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Effect of Ro 15-4513 on Ethanol-Induced Conditioned Place Preference

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RISINGER, F. O., D. H. MALOTT, A. L. RILEY AND C. L. CUNNINGHAM. *Effect of Ro 15-4513 on ethanol-induced conditioned place preference.* PHARMACOL BIOCHEM BEHAV 43(1) 97-102, 1992.—The benzodiazepine receptor inverse agonist Ro 15-4513 reverses a number of ethanol's effects, including its reinforcing properties as measured through self-administration. The present study examined the effect of this putative ethanol antagonist in a place conditioning design that has been shown to be sensitive to ethanol's rewarding properties in mice. Using an unbiased differential conditioning procedure, DBA/2J mice received, on alternate days, pairings of a distinctive floor stimulus (CS+) with either ethanol (2 g/kg), Ro 15-4513 (3 mg/kg), or a combination of ethanol and Ro 15-4513. On alternate days, a different distinctive floor stimulus (CS−) was paired with vehicle. Under these conditions, ethanol produced a conditioned place preference that was unaffected by Ro 15-4513. Ro 15-4513 alone did not produce either a place preference or aversion. Ro 15-4513 did produce reductions in locomotor activity during conditioning, indicating it was behaviorally active. These results indicate that a dose of Ro 15-4513 that alters general activity does not affect ethanol reward.

Ro 15-4513 Conditioned place preference Ethanol Inbred mice Locomotor activity Reward
DBA/2J

FOLLOWING the demonstration that the imidazobenzodiazepine Ro 15-4513 (44,56) blocked ethanol's stimulatory effect on GABA-mediated Cl- flux in synaptosomal preparations [(58); see also (23)], the antagonistic interaction between Ro 15-4513 and ethanol has been examined in a range of behavioral designs. For example, Ro 15-4513 has been reported to reverse ethanol-induced motor deficits (2,6,28,35,66), disruptions in schedule-controlled (22,33) and avoidance (21) behaviors, taste aversions (57), reductions in exploration (36,37) and soporific (35,46,60), ataxic (18,58), anticonvulsive (34,46,47), and anticonflict effects (33,58). Recently, the effects of Ro 15-4513 on the reinforcing or rewarding properties of ethanol have been examined. Animals responding for ethanol on a fixed ratio (FR) 4 schedule of reinforcement significantly reduced ethanol responding following administration of 1.0 and 3.0 mg/kg Ro 15-4513 (53). Subsequent work demonstrated that this reduction in the self-administration of ethanol is specific to ethanol in that responding for sucrose, water, or food is unaffected by doses of Ro 15-4513 that affect ethanol intake [(31,41,52); see also (1)].

The present experiment extended this assessment of the antagonist effects of Ro 15-4513 on the reinforcing properties of ethanol by examining the interaction of Ro 15-4513 and ethanol within a conditioned place preference design [for reviews, see (9,59,62)]. In this design, a drug's motivational value is assessed by measuring an animal's tendency to approach a stimulus that has previously been paired with drug in a Pavlovian conditioning paradigm (13). Although a wide range of compounds have been effective in establishing conditioned place preferences within this design [cf. (62)], ethanol has generally been ineffective in conditioning such preferences unless extensive repeated exposure to ethanol is given (8,51). In fact, ethanol has frequently been reported to condition place aversions when assessed within this preparation (11,12, 55,63). Recently, however, we found robust conditioned place preferences with ethanol in both inbred and selectively bred mice (14,15), thus allowing for an assessment of the effects of Ro 15-4513 within this procedure. If Ro 15-4513 has antagonist actions on the reinforcing properties of ethanol, it might be expected that the development of ethanol-induced conditioned place preference should be reduced or eliminated by Ro 15-4513 pretreatment.

METHOD

Subjects

Subjects were 96 experimentally naive, adult, male DBA/2J mice 60 days old at the beginning of the experiment.

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Groups of four mice were housed in polycarbonate cages (27.9 × 9.5 × 12.7 cm) with cob bedding in a Thoren rack. A 12 L : 12 D cycle was in effect with the onset of the light portion of the cycle occurring at 0700 h. Food and water were continuously available in the home cage, and the room temperature was maintained at 22 ± 2°C. Experimental procedures were conducted during the light phase of the cycle.

Drugs

Ethanol was administered in a 20% v/v solution prepared by diluting 95% ethanol with physiological saline. Ro 15-4513 was suspended in a vehicle of 4% Tween-80 and sterile distilled water.

Apparatus

The place conditioning apparatus consisted of 12 identical Plexiglas and aluminum chambers (30 × 15 × 15 cm) enclosed in ventilated light- and sound-attenuating boxes (Coulbourn Model E10-20, Coulbourn Instruments, Allentown, PA). Infrared light sources and detectors were positioned opposite each other at 5-cm intervals on the long walls of each place conditioning chamber 2.2 cm above the floor surface. Occlusion of the infrared light beams was used as a measure of general activity and to determine the animal's position (left or right side) in the chamber. Data were recorded each minute by computer (0.01-s resolution).

