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Differential Interactions Between Ethanol and Ro 15-4513 on Two Anxiety Tests in Rats

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PRUNELL, M., R. M. ESCORIHUELA, A. FERNÁNDEZ-TERUEL, J. F. NÚÑEZ AND A. TOBEÑA. *Differential interactions between ethanol and Ro 15-4513 on two anxiety tests in rats.* PHARMACOL BIOCHEM BEHAV 47(1) 147–151, 1994. — The effects of low (2 g/kg) and high (4 g/kg) doses of ethanol and their interaction with the imidazobenzodiazepine Ro 15-4513 (a partial inverse agonist of the benzodiazepine receptor) were studied in two different models of anxiety in rats: the "elevated plus-maze" test and the early acquisition of two-way (shuttlebox) avoidance. In the elevated plus-maze, ethanol (2 g/kg) increased the percentage of entries into the open arms (%EOA) and both ethanol doses increased the percentage of time spent into the open arms (%TOA), thus indicating an anxiolytic action which was reversed by Ro 15-4513 (5 mg/kg). By contrast, Ro 15-4513 did not counteract the anxiolytic effect of the low dose of ethanol in the acquisition of shuttlebox avoidance. Thus, the treatment with 2 g/kg of ethanol (plus either vehicle or Ro 15-4513) significantly increased the total number of avoidances. Conversely, animals treated with 4 g/kg of ethanol showed impaired shuttlebox avoidance acquisition, and this effect was completely reversed by Ro 15-4513. Ro 15-4513 was without effect on its own in any of the anxiety-related parameters (i.e., %EOA, %TOA, and avoidance acquisition). The results indicate a different pattern of ethanol effects and Ethanol × Ro 15-4513 interactions depending upon the task used.

Ethanol Ro 15-4513 GABA/Bz receptor Elevated plus-maze Shuttlebox avoidance Rats

SEVERAL reports have claimed that the imidazobenzodiazepine Ro 15-4513, a partial inverse agonist of the benzodiazepine receptors, could antagonize several effects of ethanol (18,20,21,23,29). While the mechanism of this antagonism is not completely clear, it has been generally attributed to the stimulant properties of Ro 15-4513, resulting from an anti-GABAergic action mediated by its particular effect (i.e., partial inverse agonism) on the $GABA_A$/benzodiazepine ($GABA_A$/BZ) receptor domain (4,14). Nevertheless, by using doses of Ro 15-4513 devoid of intrinsic effects some authors have presented evidence supporting the specificity of the antagonism of ethanol effects by Ro 15-4513 (1,14,29).

Benzodiazepines and ethanol, though acting at different sites of the $GABA_A$/BZ receptor complex, share a number of similar behavioral effects, one of which is an anxiolytic action (15,22,25). On the contrary, Ro 15-4513 at certain doses has been shown to be anxiogenic in animal tests of anxiety (4,7, 12,22). Hence, testing the antagonism of the anxiolytic actions of ethanol by Ro 15-4513 (at nonanxiogenic doses) in different models of anxiety may provide additional information about the specificity of Ro 15-4513 effects.

Therefore, the purpose of the present study was to compare the effects of two doses of ethanol (a low one, 2 g/kg PO, and a high one, 4 g/kg PO) in two particular tests of anxiety, the elevated plus-maze (24) and the early acquisition of shuttlebox avoidance (12), and to investigate their interactions with Ro 15-4513 in the same tests. We have previously established that the aforementioned doses of ethanol show clear opposite effects in rats, the 2-g/kg dose being stimulant, as measured by spontaneous motor activity and shuttlebox avoidance (in overtrained rats), and the 4-g/kg dose being clearly a depressant in the same tests (28).

