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Anxiogenic Effects of a Benzodiazepine Receptor Partial Inverse Agonist, RO 19-4603, in a Light/Dark Choice Situation

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BELZUNG, C., R. MISSLIN AND E. VOGEL. *Anxiogenic effects of a benzodiazepine receptor partial inverse agonist, RO 19-4603, in a light/dark choice situation.* PHARMACOL BIOCHEM BEHAV **36**(3) 593–596, 1990.—In a light/dark choice procedure, the imidazothienodiazepinone RO 19-4603, given alone, induced a dose-dependent decrease in the time spent by mice in the lit box as well in the number of transitions between the two boxes. These data confirm the anxiogenic intrinsic properties of inverse agonists of the benzodiazepine receptor. Since RO 19-4603 also reversed the anxiolytic effects of ethanol and exhibited proconvulsant properties, it is suggested that the antagonistic action of this drug against ethanol could be due to an additive rather than an interactive process.

RO 19-4603 Light/dark choice procedure Inverse agonist of the benzodiazepine receptor Ethanol Convulsions Mice

RO 19-4603 is an imidazothienodiazepinone derivative structurally related to RO 15-4513 that exhibits a specific high-affinity binding to the central benzodiazepine receptor and is the most potent partial benzodiazepine receptor inverse agonist found to date in the class of imidazodiazepinone derivates (26). There is considerable evidence that inverse agonists can reverse some of the behavioral effects of ethanol (2–4, 7, 8, 15, 18, 22, 28). Although it was claimed that RO 15-4513 selectively blocks the anxiolytic and intoxicating properties of ethanol in rats (28), we proposed, in accordance with other authors (18,19), that the benzodiazepine receptor inverse agonists antagonize the actions of ethanol by producing intrinsic effects (2–4, 21).

Recently, Lister and Durcan (20) showed that low doses of RO 19-4603 (0.01–0.3 mg/kg) attenuated the intoxicating effects of ethanol (2.4 g/kg) and these authors suggested that the ability of benzodiazepine receptor inverse agonists in reducing the effects of ethanol may be related to their effectiveness as inverse agonists.

A first experiment was undertaken in order to examine the effects of RO 19-4603 on activity in a free exploration test. Indeed, we previously showed that some inverse agonists induced a reduction of locomotion that could be related to pre-ictal prostration (3,22). Since we found that the dose of 10 mg/kg produced a significant reduction in activity, we exclude this dose in the other experiments. Experiment 2 investigated the effects of several doses of RO 19-4603 on the behavior of mice confronted with the light/dark choice procedure described by Crawley and Goodwin (12) and modified by one of us (R.M.). In such a situation, mice display a preference for the dark box and, after treatment with benzodiazepines, spend more time in the lit box than controls (1) and show more crossings between the two boxes (1, 5, 11, 12), while the inverse agonists β-CCM and RO 15-4513 decreased both behavioral variables (1–3, 22). Since we found anxiogenic effects of RO 19-4603, we compared the behavior of mice treated with an anxiolytic dose of ethanol and RO 19-4603 (Experiment 3). Finally, we examined the effects of a subconvulsant dose of pentylenetetrazol (PTZ) on mice pretreated with several doses of RO 19-4603 (Experiment 4).

METHOD

Animals

Male Swiss albino mice from Centre d'Elevage R. Janvier, 13 weeks of age at time of testing, were used. Prior to testing, they were housed five to a standard cage containing a constant supply of food pellets and water, and kept on a 12/12 light/dark cycle with lights on at 1 a.m. in order to observe animals in their high activity period, that is, when lights are off. In all experimental procedures, each mouse was only tested once.

Experiment 1: Effects of RO 19-4603 on Locomotor Activity

Apparatus. The apparatus consisted of a polyvinylchloride box ($30 \times 20 \times 20$ cm) covered with Plexiglas and subdivided into six equal square exploratory units, which were all interconnected by small doors. It could be divided in half lengthwise by closing three temporary partitions. The apparatus was kept on a stand in the mouse room. The experimenter stood next to the box, always at the same place.

Procedure. Approximately 24 hr before testing, each subject was placed in one half of the apparatus with the temporary partitions in place, in order to be familiarized with it. The floor of this half was covered with sawdust and the animal was given unlimited access to food and water. Next day, the subject was exposed to both familiar and novel environments by the removal of the temporary partitions without itself being taken out of the box. The subject was then observed, in red light, for 10 min. The number of units entered (locomotion) and the number of rears made by the animals were recorded.