The floor of each box consisted of interchangeable halves with one of two distinctive textures: "hole" floors were made from perforated stainless steel (16 g) with 6.4-mm round holes on 9.5-mm staggered centers; "grid" floors were composed of 2.3-mm stainless steel rods mounted 6.4 mm apart in Plexiglas rails. This combination of floor textures was selected on the basis of previous studies indicating that drug-naive control groups spend about half their time on each floor type during preference tests (15). The inside of the box and floors were wiped with a damp sponge and the litter paper beneath the floors was changed after each mouse.

Procedure

The experiment was divided into three consecutive phases: habituation (one session), conditioning (eight sessions), and testing (one session). Sessions were conducted 5 days a week with a 2-day break between the first and second four conditioning sessions.

Habituation. During habituation, all subjects received saline (15 ml/kg) and were immediately placed in the conditioning apparatus for 30 min on a smooth floor covered with paper. Subjects were not exposed to the distinctive floor textures to avoid the development of latent inhibition (40). The habituation session was intended to reduce the novelty and stress associated with handling, injection, and exposure to the apparatus.

Conditioning. During this phase, subjects were randomly assigned to one of three drug treatment groups (n = 31–32 per group) and exposed to a Pavlovian differential conditioning procedure. Specifically, on alternate days subjects in Group RE received an IP injection of Ro 15-4513 (3 mg/kg) followed 15 min later by an injection of ethanol (2 g/kg); subjects in Group TE received an equivolume injection of Tween-80 (the Ro 15-4513 vehicle) followed 15 min later by an injection of ethanol; and subjects in Group RS received an injection of Ro 15-4513 followed by an injection of saline. After the first injection, subjects were returned to the home cage. After the second injection, subjects were immediately placed in the apparatus for 5 min with either the grid or hole floor CS+. Conditioning floor assignment was randomly determined within each drug treatment condition, yielding two subgroups: Grid+ and Grid−. On alternate days (CS− trials), all subjects received vehicle injections (separated by 15 min) and were exposed to the floor type (either grid or hole) not used during CS+ conditioning. On either CS+ or CS− trials, subjects had access to both sides of the apparatus and floor texture was homogeneous [cf. (64)]. All groups received four 5-min CS+ and four 5-min CS− trials with order of exposure to drug treatment counterbalanced within groups.

Testing. During preference testing, all subjects received injections of Tween-80 and saline (15-min intervals between the two injections) before placement in the apparatus (60-min session) with half grid floor and half hole floor (left and right positions counterbalanced within groups).

Data Analysis

Two subjects died prior to the preference test (1 from Group RE, 1 from Group RS). Data from the remaining subjects were analyzed by unweighted means analysis of variance (ANOVA). The level of significance was set at $p < 0.05$.

RESULTS

Conditioning

Figure 1 displays mean (±SEM) activity counts per minute on the first drug conditioning trial (CS+) and the first vehicle conditioning trial (CS−) for each drug treatment group. Subjects in Group TE showed higher levels of activity after drug treatment (CS+ trial) than after Tween-80/saline treatment (CS− trial), indicating ethanol-induced activation. Subjects in Group RE demonstrated lower levels of activity after drug treatment, suggesting that Ro 15-4513 partially antagonized ethanol-induced activation. Subjects in Group RE displayed lower levels of activity during drug treatment compared to CS− trials, indicating that Ro 15-4513 (in the absence of ethanol) decreased motor activity. Two-way ANOVA (drug

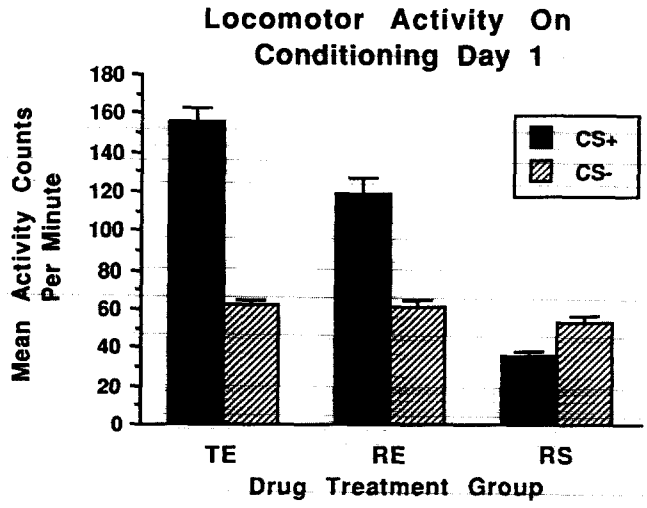

FIG. 1. Mean (±SEM) activity per minute on the CS+ and CS− associated sides during the first place conditioning trial for Groups TE, RE, and RS.