The elevated plus-maze was validated by Pellow et al. (24) as a test of anxiety and has been widely used in this way by several authors (13,19,21,22). On the other hand, the acquisition of two-way active (shuttlebox) avoidance has been generally considered as a learning-retention task used to study the disruptive effects of drugs such as ethanol (28) on these pro-

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cesses. But in the present study, the early acquisition (i.e., early trials) of shuttlebox avoidance was used as a test of anxiety, since previous work has proved 1) that anxiety interferes with the initial acquisition of shuttlebox avoidance (15,16,30,31) and 2) that anxiolytic and anxiogenic drugs (as well as anxiolytic and anxiogenic environmental treatments) respectively improve and impair two-way avoidance acquisition (2,3,9–12,30).

MATERIAL AND METHODS

Animals

Seventy-one male Sprague–Dawley rats, weighing 230–310 g (obtained from Iffa Credo, France) were used in the experiment. They were housed two to three per cage under regulated light/dark conditions (light, 0800 to 2000) with food and water freely available. The experiment was carried out from 0900 to 1400.

Behavioral Tests

Elevated plus-maze. Twenty minutes after drug administration, animals were tested for 5 min in the elevated plus-maze, which consisted of two open arms, 50 × 10 cm, and two enclosed arms, 50 × 10 × 40 cm, with an open square of 10 × 10 cm in the center of the "plus" sign. The maze was elevated to a height of 50 cm (25). Total arm entries (TE) and the number and duration of the entries into the open and enclosed arms were scored.

Shuttlebox avoidance acquisition. A shuttlebox (Letica Instruments, Barcelona, Spain) was employed. The session included 10 min of habituation, inmediately followed by a series of 40 trials of avoidance acquisition. Each trial consisted of 10 s of conditioned stimulus (CS, light and tone simultaneously), followed by a 0.6-mA foot-shock of 30 s as aversive unconditioned stimulus (US). The intertrial interval was 30 s. The number of avoidances (crossings in the presence of CS) and the total escape latencies (mean time elapsing between the CS presentation and the response) were scored. The animals were introduced into the apparatus 30 min after drug administration and 5 min after testing in the elevated plus-maze.

Preliminary experiments (unpublished) from our laboratory have indicated that the present design (involving the succesive testing of animals in the elevated plus-maze and in shuttlebox avoidance) is a valid procedure for testing anxiolytic effects of drugs, since benzodiazepines produce the expected increase in the percentage (and time) of open arm entries in the elevated plus-maze and they enhance avoidance acquisition in a way similar to when a single test procedure (either the elevated plus-maze or a suttlebox session) is used (12).

Drug Administration and Experimental Groups

Ro 15-4513 (provided by Roche S.A., Basel, Switzerland) was suspended in 1% carboxymethyl cellulose (cmc) and administered IP at a dose of 5 mg/kg in a volume of 2 ml/kg. Ethanol (Panreac, Barcelona, Spain), at doses of 2 and 4 g/kg, was given PO in a 20% v/v solution in tap water. Control animals received cmc and tap water at the corresponding volumes. The experimental groups were as follows: VEH-VEH (n = 18), control rats receiving the corresponding vehicle treatments; ETOH2-VEH and ETOH4-VEH (n = 15 and n = 10, respectively), animals receiving either acute ethanol 2 or 4 g/kg, respectively; VEH-RO5 (n = 9), animals treated with Ro 15-4513 5 mg/kg; ETOH2-RO5 and ETOH4-RO5 (both n = 9), animals treated with ethanol 2 or 4 g/kg, respectively, plus Ro 15-4513 5 mg/kg.

The present ethanol doses were selected on the basis of previous studies [(28) and unpublished results] showing that 2 and 4 g/kg (PO, in a 20% v/v solution) of the drug produced clear (but not maximal) stimulation and depression of locomotor activity [maximal depression was observed after ethanol 5 g/kg PO (28)], respectively, in rats of the same weight and strain as the present ones. The blood ethanol levels observed in two separate groups of rats (same weight and strain as in the present study; n = 6/group) 30 min after receiving ethanol 2 and 4 g/kg (PO; 20% v/v solution) were of 77.0 ± 2.2 and 121.0 ± 3.6 mg/dl, respectively (mean ± SE; blood samples obtained by cardiac puncture and analyzed using an enzymatic assay kit, Boehringer Mannheim GmbH Diagnostica, No. 123960-UV).

