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BRIEF COMMUNICATION

Failure of Ro15-4513 to Alter an Ethanol-Induced Taste Aversion

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JUNE, H. L., P. L. JUNE, K. R. DOMANGUE, L. H. HICKS, G. H. LUMMIS AND M. J. LEWIS. *Failure of Ro15-4513 to alter an ethanol-induced taste aversion.* PHARMACOL BIOCHEM BEHAV **41**(2) 455–460, 1992. – The ability of Ro15-4513, an imidazobenzodiazepine inverse benzodiazepine agonist, to attenuate/block the acquisition of an ethanol (ETOH)-induced conditioned taste aversion (CTA) was investigated in two experiments. Experiment 1 examined the effects of Ro15-4513 (3 mg/kg) on rats' consumption of a novel saccharin solution under a traditional CTA paradigm. Experiment 2 examined the effects of Ro15-4513 (3 mg/kg) on rats' consumption of a novel saccharin solution under a preexposure CTA paradigm. Under the preexposure paradigm, rats were given Ro15-4513 immediately before each of five daily consecutive preexposure treatments prior to the initial conditioning day. To obtain maximal preexposure and unconditioned stimulus effects, a 2-g/kg dose of ETOH (20% v/v) was used in the present study. As previously reported, animals given ETOH following 20-min access to a novel saccharin solution established moderate to strong aversions, with the degree of aversion being directly related to the number of conditioning days. Experiment 1 showed that Ro15-4513 failed to alter the CTA induced by ETOH. Experiment 2 further showed that Ro15-4513 failed to block the preexposure effect exerted on the ETOH-mediated CTA. The results confirm previous reports regarding the failure of Ro15-4513 to disrupt an ETOH-induced CTA. These data are in agreement with a number of behavioral studies demonstrating the failure of Ro15-4513 to antagonize certain actions of ETOH. Moreover, the present study along with a previous report suggests that ETOH-induced CTA's do not appear to be mediated via actions at the GABA-BDZ receptor complex.

Ethanol Conditioned taste aversion Benzodiazepine inverse agonist Ro15-4513

DESPITE the ability of ethanol (ETOH) to function as a reinforcer for many species (31), it can also function as an aversive stimulus in a conditioned taste aversion (CTA) paradigm (6–8,13,22). The apparent paradox that ETOH, as well as other self-administered drugs, possesses both aversive and positive reinforcing properties suggests the possibility that these effects may have a similar underlying neuromechanism of action (14).

Manipulations of the catecholamine systems have been shown to have a robust effect on the formation of a CTA [(28), see (14) for review], as well as on the self-administration of stimulant drugs (32). The role of catecholamines has also been implicated in the stimulant and rewarding properties of ETOH (1,5,23). On the other hand, dopamine, but not noradrenergic manipulations of the catecholamine systems, has been shown to be critical in the acquisition and maintenance of a CTA mediated by ETOH (28). Thus, while the catecholaminergic systems underlying the positive reinforcing properties of ETOH are well documented, less clear are the neuromechanisms mediating the aversive properties.

Recently, Smith and colleagues (29) suggested that the GABA-BDZ systems play a role in the development of an ETOH-induced CTA. These researchers have shown that both picrotoxin, a GABA antagonist (1 mg/kg), and Ro15-4513, a partial inverse BDZ agonist (3 mg/kg), significantly attenuated the development of an ETOH CTA. In contrast, Jeffrey and colleagues (15) employed similar procedures and failed to observe any disruption of the acquisition or maintenance of an ETOH-induced aversion over a broad dose range of Ro15-4513 (0.5–3.0 mg/kg). To further investigate the possibility

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that GABA-BDZ mechanisms play a role in ETOH-mediated CTA's, the present study examined the effects of Ro15-4513 (3.0 mg/kg) on rats' saccharin consumption in both a traditional and a preexposure CTA paradigm. Since it is well documented that preexposure treatment attenuates ETOH CTA's (6,14), determining if Ro15-4513 would block the preexposure treatment effect would provide further information concerning the role of GABA-BDZ mechanisms in ETOH-induced CTA.

