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The Effects of Ro 15-4513 on the Behavioral Actions of Ethanol in an Operant Reaction Time Task and a Conflict Test

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Division of Preclinical Neuroscience and Endocrinology, Department of Basic and Clinical Research Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037 Department of Psychiatry, Veterans Administration Medical Center and UCSD School of Medicine, La Jolla, CA 92161

KOOB, G. F., L. PERCY AND K. T. BRITTON. *The effects of Ro 15-4513 on the behavioral actions of ethanol in an operant reaction time task and a conflict test.* PHARMACOL BIOCHEM BEHAV 31(3) 757–760, 1988.—Ro 15-4513, an analogue of the benzodiazepine receptor antagonist Ro 15-1788, has been reported to selectively block the anxiolytic and intoxicating properties of ethanol in rats (16). To examine the specificity and selectivity of this ethanol antagonism, the effects of Ro 15-4513 were tested on the actions of ethanol in an operant reaction time and conflict test in rats. The operant reaction time task involved holding down a lever for 0.25–2.0 seconds to obtain food, and animals treated with 1 g/kg of ethanol showed a significant disruption in performance. This disruptive effect was reversed by Ro 15-4513 in doses of 1.5–5.0 mg/kg. Ro 15-4513 was also tested in an operant conflict paradigm sensitive to alcohol effects. Ro 15-4513 (0, 1.5, 3.0, 6.0 mg/kg) produced a significant decrease in both punished and unpunished responding in the conflict test. Ethanol (0.75 g/kg), pentobarbital (5 mg/kg) and chlordiazepoxide (5 mg/kg) all produced a significant release of punished responding that was blocked by pretreatment with 6.0 mg/kg Ro 15-4513, but again Ro 15-4513 suppressed responding on its own at this dose. These results suggest that Ro 15-4513 has inverse agonist properties that may explain its ethanol-antagonist action.

Ethanol Conflict Reaction time Ro 15-4513 Pentobarbital Chlordiazepoxide

RECENT work with the imidazodiazepine Ro 15-4513 (ethyl 8 azido-5,6,dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4] benzodiazepine-3 carboxylate) renewed a significant amount of interest in the possibility of a drug antagonist for ethanol's actions and the role of the GABA benzodiazepine ionophore complex in the actions of ethanol. This compound, Ro 15-4513, is a structural analog of the benzodiazepine receptor antagonist Ro 15-1788 and was reported to antagonize some of the behavioral and physiological effects of ethanol (2,14).

These findings were confirmed and extended with the report that Ro 15-4513 not only reversed the behavioral effects of ethanol but also blocked ethanol-stimulated chloride uptake into brain vesicle preparations (16). This antagonism was selective and specific in that Ro 15-4513 blocked ethanol's action but did not block that of pentobarbital, and Ro 15-4513 blocked ethanol's actions at doses that produced no behavioral actions on its own (16). A similar antagonism of ethanol's anticonflict actions was observed in our laboratory using the beta carboline compound, FG 7142 (N-methyl-B-carboline-3 carboxamide) which is an inverse agonist at the benzodiazepine ionophore complex (7). However, these anticonflict ethanol effects were not selective for ethanol and occurred only at doses that produced an "anxiogenic-like" suppression of punished responding.

The purpose of the present series of studies was to examine the effects of Ro 15-4513 in two behavior tasks designed to measure motor performance and sensitivity to conflict. We show that Ro 15-4513 can reverse ethanol actions in an operant reaction time task at doses that fail to alter behavior when administered alone. In an operant conflict test, these same doses block ethanol, pentobarbital, and chlordiazepoxide anticonflict effects, but when Ro 15-4513 is injected alone, these same doses produce a further suppression of punished responding.

METHOD

Subjects

For the reaction time experiment seventeen male albino Wistar rats were used weighing 180–200 g at the start of the experiment and 350–450 g at time of first injection. They were group housed, three per cage, in a temperature-, light-