Statistical Analysis

Two-way analysis of variance (ANOVA, factors: Ethanol and Ro 15-4513) was applied to the total arm entries (TE), the

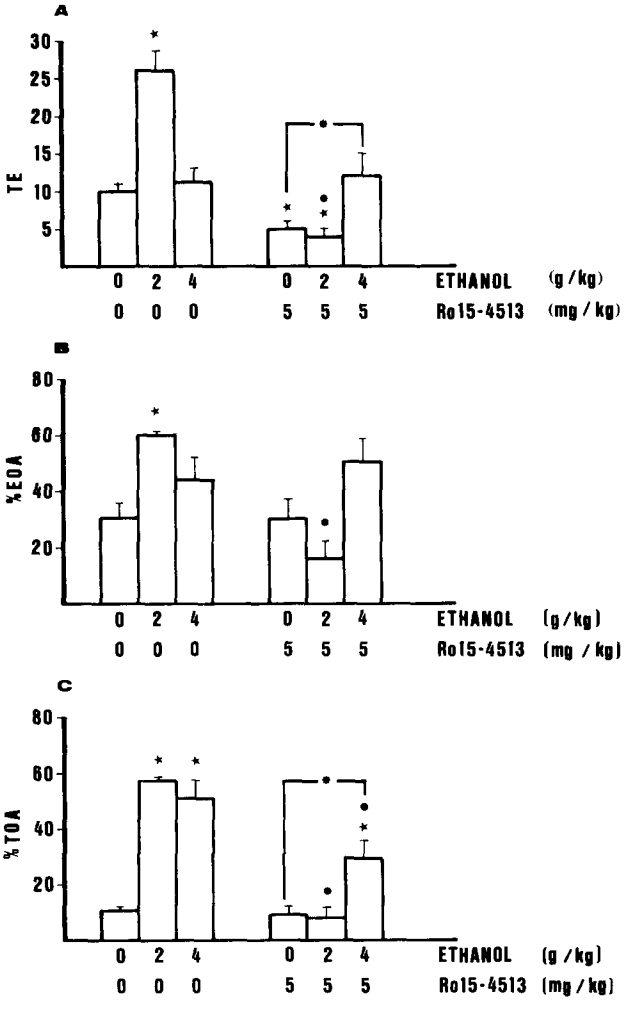

FIG. 1. Means ± SE of plus-maze measures: (A) Total arm entries (TE), (B) percentage of open arm entries (%EOA), and (C) percentage of time spent in the open arms (%TOA). ★$P < 0.05$ vs. VEH-VEH group; ●$P < 0.05$ vs. the respective ETOH-VEH group; *$P < 0.05$ between the groups indicated (Duncan's tests).

percentage of open arm entries (%EOA = open arm entries/TE * 100), and the percentage of time spent in the open arms (%TOA = time open arms/[time open arms + time enclosed arms] * 100). Total avoidances and escape latencies during shuttlebox avoidance acquisition were also analyzed with two-way ANOVA. Duncan's multiple range tests were used for comparisons between groups.

Analysis of covariance (ANCOVA) was performed to determine whether the effects in open arm entries were influenced by nonspecific changes in locomotor activity. Thus, covariance analysis was carried out with open arm entries (dependent variable) and enclosed arm entries as the covariate.

