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Effects of Ro 15-4513, Alone or in Combination With Ethanol, Ro 15-1788, Diazepam, and Pentobarbital on Instrumental Behaviors of Rats

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HILTUNEN, A. J. AND T. U. C. JÄRBE. *Effects of Ro 15-4513, alone or in combination with ethanol, Ro 15-1788, diazepam, and pentobarbital on instrumental behaviors of rats.* PHARMACOL BIOCHEM BEHAV **31**(3) 597–603, 1988.—Intraperitoneally administered Ro 15-4513 and ethanol (ETOH), singly and in combination, were examined in rats. Leaping, climbing, bar-pressing, open-field (O-F) activity, as well as concentrations of ETOH in rebreathed air, were studied. Rats in the ETOH (1.2 g/kg) plus Ro 15-4513 (3 mg/kg) condition evinced a jumping performance significantly better than that of the ETOH singly-treated rats; the ETOH (1.2 g/kg) plus Ro 15-4513 (10 mg/kg) condition was intermediate to those of the ETOH and vehicle conditions. In the climbing and bar-pressing experiments, Ro 15-4513 did not attenuate the ETOH-induced impairments. Yet, ETOH improved performance of the Ro 15-4513 high dose (10 mg/kg) condition in the climbing situation. Additional findings were that a) intrinsic activity was noted with Ro 15-4513 in the climbing and bar-pressing situations. and b) the Ro 15-4513/ETOH combination in the O-F test resulted in reduced defecation (antagonism) and rearing activity similar to that of the ETOH-treated rats (lack of antagonism). Concentrations of ETOH in rebreathed air suggested no significant differences between the ETOH singly as compared to the ETOH plus Ro 15-4513 groups. Thus the antagonism of ETOH by Ro 15-4513 was dependent on the parameter examined. Additional experiments examined combinations of Ro 15-4513, Ro 15-1788, diazepam, and pentobarbital in the bar-pressing situation. Results were compatible with the view that Ro 15-4513 acts as a partial benzodiazepine inverse agonist.

Ethanol Ro 15-4513 Ro 15-1788 Diazepam Pentobarbital Drug combinations Rats

ANTAGONISM of ethanol- (ETOH) induced effects by the imidazo benzodiazepine Ro 15-4513 was recently reported [for overview, see (19)]. Thus, electrophysiological effects of ETOH were reported to be antagonized by Ro 15-4513 (20,25), as were the purported anxiolytic effects of ETOH in conflict procedures (2,26). Furthermore, in the wire test, the effects of ETOH were attenuated (1), as was the protection by ETOH of the increase in cortical 3,4-dihydroxy-phenyl acetic acid (DOPAC) levels induced by electric shock (5). However, Hellevuo and Korpi (11) recently reported failures in replicating some of the above data in Wistar rats as well as in rat lines selected for differential sensitivity to ETOH.

Previously we investigated whether or not the discriminative stimulus effects of ETOH would be blocked by Ro 15-4513 (12). Since we found no antagonism of the ETOH stimulus effects by Ro 15-4513 in our drug discrimination learning (DDL) situation, we investigated whether or not ETOH-induced impairments in other instrumental tasks such as leaping, climbing, and bar-pressing would be antagonized by Ro 15-4513. Previous research indicated these methods to be sensitive to drug effects (3, 8, 28). Concentrations of ETOH were determined in rebreathed air in the rats participating in the leaping experiment. In order to be able to investigate several "spontaneous" behaviors, open-field (O-F) testing was also included. In previous experiments, rearing in particular has proven to be a sensitive indicator of the effects of ETOH [e.g., (14)].

The intrinsic activity of the proposed (25) partial inverse benzodiazepine (BDZ) agonist Ro 15-4513 is not well characterized. Rats trained the operant bar-pressing task were used to also examine combinations of Ro 15-4513 and anxiolytics/sedatives such as diazepam (DZP) and pen-

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tobarbital (P-BARB) as well as the BDZ receptor antagonist Ro 15-1788.

METHOD

Subjects

Female Sprague-Dawley rats (ALAB AB, Sollentuna, Sweden) were used throughout. The animals were housed individually, except for the O-F animals which were housed in groups of 4 rats to a cage, under standard laboratory conditions (temperature 20–22°C; relative humidity of about 50–60%; and 12 hr light-dark cycle, lights on 7 a.m.).

In the leaping and O-F experiments 10 and 30 rats were used, respectively. For these animals, water and food pellets were available continuously. The average (±SEM) weights were 257±2.4 g, and 250±2.2 g, respectively.

In the climbing and bar-pressing experiments, 15 and 8 rats, respectively, were deprived of water 23.5 hr per day. Food pellets (type R3, Ewos AB, Södertälje, Sweden) were freely available in the home cages. The average (±SEM) free-feeding weights were 269±3.0 g, and 244±4.9 g.

