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Interactions Between RO 15-4513 and Ethanol on Brain Self-Stimulation and Locomotor Activity in Rats

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SCHAEFER, G. J. AND R. P. MICHAEL. *Interactions between RO 15-4513 and ethanol on brain self-stimulation and locomotor activity in rats.* PHARMACOL BIOCHEM BEHAV **34**(4) 785–790, 1989.—Experiments were conducted to elucidate the behavioral effects of RO 15-4513, a putative alcohol antagonist, when administered alone or in combination with alcohol. Two groups of animals were trained to lever-press for brain self-stimulation (ICSS) on either a fixed ratio:15 or a fixed interval:15 second schedule of reinforcement. RO 15-4513 (0.1–3.0 mg/kg) reduced the rate of lever-pressing for ICSS in both groups. RO 15-4513 (1.0 mg/kg) further reduced rates when combined with alcohol (0.1–1.7 g/kg), and this effect was especially marked in the fixed ratio paradigm. Other groups of animals were tested in a locomotor activity apparatus. In contrast to the depression of lever-pressing in the ICSS experiments, RO 15-4513 produced a graded increase in locomotor activity. When combined with alcohol (0.1–1.7 g/kg), 1.0 mg/kg RO 15-4513 also increased locomotor activity. Thus, the depression in schedule-controlled behavior was not associated with a generalized behavioral depression. These results demonstrated that RO 15-4513 has potent behavioral effects of its own that are consistent with its classification as an anxiogenic compound.

RO 15-4513 Brain self-stimulation Fixed ratio:15 schedule Fixed interval:15 second schedule Locomotor activity Anxiogenic drug

THERE is widespread interest in the development of clinically effective alcohol antagonists (9). An important drug now being investigated in this regard is RO 15-4513, a partial inverse agonist at benzodiazepine receptors (5, 12, 27). Behavioral investigations have emphasized the effects of RO 15-4513 alone and in combination with alcohol on various measures of motor activity and motor coordination. Less well-studied are the effects of RO 15-4513 on more complex operant conditioning measures which have relevance for the human situation where a drug or drug combination may have subtle but critical effects on, for example, the behavior involved in operating machinery or driving an automobile.

We describe here the effects of RO 15-4513 either alone or in combination with alcohol on operant behavior reinforced by intracranial self-stimulation (ICSS). In a previous study the effects of alcohol on responding for ICSS depended upon the schedule of reinforcement used (24); at equal doses, alcohol produced a greater decrease in response rates in a fixed ratio than in a fixed interval schedule. In addition, intermittent schedules are very sensitive to the rate-altering properties of the opiate antagonists (21), and we have applied the analysis of schedule-controlled behavior to the study of RO 15-4513. It was also of interest to determine if, in our hands, RO 15-4513 would alter locomotor activity and if this might account for any changes in operant behavior. Results were consistent with the classification of RO 15-4513 as an anxiogenic compound with marked behavioral effects which are independent of its anti-alcohol properties.

METHOD

Animals

A total of 43 adult male Sprague-Dawley rats bred from stock purchased from Charles River Laboratories (Wilmington, MA) were used. Animals used for brain self-stimulation (ICSS) weighed 388–615 g and were 70–140 days old at the time of electrode implantation, and those used in the locomotor activity experiments weighed 555–715 g and were 115–140 days old at the beginning of testing. All animals were housed 3–4 to a cage with fresh food and water available ad lib. When not being tested, the animals were maintained in a colony room where the lights were on from 07:00 to 19:00 hr. All housing and experimental procedures were conducted according to the Institutional regulations and the *NIH Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23, revised 1985).

