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Effects of Ro 15-4513 on Ethanol Discrimination in C57BL/6 Mice

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MIDDAUGH, L. D., K. BAO, H. C. BECKER AND S. S. DANIEL. *Effects of Ro 15-4513 on ethanol discrimination in C57BL/6 mice.* PHARMACOL BIOCHEM BEHAV **38**(4) 763–767, 1991.— Ro 15-4513, a partial benzodiazepine receptor inverse agonist, counteracts many of the effects of ethanol, however, its effects on ethanol discrimination in operant paradigms remains unclear. The present study examined the effects of Ro 15-4513 on ethanol discrimination by female C57BL/6 mice in a food-reinforced behavior-operant paradigm. Under the time and dosing conditions used in previous reports, Ro 15-4513 did not alter ethanol discrimination whether given prior to or after ethanol exposure. The drug did, however, attenuate ethanol discrimination for brief periods (<8 min) when injected after ethanol and at doses and postinjection times which also disrupted responding. The present study confirmed that Ro 15-4513 attenuated ethanol discrimination, but not to the extent as previously reported. The results indicate that postinjection time is a very critical factor in whether Ro 15-4513 attenuates ethanol discrimination.

Ethanol Ro 15-4513 Benzodiazepine inverse agonist Drug discrimination Mice

SEVERAL studies indicate that many behavioral effects of ethanol can be blocked or attenuated by the partial benzodiazepine receptor inverse agonist Ro 15-4513. For example, Ro 15-4513 has been reported to antagonize the anticonvulsant (10,17), anxiolytic (3, 5, 9, 12, 20), motor incoordinating (4, 7, 11), motor depressant (1), sedative/hypnotic (11,21), and amnesic (16) properties of ethanol. The drug has also been shown to exacerbate symptoms associated with ethanol withdrawal (2,14), as well as suppress oral self-administration of ethanol (15,19). In contrast, ethanol-induced motor stimulation (1,13) and hypothermia (7,21) do not appear to be influenced by Ro 15-4513, suggesting that the compound does not antagonize all of ethanol's pharmacological effects.

Whether Ro 15-4513 alters the interoceptive effects of ethanol which are used for ethanol discrimination in operant paradigms is unclear. Ro 15-4513 was reported to have no effect on ethanol discrimination by female rats when injected prior to ethanol (6); however, the compound reportedly attenuated the ethanol discrimination by male CD-1 mice when injected after the ethanol injections (18). These two studies differed in the species and gender of the subjects, as well as the relative order of ethanol and Ro 15-4513 injections. Clearly, information is insufficient for conclusions about the effects of Ro 15-4513 on ethanol discrimination.

The present study consisted of two experiments on the effects of Ro 15-4513 on ethanol discrimination by female C57BL/6 mice. In the first experiment, the influence of the order of drug presentation on the effects of Ro 15-4513 on ethanol discrimination was examined using doses and time parameters reported to effectively attenuate the discrimination. In the second experiment we determined the time course of Ro 15-4513 effects on ethanol discrimination.

METHOD

EXPERIMENT 1

Subjects

Subjects were 6 female C57BL/6cr mice. They were housed singly in a colony room adjacent to the behavioral laboratory. The colony room was maintained on a 12-h light/dark cycle with lights on at 0700 h and temperature was regulated at 70 ± 3°F. The study began when the mice were 90 days of age and was completed over a 6-month period.

Apparatus

Three operant chambers enclosed in sound- and light-controlled boxes were used. The operant chambers (16 × 16 × 11.4 cm) were constructed of grey Plexiglas with stainless steel grid floors. A food tray with a 1.9 × 2.5 cm opening was centrally located on one wall at floor level. Light was provided by a miniature lamp (GTE 18-19) located directly above the food tray. Rodent levers (Model SRL-003, BRS/LVE, Laurel, MD) were