Drugs. Drugs were administered intraperitoneally, 20 min before testing, in concentrations given an injection volume of 10 ml/kg body wt. Mice were randomly allocated to groups receiving vehicle (saline with a drop of Tween 80) or RO 19-4603 (0.1, 0.25, 0.5, 1 and 10 mg/kg); n = 10 in all experimental groups.

Experiment 2: Effects of RO 19-4603 on the Behavior of Mice in the Light/Dark Choice Procedure

Apparatus. The apparatus consisted of two polyvinylchloride boxes (20 × 20 × 14 cm) covered with Plexiglas. One of these boxes was darkened; a light from a 100 W desk lamp above the other box provided the only room illumination. An opaque plastic tunnel (5 × 7 × 10 cm) connected the dark box to the lit one. During observation, the experimenter sat always at the same place, next to the apparatus.

Procedure. The subjects were individually tested in 5-min sessions in the apparatus described above. Testing was performed between 2 p.m. and 4 p.m. Mice were placed in the lit box to start the test session. The amount of time spent in the lit box and the number of transitions through the tunnel were recorded, minute per minute, during 5 min, after the first entry in the dark box. A mouse whose four paws were in the new box was considered as having changed boxes. Mice were naive to the apparatus.

Drugs were administered as in Experiment 1. Mice were divided into five experimental groups: vehicle control (n = 15), RO 19-4603 (0.1 mg/kg, n = 15; 0.25 mg/kg, n = 25; 0.5 mg/kg, n = 25; 1.0 mg/kg, n = 15, in vehicle).

Experiment 3: Effects of Ethanol Given Alone or in Combination With RO 19-4603 on the Behavior of Mice in the Light/Dark Procedure

Drugs. Drugs were administered intraperitoneally, 20 min before observation. Mice were randomly allocated to three groups: vehicle control (saline with a drop of Tween 80, n = 29), ethanol-treated mice (1.5 g/kg in vehicle, n = 35), mice injected with a combination of ethanol (1.5 g/kg) and RO 19-4603 (1.0 mg/kg, n = 15). This dose of RO 19-4603 was chosen because it did not produce a decrease of locomotion and had obvious anxiogenic effects in the light/dark choice test.

Experiment 4: Proconvulsant Effects of RO 19-4603

Procedure. RO 19-4603 (0.0, 0.1, 0.25, 0.5, 1.0, 5.0 and 10.0 mg/kg; n = 15) was administered intraperitoneally to mice 2 min before PTZ (30 mg/kg). The number of mice showing full tonico-clonic seizures during a 5-min period after the injection of PTZ was recorded.

Statistical analysis. Statistical significance of differences between control and treated groups was ascertained by a combined analysis of variance and an unpaired two-tailed range *t*-test using the Newman-Keuls method in Experiments 1, 2, 3 and by the chi-square test in Experiment 4.

TABLE 1

EFFECTS OF RO 19-4603 ON ACTIVITY

	Doses (mg/kg)					
	0	0.10	0.25	0.50	1.0	10.0
Locomotion	125.00	106.80	96.90	99.80	105.00	52.00*
SEM	6.0	7.0	6.3	12.6	11.2	5.9
Rears	55.10	55.30	53.00	40.70	56.50	20.30*
SEM	4.3	2.7	6.0	9.2	7.2	3.7

*$p<0.01$ (Newman-Keuls test).

RESULTS

Experiment 1: Effects of RO 19-4603 on Locomotor Activity in a Free Exploration Procedure

Table 1 shows that RO 19-4603 produced a dose-dependent decrease in locomotion, $F(54.5) = 8.84$, $p<0.01$, and in the number of rears, $F(54.5) = 6.39$, $p<0.01$, but the Newman-Keuls test indicates that only the highest dose significantly affected both variables.

Experiment 2: Effects of RO 19-4603 on the Behavior of Mice in the Light/Dark Choice Test (Fig. 1)

RO 19-4603 produced a dose-dependent decrease in the time spent by mice in the lit box, $F(90.4) = 6.87$, $p<0.001$, as well as in the number of transitions, $F(90.4) = 10.24$, $p<0.001$.

Experiment 3: Effects of Ethanol Given Alone or in Combination With RO 19-4603 on the Behavior of Mice in the Light/Dark Choice Procedure (Fig. 2)

Analysis of variance revealed significant differences among the groups with respect to the time spent in the lit box, $F(76.2) = 9.38$, $p<0.001$, and to the number of transitions, $F(76.2) = 4.43$, $p<0.001$.