Apparatus

Dimensions of our masonite leaping box were 80×40×20 cm (height × length × width), and the grid floor area was 23×20 cm (length × width). The animals, one at the time, had to leap from the electrified grid floor (26 μA) to a safe platform (17×20 cm) which was manually fixed at different heights before leaping training. The idea of the leaping experiment originated from the study by Tullis and Sargent (28), showing leaping behavior to be sensitive to the effects of ETOH.

The climbing situation, adapted after Carlini (3), used a rope (3 cm diameter) attached to two different platforms (41× 41 cm each). The climbing distance was 150 cm. The rope got through the upper platform along a hole located in the middle of the platform where the animals could climb through. On the upper platform two drinking bottles were placed, one containing plain tap water and the other also saccharin (0.1%).

The experimental chambers (Skinner boxes) used in the bar-pressing experiments were adapted after Ferster and Skinner (6). The chambers contained one response lever and next to it there was a recess in which water rewards (a 4 sec access to sweetened water, saccharin 0.1% in tap water) could be presented by a retractable drinking cup. Conventional electromechanical relay programming and recording equipment were used.

The O-F arena was a wooden box (60×60×50 cm) with an open top and the floor divided into 16 squares (15×15 cm). A circle was marked in the center of the field. The squared floor was covered with an acrylic plate (60×60 cm). Illumination was provided by the normal room lighting (215 lux at the floor level of the O-F box according to measurements using a Spectra Photometer, model 301). O-F behaviors were video recorded with a camera placed 245 cm above the O-F arena.

Procedures

Leaping. Initially the animals were trained to move from the grid floor to the "safe" platform placed 10 cm above. The distance of the platform was then gradually extended (5 cm each time) until 50 cm was reached. The animals were placed on the activated grid floor, their nose facing towards the wall containing the safe platform. The rats were trained until each of the animals jumped to the platform already on the daily first trial. This initial training occurred for 5 consecutive days before the first test session. When different doses of the drugs were tested the animals were allowed to make up to 5 leaping trials during 30 sec; if they failed to reach the platform or if they did not leap at all during this time period they were removed from the box, and the test trial labeled 'incorrect.' Data are expressed as mean percent correct responding.

Concentrations of ETOH in rebreathed air was measured analogous to the procedure by Pohorecky and Brick (23). ETOH (1.2 g/kg) was administered with and without Ro 15-4513 (3 and 10 mg/kg) and examined in the animals participating in the leaping experiment. The rats were held tight against a plastic cylinder (3 cm diameter, volume 21 ml) during 20 sec with the nose poking into the enclosure. While still holding the animal against the cylinder, air (1 ml) was withdrawn with a Hamilton syringe attached to the cylinder through a rubber septum. The concentrations of ETOH were determined using a gas chromatograph (Perkin Elmer, F11) equipped with a flame ionization detector with known concentrations of ETOH used as the standard curve. Data are expressed as μg ETOH per ml air. Under our experimental conditions the conversion factor between rebreathed air and trunk blood samples was 3241, estimated analogues to the method by Pohorecky and Brick (23).

Climbing. In the climbing experiments, the animals were trained to climb from the lower to the higher platform. On the upper platform the animals had free access to regular tap water or sweetened tap water during 10 sec after initiation of drinking; if a drinking bout did not occur within 60 sec after reaching the platform the animal was removed. The animals were trained in batches of 3 animals and were given 3 trials per training day, 5 days a week. After the initial training of two weeks of duration, tests were introduced twice a week, and baseline (see below) determinations occurred once a week. On test days as well as on baseline recording days, only two trials were run viz. 7.5 and 15 min after injections. The cut-off time was 60 sec; thus, if an animal had not reached the upper platform within the allotted time it was given the score 60 (sec). Two experiments (Experiments 1 and 2) were conducted, the main difference being the dose of Ro 15-4513 (3 and 10 mg/kg) examined. Data are expressed as a ratio between the time used in baseline sessions divided by the time used in test sessions. Values lower or higher than 1.0 indicate increased or decreased climbing time, respectively. Baseline sessions were identical to test sessions except that the vehicles only were administered throughout.

Bar-pressing. After initial shaping, the animals were trained to press the lever 10 times to produce a reward (FR-10). The rats were trained in 20 min sessions, 5 days a week (Monday through Friday), and injections of saline (5 ml/kg) preceded daily session onset by 15 min.