Apparatus

The operant chambers used for ICSS measured 31 cm long, 30

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cm wide and 29 cm high (inside dimensions). A single conventional lever (Model G6312, Ralph Gerbrands, Arlington, MA) was positioned 10 cm above a grid floor on one side of the aluminum walls. The chamber was placed inside a ventilated box that was sound attenuated and light proof. Electrical pulses were produced by a biphasic, constant-current stimulator (22), and consisted of 200 msec trains of square-wave pulses at 100 Hz with a pulse duration of 0.5 msec. Stimulus currents ranged from 26–500 μA (median = 50 μA) in the fixed-ratio studies, and from 100–420 μA (median = 210 μA) in the fixed interval studies. The stimuli were delivered to the animal's brain through a two-channel commutator (Model 590, Mercotac, San Diego, CA), and spring-shielded hearing-aid wire (Plastic Products, Roanoke, VA) that allowed the animal freedom of movement about the chamber. Programming the test sessions and data collection were obtained with a combination of +28 and +5 volt DC modules.

An OmniTech Digiscan RXY activity monitor (Columbus, OH) interfaced with a Behavioral Control Unit was used to measure activity. During test sessions the animal was placed in a Plexiglas box (39.4 × 39.4 × 30.5 cm high, inside dimensions) in the Digiscan. The Digiscan monitor and Plexiglas box were placed inside a sound-attenuating chamber illuminated with a 25 watt red light bulb which produced 32 lux in the center of the floor of the Plexiglas box. A fan provided fresh air and also served as a source of masking noise. Horizontal activity was measured by counting the total number of interruptions of infrared photobeams. Ambulatory activity was also measured by the number of beam interruptions, but repetitive breaking of the same beam was ignored. In addition, the Behavioral Control Unit measured the amount of time (to the nearest 1/10 second) that animals were inactive (Channel 1) as well as moving at one of three pre-selected speeds (Channel 2 = slow, Channel 3 = moderate, Channel 4 = fast). The settings for Channels 2, 3 and 4 were based on previous work with amphetamine and haloperidol, and they allowed us to demonstrate both increases and decreases in activity (23); this was an important consideration since it could not be anticipated what the effects of RO 15-4513 might be. The channels' analysis has also been shown to correlate highly both with horizontal activity and with an observational method.

Surgery and Histology

Animals were deeply anesthetized with sodium pentobarbital (50 mg/kg, IP) and given atropine sulfate (0.25 mg, SC) to reduce any respiratory distress. After placement in a stereotaxic device, the skull was exposed and 4–5 stainless screws were inserted. A burr-hole was made in the skull, the dura incised, and a bipolar platinum electrode (tip diameter = 0.125 mm, Plastic Products Co.) was lowered into the lateral hypothalamus (medial forebrain bundle) using coordinates from the Pellegrino *et al.* (17) system (AP 5.2, L 1.7, H −2.2). To obtain a secure foundation on the skull, cranio-plastic cement was applied to the screws and electrode. The animals were given an IM injection of 100,000 U of benzathine penicillin G and procaine penicillin G postoperatively together with 1 mg/kg flunixin meglumine (Banamine®, Schering, Kenilworth, NJ) to prevent any postoperative discomfort. When ICSS testing was completed, animals were killed with a large overdose of sodium pentobarbital and perfused via the heart with 10% formalin. The tissue was fixed, and frozen sections were cut at 50 μm. Alternate sections were stained with cresyl violet and Weil's stain and viewed under a microprojector to locate the sites of the electrode tips.

Procedure

Animals were allowed at least one week to recover from surgery, and were then placed in the operant chamber and trained to press a lever to deliver electrical stimuli to their brains. Animals in one group were trained to lever press for ICSS on a fixed ratio:15 (FR:15) schedule. When responding was stable to within ± 10% of a weekly mean rate, these animals (n = 11) were injected intraperitoneally (IP) with vehicle (Mondays and Thursdays) and on Tuesdays and Fridays were injected with RO 15-4513 in a nonsystematic order (0.1, 0.3, 1.0, 3.0 mg/kg). Following this, animals were tested with vehicle on Mondays and Thursdays, and on Tuesdays and Fridays were injected IP with either alcohol (0.1, 0.3, 0.56, 1.0, 1.7 g/kg) or with 1.0 mg/kg RO 15-4513 plus graded doses of alcohol. The baseline response rates of two animals became unstable and they were replaced with two other animals, so the total of 11 animals remained unchanged. Alcohol was given in a volume of 10 ml/kg and RO 15-4513 was given in a volume of 1.0 ml/kg. Both drugs were administered five minutes before a 20-minute test session was begun.