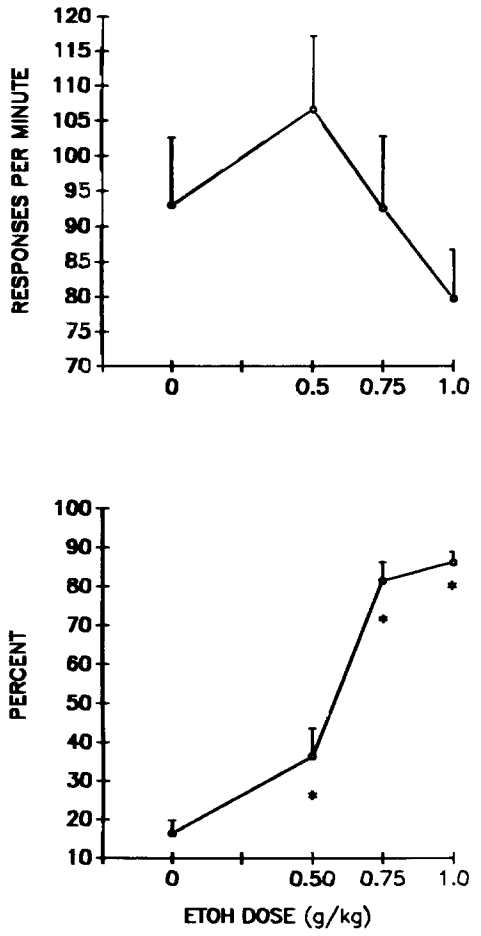

FIG. 1. Response rates (upper graph) and percentage of responses on the ethanol lever (lower graph) for C57 female mice during 2-min tests beginning 5 min after IP injections of the various ethanol doses. Data are expressed as mean ± SE and *denotes statistically significant differences from the vehicle control.

located 4 cm to each side of the food tray and 3 cm above the floor. Depressing a lever (8 g dead weight) defined a response and served as a signal for appropriate solid-state programming equipment. The responses made on each lever were recorded on electromechanical counters and a programmable printout recorder.

Procedures

Deprivation. Animals were weighed and deprived of all food for 24 h at the start of the experiment. They were then weighed daily throughout the experiment and appropriate amounts of food were given to maintain body weight at 80 ± 3% of ad lib levels.

Lever-response acquisition. For lever response acquisition, animals were placed in the chamber with alternate levers exposed daily. Five pellets were available in the food tray at the start of each session and an additional pellet was dispensed for each lever press. This procedure was continued for four sessions, allowing a maximum of 10 responses or 15 minutes per session. After all animals had learned the lever-press response (Day 4), they were trained 20 min per day, five days a week for the rest of the study. Initially, they were placed on a fixed ratio (FR) 5 reinforcement schedule. After five days, the schedule was changed to FR 20. During the FR 5 and the first 5 days of FR 20, the available lever was alternated daily. Drug discrimination training began 10 days after the start of the FR 20 schedule with both levers available during each 20-min session.

Ethanol discrimination training. Animals were injected intraperitoneally (IP), 0.02 ml/g body weight, five min before each training session with either ethanol (1.0 g/kg) or its vehicle (distilled water). Ethanol and vehicle injections were given according to a schedule which avoided daily drug alternation or exposure to the same drug condition on more than two consecutive sessions, but which provided equal exposure to the two conditions over a 10-day period. The levers designated as water or ethanol levers were counterbalanced across the 6 subjects. During the training sessions, responses made on the appropriate lever were reinforced on an FR 20 schedule. The number of responses on each lever was recorded daily and a discrimination index (DI) was calculated based on correct responses/total responses made prior to delivery of the first reinforcement. Three consecutive days with a DI of 0.80 or better was used as a criterion for ethanol discrimination.

Ethanol discrimination testing. After reaching the ethanol discrimination criterion, the animals were given several drug generalization tests. All test sessions were for 2 min and no reinforcement was given for responses on either lever (extinction conditions). After testing, the animal was returned to the home cage until the next daily training session. The number of responses on each lever was recorded and the percentage of response on the drug lever was calculated. The animal was required to meet the discrimination criterion between each test. The test drugs were injected IP at the intervals described below and all animals were tested under each of the test conditions.

In the first set of tests, the discriminability of decreasing doses of ethanol was tested. Ethanol (1.0, 0.75 and 0.5 g/kg) or water were injected 5 min prior to testing. In the second set of tests, we examined the effect of pretreating the animals with Ro 15-4513 on ethanol discrimination. Ro 15-4513 (0.375, 0.75, or 1.5 mg/kg) or its vehicle were injected 5 min prior to an injection of either EtOH (1.0 g/kg) or its water vehicle and the animal was tested 5 min later. Ro 15-4513 (Hoffmann-La Roche, Basel, Switzerland) was suspended via sonification in a Tween-20 solution. The solution was prepared immediately prior to testing and kept in the dark during testing. In the third set of tests, we examined the effects of giving Ro 15-4513 subsequent to ethanol injections on ethanol discrimination. The drug doses and injection time parameters for this phase of the experiment were based on the earlier report in which Ro 15-4513 attenuated ethanol discrimination in CD-1 mice (18). After completing 10 training trials at criterion performance with water or ethanol (1 g/kg) injected 20 min prior to the training session, the animals were tested. During test sessions Ro 15-4513 (0, 0.15 or 0.375 mg/kg) was injected 10 min later.