treatment × CS trial type) yielded significant effects of drug treatment, $F(2, 91) = 84.3$, CS trial type, $F(1, 91) = 131.0$, and drug treatment × CS trial type, $F(2, 91) = 71.9$. Separate follow-up analyses of each trial type indicated reliable drug treatment effects both for the CS+ trial, $F(2, 91) = 89.7$, and for the CS− trial, $F(2, 91) = 4.1$. Posthoc comparisons showed that Groups TE and RE differed on the CS+ trial, $F(1, 61) = 11.7$, but not on the CS− trial ($F < 1$). Separate follow-up analyses of trial type within each group revealed a significant difference between CS+ and CS− trials in all three drug treatment groups ($Fs > 30.2$).

The mean (±SEM) activity levels for each CS+ trial are given in Table 1. Activity in the two ethanol-treated groups (TE and RE) generally increased over trials, suggesting sensitization to ethanol's activating effect. Mice in Group RS continued to display relatively low levels of activity on CS+ trials (i.e., after Ro 15-4513 + saline), with only a slight decrease over trials. Two-way analysis (drug treatment × trial) of activity levels over CS+ trials showed significant effects of drug treatment, $F(2, 91) = 199.7$, and drug treatment × trial, $F(6, 273) = 7.2$. Separate follow-up analyses of the trial effect within each group indicated a significant trial effect in Groups TE, $F(3, 93) = 8.6$, and RE, $F(3, 90) = 15.2$, but not in Group RS.

Testing

Figure 2 depicts the mean (±SEM) seconds per minute on the grid floor for both subgroups within each drug treatment condition. As indicated by the difference between the Grid+ and Grid− subgroups, mice in Groups TE and RE displayed a preference for the ethanol-paired floor whereas mice in Group RS showed no evidence of place conditioning. Overall analysis (drug treatment × conditioning group) produced significant effects of conditioning group (Grid+ vs. Grid−), $F(1, 88) = 54.1$, and drug treatment × conditioning group, $F(2, 88) = 10.9$. A separate analysis comparing Groups TE and RE showed no effect of drug treatment or drug treatment × conditioning group ($Fs < 1.0$), indicating that Ro 15-4513 did not alter the magnitude of place conditioning. Subsequent comparisons of the conditioning groups within each drug treatment showed preference for the ethanol-paired floor in Groups RE, $F(1, 29) = 33.2$, and TE, $F(1, 30) = 38.2$. In contrast, no conditioning effect was observed in Group RS, $F(1, 29) = 0.2$.

Activity levels during the test session were initially high in all groups and decreased over the session. Analysis indicated a significant drug treatment × time interaction, $F(118, 5369) = 1.7$. This result was due primarily to group differences in activity during the initial minute of testing. Subjects in Group TE had higher levels of activity during the first minute of testing (mean = 98.2 ± 9.0) than subjects in Group RE (mean = 81.3 ± 7.2) or Group RS (mean = 63.9 ± 5.3). Analysis of first-minute activity showed a significant effect of drug treatment, $F(2, 91) = 10.8$. Follow-up comparisons indicated that Groups RE and TE each had higher levels of activity than Group RS, ($Fs > 7.6$). Moreover, Group TE demonstrated greater activity than Group RE, $F(1, 61) = 4.3$.

TABLE 1

MEAN ACTIVITY PER MINUTE (± SEM) ON CS+ TRIALS

Group	Trial 1	Trial 2	Trial 3	Trial 4
TE	156.3 (6.9)	177.1 (10.1)	205.5 (9.4)	197.1 (9.4)
RE	119.1 (8.3)	136.2 (12.1)	191.4 (6.1)	162.0 (11.9)
RS	35.3 (2.9)	33.2 (2.9)	31.7 (2.1)	30.7 (2.3)

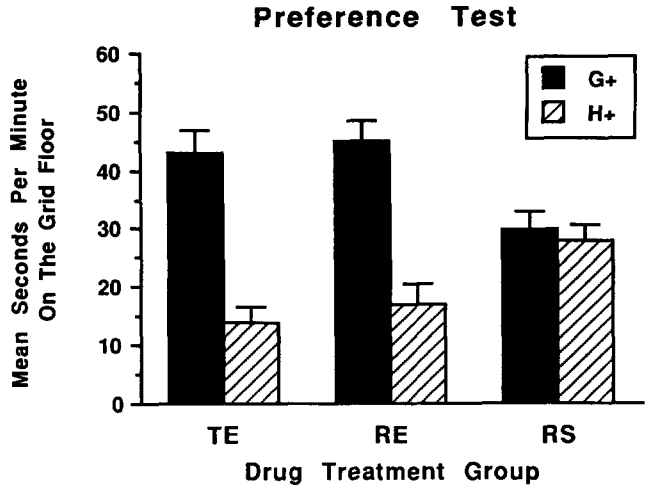

FIG. 2. Mean (± SEM) seconds per minute spent on the grid floor during choice testing (60-min session) for Groups TE, RE, and RS. Grid+ and Grid− refer to the subgroups within each drug treatment group that had previously received either the grid floor (Grid+) or hole floor (Grid−) during CS+ conditioning trials. The opposite floor type was paired with saline on CS− conditioning trials.

