethanol or saline injections were given at 60 min on day 1 in each group. Rapid tolerance to ethanol was assessed on day 2, when all groups received a challenge dose of ethanol. Group EE received ethanol on day 1 and the test day whereas the SE group received saline on day 1 and ethanol on the test day. Animals in the control group were given vehicle (saline), those in the CS group were given CS, and those in the MK-CS group were given (+)MK-801 30 min prior to CS (see the Method section for details). Results shown are means ± SEM; $n = 8$ animals per group.

$F(2, 42) = 211$, $p < 0.0016$, but CS did not. This was confirmed by posthoc Duncan's multiple-range tests for maximum percent impairment (MK-CS > S-CS and MK-CS > S-S, $p < 0.05$ in each case). Ethanol produced the expected degree of impairment, $F(1, 42) = 345$, $p < 0.0001$, but there was no significant interaction with pretreatment. The results of the rapid tolerance test on day 2 showed that (+)MK-801 significantly blocked CS enhancement of rapid tolerance to ethanol. A two-way GLM ANOVA of maximum percent impairment confirmed this with significant pretreatment × treatment interaction, $F(2, 42) = 12.15$, $p < 0.0001$. Maximum percent impairment, however, was significantly different when SE vs. EE groups were compared for only CS-pretreated groups, $t(14) = 6.67$, $p < 0.001$, suggesting the development of rapid tolerance to ethanol. Blood ethanol levels on day 2 at 120 min were again not different among various groups as neither pretreatment effect, $F(2, 41) = 2.55$, $p > 0.091$, nor pretreatment × treatment interaction, $F(2, 41) = 2.43$, $p > 0.1004$, was significant.

DISCUSSION

The results of this study indicate that administration of CS, which facilitates glutamatergic transmission, being a positive modulator at the glycine recognition site on the NMDA receptor complex, enhances the rate of development of rapid tolerance to ethanol on the tilt-plane test. The day-1 dose of ethanol employed in this study did not produce rapid tolerance by itself on day 2. Therefore, it is not possible to state whether CS would enhance tolerance only when the alcohol doses are insufficient to do so or whether it will enhance tolerance even if this is already proceeding maximally (i.e., with higher day-1 doses). Whether the facilitatory effects of CS on tolerance development are general or task specific (i.e., learned and unlearned tasks) requires investigation. Previous work had already shown that administration of (+)MK-801 on day 1 would prevent rapid tolerance to ethanol if the dose of ethanol was large enough to produce it. In the present study, the ethanol dose was purposely chosen to be too small to produce tolerance alone so that there was no need for a (+)MK-801-saline pretreatment group. In a later study with ketamine instead of (+)MK-801 (Khanna et al., unpublished), a ketamine-saline pretreatment group was included but did not alter the conclusions in any way: Ketamine clearly blocked the ability of CS to promote rapid tolerance to a low dose of E without having any effect on the control group.

At least four possible mechanisms of this enhancement must be considered: a) CS might alter the pharmacokinetics of E in some way that caused the same dose of E to yield a lower blood alcohol level on day 2 than on day 1; b) CS might modify a direct action of E on the NMDA receptor complex, increasing the acute motor impairment produced by E and thus increasing the stimulus to development of tolerance; c) CS might independently cause a long-lasting change in sensitivity of the NMDA complex, making it "cross-tolerant" to E given the following day; d) CS might increase specifically the NMDA receptor role in retaining the task-related learning component of tolerance to the effects of E given on day 1.

The first possibility appears to be excluded by the fact that day-1 CS treatment did not alter blood ethanol levels on day 2

after the last measurement of motor impairment: There was no significant difference between the CS-E animals and the other groups. Therefore, the enhancement of ethanol tolerance by CS and the blockade of tolerance by (+)MK-801 cannot be attributed to changes in the pharmacokinetic properties of ethanol. The second possible explanation is not supported because CS did not alter the day-1 response to E. Moreover, later work has shown that when performance was also measured after the second injections of CS and E on day 1 there was again no difference in impairment. The third possibility is excluded by the fact that CS treatment on day 1 did not decrease the initial response to E in the SE group on day 2 compared to the SE group that had received saline instead of CS on day 1.

Evaluation of the fourth possibility will require more detailed investigation of the temporal relation between CS and task performance under E. Recent studies showed that administration of NMDA antagonists [(+)MK-801 or ketamine] before behavioral testing on day 1 blocked the development of rapid tolerance on day 2. However, administration of (+)MK-801 on day 1 but after behavioral testing with ethanol did not block tolerance to ethanol on day 2 (7). Whether CS would enhance rapid tolerance by enhancing some adaptation that occurs during intoxicated practice remains to be determined. In one study on memory and learning (15), CS was effective in enhancing performance when administered both before and after the shock. Systematic examination of different doses of CS and ethanol and different test systems, in the presence and absence of intoxicated practice, is currently being undertaken.

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