Data analysis. The data collected included the number of responses and the percentage of responses made on the drug lever during each 2-min test. These data were subjected to either single or multifactor repeated measures analyses of variance (ANOVA) with ethanol and Ro 15-4513 as factors. Differences between means were statistically assessed with Duncan's test for multiple comparisons.

EXPERIMENT 2

Subjects and Apparatus

Twelve female C57BL/6 drug-naive mice were used. The apparatus was as described above except three additional chambers were used and programming and data acquisition were controlled by an XT computer.

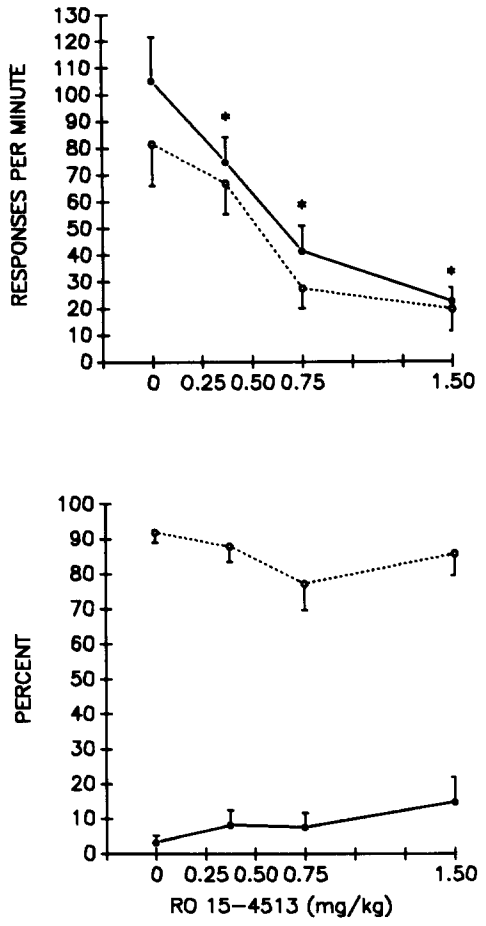

FIG. 2. Response rates (upper graph) and percentage of responses on the ethanol lever (lower graph) during 2-min tests 5 min following injections of either water (solid lines) or ETOH (1.0 g/kg, broken lines) which were preceded by injections of various doses of Ro 15-4513. Data are expressed as mean ± SE and *denotes differences from the Ro vehicle condition.

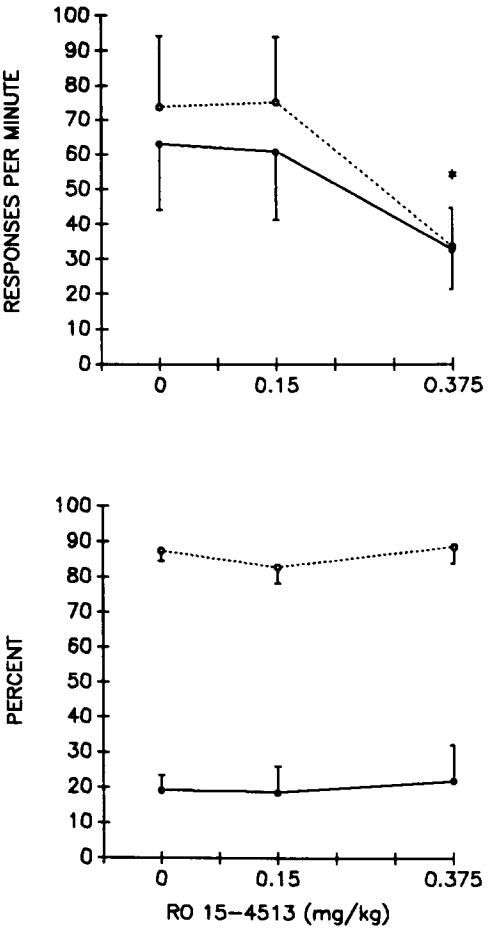

FIG. 3. The legend is the same as for Fig. 2 except that the Ro 15-4513 injections followed rather than preceded water or ETOH injections.