Ethanol given alone at a dose of 1.5 g/kg produced a significant increase in the time spent by animals in the lit box without significantly modifying the number of transitions. RO 19-4603, injected at a dose of 1.0 mg/kg in combination with ethanol dosed at 1.5 g/kg, antagonized the increasing effects of the latter drug on the time spent by mice in the lit box and significantly reduced the number of transitions.

Experiment 4: Proconvulsant Properties of RO 19-4603

As can be seen from Table 2, there were significant differences between the mice treated with PTZ and those pretreated with RO 19-4603 at 1.0, 5.0 and 10.0 mg/kg (respectively $\chi^2 = 7.77$, $p<0.01$; $\chi^2 = 5.4$, $p<0.02$; $\chi^2 = 10.9$, $p<0.001$).

DISCUSSION

We showed in this study that the inverse agonist of benzodiazepine receptors, RO 19-4603, induced a dose-dependent decrease in the time spent by mice in the lit box as well in the number of transitions between the dark box and the lit one. It appeared that this drug had opposite effects to those of benzodiazepines insofar as these latter drugs tend to disinhibit animals' behavior blocked by the aversive properties of the well-illuminated box. These

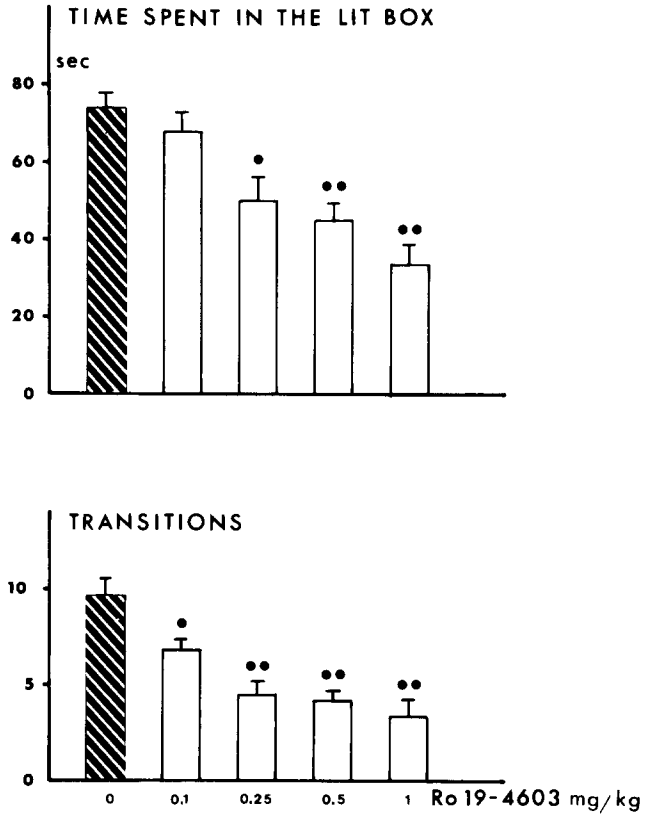

FIG. 1. Effects of RO 19-4603 on the time spent by mice in the lit box and on the number of transitions in a light/dark choice procedure (mean ± SEM). The drug was administered 20 min before testing. ●$p<0.05$; ●●$p<0.01$.

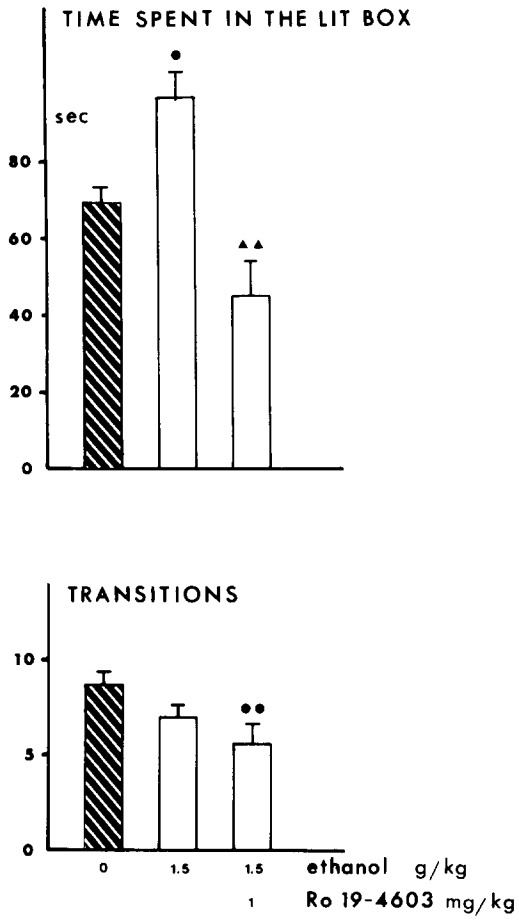

FIG. 2. Effects of ethanol given alone or in combination with RO 19-4603 on the time spent in the lit box and on the number of transitions (mean ± SEM). Drugs were administered IP 20 min before testing. ●$p<0.05$; ●●$p<0.01$: treated mice versus controls; ▲▲$p<0.01$: ethanol versus drug combination-treated mice.