Once bar-pressing behavior was stable, i.e., after an average of 15 sessions of FR-10, test sessions were introduced. Each test as well as training session ended after the animals had received 50 rewards, or a preset time (20 min) had elapsed since the initiation of the session. Tests were conducted twice a week (Tuesdays and Fridays), and Thursdays served as control days. Sessions were initiated 15 min after the ETOH (or the corresponding volume of saline) administrations in the two phases of the first experiment (Experiment 1); a difference between the phases being the ETOH dose used (1.2 or 0.9 g/kg). Thereafter, combinations of 10

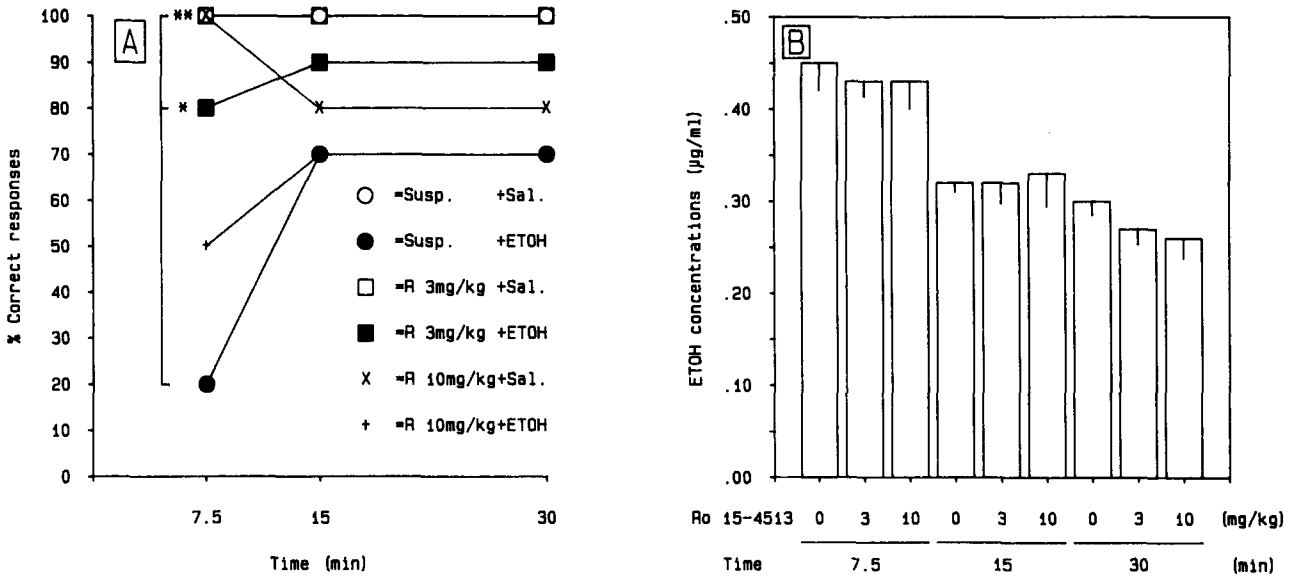

FIG. 1. Leaping behavior (A) and concentrations of ETOH in rebreathed air (B). The rats were tested 7.5, 15 and 30 min after injections of ETOH or saline (Sal.); suspension (Susp.) or Ro 15-4513 injections were given 5 min prior to the ETOH or saline administrations. The combinations of ETOH (1.2 g/kg) and Ro 15-4513 (R, 3 and 10 mg/kg) tested are indicated in (A); in (B) all the animals were injected with 1.2 g/kg of ETOH, with or without Ro 15-4513 (3 and 10 mg/kg). Y-axis, percentage of rats which succeeded to leap to the safe platform (A) and concentrations of ETOH expressed as µg/ml air (B). X-axis, time intervals tested (A and B) and doses of Ro 15-4513 (B). Vertical lines represent SEM; $*p<0.05$ and $**p<0.02$ (McNemar's test).

mg/kg of Ro 15-4513 and various doses of Ro 15-1788, DZP, and P-BARB were examined (Experiment 2). Data are expressed as in the climbing experiment. Hence values lower or higher than 1.0 indicate increased or decreased response rate, respectively.

Open-field. On the day of examination, the rats were placed in individual macrolone cages. After the injections the animal was placed in the center of the O-F arena; the rat was then allowed to explore the field during 5 min. Records were kept on the following behaviors: *Ambulation*=the number of squares crossed with all four feet; *Rearing*=the number of times the animal stood on its hind feet; *Defecation*=the number of fecal boli deposited; *Urination*=the number of urination spots deposited; Latency= time in sec before leaving the center circle with all four feet; and *Grooming*=the number of cleaning bouts, including washing of the face with front paws and trimming of the fur. The acrylic plate was rinsed with water between trials to minimize odors from the previously tested animal. Data are expressed as the total counts for the 5 min observation period.

Data Analysis

In the leaping experiment the nonparametric overall analysis of the Cochran's Q-test followed by an additional analysis of McNemar's test (24) were used. Concentrations of ETOH in rebreathed air were evaluated using a multiple stepwise regression analysis, and Student's *t*-test (4). Analysis of variance (ANOVA) was used for the climbing (RB-design), bar-pressing (RB-design), and O-F experiments (CR-design) followed by post hoc (Tukey's HSD) statistical procedures (15), the abbreviations being explained in the citation.