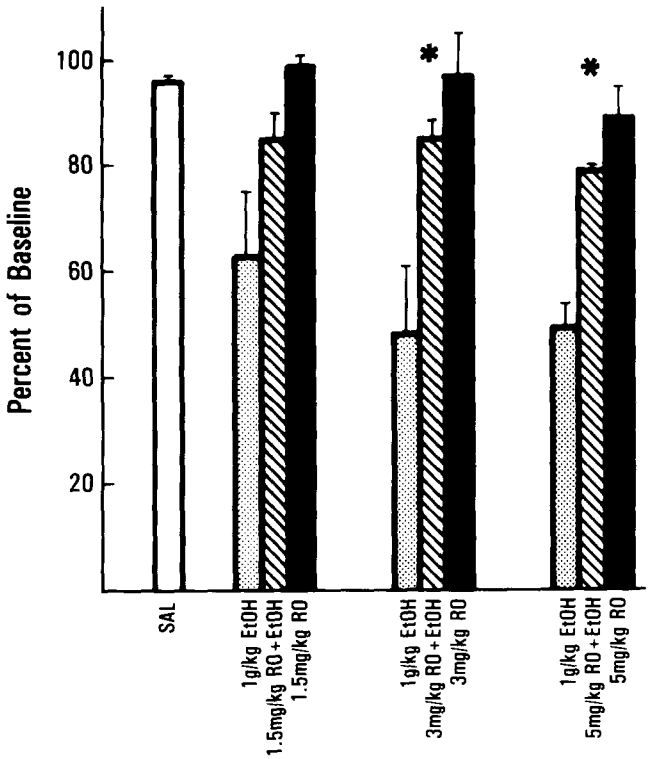

FIG. 1. The effects of Ro 15-4513 on the disruption in reaction time task performance produced by ethanol in rats. Hungry rats were trained to hold a lever down for 0.25 to 2.0 sec and release the lever within 0.6 sec of onset of a light signal in order to obtain a 45 mg food pellet. Ethanol in a dose of 1.0 g/kg disrupts this performance. Ro 15-4513 reversed these disruptive effects of ethanol at doses of 3.0 and 5.0 mg/kg. Design was within subjects, each of three separate groups N=6, received a different dose of Ro or repeated dosing with ethanol once per week in a descending order, i.e., 5, 3, and 1.5 mg/kg.

controlled environment (lights on 0700–1900 hr). For the conflict experiment 24 rats were used. Rats were maintained at 85% of their free feeding weight by restricting their food to 15 g/rat/day after each experimental session.

Procedure Reaction Time Task

Rats were food deprived with access to water for a two-day period, then trained to lever press for food pellets (Noyes Pellets, 45 mg) on a continuous reinforcement schedule until earning 100 pellets. They were then trained on a reaction time task (1) in which a lever was held until a light cue (conditioned stimulus, three lights above the lever) illuminated, then released as quickly as possible (reaction time). All training was done in sound-proof operant chambers (Coulbourn Instruments, Inc.) equipped with a food-pellet dispenser (Ralph Gerbrands Inc., Arlington, MA, model D-1) and a retractable lever (BRS/LVE Division of Technical Service Inc., Beltsville, MD, model RRL-005).

A session consisted of 100 trials and a trial was initiated only after the lever was held down long enough for the conditioned stimulus (CS). There were four possible time periods (delays), which were randomized, that the lever had to be held: D_1 (0.25 sec), D_2 (0.50 sec), D_3 (1.0 sec), D_4 (2.0 sec). If the lever was released before the CS there was no reward given and a new trial began with a different delay. If the lever was held down long enough, there was a 1 second period (restriction time) in which it had to be released for the rat to be rewarded. If the lever was not released in time, no reward was given. The restriction time was shortened to 0.7–0.6 seconds during the 40 training sessions, which was dependent upon the rat's performance—the minimum percent correct reaction time was set at 85% (number of correct trials divided by the number of trials when CS was presented).

Rats were divided into 3 balanced groups (ETOH n=5, Ro n=5, Ro/ETOH n=6; based upon their reaction time after being injected with 2 ml saline (0.9% saline) intraperitoneally (IP) 15 min prior to testing. Rats in these groups were only injected with those drugs throughout the experiment.

On a test day, rats were injected with Ro 15-4513 IP 20 min prior to testing and/or with 10% ETOH IP 15 min prior to testing then returned to their home cage. Test days were separated by at least a week. ETOH was kept at a constant dose of 1 g/kg and Ro was tested at 1.5 mg/kg, 3 mg/kg, and 5 mg/kg.

Procedure Conflict Test

Twenty-four animals were first trained to lever press for 45 mg Noyes food pellets on a continuous reinforcement schedule in sound-proof operant chambers equipped with stainless steel bars on the floor through which electric shock could be delivered. The rats were subsequently switched to a random interval 30-sec reinforcement schedule and finally trained on a multiple-schedule incremental shock conflict test (15). The multiple-schedule conflict test consisted of three components: a pure reward component (unpunished component), a time-out component, and a conflict component. Responses made during the reward component were reinforced on a random interval 30-sec schedule in a darkened chamber. The chamber was illuminated with a house light during the time-out component, and responses were not reinforced. The third component (conflict) was signalled by three flashing lights above the lever (one per second), and responses were both rewarded with food and punished with footshocks (biphasic square wave) on a continuous reinforcement schedule, thus minimizing the motor response requirements during this component, i.e., the number of lever presses required to reach an unacceptable level of shock in untreated rats was minimal (approximately 5–6 per min).