Procedures

The deprivation, lever-response acquisition, and ethanol discrimination training phases of the experiment were as described above. The training dose of ethanol in this experiment was 1.5 g/kg and was injected 20 min prior to the training trial. A previous report (18) indicated discrimination of ethanol at this dose was effectively blocked by Ro 15-4513. Ro 15-4513 (0.40 mg/kg) was injected either 2, 4, or 8 min prior to testing. These time periods were selected on the basis of the absence of Ro 15-4513 effect on ethanol discrimination at 10 min postinjection in Experiment 1 and unpublished data suggesting that Ro 15-4513 effects were of very short duration. The testing procedures and data analysis were as described for Experiment 1.

RESULTS

EXPERIMENT 1

Ethanol Discrimination: Dose-Response

The effect of the various doses of ethanol on response rates and the distribution of responses on the two levers during the two-minute test periods is shown in Fig. 1. Although response rates after the 0.5 and 1.0 g/kg dose of ethanol differed ($p<0.05$, Duncan's t-test), the rates under either of these doses did not differ statistically from vehicle rates. The percentage of responses made on the drug lever increased systematically across doses from approximately 15% on the water test to 85% for the 1.0 g/kg dose, $F(5,15)=53.884$, $p<0.001$. Duncan's test on these data established significant increases for all ethanol doses compared to the vehicle.

Effect of Preethanol Ro 15-4513 Injections on Ethanol Discrimination and Response Rates

These data are summarized in Fig. 2. Ro 15-4513 produced a dose-responsive disruptive effect on response rates, $F(3,15)=17.779$, $p=0.0001$. Although mean response rates after ethanol injections tended to be lower than those after water the effect was only marginally significant, $F(1,5)=6.064$, $p=0.0564$. Importantly, there was no interactive effect of the Ro 15-4513 vs. ethanol/vehicle factor on the response rate measure, $F(3,15)=0.675$. In addition, Ro 15-4513 did not influence the distribution of responses on the two levers, $F(3,15)=1.079$, $p=0.389$, and the drug had no interactive effect with ethanol/water on this measure, $F(3,15)=1.351$.

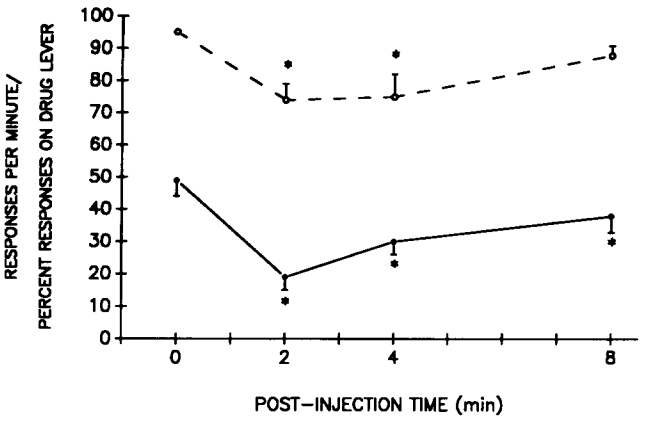

FIG. 4. Response rates (●) and percent responses on the ethanol lever (○) for female mice during 2-min tests beginning 20 min after injections of ethanol (1.5 g/kg) and 2, 4, or 8 min after injections of Ro 15-4513. *Denotes significant differences from the vehicle control (time 0).

Effect of Postethanol Ro 15-4513 Injections on Ethanol Discrimination and Response Rates

These data are summarized in Fig. 3 and are similar to the results illustrated in Fig. 2. Under this testing procedure, Ro 15-4513 again disrupted lever-responding, $F(1,10) = 10.101$, $p = 0.0043$; however, it did not influence the distribution of responses on the two levers, $F(2,10) = 0.228$, and did not interact with ethanol on either measure.