Drugs

The drugs used were: Ro 15-4513 (i.e., ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5-a][1,4] benzodiazepine-3-carboxylate); Ro 15-1788 (flumazenil), diazepam base, DZP (Valium®), pentobarbital sodium, P-BARB (Nembutal®), and ethanol, ETOH. Ro 15-4513 (lot 4323, 132 A), Ro 15-1788 (lot 405, 001), and DZP, were kindly provided by Hoffmann-La Roche & Co. Ltd., Basel, Switzerland. P-BARB and ETOH (99.5%) were purchased from The Academic Hospital, Uppsala, Sweden, and AB Svensk Sprit, Stockholm, Sweden, respectively. P-BARB and ETOH were dissolved in normal saline (0.9%). Ro 15-4513 and Ro 15-1788 were suspended in saline plus tween-80 (4%, v/v) and distilled water plus tween-80 (two drops/10 ml), respectively. All injections were given intraperitoneally (IP). The doses of ETOH (10%, w/v) were achieved by varying the volumes administered (17). The volume for Ro 15-4513 was 4 ml/kg; the latter agent was always administered 5 min prior to ETOH [see Suzdak *et al.* (26)]. Vehicle injections for the Ro 15-4513 and ETOH studies contained tween-80 (4%) and physiological saline, and were administered in amounts corresponding to those of the respective drug testing session. The volumes for the other agents varied between 1 to 4 ml/kg. Test drugs and doses were studied in an unsystematic, mixed order in each of the experiments.

RESULTS

Leaping

Figure 1A shows the results of the leaping experiment during three different test intervals, viz. 7.5, 15 and 30 min after ETOH. When the animals were treated with vehicle, or

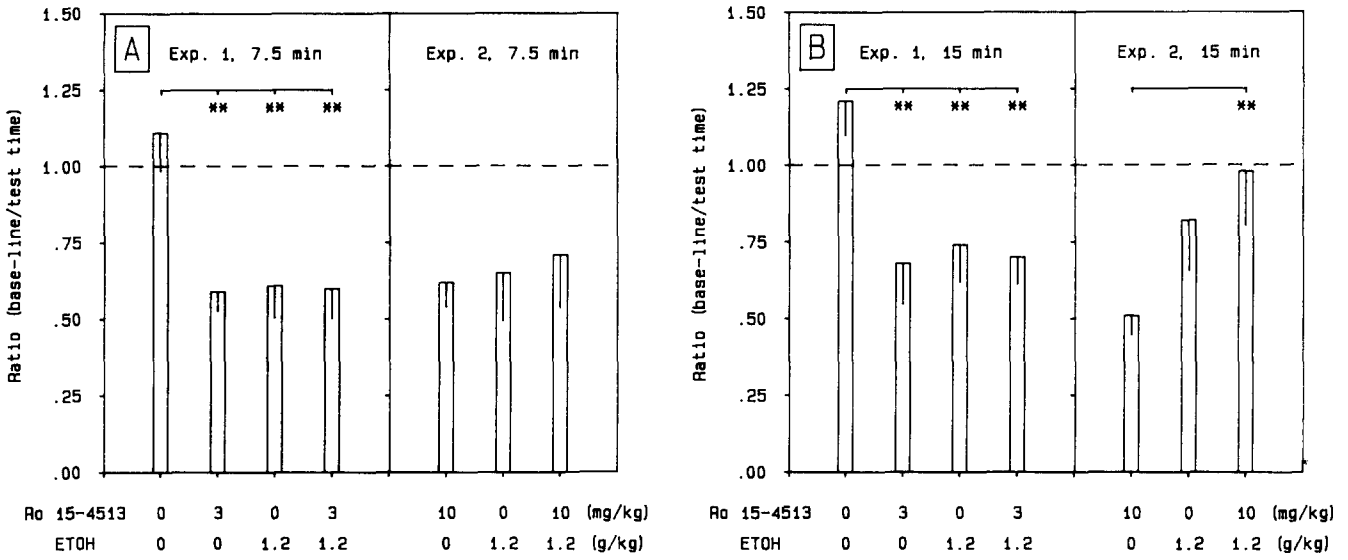

FIG. 2. Climbing behavior with combinations of ETOH (1.2 g/kg) and Ro 15-4513 (3 mg/kg and 10 mg/kg) in two test intervals viz. 7.5 min (A), and 15 min (B) after the ETOH injections, respectively. Y-axis, the time taken to reach the upper platform, expressed as a ratio between the time used in baseline sessions divided by the time used in the test sessions. X-axis, doses of Ro 15-4513 and ETOH; administrations of Ro 15-4513 preceded ETOH by 5 min. Vertical lines represent SEM; $^{**}p<0.01$ (Tukey's HSD).

with the dose of 3 mg/kg of Ro 15-4513 singly, all the animals succeeded to leap to the safe platform in all test intervals. During the first interval a significant, $Q(5)=34.69$, $p<0.001$, overall effect was obtained. Subsequent analysis suggested a significant reduction in leaping after ETOH singly (1.2 g/kg) as compared to the vehicle treatment, as only two of the ETOH-injected animals succeeded in reaching the platform. A significant improvement was observed when 3 mg/kg of Ro 15-4513 preceded the ETOH injection. Effects of 10 mg/kg of Ro 15-4513 alone or together with ETOH were not significantly ($p>0.05$) different from the ETOH treatment.

