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Does RO 15-4513 Reverse the Anxiolytic Effects of Ethanol by its Intrinsic Properties?

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BELZUNG, C., R. MISSLIN AND E. VOGEL. *Does RO 15-4513 reverse the anxiolytic effects of ethanol by its intrinsic properties?* PHARMACOL BIOCHEM BEHAV **30**(4) 867–870, 1988.—In order to better understand the antagonistic effects of the partial inverse agonist of benzodiazepine receptors, RO 15-4513, against the disinhibitory action of ethanol, we examined the effects of RO 15-4513 at a dose (2.0 mg/kg) that did not alter locomotor activity, given alone or in combination with ethanol, on the behavior of mice confronted with the light/dark choice procedure and the staircase test. At this dose, RO 15-4513 given alone was found to have slight anxiogenic properties and when given in combination with ethanol, to completely reverse the disinhibitory effects of ethanol. Since we previously observed postictal depression after higher doses of RO 15-4513 given alone and antagonistic effects of these same doses on the action of ethanol, it can be suggested that the antagonistic effects of RO 15-4513 against ethanol are due to its anxiogenic or depressive properties depending on doses. However, this hypothesis can only be regarded as being in early stages of development at the present time since these results do not parallel with those of several other studies and the question whether the antagonistic action of RO 15-4513 against ethanol is additive or interactive remains open.

RO 15-4513 Light/dark choice procedure Staircase test Locomotion Mice Convulsions

RO 15-4513 (ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5-a] [1,4] benzodiazepine-3-carboxylate) has a high affinity for central benzodiazepine binding sites and has been used in photoaffinity labelling studies of these receptors [14]. Recently, this drug has been shown to reverse some of the actions of ethanol. For example, it reversed the impairment of motor performance, reduced the level of intoxication caused by ethanol [3, 4, 6, 16] and antagonized the disinhibitory effects of this latter drug in a conflict paradigm [16], in a holeboard test [8] and in the light/dark choice procedure [13]. Moreover, there are also reports that RO 15-4513 possesses some intrinsic activities: it has partial inverse agonist activity at benzodiazepine receptors [14] and decreased exploratory behavior in a holeboard apparatus [8,9]. Therefore, one can suggest that the reversal of ethanol's anticonflict activity by RO 15-4513 is related to anxiogenic properties and thus, it would act in an additive, rather than interactive manner [10]. In addition, RO 15-4513 has also been found to lower seizure threshold to bicuculline and pentylenetetrazol [3, 13, 15]. Since we recently observed [13] that soon (1–2 min) after injection of doses of RO 15-4513 inducing convulsions, mice presented a postural pattern characterized by ataxia that could be related to a postictal prostration, we suggested that the antagonistic properties of RO 15-4513 against disinhibitory effects of ethanol could also be due to its depressive action. However, other authors did not find a reduction of locomotion in animals treated with RO 15-4513 alone [8]. This discrepancy can be related to the differences in doses, in species and perhaps in experimental designs used by the different experimenters. In order to partially clarify this question, we first examined the effects of different doses, from 0.5 to 12.0 mg/kg of RO 15-4513 in a free exploration test. Since we found that the dose of 2 mg/kg did not produce a decrease of locomotion, we investigated the effects of ethanol and RO 15-4513 (2 mg/kg), given alone or in combination, on the behavior of mice confronted with two experimental procedures: the light/dark choice paradigm described by Crawley and Goodwin [5] and modified by one of us (R.M.) and the staircase test described by Thiébot *et al.* [18]. Finally, we also examined the proconvulsant activity of RO 15-4513 (2 mg/kg) in mice treated with a subconvulsant dose of pentylenetetrazol.

METHOD

Male Swiss albino mice from Centre d'Elevage Iffa Credo, 13 weeks of age at time of testing, were used. Prior to experimental testing, they were housed five to a standard cage containing a constant supply of food pellets and water, and kept on a 12/12 hr 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.