EXPERIMENT 1

METHOD

Subjects

Forty naive, male Sprague-Dawley rats (Charles River Breeding Laboratory, Ltd.) weighing between 280–360 g were used in the study. All animals were maintained in a temperature-controlled vivarium and kept on a 12L:12D cycle (lights on at 0700). Subjects had ad lib access to food (Purina Lab Chow), but were water deprived throughout the experiment. Animals were handled and given 1 week to habituate to the animal colony room prior to experimentation.

Apparatus

Subjects were individually housed in stainless steel wire mesh cages. In front of each cage, a graduated drinking tube for the presentation of saccharin and water was attached with stainless steel clips.

Drugs and Solutions

Ro15-4513 was prepared as an emulsion by power agitation (Fisher Scientific Mixer) in a vehicle of 4% Tween-80 in 0.9% sodium chloride solution to a fixed volume. Ethanol was administered as a 20% (v/v) solution, prepared by mixing 95% ETOH in 0.9% sodium chloride solution. All drugs were administered IP and ETOH was injected in a volume sufficient to administer a 2-g/kg dose. Saccharin solutions were 0.10% (w/v) (sodium saccharin, Fisher Purified).

Procedures

Acclimation phase. Animals were placed on a 23-h, 40-min water deprivation schedule for 2 weeks following habituation to the animal colony room. To acclimate the animals to the injection procedures that would be used in the study, rats were injected with Tween-80 vehicle and saline, respectively, every other day immediately after their daily water regimen.

Conditioning phase. Following a 2-week adaptation period to the water deprivation schedule, animals were presented with a novel saccharin solution for 20 min (day 15). Animals were then assigned to one of four groups matched according to their baseline saccharin intake: 1) vehicle + saline; 2) vehicle + ETOH; 3) Ro15-4513 + saline; 4) Ro15-4513 + ETOH. Following saccharin intake, Groups 1 and 2 were given IP injections of Tween-80 vehicle followed 5 min later by an injection of saline or ETOH (2.0 g/kg). Groups 3 and 4 were given injections of Ro15-4513 (3.0 mg/kg) followed 5 min later by injections of saline or ETOH (2.0 g/kg). The interval between Ro15-4513 and ETOH injections was chosen based on several reports from our laboratory (17,18) demonstrating antagonism of ETOH's actions in a number of paradigms. On days 16 and 17, all animals were given 20-min access to tapwater. No injections were given on these water recovery days. This conditioning and 2-day water recovery cycle continued through day 27. Thus, animals received five conditioning trials, with baseline saccharin intake measured prior to conditioning day 1. On the final conditioning day (day 27), animals were presented with saccharin for 20 min but were given no drug injections.

RESULTS

Animals were initially exposed to saccharin on day 15. Mean intake on day 15 represented the baseline intake day; the latter four conditioning days represented true conditioning days.

The major findings of Experiment 1 presented in Fig.1, which shows mean saccharin intake for the four groups during the baseline and across the four conditioning days. These data show that following the initial exposure to saccharin (baseline day) the four groups consumed similar amounts of the saccharin solution. As early as conditioning day 1, a clear reduction in saccharin intake was apparent for both the VEH + ETOH and Ro15-4513 + ETOH groups. This reduction in saccharin intake continued throughout conditioning day 4. In contrast, intake of the VEH + SAL and Ro15-4513 + SAL groups were relatively similar to their baseline across each of the four conditioning days. These patterns resulted in a significant drug treatment X conditioning day interaction, $F(12,144) = 14.62$, $p < 0.01$. Significant simple main effects indicated a significant main effect of treatment for conditioning days 1-4, reflecting the saccharin-attenuation effect of both the VEH + ETOH and Ro15-4513 + ETOH treatments ($p < 0.01$ and $p < 0.01$, respectively). No simple main effect was observed for treatment at the level of the baseline day ($p > 0.05$), indicating that intake of the four groups were similar following initial saccharin exposure. Significant simple main effects also revealed a significant main effect of day at both the VEH + ETOH and Ro15-4513 + ETOH levels ($p < 0.01$ and $p < 0.01$, respectively). This finding was due to both VEH + ETOH and Ro15-4513 + ETOH pretreated groups exhibiting greater aversions across conditioning days. Pairwise comparisons confirmed that animals pretreated with