Concentrations of ETOH in rebreathed air when ETOH was administered with and without Ro 15-4513 are illustrated in Fig. 1B (n=5–10). Multiple stepwise regression analysis suggested that ETOH concentrations (dependent variable) correlated significantly, $t(41)=-7.06$, $p<0.001$, with elapsed time after administrations of ETOH but not with presence of Ro 15-4513. The lack of significance, $t(41)=0.24$, $p>0.05$, in the latter instance indicates that ETOH concentrations in rebreathed air for ETOH alone are similar to those where ETOH was combined with Ro 15-4513.

Climbing

Data from the climbing experiments are shown in Fig. 2A (tests 7.5 min after ETOH injections), and in Fig. 2B (tests 15 min after ETOH administrations). The animals received ETOH (1.2 g/kg) and either 3 mg/kg (Experiment 1, n=15), or 10 mg/kg (Experiment 2, n=14) of Ro 15-4513. The difference in the number of animals in the two experiments is due to an increased variability in climbing time of one of the rats. Four ANOVAs were conducted and the overall significances between treatments were as follows: $F(3,42)=10.71$, $p<0.01$ (Ro 15-4513, 3 mg/kg, 7.5 min); $F(3,42)=6.87$, $p<0.01$ (Ro 15-4513, 3 mg/kg, 15 min); $F(2,26)=0.18$, $p>0.05$ (Ro 15-4513, 10 mg/kg, 7.5 min); $F(2,26)=5.74$, $p<0.01$ (Ro 15-4513, 10 mg/kg, 15 min). Both ETOH (1.2 g/kg) and Ro 15-4513 (3 mg/kg) alone increased climbing time significantly as compared to the vehicle session; ETOH together with Ro 15-4513 also significantly increased the time to climb, 7.5 min after the ETOH injection. Only the comarisons between the vehicle session data and drug sessions were significant 15 min after the ethanol administrations (cf., Fig. 2B, Experiment 1, 15 min). Similar tendencies were noted in Experiment 2, in tests 7.5 min after the ETOH administrations but not verified statistically as indicated above ($p>0.05$). Figure 2B, however, indicates that the higher dose of Ro 15-4513 (10 mg/kg) alone exerted stronger effects than when combined with ETOH in the latter test period, as evinced by a significant difference between these two conditions.

Bar-Pressing

The results of the bar-pressing experiments are shown in Fig. 3 (Experiment 1), and in Fig. 4 (Experiment 2). In both phases of Experiment 1, significant overall scores (ANOVA) between treatments were obtained: $F(5,35)=26.01$, $p<0.01$, for 1.2 g/kg of ETOH (Fig. 3A, n=8); and $F(7,35)=11.40$, $p<0.01$, for 0.9 g/kg of ETOH (Fig. 3B, n=6), the difference in the n's being explained below.

The vehicle condition in the first phase of Experiment 1 (cf., Fig. 3A) yielded scores significantly different than any of the other conditions examined. In addition, 1 and 3 mg/kg of Ro 15-4513 were significantly different from the combination of 3 mg/kg of Ro 15-4513 plus 1.2 g/kg of ETOH.

In the second phase of Experiment 1 (cf., Fig. 3B) the vehicle condition was significantly different from all other conditions except 1 mg/kg of Ro 15-4513 singly ($p>0.05$). In addition, 1 mg/kg of Ro 15-4513 was significantly different from 10 mg/kg of Ro 15-4513 singly. ETOH (0.9 g/kg) together with 10 mg/kg of Ro 15-4513 differed significantly from all other conditions except from Ro 15-4513 (10 mg/kg) singly, or Ro 15-4513 (3 mg/kg) together with ETOH (0.9 g/kg).