EXPERIMENT 2

Effect of Postethanol Ro 15-4513 Injections on Ethanol Discrimination and Ethanol Disruption of Lever-Responding: Time Course

In this experiment, the effects of Ro 15-4313 on ethanol discrimination were examined beginning 2, 4, and 8 min after injection. These data are summarized in Fig. 4. Compared to performance at 2 min after injection with the Tween 20 vehicle, Ro 15-4513 reduced the percentage of responses on the drug lever, $F(3,47) = 5.999$, $p = 0.002$. Post hoc tests indicated that the percentage of drug lever responses was lower at the 2- and 4-min postinjection tests than at 8-min test or after the vehicle injection. Ro 15-4513 also reduced the number of responses made during the 2-min test period compared to the vehicle control, $F(3,47) = 21.115$, $p = 0.001$. Post hoc tests indicated significant reductions at all three postinjection times.

DISCUSSION

The present study indicates that Ro 15-4513 can attenuate the discrimination of ethanol and disrupt lever responding of C57 mice. Ro 15-4513 disrupted responding at doses and postinjection times which did not influence ethanol discrimination. When tested 10 min after injection, Ro 15-4513 (0.375–1.5 mg/kg) reduced responding of female mice during discrimination testing but had no effect on ethanol discrimination whether given before or after ethanol (Experiment 1). When tested at earlier postinjection times (2–4 min), the drug slightly attenuated ethanol discrimination (Experiment 2); however, only at doses and postinjection times which also disrupted lever responding. That lever responding was disrupted at doses and postinjection times during which ethanol discrimination was unaltered indicates that separate mechanisms must mediate these two effects of Ro 15-4513 and that the response rate was the more sensitive measure.

The present result that Ro 15-4513 given prior to ethanol had no effect on ethanol discrimination was also reported in an earlier study using female rats (6). The absence of a drug effect on ethanol discrimination in each of these studies could be due to the short duration of action of Ro 15-4513 on this measure (<8 min), as noted in Experiment 2. In both the present and earlier study (6), however, Ro 15-4513 reduced responding at the time of discrimination testing indicating that it was biologically active.

In the only other published study of Ro 15-4513 effects on ethanol discrimination, the drug was injected after ethanol exposure, and substantially attenuated ethanol discrimination at doses and time parameters which had little influence on response rates (18). The present study also indicated that Ro 15-4513 given after ethanol attenuated ethanol discrimination; however, the effect was of considerably less magnitude than previously reported and occurred only at doses which disrupted lever-responding. When given at doses of ethanol (1.0 g/kg) and Ro 15-4513 (0.15–0.375 mg/kg) and with injection times similar to those used in the earlier report (18), Ro 15-4513 in our experiment had the opposite effect; it reduced responding but had no influence on ethanol discrimination. The compound attenuated ethanol discrimination in our study only at shorter postinjection times. In spite of the absence of a Ro 15-4513 effect on ethanol discrimination during the later tests in our study, the drug was biologically active as indicated by substantial reductions in response rate (approximately 50% for the highest dose). This degree of response rate reduction was observed in the earlier report (18) only at a dose of 10 mg/kg or higher.

The use of a different gender and mouse strain might contribute to the greater effects of Ro 15-4513 on response rates during discrimination testing in our study compared to the earlier report (18), however, a procedural difference in the drug generalization tests is more likely accountable. In our drug discrimination experiment, mice were tested under extinction conditions, and thus, were never reinforced for responding under the Ro 15-4513 condition. In the earlier study (18), however, reinforcers were given for responses on either the correct or the incorrect lever during the drug test. The latter reinforcement procedure (18) should produce higher response rates under Ro 15-4513, and thus might require higher doses of Ro 15-4513 to reflect a reduction in response rates. This interpretation is supported by another experiment (unpublished) which indicated that Ro 15-4513 disrupted lever-responding maintained by an FR 20 schedule of food reinforcement to about the same extent as that reported for male CD-1 mice (18).

In summary, the present study confirms previous reports that Ro 15-4513 disrupts operant behavior (6, 8, 22). The disruptive effect occurs at low doses and depends on the dose and time after injection. The results are consistent with previous work indicating that the duration of action for Ro 15-4513 is very short. The present study also indicated that Ro 15-4513 can attenuate ethanol discrimination; however, the duration and magnitude of this effect were considerably less than previously reported (18). Although the present study does not resolve the disparity in the literature regarding the effects of Ro 15-4513 on ethanol discrimination, it does suggest that neither gender nor relative order of Ro 15-4513 vs. ethanol injection are likely to account for this disparity. The present results indicate that the Ro 15-4513 effect on ethanol discrimination is of extremely short duration and that postinjection time is a critical variable in these studies.

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