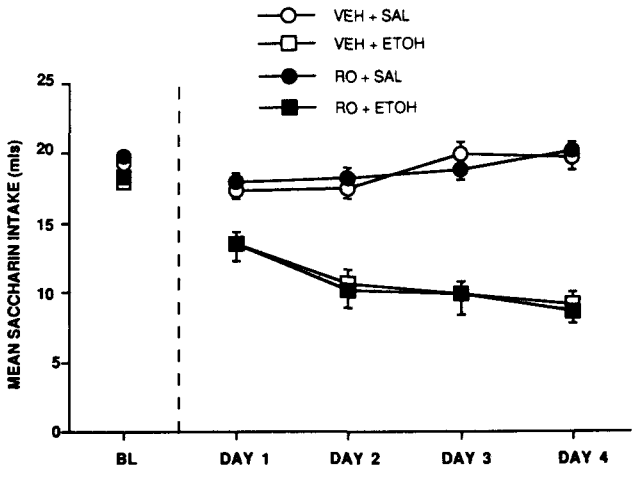

FIG. 1. Mean saccharin consumption (ml) for animals in Groups 1) Tween-80 vehicle + saline (VEH + SAL), 2) Tween-80 vehicle + ETOH (VEH + ETOH), 3) Rol5-4513 + saline (RO + SAL), and 4) Rol5-4513 + ETOH (RO + ETOH) on the baseline and the four conditioning trials. All groups were tested for 20 min. Bars represent ± SEM.

VEH + ETOH established a stronger aversion at day 4 (mean = 7.5 ± 0.91) that was greater than day 3 (mean = 8.9 ± 1.4) ($p < 0.05$), which in turn was greater than day 2 (mean = 9.8 ± 1.1) ($p < 0.05$) and day 1 (mean = 12.0 ± 1.2) ($p < 0.05$). A similar pattern occurred for Ro15-4513 + ETOH pretreated group (see Fig. 1), with the exception being that during conditioning days 2 and 3 animals' intake of saccharin was relatively similar (mean = 9.3 ± 1.2 and 8.7 ± .93, respectively).

EXPERIMENT 2

The results of Experiment 1 demonstrated that Ro15-4513 was ineffective in blocking the acquisition of an ETOH-induced CTA in rats. To further examine the potential blocking properties of Ro15-4513 in a CTA paradigm, Experiment 2 investigated the effects of Ro15-4513 on the ETOH preexposure effect. Employing these procedures, we attempted to characterize potential blocking properties of Ro15-4513 on ETOH-mediated taste aversions not detectable by previous investigators (15,29) when using a traditional CTA paradigm [see (6)].

METHOD

Subjects and Apparatus

Thirty naive, male Sprague-Dawley rats of similar age and body weight as Experiment 1 were used in Experiment 2. Fluid intake was determined in a manner similar to that of Experiment 1.

Drugs

All drugs and solutions were prepared in a similar manner to Experiment 1.

Procedures

Preexposure treatment phase. The initial acclimation procedures for Experiment 2 were identical to those of Experiment 1; however, 5 days before the baseline day, 10 of the 30 animals matched according to their average water consumption on day 9 were assigned to one of two ETOH groups. These 10 animals were given five daily consecutive preexposure injections of ETOH (2.0 g/kg, IP) (days 10–14), while the other 20 were given an equivalent volume of saline. Immediately prior to these injections, one half of the ETOH injected animals ($n = 5$) were randomly assigned to the Ro15-4513 group and the other half ($n = 5$) to the Tween-80 vehicle group. Animals assigned to the Ro15-4513 group were given a daily Ro15-4513 (3.0 mg/kg) injection 5 min prior to each ETOH preexposure treatment, while animals assigned to the vehicle group were given injections of Tween-80 vehicle. The remaining 20 animals were administered a second injection of saline.