Footshock consisted of a scrambled constant current, biphasic square wave produced by a SGS-003 stimulator (BRS/LVE Division of Tech Serv, Inc.). The stimulator was modified by the addition of a stepping mechanism which allowed the shock to be incremented after each shock in 0.15 mA steps to a maximum of 3.3 mA. A testing session consisted of two cycles of a 5-min reward period, 2-min time out, and 2-min conflict period presented in succession, giving a total daily session duration of 18 min. The animals were tested 5 days a week at the same time of day.

The rats were then randomly divided into four groups and injected with 0, 1.5, 3.0 or 6 mg/kg Ro 15-4513. The same rats were then randomly reassigned to four groups and injected with saline, ethanol (0.75 g/kg), Ro 15-4513 (3 mg/kg) or a combination of ethanol (0.75 g/kg) and Ro 15-4513 (3 mg/kg). This same design was repeated for ethanol (0.75 g/kg), pentobarbital (5 mg/kg) and chlordiazepoxide (5 mg/kg) interactions with Ro 15-4513 (6 mg/kg).

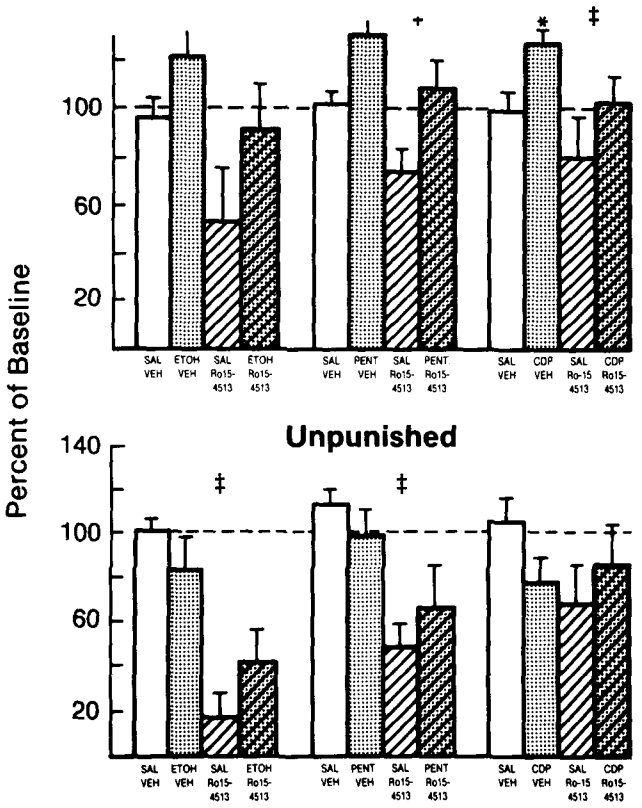

FIG. 2. The interaction of Ro 15-4513 with ethanol (0.75 g/kg), pentobarbital (5 mg/kg) and chlordiazepoxide (5 mg/kg) on punished (conflict) and unpunished (random interval) responding in the conflict test. N=6 rats/group except for chlordiazepoxide/vehicle and saline/vehicle groups where n=7. Results are expressed as percent of baseline responding from previous two days (mean±SEM). For conflict responding, a 2-factor analysis of variance (ANOVA) revealed significant main effects for ethanol, pentobarbital, and chlordiazepoxide, and a significant main effect for Ro 15-4513. Ethanol, pentobarbital and chlordiazepoxide significantly increased punished responding (ANOVA, main effect drug, $p<0.05$) and Ro 15-4513 significantly decreased punished responding (main effect Ro, $p<0.05$). There were no drug × Ro interactions. For unpunished responding there was a main effect of Ro 15-4513 in suppressing responding with ethanol and pentobarbital, but not with chlordiazepoxide (ANOVA, main effect Ro, $p<0.05$). Taken with permission from (4).

Drugs

Ro 15-4513 was provided by Dr. W. E. Haefely, Hoffmann-La Roche, Inc. (Basel) and chlordiazepoxide was provided by Dr. W. E. Scott, Hoffmann-La Roche, Inc. (USA). Ro 15-4513 was dissolved in 99% saline (by volume), 0.5% ETOH (10% solution) and 0.5% emulfer, then sonicated for 1 minute. Pentobarbital and chlordiazepoxide were dissolved in saline.