Animals in a second group (n = 10) were trained to lever-press for ICSS on a fixed interval:15 second (FI:15 sec) schedule. When response rates were stable, they were tested with vehicle, RO 15-4513, alcohol and combinations of RO 15-4513 and alcohol using the same procedure as for the FR:15 experiments.

Locomotor activity was assessed by placing animals individually in the activity apparatus and allowing them to habituate for 30 minutes. The apparatus was always thoroughly cleaned before the next animal was tested. Animals in one group (n = 10) were injected IP either with vehicle or with RO 15-4513 (0.1, 0.3, 1.0, 3.0 mg/kg). Animals in a second group (n = 10) were injected IP either with vehicle, alcohol (0.1, 0.3, 1.0, 1.7 g/kg) or 1.0 mg/kg RO 15-4513 in combination with graded doses of alcohol. Animals were returned to the test apparatus immediately after receiving injections and activity was measured for the next 60 minutes; data from the first 30 minutes were used for statistical analyses because of the relatively short half-life of RO 15-4513 (13).

Drugs

Absolute ethyl alcohol was first diluted with 0.9% saline to give a stock solution of 30% w/v. From this, dilutions of 0.17, 0.10, 0.056, 0.03 and 0.01 g/ml were made by the addition of 0.9% saline to aliquots of the stock. RO 15-4513 was suspended in a 1:1 mixture of 15% propylene glycol and 1% Tween 80 (Sigma, St. Louis, MO). The suspension was dispersed by ultrasound prior to an IP injection.

Data Analysis

The total number of lever presses during 20-minute test sessions provided the data for analysis. Scores for all vehicle days (4–5 determinations) were averaged and response rates for drugs were expressed as percentages of the mean vehicle score. To evaluate the effects of RO 15-4513 alone, analyses of variance for repeated measures (ANOVA) were performed on the response rates (SPSS/PC+, SPSS Inc. Information Analysis Systems, Chicago, IL). To compare alcohol alone and in combination with RO 15-4513, an overall ANOVA was performed, followed by ANOVAs for the main effect of dose in each drug combination. Dunnett's test (two-tailed) was then used to compare response rates during vehicle administration with those following graded doses of RO 15-4513 alone, alcohol alone and combinations of alcohol and 1.0 mg/kg RO 15-4513.

In tests of locomotor activity, data from the first 30 minutes of the 60-minute session were used. For each of the six measures of activity, scores for vehicle and graded doses of RO 15-4513 alone

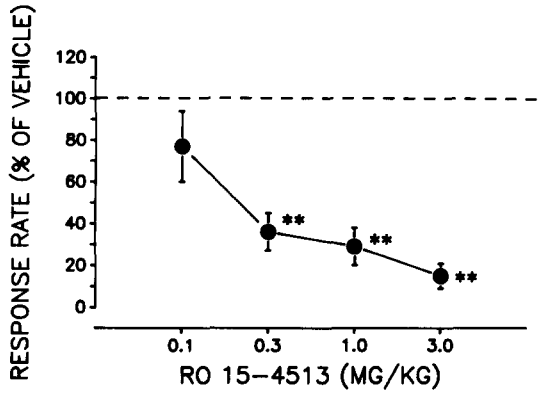

FIG. 1. Effects of graded doses of RO 15-4513 on rates of lever-pressing for ICSS on a fixed ratio:15 schedule of reinforcement. Data are expressed as percentages of the mean score when vehicle was administered (----). Vertical bars give standard errors of means (SEM). The mean ± SEM response rate for all vehicle sessions was 871 ± 116 presses per 20-minute session. N = 11. **Significantly different from vehicle at $p<0.01$ by Dunnett's test.