Conditioning phase. On day 15, all animals were presented with a novel saccharin solution for 20 min. Following baseline saccharin intake, the 20 animals who received saline alone during the preexposure phase were matched according to their baseline saccharin intake and assigned to one of four groups ($n = 5$): 1) vehicle + 5 saline pretreatments + saline; 2) vehicle + 5 saline pretreatments + ETOH; 3) Ro15-4513 + 5 saline pretreatments + saline; 4) Ro15-4513 + 5 saline pretreatments + ETOH. The 10 animals given ETOH preexposure treatment were assigned to the Ro15-4513 + 5 ETOH pretreatments + ETOH (Group 5) or vehicle + 5 ETOH pretreatments + ETOH (Group 6) groups ($n = 5$) depending on their preexposure treatment. On days 16 and 17, all animals were given 20 min access to tapwater. No injections were given on these water recovery days. This conditioning and 2-day water recovery cycle continued throughout day 27. Hence, animals received five conditioning trial days with baseline saccharin intake being measured prior to conditioning day 1. On day 27, animals were presented with saccharin for 20 min but were given no injections.

Method of Analysis

The design comprised a 6 × 5 mixed analysis of variance (ANOVA) with the first factor representing treatment condition (vehicle + 5 saline pretreatments + saline; vehicle + 5 saline pretreatments + ETOH; Ro15-4513 + 5 saline pretreatments + saline; Ro15-4513 + 5 saline pretreatments + ETOH; Ro15-4513 + 5 ETOH pretreatments + ETOH; vehicle + 5 ETOH pretreatments + ETOH) and the second day (baseline day and conditioning days 1–4). Repeated measures were made of the day factor.

RESULTS

Figure 2 shows mean saccharin intake for the six groups ($n = 5$) during the baseline and across the four conditioning days of Experiment 2. These data show that following the initial exposure to saccharin (baseline day) no significant differences were observed among the six groups ($p > 0.05$). Similar to Experiment 1, as early as conditioning day 1 a clear reduction in saccharin intake was observed among the vehicle + 5 saline pretreatments + ETOH and the Ro15-4513 + 5 saline pretreatments + ETOH groups. In contrast, ETOH

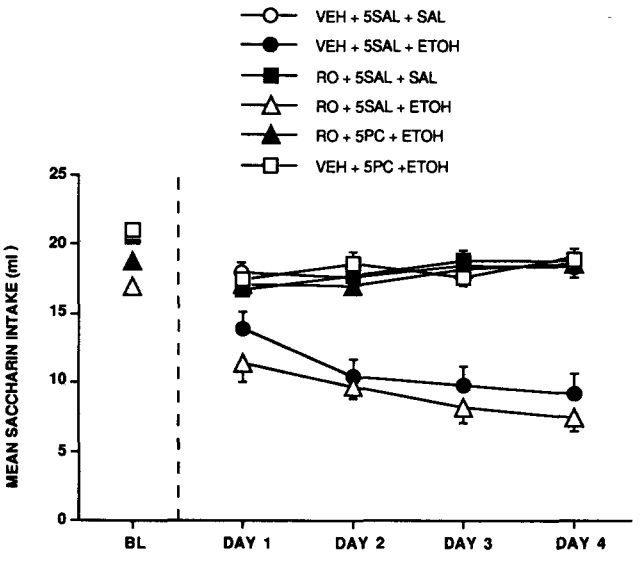

FIG. 2. Mean saccharin consumption (ml) for animals in Groups 1) Tween-80 vehicle + five saline pretreatment + saline (VEH + 5SAL + SAL), 2) Tween-80 vehicle + five saline pretreatments + ETOH (VEH + 5SAL + ETOH), 3) Rol5-4513 + five saline pretreatments + saline (RO + 5SAL SAL), 4) Rol5-4513 + five saline pretreatments + ETOH (RO + 5SAL + ETOH), 5) Rol5-4513 + five ETOH pretreatments + ETOH (RO + 5PC + ETOH) and 6) Tween-80 vehicle + five ETOH pretreatments + ETOH (VEH + 5PC + ETOH) on the baseline and the latter four conditioning days. All groups were tested for 20 min. Bars represent ± SEM. Outer error bars not shown do not expand beyond the radius of the symbol.