The effects of Ro 15-1788 (Fig. 4A), DZP (Fig. 4B), and

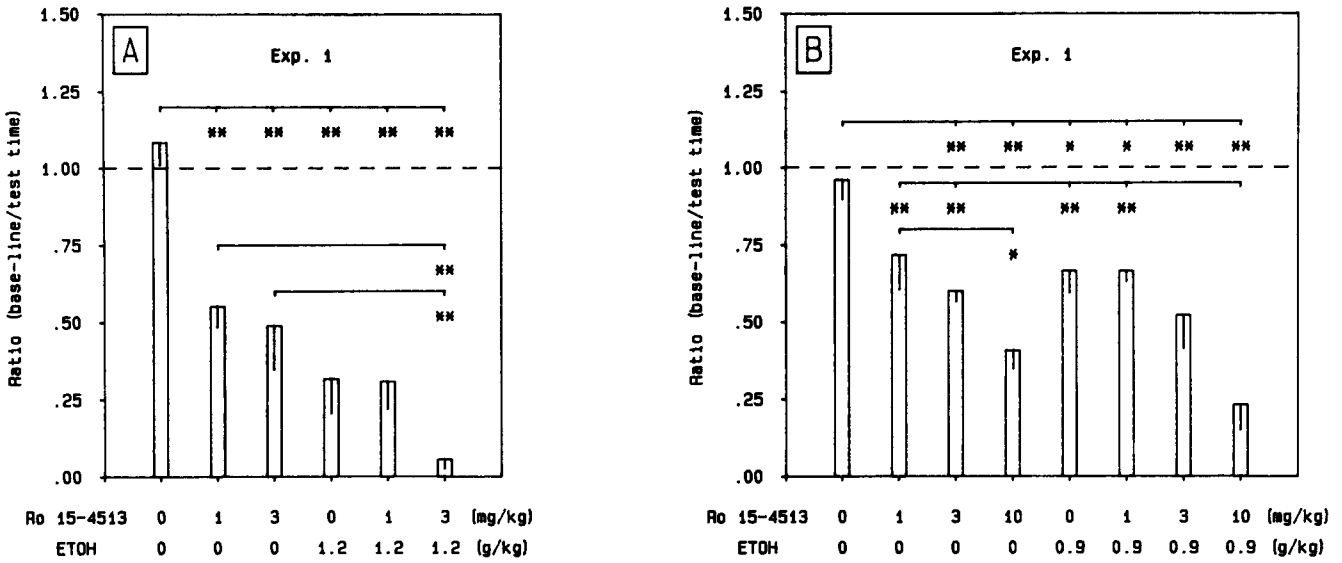

FIG. 3. Bar-pressing behavior with combinations of ETOH [1.2 g/kg (A); and 0.9 g/kg (B)] and Ro 15-4513 (1–10 mg/kg). Y-axis, time used for 50 reinforcements, expressed as a ratio between the time used in baseline sessions divided by the time used in the test sessions. X-axis, doses of Ro 15-4513 and ETOH, the administrations of Ro 15-4513 occurring 5 min prior to the ETOH injection, and tests were done 15 min after ETOH. Vertical lines represent SEM; *$p<0.05$, and **$p<0.01$ (Tukey's HSD).

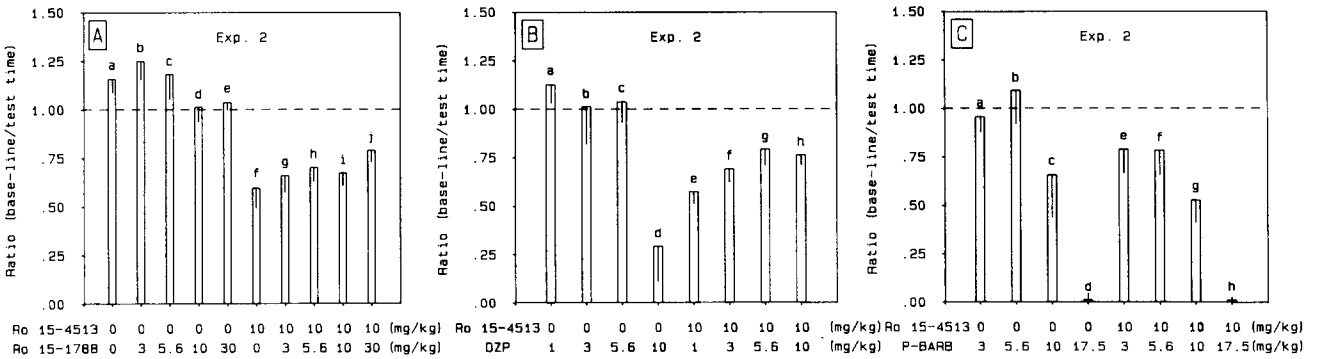

FIG. 4. Bar-pressing behavior with combinations of Ro 15-1788 [0–30 mg/kg (A)], DZP [1–10 mg/kg (B)], P-BARB [3–17.5 mg/kg (C)] together with Ro 15-4513 (0 and 10 mg/kg). Y-axis, time used for 50 reinforcements, expressed as a ratio between the time used in baseline sessions divided by the time used in the test sessions. X-axis, doses of Ro 15-4513, Ro 15-1788, DZP, or P-BARB, the administrations of Ro 15-4513 occurring 5 min prior to the injections of the other drugs; tests were initiated 15 min after application of the second drug. Vertical lines represent SEM. The following differences between means were significant: a,b,c≠f,g,h,i; d,e≠f; b,c≠j ($p<0.01$); a≠j; d≠g,i; e≠g,h,i ($p<0.05$) in (A); a≠d,e; b,c≠d ($p<0.01$); d≠g,h; b,c≠e ($p<0.05$) in (B); a,b,c,e,f≠d,h ($p<0.01$); b,d,h≠g ($p<0.05$) in (C) (Tukey's HSD).