RESULTS

The results from the elevated plus-maze test are represented in Fig. 1. The two-factor ANOVA revealed an overall effect of ethanol on TE, $F(2, 69) = 21.69$, $P = 0.000$; %EOA, $F(2, 69) = 4.68$, $P < 0.02$; and %TOA, $F(2, 69) = 48.89$, $P = 0.000$, mainly reflecting overall increases (induced by ethanol) in the three measures (although ethanol 4 g/kg produced a significant effect only in %TOA; Fig. 1c). ANOVA analysis also showed an opposite overall effect of Ro 15-4513 on TE, $F(1, 70) = 9.59$, $P = 0.000$; %EOA, $F(1, 70) = 9.59$, $P < 0.01$; and %TOA, $F(1, 70) = 56.83$, $P = 0.000$, indicating that Ro 15-4513 reduced ethanol effects in those three parameters. As a consequence of that, the two-way Ethanol × Ro15-4513 interactions were also significant in all the three measures: TE, $F(2, 69) = 27.06$, $P = 0.000$; %EOA, $F(2, 69) = 9.71$, $P = 0.000$; %TOA, $F(2, 69) = 21.20$, $P = 0.000$. More specifically, Ro 15-4513 clearly reversed the increase in TE, %EOA, and %TOA ($P < 0.05$ between ETOH2 and ETOH2 + RO5, Duncan's test, Fig. 1) induced by 2 g/kg ethanol. Moreover, Ro 15-4513 partially (but significantly) counteracted the increase of %TOA produced by 4 g/kg ethanol ($P < 0.05$ between ETOH4 and ETOH4 + RO5, Duncan's test, Fig. 1c).

ANCOVA analysis of entries into the open arms, by taking entries into the enclosed arms as the covariate, showed that the covariate was significant, $F(1, 70) = 115.65$, $P = 0.000$, but all the main and interaction effects on entries into the open arms were still significant—ethanol effect, $F(2, 69) = 26.44$, $P = 0.000$; Ro 15-4513 effect, $F(1, 70) = 5.42$, $P < 0.03$; two-way Ethanol × Ro 15-4513 interaction, $F(2, 69) = 25.74$, $P = 0.000$—thus indicating that the influence of treatments on entries into the open arms can not be accounted by their effects on general motor activity.

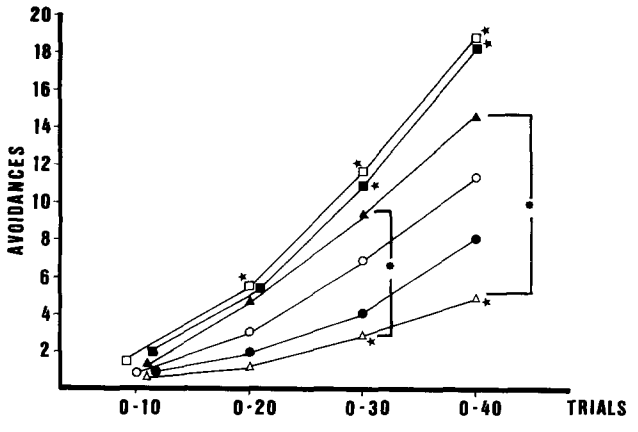

FIG. 2. Means of accumulated avoidances (in blocks of 10 trials) in the 40-trial session of two-way active avoidance acquisition. ○—○ VEH-VEH, □—□ ETOH2-VEH, △—△ ETOH4-VEH, ●—● VEH-RO5, ■—■ ETOH2-RO5, ▲—▲ ETOH4-RO5. ★$P < 0.05$ vs. VEH-VEH group; *$P < 0.05$ between the groups indicated (Duncan's tests).

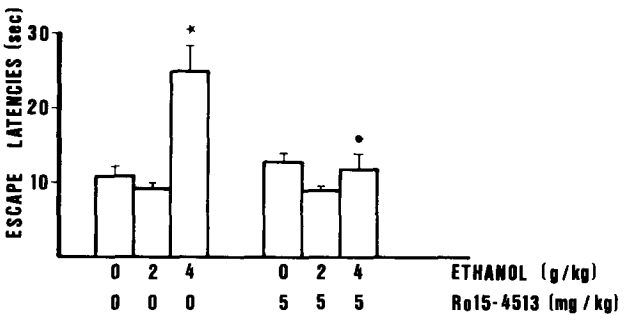

FIG. 3. Means ± SE of "escape latencies" (calculated for each animal as the mean escape/avoidance latency of the 40 trials). ★$P < 0.05$ vs. VEH-VEH group; ●$P < 0.05$ vs. the corresponding ETOH-VEH group (Duncan's tests).