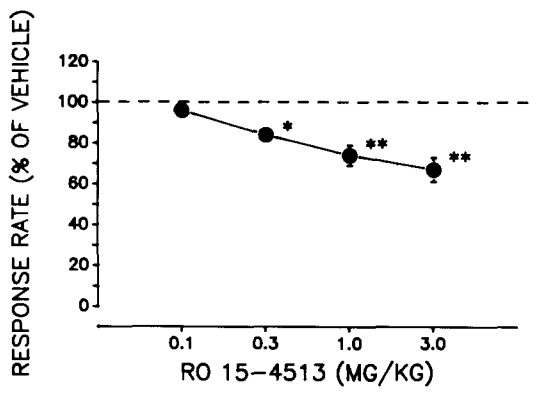

FIG. 3. Effects of graded doses of RO 15-4513 on rates of lever-pressing for ICSS on a fixed interval:15 second schedule of reinforcement. Data are expressed as percentages of the mean score when vehicle was administered. The mean ± SEM response rate for all vehicle sessions was 440 ± 34 presses per 20-minute session. N = 10. *Significantly different from vehicle at $p<0.05$; **$p<0.01$ by Dunnett's test.

and for the combination of 1.0 mg/kg RO 15-4513 and graded doses of alcohol were analyzed using ANOVAs followed by Dunnett's test.

RESULTS

Figure 1 shows that RO 15-4513 when administered alone produced a dose-related decrease in the rate of responding on a FR:15 schedule of reinforcement, $F(4,40) = 15.8$, $p<0.001$. These reductions were significant at doses of 0.3 mg/kg and above ($p<0.01$, Dunnett's test). At the highest dose, the rate of responding was less than 20% of control rates. The effects of alcohol alone and in combination with 1.0 mg/kg RO 15-4513 are shown in Fig. 2. The overall ANOVA showed significant main effects of drug, $F(1,50) = 142.8$, $p<0.001$, and of dose, $F(5,50) = 100.2$, $p<0.001$, and a significant drug by dose interaction, $F(5,50) = 29.8$, $p<0.001$. When alcohol was administered alone, a significant depression of lever-pressing was not observed until the 0.56 g/kg dose was given. In marked contrast, when RO 15-4513 was administered in combination with graded doses of alcohol, there were depressions at all doses. Thus, the two dose-response curves were quite dissimilar, and the decreases in response rates produced by alcohol at higher doses were not antagonized by RO 15-4513.

RO 15-4513 appeared to have a smaller effect on the rate of lever-pressing for ICSS on the fixed interval schedule (FI:15 sec), but this effect was dose-dependent (Fig. 3), $F(4,36) = 12.4$, $p<0.001$. At the highest dose, rates were reduced to 67 ± 6% of control values. Figure 4 shows the effects of graded doses of alcohol alone and of alcohol in combination with 1.0 mg/kg RO 15-4513. By itself, alcohol produced significant decreases in response rates at doses of 0.56–1.7 g/kg, but the decreases were

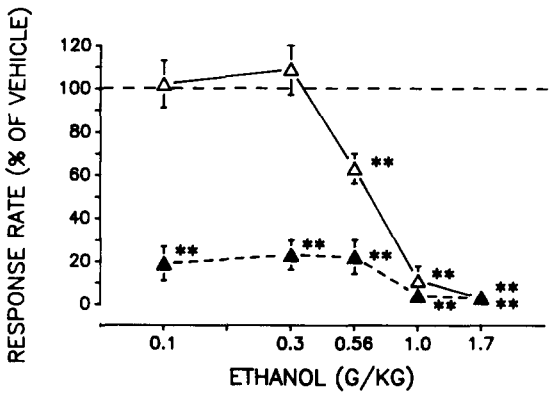

FIG. 2. Effects of graded doses of alcohol alone (△) and of 1.0 mg/kg RO 15-4513 with graded doses of alcohol (▲) on rates of lever-pressing for ICSS on a fixed ratio:15 schedule of reinforcement. The depression of lever-pressing by higher doses of alcohol was not antagonized by RO 15-4513. Data are expressed as percentages of the mean score when vehicle was administered. Where standard error bars are missing in this and subsequent figures, they were smaller than the symbol. The mean ± SEM response rate for all vehicle sessions was 1178 ± 87 presses per 20-minute session. N = 11. **Significantly different from vehicle at $p<0.01$ by Dunnett's test.