P-BARB (Fig. 4C) in combination with Ro 15-4513 were investigated in Experiment 2 (n=7). In all three phases of Experiment 2, significant overall scores between treatments were obtained: $F(9,54)=12.26$, $p<0.01$, for Ro 15-1788 plus Ro 15-4513 (Fig. 4A); $F(7,42)=7.90$, $p<0.01$, for DZP plus Ro 15-4513 (Fig. 4B); and $F(7,42)=13.05$, $p<0.01$, for P-BARB plus Ro 15-4513 (Fig. 4C).

The critical values for significant differences between treatments according to Tukey's HSD test were estimated to be: 0.331, 0.442, and 0.504 ($\alpha=0.05$), or 0.390, 0.527, and 0.602 ($\alpha=0.01$) in the first, second, and third phase of Experiment 2, respectively; i.e., any difference (in Fig. 4) between treatments which exceeded the critical value is significant. Details of the pairwise comparisons are given in the legend of Fig. 4.

Thus, in Fig. 4A, no significant ($p>0.05$) differences occurred for the treatments involving no Ro 15-4513, or the treatments concerning 10 mg/kg of Ro 15-4513 together with different doses of Ro 15-1788. Most of the treatments with no Ro 15-4513 differed from the treatments involving 10 mg/kg of Ro 15-4513.

In Fig. 4B, 10 mg/kg of DZP differed significantly from the other single doses of DZP (1, 3 and 5.6 mg/kg), as well as from the combinations of 10 mg/kg of Ro 15-4513 together with DZP (5.6 or 10 mg/kg).

In Fig. 4C, 17.5 mg/kg of P-BARB singly or in combination with 10 mg/kg of Ro 15-4513 differed significantly from all the other treatments. P-BARB (5.6 mg/kg) also differed significantly from the P-BARB/Ro 15-4513 (10 plus 10 mg/kg) combination.

TABLE 1

RESULTS [MEANS (±SEM)] OF O-F TESTING WITH Ro 15-4513 AND ETOH

Ro 15-4513 (mg/kg)	ETOH (g/kg)	n	Rearing	Ambulation	Defecation
0	0	10	39.7 (4.7)	107.7 (8.3)	0.6 (0.4)
0	1.2	10	6.9 (2.1)*	108.8 (18.0)	1.5 (0.4)
3	1.2	10	9.1 (2.8)*	115.9 (10.5)	0.3 (0.2)†

*Significantly ($p<0.01$) different from controls (Tukey's HSD); †significantly ($p<0.05$) different from ETOH singly (Tukey's HSD).

Additionally, tests with combinations of Ro 15-1788 (30 mg/kg) together with 10 mg/kg of DZP (both injections were given simultaneously, and the test occurred 15 min later) resulted in mean±SEM response output of 0.83±0.08 (n=6). This is quite similar to the output ratio noted for the combination of 10 mg/kg of Ro 15-4513 together with 30 mg/kg of Ro 15-1788 (mean±SEM=0.79±0.04, n=7). A reevaluation of the combination of 10 mg/kg of Ro 15-4513 plus 0.9 g/kg of ETOH yielded the following output ratio viz. 0.45±0.04 (n=7); the order of treatments were as described in Fig. 3. The difference in the number of animals participating in the bar-pressing experiments is 1) due to the death of one animal and, 2) due to apparatus failures during the second phase of Experiment 1.

Open Field

The O-F data disclosed overall significant ANOVA effects for the rearing, $F(2,27)=32.98$, $p<0.01$, and defecation, $F(2,27)=3.63$, $p<0.05$, parameters. The mean scores for rearing, ambulation and defecation are presented in Table 1. The control animals reared significantly more than the animals treated with ETOH, either when given singly, or when combined with Ro 15-4513. The animals treated with ETOH singly defecated significantly more than the animals treated with the combination of ETOH and Ro 15-4513. No significant ($p>0.05$) differences were observed in the remaining parameters (ambulation, grooming, latency, and urination).

DISCUSSION

In the present study the effects of Ro 15-4513 and ETOH alone and in combination were studied employing leaping, climbing, and bar-pressing behaviors as well as O-F parameters. The only instance where Ro 15-4513 significantly antagonized the behavioral impairments caused by ETOH was in the leaping procedure. Antagonism occurred with the lower dose of Ro 15-4513 in the first test interval where the impairment by ETOH singly was strongest. That the highest dose of Ro 15-4513 failed to produce clear-cut antagonism of ETOH may be due to intrinsic effects of Ro 15-4513 (see below). The behavioral effects of ETOH singly decreased rapidly over time, hence, antagonism not being observable in the latter test intervals.