Effects of RO 15-4513 on Locomotor Activity

The apparatus consisted of a polyvinylchloride box (30×20×20 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

TABLE 1
EFFECTS OF RO 15-4513 ON LOCOMOTOR ACTIVITY

Doses (mg/kg)	0	0.5	1	2	3	6	12
Locomotion	111.60	111.90	97.50	102.70	69.60*	67.50*	58.40†
SEM	6.6	13.9	5.6	12.9	10.2	9.0	11.1

RO 15-4513 was administered IP 20 min before testing. Dosed at 3.0, 6.0 and 12.0 mg/kg, RO 15-4513 significantly reduced locomotion, *$p<0.05$, †$p<0.01$ (Newman-Keuls test).

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.

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 familar 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 by the animals was recorded and defined as locomotor activity.

Drugs were administered intraperitoneally, 20 min before testing, in concentrations giving an injection volume of 10 ml/kg of mouse. Mice were randomly divided into 7 groups receiving vehicle (saline with a drop of Tween 80; n=10) or RO 15-4513 (0.5, 1.0, 2.0, 3.0, 6.0 and 12.0 mg/kg; n=10).

The Light/Dark Choice Procedure

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

The subjects were individually tested in five-minute 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 minutes, 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 above. Mice were randomly allocated to the following groups: vehicle (saline with a drop of Tween 80), ethanol (1 g/kg in saline), ethanol (1 g/kg) plus RO 15-4513 (2 mg/kg), RO 15-4513 (2 mg/kg) given alone. The size of all groups was the same: n=25.

The Staricase Test

The apparatus was a staircase that consisted of a white PVC enclosure with five steps (3.0 cm high, 10.0 cm wide, 7.5 cm deep) surrounded by a wall 23.0 cm high. A light from a 100 watt desk lamp above the staircase provided the only room illumination.

Mice were placed singly on the floor of the box, facing the steps. Two behavioral parameters were measured: the number of rears and the number of steps climbed; a step was considered as climbed only if the animal placed its four paws on it. The duration of the test was 5 min.

Drugs were as in the light/dark procedure. The size of all groups was the same: n=12.

Proconvulsant Effects of RO 15-4513

Saline (n=14) or RO 15-4513 (in saline with a drop of Tween 80, 2 mg/kg; n=14) were administered intraperitoneally to mice 2 min before pentylenetetrazol (in saline, 39 mg/kg) in concentrations giving an injection volume of 10 ml/kg. The number of mice showing full tonico-clonic seizures in a 5-min period after injection of pentylenetetrazol was recorded.

Statistical Analysis

Statistical significance of differences between control and treated groups was ascertained by a combined analysis of variance and a Newman-Keuls a posteriori test. The chi-square test was used for comparisons in experiment devoted to the proconvulsant effects of RO 15-4513.

RESULTS

Locomotor Activity

Table 1 shows that RO 15-4513 produced a significant decrease in the locomotion of animals treated with the doses of 3.0, 6.0 ($p<0.05$) and 12.0 mg/kg ($p<0.01$). However, there were no significant differences between controls and mice treated with RO 15-4513 at the doses of 0.5, 1.0 and 2.0 mg/kg.

The Light/Dark Choice Procedure

As can be seen from Fig. 1, ethanol significantly increased both the time spent by mice in the lit box and the number of transitions ($p<0.05$). With respect to these parameters, animals treated with the combination of ethanol and RO 15-4513 did not differ from controls, but they significantly differ from ethanol treated mice ($p<0.01$). Furthermore, when given alone, RO 15-4513 produced a reduction in both behavioral components of the test, but only the decrease of the time spent in the lit box reached significance ($p<0.01$)

The Staircase Test

Figure 1 shows that ethanol at 1 g/kg produced a significant increase in the number of rears and in the number of steps climbed by animals ($p<0.01$). This effect was reversed by RO 15-4513, since mice treated with a combination of