preexposure treatment (e.g., groups Ro15-4513 + 5 ETOH pretreatments + ETOH & vehicle + 5 ETOH pretreatments + ETOH) completely blocked the saccharin-attenuation effect as was observed among the vehicle + 5 saline pretreatments + ETOH and the Ro15-4513 + 5 saline pretreatments + ETOH groups (see Fig. 2). These patterns resulted in a significant drug treatment X conditioning day interaction, $F(20,96) = 3.71$, $p < 0.01$. Significant simple main effects indicated a significant main effect of treatment for conditioning days 1-4, reflecting the saccharin-attenuation effect for the vehicle + 5 saline pretreatments + ETOH and Ro15-4513 + 5 saline pretreatments + ETOH groups. Mean intake of the vehicle + 5 saline pretreatments + saline, Ro15-4513 + 5 saline pretreatments + saline, vehicle + 5 ETOH pretreatments + ETOH and Ro15-4513 + 5 ETOH pretreatments + ETOH groups was similar at baseline compared to each of the four conditioning days ($p > 0.05$, $p > 0.05$, $p > 0.05$, and $p > 0.05$, respectively). Thus, animals treated with Ro15-4513 immediately prior to receiving ETOH preexposure treatment failed to exhibit a reduction in saccharin intake across any of the four conditioning days. In contrast, mean intake for the vehicle + 5 saline pretreatments + ETOH and the Ro15-4513 + 5 saline pretreatments + ETOH groups across the four conditioning days was substantially lower than during baseline day ($p < 0.05$, $p < 0.05$, $p < 0.05$, and $p < 0.05$, respectively). These findings were confirmed by significant simple main effects of day at the vehicle + 5 saline pretreatments + ETOH and Ro15-4513 + 5 saline pretreatments + ETOH levels ($p < 0.05$ and $p < 0.05$, respectively) and were due to the fact that both groups showed greater aversions across conditioning days compared to their baseline intake (see Fig. 2).

GENERAL DISCUSSION

The results of the present study showed that similar to previous research (6,21,22) the aversive properties of ETOH (2 g/kg) can be demonstrated reliably using a CTA paradigm. The primary purpose of the present study, however, was to investigate if GABA-BDZ mechanisms play a role in mediating these effects.

Experiment 1 demonstrated that Ro15-4513 was completely ineffective in attenuating the establishment of an ETOH-induced aversion; animals treated with Ro15-4513 + ETOH demonstrated a saccharin aversion identical to that of the animals treated with ETOH alone (Fig. 1). Moreover, Ro15-4513 failed to exert any intrinsic activity of its own on saccharin intake. This latter finding is consistent with several reports from our laboratory, as well as others, demonstrating a general lack of intrinsic activity by Ro15-4513 on the fluid consumption in deprived (15,16,19) and nondeprived rats (17,27).

The results of Experiment 2 showed that similar to previous reports demonstrating the robustness of the preexposure effect (6) five daily consecutive ETOH preexposure treatments completely prevented the acquisition of an ETOH CTA using a 2 g/kg conditioning dose. When Ro15-4513 was administered prior to preexposure treatments in animals subsequently conditioned with ETOH, animals' intake of saccharin was similar to that of animals who received 5 ETOH preexposures and no Ro15-4513 (vehicle + 5 preexposures + ETOH) or animals given control injections. This finding clearly suggests that Ro15-4513 was ineffective in attenuating/blocking the ETOH preexposure effect on the ETOH CTA. Similar to Experiment 1, Experiment 2 also showed that Ro15-4513 appeared devoid of any intrinsic activity during the preexposure phase of the study.

The findings of the present study are consistent with those of Jeffrey and colleagues (15), who showed that Ro15-4513 was ineffective in blocking an ETOH-mediated CTA at doses (0.5-3.0 mg/kg) previously reported to be effective in blocking GABA-mediated effects [for review, see (11) and (30)]. In contrast, the results of the present study are at variance with those of Smith and colleagues (29), who found that Ro15-4513 (3 g/kg) significantly attenuated the aversion induced by an ETOH conditioning dose of 1.2 g/kg. The methods employed by the three studies were relatively similar, with the exception of the conditioning doses and delay interval between Ro15-4513 administration and ETOH injections. Specifically, all three studies used a 20% v/v concentration of ETOH for the conditioning dose and a similar pretreatment dose of Ro15-4513 (3 mg/kg). It is possible, however, that the ETOH-induced aversions were greater in the Jeffrey et al. and the present study than those in the Smith et al. study. Thus, Ro15-4513 may have been capable of attenuating these weaker aversions but unable to block the stronger ones. The Jeffrey et al. and the present study employed a 1.75 and a 2.0 g/kg conditioning dose, respectively, while the conditioning dose used by Smith et al. was slightly lower (1.25 g/kg). A number of reports have demonstrated dose-dependent CTA's with ETOH (2,21,26). Thus, it is possible that higher doses of Ro15-4513 might attenuate/block an ETOH-mediated CTA.