RESULTS

In the reaction time task the data were pooled across delays and expressed as a percent correct responding in the trials where the lever was held down long enough to obtain the conditioned stimulus. In this case errors that reduced performance were those where the animal failed to release the lever within the 0.6–0.7 sec restriction period. Using this procedure control performance ranged on average above 90% (see Fig. 1). Ethanol significantly disrupted this performance at a dose of 1 g/kg and this effect was reversed by doses of 3 and 5 mg/kg Ro 15-4513; Ro 15-4513 had no effect when injected alone on this measure (see Fig. 1). Analysis of variance revealed a significant group × dose interaction, $F(6,42)=4.32$, $p<0.01$, and the Ro plus ethanol group was significantly different from the ethanol alone group at the 3 and 5 mg/kg doses ($p<0.05$, Newman-Keuls test).

In the conflict test, Ro 15-4513 produced a significant dose-dependent decrease in both punished and nonpunished responding (4). When these same rats were then randomly reassigned to four groups and injected with saline, ethanol (0.75 g/kg), Ro 15-4513 (6 mg/kg) or both ethanol (0.75 g/kg) and Ro 15-4513 (6 mg/kg), ethanol produced a significant increase in responding during the punished component and this was blocked by Ro 15-4513 (see Fig. 2). However, this antagonism was only observed at doses of Ro 15-4513 that produced a significant decrease in unpunished responding (see Fig. 2). Similar effects were observed with Ro 15-4513 versus pentobarbital and chlordiazepoxide. Two-factor analyses of variance revealed significant main effects (drug) for ethanol, pentobarbital and chlordiazepoxide, $F(1,20)=4.98$; $F(1,20)=15.17$; and $F(1,22)=4.70$, respectively. Two-factor analyses of variance also revealed significant main effects (Ro) for ethanol, pentobarbital and chlordiazepoxide, $F(1,20)=5.60$; $F(1,20)=11.4$; $F(1,22)=4.33$, respectively. A lower dose of Ro 15-4513 (3 mg/kg) had no effect on its own on punished responding but also failed to antagonize the anticonflict effects of ethanol (ANOVA, main effect of ethanol only).

DISCUSSION

These results indicate that Ro 15-4513 is effective in reversing some of the behavioral effects of ethanol as previously reported (16). Ro 15-4513 reversed the response disruptive effects of ethanol in an operant, appetitively motivated reaction time task at doses that failed to alter responding by themselves. These data, alone, support the hypothesis that Ro 15-4513 has specific antiethanol actions.

However, in an operant conflict test, Ro 15-4513 reversed ethanol's anticonflict actions but only at doses that when injected alone produce opposite effects, i.e., an enhanced suppression of punished responding. Further, Ro 15-4513 produced virtually identical results when combined with pentobarbital and chlordiazepoxide. These results cast some doubt on both the selectivity and specificity of this compound.

This profile of behavioral actions is similar to that of the beta carboline, FG 7142 which is a benzodiazepine inverse agonist. FG 7142 reverses the effects of ethanol and chlordiazepoxide on conflict responding but only at doses that produce a proconflict action (7). These proconflict effects of FG 7142 are also reversed by the benzodiazepine antagonist Ro 15-1788 (7).

Further evidence suggesting an inverse agonist profile for Ro 15-4513 is that it shows proconvulsant activity against pentylenetetrazole (3), and bicuculline (11–13). Ro 15-4513 (1.45–6.0 mg/kg) also produces abnormal EEG activity as shown by increases in slow sharp waves and high amplitude EEG seizures in rats being recorded from dorsal hippocampal electrodes (5). These ictal episodes were 5–10 seconds in length and occurred regularly over a one hour period.

While these data cast some doubt on the selectivity and specificity of Ro 15-4513 as an ethanol antagonist, particularly for clinical use, they add further support for the hypotheses relating the function of the GABA benzodiazepine ionophore (GBI) complex in ethanol actions. Some of the most effective ethanol antagonists are GABA antagonists (6, 8–10) and it has been hypothesized that ethanol may interact with the picrotoxinin barbiturate site on the GBI complex (17). Our recent data showing that very low doses of isopropylbicyclophosphate, a picrotoxinin receptor ligand, block ethanol's anticonflict effects support this hypothesis (8). This compound, however, also has proconflict and proconvulsant actions at higher doses. Unknown at this time is whether antiethanol effects can ever be separated from the actions of these drugs on their own at the GBI complex.

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