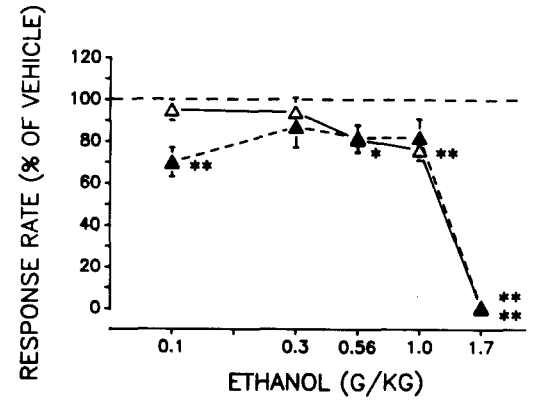

FIG. 4. Effects of graded doses of alcohol alone (△) and of 1.0 mg/kg RO 15-4513 with graded doses of alcohol (▲) on rates of lever-pressing for ICSS on a fixed interval:15 second schedule of reinforcement. In this paradigm there were no significant differences between the effects of alcohol alone and of alcohol and RO 15-4513 combined except at the 0.1 g/kg dose. Data are expressed as percentages of the mean score when vehicle was administered. The mean ± SEM response rate for all vehicle sessions was 415 ± 24 presses per 20-minute session. N = 10. *Significantly different from vehicle at $p<0.05$; **$p<0.01$ by Dunnett's test.

TABLE 1

EFFECTS OF GRADED DOSES OF RO 15-4513 ON DIFFERENT MEASURES OF LOCOMOTOR ACTIVITY OF RATS IN THE DIGISCAN APPARATUS DURING THE FIRST 30 MINUTES OF THE TEST SESSION

	Dose of RO 15-4513 (mg/kg)						
Measure*	Vehicle	0.1	0.3	1.0	3.0	F-ratio	p
Horizontal Activity (Beam interruptions)	556 ±125	647 ±115	558 ±111	785 ±95	857 ±116	2.3	n.s.
Ambulatory Activity (Beam interruptions)	224 ±63	276 ±60	222 ±58	342 ±50	359 ±58	2.1	n.s.
Channel 1 (Seconds)	1647 ±31	1618 ±33	1644 ±29	1590 ±25	1555 ±34	2.4	n.s.
Channel 2 (Seconds)	109 ±18	136 ±22	120 ±18	148 ±6	187† ±26	3.2	0.025
Channel 3 (Seconds)	36 ±11	43 ±11	34 ±12	56 ±10	59 ±9	1.7	n.s.
Channel 4 (Seconds)	5 ±2	3 ±1	2 ±1	6 ±1	5 ±1	1.5	n.s.

*See the Apparatus section for description of units. Values are numbers of beam interruptions or seconds ± standard errors of mean (SEM). †Significantly different from vehicle by Dunnett's test, $p<0.01$.

less at 0.56 and 1.0 g/kg on the fixed interval schedule than on the fixed ratio schedule. When alcohol was administered together with RO 15-4513, the dose-response profile remained virtually unchanged. While the main effect of drugs was not significant, the effect of doses was highly significant, $F(5,45)=132.8$, $p<0.001$. The drug by dose interaction was also significant, $F(5,45)=3.2$, $p<0.02$, because of the effect at 0.1 g/kg alcohol, where the drug combination produced a significant decrease in rate.