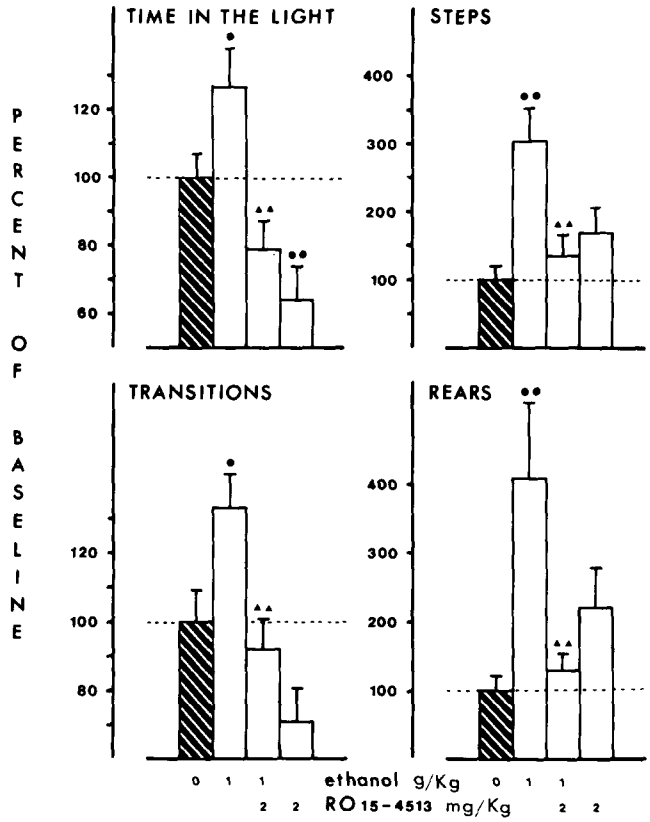

FIG. 1. Ethanol (1 g/kg) administered IP 20 min before testing tended to increase all the behavioral parameters that were recorded. RO 15-4513, dosed at 2.0 mg/kg, antagonized these effects. When given alone, RO 15-4513 (2.0 mg/kg) significantly reduced the time spent by mice in the lit box. ●$p<0.05$; ●●$p<0.01$: treated mice versus controls. ▲▲$p<0.01$: ethanol (1 g/kg) combined with RO 15-4513 (2.0 mg/kg) versus ethanol (1 g/kg) mice.

ethanol and RO 15-4513 did not significantly differ from controls while they differed from ethanol treated mice ($p<0.01$). When RO 15-4513 was administered alone, it produced a slight, but not significant, increase of both parameters.

Proconvulsant Effects of RO 15-4513

A dose of 39 mg/kg of pentylenetetrazol did not induce tonico-clonic convulsions in the 14 mice injected. In contrast, this drug administered 2 minutes after RO 15-4513 at a dose of 2.0 mg/kg induced full tonico-clonic convulsions in 7 out of 14 mice. These two groups significantly differ ($chi^2=6.85$, $p<0.01$).