results confirm the anxiogenic effects of several other inverse agonists of benzodiazepine receptors such as FG 7142 (16,17), β-carbolines (1, 9, 13, 23, 25, 27) and RO 15-4513 (3, 8, 14, 18, 22).

In addition, we found (Experiment 3) that RO 19-4603 dosed at 1.0 mg/kg completely reversed the significant increase of the time spent by mice in the lit box induced by ethanol dosed at 1.5 g/kg. This result is in agreement with the findings of the study of Lister and Durcan (20). Indeed, these authors showed that low doses of RO 19-4603 (0.01–0.3 mg/kg) partially reversed the intoxicating effects of a high dose of ethanol (2.4 g/kg).

Since RO 19-4603, at a dose of 1.0 mg/kg, elicit intrinsic anxiogenic effects in the light/dark choice procedure, it can be suggested that this drug acts in an additive rather than in an interactive manner against the behavioral effects of ethanol. This hypothesis is strengthened by the observation that mice treated with the combination of RO 19-4603 and ethanol exhibited less transitions then controls, while ethanol given alone did not significantly affect this variable. On the other hand, it is interesting to note that the doses of RO 19-4603 used by Lister and Durcan (20) are lower than these we used here and that Bonetti (personal communication) reported that high doses of this compound were unable to antagonize the intoxicating effects of ethanol. This can be explained by the fact that high doses of RO 19-4603 produce a reduction of locomotion and rears (see Experiment 1) and this is a new argument in favour of the hypothesis that ethanol and RO 19-4603 may act in an additive way. According to this, the present results confirm the assumption that the ability of the benzodiazepine inverse agonists to reverse some of ethanols' actions may be due to their intrinsic effects rather than to a specific and selective antagonism. This is in agreement with the results of several studies (2–4, 16, 18) but contrasts with those of others (28,29). The latter authors noted that there are data demonstrating

TABLE 2

PROCONVULSANT EFFECT OF RO 19-4603

	Doses (mg/kg)						
	0	0.10	0.25	0.50	1.00	5.00	10.0
Number of mice showing full tonico clonic convulsions	4	11	11	10	14†	13*	15‡
N	15	15	15	15	15	15	15

RO 19-4603 was administrated 2 min prior to PTZ (30 mg/kg).
*$p<0.02$; †$p<0.01$; ‡$p<0.001$ (chi-square test).

that RO 15-4513, for example, reverses some actions of ethanol at doses that do not alter baseline performance. Indeed, we have also found that RO 15-3505 (2) as well as RO 15-1788 (3) were capable of reversing some behavioral effects of ethanol in mice, without showing obvious intrinsic effects. Moreover, Treit (30) observed that low doses of anxiogenic drugs other than inverse agonists of benzodiazepine receptors such as picrotoxin and PTZ were able to counteract the anxiolytic effects of chlordiazepoxide in the defensive burying test without exhibiting intrinsic effects. Thus, as we already noted elsewhere (2), this difficulty perhaps reflects the fact that animal models of anxiolytic/anxiogenic drug action are not equally sensitive in revealing possible anxiogenic actions.

It is well established that the inverse agonists of benzodiazepine receptors are proconvulsant (2, 3, 6, 10, 24, 26). In Experiment 4, we also found that RO 19-4603 lowered the seizure threshold to PTZ and so we confirmed the proconvulsant properties of this compound that were first observed by Pieri (26) and by Lister and Durcan (20). Massoti (21), using electroencephalographic investigations, noted that low doses of proconvulsant drugs such as FG 7142 and β-CCE elicited changes in EEG similar to those observed after administration of bicuculline, but that higher doses can paradoxically induce an hypersynchronous cortical pattern, the animals showing muscle relaxation and head drop and so it is likely that the reduction of activity (locomotion and rears) induced by the highest dose (10.0 mg/kg) of RO 19-4603 in the free exploration test may be related to a pre-ictal prostration. Indeed, we did not observe convulsions when the compound was injected alone, not even with the highest dose used (10 mg/kg).

Taken together, these results suggest the ability of inverse agonists to block some of actions of ethanol are due to the analeptic properties of these drugs.

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