DISCUSSION

As shown previously (15), ethanol was effective in conditioning a place preference in DBA/2J mice. However, pretreatment with Ro 15-4513 had no effect on this conditioning. Ro 15-4513 did suppress motor activity (both when given alone and in combination with ethanol), an effect consistent with other reports of the behavioral suppressant effects of Ro 15-4513 (3,20) and one that indicates the compound was behaviorally active in this design. Its activity did not appear aversive, however, in that there was no evidence of place aversion in subjects administered saline and Ro 15-4513. Several reports have noted that other inverse agonists of the benzodiazepine receptor induce place aversion at high doses (17, 19,65).

The failure to antagonize the ethanol-induced conditioned place preference is not consistent with earlier reports assessing the effects of Ro 15-4513 on ethanol self-administration. As noted above, at doses that had no effect on general fluid or food consumption Ro 14-4513 markedly suppressed ethanol intake (31,41,52). The present data, however, are consistent with several other reports assessing the effect of Ro 15-4513 on other alleged measures of the rewarding properties of ethanol. For example, Ro 15-4513 does not affect ethanol-induced reductions in selected current duration or response rate in an intracranial self-stimulation design [(ICSS); (45,54)]. Further, Ro 15-4513 fails to antagonize the stimulus properties of ethanol in the drug discrimination learning procedure [(DDL); (26,27,32,42), but see (50)]. Both the ICSS and DDL designs have been utilized often as methods for the assessment of the rewarding properties of drugs of abuse (7).

The basis for the differences in the effects of Ro 15-4513 on these various indices of the rewarding properties of ethanol

is unknown. One conclusion may be that ethanol self-administration is the only valid index of ethanol reinforcement. Given that Ro 15-4513 antagonizes ethanol self-administration, one could then conclude that the reinforcing properties of ethanol are reversed by Ro 15-4513. Although possible, there is no clear basis for such a conclusion given the general ability of the other behaviors to index the rewarding properties of ethanol. It might also be concluded that each of the behaviors is maintained by a different component of the reinforcing properties of ethanol, some sensitive to antagonism by Ro 15-4513 and others mediated through some undefined neurochemical system insensitive to such antagonism.

Consideration must be given to two additional possibilities in the interpretation of the present results. Previous demonstrations of the ability of Ro 15-4513 to antagonize the rewarding effects of ethanol have generally used rats (1,31, 52,53). Therefore, the present failure of Ro 15-4513 to antagonize ethanol-induced conditioned place preference in mice may simply represent a species difference. However, given the broad similarity between mice and rats this conclusion seems unlikely. Another consideration is the procedural differences between place conditioning and drug self-administration. For example, it may be that the outcome of previous drinking and self-administration studies was due more to the effect of Ro 15-4513 on the expression of conditioned reward than an effect on the primary rewarding properties of ethanol. Because Ro 15-4513 was not administered until after the ethanol-reinforced behavior was well established in those studies, one might attribute the antagonism of ethanol reinforcement to interference with the expression of a conditioned motivational effect. In contrast, the present study attempted to determine the effect of Ro 15-4513 on ethanol's primary rewarding properties experienced during the process of conditioning.

Independent of the basis for these differences in the effects of Ro 15-4513 on the rewarding properties of ethanol, the failure to antagonize ethanol-induced place preference adds another measure to the growing list of ethanol-induced effects that are not antagonized by Ro 15-4513 (5,10,16,23–25,29, 30,48,49,60,61,67). The basis for the response specificity of the Ro 15-4513/ethanol interaction is not known. Some have suggested that the failure to antagonize an ethanol-mediated effect by Ro 15-4513 is due to the fact that only some ethanol-induced behavioral changes are mediated via the GABA/benzodiazepine receptor complex (23,25,30,60) and as such only these responses would be sensitive to antagonism. Others have argued that antagonism is dependent upon the degree of intrinsic activity induced by Ro 15-4513 and the degree to which this intrinsic activity interacts with the effects of ethanol [i.e., the degree of physiological antagonism; (3,4,20,29,33, 38,39,43,47)]. Although the mechanism may be unknown, it is clear that Ro 15-4513 is not an ubiquitous antagonist of ethanol.

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