DISCUSSION

In accordance with previous studies [2,13], ethanol caused disinhibitory effects on the behavior of mice confronted with a light/dark choice procedure and with the staircase test. These effects closely resemble those obtained with benzodiazepines [1, 5, 12]. RO 15-4513, at a dose of 2.0 mg/kg, completely reversed these effects. Furthermore, when given alone, this drug induced a significant decrease in the time spent by mice in the lit box without significantly affecting neither the transitions between the light box and the dark one nor the behavior in the staircase test. Therefore, it can be suggested that, dosed at 2.0 mg/kg, RO 15-4513 exhibited only slight anxiogenic properties when compared, for instance, to the effects of the benzodiazepine receptor inverse agonist β-CCM that reduced the time spent by mice in the lit box as well as the behavioral parameters in the staircase [1,2]. Support for this hypothesis is that no significant effects on the locomotor activity were found when RO 15-4513 was administered at 2.0 mg/kg, while this drug significantly decreased locomotion at higher doses (3.0, 6.0 and 12.0 mg/kg). Massotti [11], 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. It is likely that the reduction of locomotion induced by high doses of RO 15-4513 in the present study can be related to a postictal prostration insofar as we previously found that the high doses of RO 15-4513 induced full tonico-clonic seizures in all mice treated with a subconvulsant dose of pentylenetetrazol [13]. In contrast, the dose of 2.0 mg/kg seems to present less pronounced proconvulsant properties since only 7 out of 14 mice exhibited full tonico-clonic seizures when treated with a subconvulsant dose of pentylenetetrazol. This can explain why at this dose RO 15-4513 did not reduce locomotion of mice. The findings of the present study are in agreement with those observed by Lister [8] showing that RO 15-4513, given alone at doses of 0.75, 1.5 and 3.0 mg/kg, significantly reduced exploratory head-dipping in mice confronted with the holeboard test, without altering locomotor activity. Insofar as RO 15-4513 at these doses did not modify locomotion, it can be suggested that this drug induced an inhibitory action on the behavior of animals by exhibiting opposite effects of those of benzodiazepines. Furthermore, since we previously observed a postictal depression after higher doses of RO 15-4513, we suggested that the ability of this drug in reversing some of ethanol's actions can in some cases depend on its depressive properties. According to this, the antagonistic effects of RO 15-4513 against ethanol can be considered as an additive action rather than an interactive one per se. The present results are similar to those obtained by Koob *et al.* [7] with the well known inverse agonist FG 7142 and to those we observed with the β-carboline, β-CCM [2].

However, for different reasons, the question seems to be more complex. First, we observed that RO 15-4513 was able to counteract the anxiolytic effects of ethanol in the staircase test at a dose that was devoid of intrinsic properties. There are some clear similarities between this later result and those we obtained using another inverse agonist, RO 15-3505, in the staricase test as well as in the light/dark procedure [2]. Lister [9] also reported that FG 7142 had antagonistic effects at low as well as high doses but was devoid of intrinsic properties alone at low doses. Treit [19] observed that low doses of picrotoxin and pentylenetetrazol are able to counteract the anxiolytic effects of chlordiazepoxide in the defensive burying test without exhibiting intrinsic effects. Finally, we noted that RO 15-4513, at a dose of 3.0 mg/kg, presents intrinsic effects in the light/dark procedure, but not in a running wheel test, while it antagonizes ethanol's effects in both situations [13]. This may lead us to suggest that it is not absolutely necessary to observe obvious intrinsic effects with the inverse agonists so as to observe antagonistic properties against ethanol. For example, Treit [19] in his recent study also suggested that the different animal paradigms of

anxiolytic drug effects are not equally sensitive to the possible anxiogenic effects of benzodiazepine receptor inverse agonists. Second, there are conflicting data concerning the ability of inverse agonists in reversing some of ethanol's effects. For example, Suzdak *et al.* [16] reported that FG 7142 did not reverse the anticonflict effects of ethanol in the Vogel test, whereas Koob *et al.* [7] found such effects. Moreover, Bonetti *et al.* [4] observed only a slight activity of β-CCM against ethanol in the horizontal wire test, while we found that the same drug completely reversed the anxiolytic effects of ethanol [2]. Finally, while Suzdak *et al.* [16] have demonstrated that RO 15-4513 was able to reduce the level of sedation induced by a high dose of ethanol, we did not observe such effects [13]. Taken together, these data suggest, therefore, that the effects of the interaction between the inverse agonists and ethanol can depend in part on the behavioral paradigms, species of animal as well as doses used.

In conclusion, the present data suggest that some of the antagonistic properties of RO 15-4513 against ethanol might be due to a functional opposition of behavioral effects. Nevertheless, the question remains open whether inverse agonists act in an additive or in an interactive manner in their antagonistic properties against ethanol. Although Suzdak *et al.* [17] recently showed that ethanol is able to stimulate GABA/barbiturate receptor-mediated Cl^- transport in rat brain synaptoneurosomes, the exact mechanism(s) for this action is unknown and that can explain in part why it is difficult at this time to account for the antagonistic properties of benzodiazepine inverse agonists against ethanol.

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