When animals were administered RO 15-4513, increases in motor activity occurred. Table 1 shows data for six measures of locomotor activity during the first 30 minutes of the test session. Increases were most reliably observed in movements at slow speed (Channel 2), $F(4,36)=3.2$, $p<0.025$, and the 3.0 mg/kg dose produced a significant increase above that during the administration of vehicle (Dunnett's test, $p<0.01$). When 1.0 mg/kg RO 15-4513 was administered together with graded doses of alcohol, activity was increased at all dose-levels compared with the administration of alcohol alone (Fig. 5). For horizontal activity (left panel) the two lower doses of alcohol together with RO 15-4513 showed marked increases compared with that of alcohol alone. The overall ANOVA produced a significant main effect of drug, $F(1,36)=47.9$, $p<0.001$, and of dose, $F(4,36)=2.7$, $p<0.05$. Alcohol appeared to counteract some of the effects of RO 15-4513; the combination of 1.0 mg/kg RO 15-4513 and 1.7 g/kg alcohol resulted in a smaller increase in activity than did that of 1.0 mg/kg RO 15-4513 alone ($p<0.05$). A similar pattern of results was seen for Channel 2 (right panel). The overall ANOVA produced a significant main effect of drug, $F(1,36)=120.6$, $p<0.001$, and of dose, $F(4,36)=4.1$, $p<0.01$, and a significant drug by dose interaction, $F(4,36)=2.7$, $p<0.05$. No significant changes in activity occurred over the entire dose-range 0.1–1.7 g/kg alcohol alone.

For the 23 animals used in the ICSS experiments, 18 electrodes terminated in the lateral hypothalamus and 5 terminated in the adjacent zona incerta. Termination sites overlapped for animals in the fixed ratio and fixed interval groups, and overall there was a lateral distribution from the fornix to the internal capsule, and a vertical distribution from the zona incerta to the ventral premamillary nucleus of the hypothalamus.

DISCUSSION

These experiments demonstrated that RO 15-4513, an inverse agonist at benzodiazepine receptors, generally depresses behavior, but under certain conditions can also stimulate it. By itself, RO 15-4513 tended to increase locomotor activity (Table 1) and it also increased activity in animals that were administered alcohol (Fig. 5). In contrast, it decreased the rate of lever-pressing for ICSS (Figs. 1 and 3), and further decreased responding in the presence of alcohol (Figs. 2 and 4). The changes in operant behavior occurred both in the fixed interval paradigm, where control rates were relatively low (0.3 responses per second), and in the fixed ratio paradigm where control rates were 2–3 times higher. However, there were quantitative differences in these effects, and the reductions in rate brought about by RO 15-4513 were greater in the fixed ratio than in the fixed interval paradigm. When administered

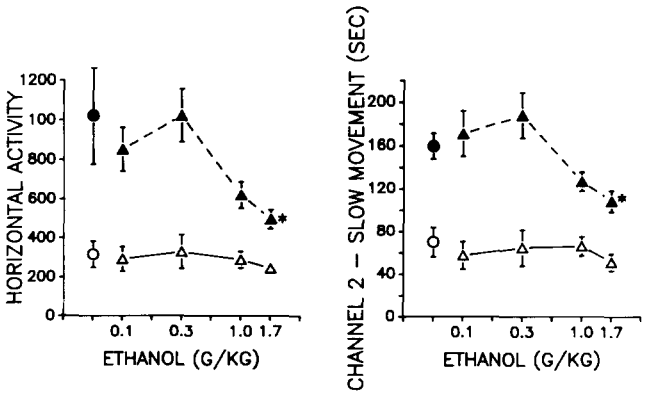

FIG. 5. Effects of vehicle (○), 1.0 mg/kg RO 15-4513 (●), graded doses of alcohol alone (△) and of 1.0 mg/kg RO 15-4513 together with graded doses of alcohol (▲) on Horizontal Activity (left panel) and on the amount of time animals were moving at a slow speed (Channel 2) (right panel) in the Digiscan activity apparatus. N = 10. *Significantly different from 1.0 mg/kg RO 15-4513 alone at $p<0.05$ by Dunnett's test.