In the Smith et al. study, the interval between administration of Ro15-4513 and ETOH was 50 min, while in the Jeffrey et al. and the present study the delay interval was 0 and 5 min, respectively. A number of studies have suggested that Ro15-4513 has a rapid and short duration of action (9,16, 17,24,25). Because the half-life of Ro15-4513 is not yet known, it is not clear whether its absorption/distribution time course overlapped the aversive properties induced by ETOH. However, we (16,17,19,20) as well as others (25) have characterized the time course of Ro15-4513's antagonistic actions on ETOH self-administration in a number of rodent species. While a number of these reports have shown that Ro15-4513 substantially attenuates ETOH intake across 30- to 60-min consumption intervals, Ro15-4513 was always administered 5-10 min prior to the experimental session. Thus, while the delay interval between Ro15-4513 and ETOH injection may be an important factor in the contrasting results between the above studies, it is also possible that our failure to block the ETOH-induced aversion may be related to the uniqueness of the CTA paradigm. Hence, the CTA paradigm may represent a behavioral measure that is not as sensitive to the ETOH antagonistic actions of Ro15-4513 as numerous other tasks that have been reported in the literature.

Jeffrey et al. (15) suggested that one possible explanation of Ro15-4513's failure to block an ETOH-mediated aversion may be related to its lack of intrinsic activity in this paradigm. This explanation, however, is based on the finding of previous research (4,22) demonstrating that the intrinsic effects of Ro15-4513 are in opposition to that of ETOH. While this interpretation supports an additivity hypothesis regarding the blocking of ETOH's action [see (4), (15), and (24)], several investigators have shown that Ro15-4513 is capable of antagonizing the actions of ETOH in a number of paradigms without exerting any intrinsic actions of its own (16-20,23). Specifically, we (17) as well as McBride and colleagues (25) demonstrated that using a 5-min postinjection interval, similar to that used in the present study, Ro15-4513 attenuated the self-administration of ETOH in both randomly (17) and genetically bred alcohol-preferring and nonpreferring rats (25)

across 60- and 120-min consumption intervals, respectively. Similar results regarding the failure of Ro15-4513 to exert any intrinsic activity in a self-administration paradigm have also been obtained by Samson and colleagues (28) using operant procedures. Considering the previous research, this explanation does not seem sufficient to account for the results of the present study.

Finally, several investigators have reported that GABA-BDZ receptor ligands do not appear to alter ETOH-induced behaviors believed to be mediated primarily via catecholamines (3,10,12). As mentioned previously, however, some catecholaminergic manipulations (e.g., noradrenergic) also do not have a disruptive effect on the acquisition of a CTA mediated by ETOH (28). It is possible then that the aversive properties of ETOH may in part be mediated via other neuromechanisms than those involving the catecholamines or GABA-BDZ receptor complex.

In summary, the present study demonstrated that Ro15-4513 was ineffective in preventing the acquisition or disruption of an ETOH-induced CTA in rats. The present study also showed that Ro15-4513 failed to attenuate/block the preexposure effect typically observed following preexposure treatment with ETOH (6). The failure of Ro15-4513 to block this effect further suggests that Ro15-4513 does not interfere with the acquisition of an ETOH CTA. The results confirm the findings of previous investigators (15) regarding the failure of Ro15-4513 to alter an ETOH-induced aversion. Moreover, the results suggest that while GABA-BDZ mechanisms may play a significant role in the reinforcing properties of ETOH [see (27)] they do not appear to be as critical in mediating the aversive properties of ETOH when assessed by a traditional or preexposure CTA paradigm.

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