As the doses, routes of administrations, injection time intervals and suspensions used were similar in all of our procedures, reasons for the seemingly different outcomes must be sought elsewhere. One difference between the leaping, climbing and bar-pressing procedures concerns the incentive, shock escape, in the first procedure and water deprivation in the two other instrumental procedures. If this is critical, it is possible that Ro 15-4513 more readily attenuates the behavioral effects of ETOH in conflict and stressful situations. ETOH is known to produce anxiolytic effects and counteract stress-induced responses in some instances (22). For example, ETOH prevents stress-induced increases in cortical DOPAC (5), attenuates Ro 15-4513 induced proconvulsant activity (19,20), and modulates alterations in the metabolism and function of GABA neurons due to immobilization of rats (16). Interestingly the antagonistic effects of Ro 15-4513 against ETOH reported by Britton *et al.* (2) and Suzdak *et al.* (26) were studied in aversively- (shock) motivated situations. Concentrations of ETOH in rebreathed air were the same in the ETOH singly and the ETOH plus Ro 15-4513 conditions. This is in keeping with previous observations to that account [(12,26); however, see also (10)].

The results with regard to the ETOH-induced decrease of rearing activity indicated lack of antagonism by Ro 15-4513. This suggests that the significant improvement of leaping performance by 3 mg/kg of Ro 15-4513 in the ETOH-treated rats may not simply be explained by an improved ability of the animals to stand on the hind legs. An ancillary finding was that treatment with Ro 15-4513 together with ETOH yielded the lowest defecation score in the O-F test. One explanation might be reduced intestinal motility induced by Ro 15-4513.

In challenge tests in the DDL experiment (12), the combination of Ro 15-4513 (3 and 10 mg/kg) and ETOH (0.9 and 1.2 g/kg) clearly reduced response rate without affecting the ETOH discriminative choice suggesting intrinsic activity of Ro 15-4513. Other workers [e.g., (2, 9, 19)] have observed intrinsic activity with Ro 15-4513. Consistent changes were observed with Ro 15-4513 singly in the bar-pressing and climbing experiments. It remains to be determined if this represents a specific interaction with the incentive common to these tests or if the two situations are differentially susceptible to the actions of Ro 15-4513. The significant improvement in the ETOH/Ro 15-4513 climbing situation (Experiment 2, 15 min) would support a differential interaction with ETOH. It should be stressed that ETOH antagonized the effects of Ro 15-4513 (rather than the opposite) in the climbing experiment. In fact, in both bar-pressing situations (DDL and FR-10), combinations of the two drugs resulted in larger rate decreases than either of the drugs alone. Apparently this was not the case in the climbing experiment. Thus the enhancement of the ETOH-induced inhibition of bar-pressing by Ro 15-4513 is not simply due to a general suppresion of behavior as indicated by both the climbing and O-F data. A similar pattern of results were recently described by Misslin *et al.* (21).

Ro 15-4513 has been characterized as a partial inverse BDZ agonist (1, 20, 25). Consistent with this view, Ro 15-1788 attenuated the behavioral effects of Ro 15-4513; the

effect of DZP (10 mg/kg) was also attenuated by Ro 15-1788 (30 mg/kg). Whereas certain combinations of DZP and Ro 15-4513 yielded augmented response outputs (i.e., antagonism), this did not occur when Ro 15-4513 was combined with P-BARB. Hence previous observations (1, 13, 18) of an antagonism between Ro 15-4513 and barbiturates were not corroborated.

Furthermore, it seemed that combinations of Ro 15-4513 together with ETOH decreased response output more than combinations of P-BARB and Ro 15-4513. The normalization of the rate of bar-pressing with combinations of DZP and Ro 15-4513 points towards a differentiation of the behavioral effects of the three CNS "depressants" as revealed by their interaction with Ro 15-4513. This is in contrast to the studies by Britton *et al.* (2) and Lister (18). Assuming that Ro 15-4513 is anxiogenic (7,9), the reversal of presumed anxiolytic activity induced by ETOH, P-BARB and chlordiazepoxide is reasonable (2). The lack of a differential interaction between Ro 15-4513 and ETOH, P-BARB, and DZP in the holeboard test (18) is less obvious. Since the animals in Lister's study (18) were confronted with the test apparatus only at the time of examination, the similar results with all of the three sedatives might also be due to anxiolytic/anxiogenic activity. Apparently, in operant procedures, the animals are familiar with the experimental chamber at the time of testing.

The interactions between Ro 15-4513 and ETOH, Ro 15-1788, DZP, or P-BARB are based on a single behavior, i.e., a FR-10 schedule of reinforcement. Although recognizing this limitation, we have provided supporting evidence to characterize Ro 15-4513 as a partial BDZ inverse agonist, and to indicate that the interaction between ETOH and Ro 15-4513 is not universal. Rather it would appear that the antagonism between ETOH and Ro 15-4513 is differentially affected by the parameter studied as shown here as well as elsewhere (13,27). Obviously this is the case also with respect to the interaction between Ro 15-4513 and barbiturates. Furthermore, we have emphasized that Ro 15-4513 produces measurable effects and, hence, exerts intrinsic activity, worth studying in its own right.

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