together with alcohol, RO 15-4513 did not reverse the decreases produced by high doses of alcohol, and it even produced decreases at doses of alcohol which did not significantly alter baseline rates. The schedule of reinforcement was again important. In the fixed ratio experiment, 1.0 mg/kg RO 15-4513 produced marked decreases at all alcohol dose-levels, but this did not occur in the fixed interval paradigm. The decreases in operant behavior, however, were the opposite of those observed in the locomotor activity studies; a noteworthy contrast (Figs. 2 and 4). The schedule of reinforcement has long been recognized as important in assessing the behavioral effects of drugs (15), and the previous observation of schedule-induced differences with alcohol (24) was utilized in the current work with RO 15-4513. The demonstration that another inverse agonist, FG 7142, also decreased responding for ICSS on a variable interval schedule (18) is consistent with the results of the present study. In that report, baseline rates varied from 0.25–0.50 responses per second, and FG 7142 produced a rather steep decline in lever-pressing.

Several investigators have now reported that RO 15-4513 alone will produce decreases in operant behavior (3, 7, 19, 28). In addition, it has been demonstrated that RO 15-4513 produces decreases in fixed ratio lever-pressing for ethanol reward (20), and that it also reduces ethanol, but not water intake in a free-choice procedure (14). This might suggest that RO 15-4513 blocks alcohol reward per se. However, other investigators have reported that RO 15-4513 decreased both ethanol and water intake in a choice test; thus, RO 15-4513 did not alter preferences for solutions, but merely reduced fluid intake (25). All of these results suggest that RO 15-4513 reduces operant behavior for several types of reinforcement, including food, water, alcohol and ICSS. The rate reductions may depend on the anxiogenic properties of RO 15-4513: a strong case has already been made that the changes in ICSS induced by inverse agonists are due to an anxiogenic effect (8).

The reported effects of RO 15-4513 on motor activity are variable. Within the dose-range used here, RO 15-4513 did not alter wheel-running activity (16), although decreases in exploratory head-dipping and horizontal locomotor activity have been described (2,10). In these three studies mice were used. Another study on the activity of mice in a Digiscan activity monitor reported increases in activity at 3.0 mg/kg RO 15-4513 (1), supporting the present findings in rats. The precise procedures used to assess activity are clearly important, as is the degree of habituation to the apparatus. In this work animals were allowed 30 minutes to habituate, and the baselines were low. Under these conditions, RO 15-4513 increased motor activity in general (horizontal activity), and in particular the time spent moving at a slow speed (Channel 2); it also increased activity in alcohol-pretreated animals, but this increase was less marked at higher doses of alcohol. Since there was little evidence of an alcohol-induced decrease in activity, the view that RO 15-4513 reverses the effects of alcohol may not be correct. The lack of a reversal of low-dose alcohol-induced increases in activity (1) and the lack of a reversal of very high-dose alcohol-induced depressions in activity (6,16) suggests a need to reevaluate the "antagonist" activity of RO 15-4513. We have also observed that 1.0 mg/kg RO 15-4513 will increase activity in both diazepam, and to a lesser extent, in pentobarbital-treated rats (unpublished). Clearly, a modest dose of RO 15-4513 stimulates motor activity.

Although a range of doses (0.1–3.0 mg/kg) was used to test the effects of RO 15-4513 alone, only one dose, 1.0 mg/kg, was used in combination with alcohol. Previous studies of the effects of RO 15-4513 alone and together with alcohol have used a rather broad dose-range (0.01–100 mg/kg) and the majority has used doses greater than 1.0 mg/kg (5, 12, 27); so the animals in the present study were not overdosed.

We conclude that the stimulatory and anxiogenic properties of RO 15-4513 largely obscure whatever alcohol antagonism it may have. As demonstrated here, RO 15-4513 can increase activity both alone and together with alcohol. Its anxiogenic effects may be responsible for the decreases in operant behavior we have observed and they are also manifest in animal models of anxiety, such as the pentylenetetrazol drug discrimination paradigm (4) and the plus-maze (11). Where it appears to reverse the effects of alcohol, the interaction may not be specific to alcohol, but also applies to the benzodiazepines (19). While there is good evidence for a biochemical antagonism between the effects of alcohol and RO 15-4513 (26), it is doubtful that this biochemical interaction is a good predictor of the interactions of the two drugs on behavior.

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