With respect to shuttlebox avoidance training, there was a significant ethanol effect all along the process of avoidance acquisition (ANOVAs tests—not shown—of the first 20 and 30 trials indicated significant effects of ethanol 2 and 4 g/kg; the overall ethanol effect on total avoidances was $F(2, 69) = 14.18$, $P = 0.000$; Fig. 2). Thus, 2 g/kg ethanol enhanced ($P < 0.05$ vs. VEH-VEH group, Duncan's test, Fig. 2), and 4 g/kg ethanol impaired ($P < 0.05$ vs. VEH-VEH group, Duncan's test), avoidance acquisition. There was also a significant Ethanol × Ro 15-4513 interaction effect on avoidances, $F(2, 69) = 5.41$, $P < 0.01$. Thus, Ro 15-4513 completely reversed the avoidance deficit induced by the highest dose of ethanol ($P < 0.05$ between ETOH4-VEH and ETOH4-RO5 groups, Duncan's test, Fig. 2). Nevertheless, Ro 15-4513 did not alter the avoidance enhancement induced by 2 g/kg ethanol (Fig. 2). Concerning the total escape latency (Fig. 3), there were significant ethanol effects, $F(2, 69) = 19.55$, $P = 0.000$; Ro 15-4513 effects, $F(1, 70) = 5.25$, $P < 0.03$; and Ethanol × Ro 15-4513 effects, $F(2, 69) = 10.72$, $P = 0.000$. Duncan's comparisons between groups showed that 4 g/kg ethanol induced the highest latencies ($P < 0.05$ vs. VEH-VEH group, Fig. 3), but this effect was antagonized (as for avoidances) by Ro 15-4513 ($P < 0.05$ between ETOH4-VEH and ETOH4-RO5 groups, Fig. 3). Finally, Ro 15-4513 did not show any significant effect (on either avoidances or escape latency) on its own.

DISCUSSION

The present results show that the low dose of ethanol (2 g/kg) increased TE, %TOA, and the %EOA in the elevated plus-maze and improved shuttlebox avoidance acquisition. The high ethanol dose (4 g/kg) increased %TOA, whereas, on the contrary, it impaired avoidance acquisition. The results obtained with the dose of 2 g/kg are overall consistent with the contention that moderate doses of ethanol display anxiolytic effects, both in the "elevated plus-maze" (20) and in shuttlebox avoidance acquisition (6,15). This anxiolytic action of ethanol could not be exclusively attributed to a nonspecific increase in motor activity, since in the elevated plus-maze test 1) the 4-g/kg dose increased %TOA without affecting TE [in

agreement with previous unpublished observations showing that ethanol 4 g/kg PO does not depress TE in the plus-maze (28)] and 2) covariance analysis showed that the increase of open arm entries produced by ethanol was independent of that produced on the enclosed arm entries.

It is worth noting that in the shuttlebox avoidance acquisition animals treated with 2 g/kg of ethanol showed enhanced acquisition (as compared to the VEH-VEH group) already in the first 20 trials, this being consistent with the effect found [several times (3)] in that task after administration of anxiolytic benzodiazepines. Thus the typically observed increase in two-way avoidance acquisition after administering anxiolytic benzodiazepines is especially prominent during the early trials of the acquisition session, when the conflict between passive and active avoidance is greater [thus when anxiety is more evident (15)]. Conversely, the 4-g/kg dose of ethanol appeared to have impairing effects on avoidance acquisition which are likely interpretable in terms of its disruptive action on the perceptual and associative processes involved in the initial stages of this complex learning task. Such disruptive action of 4 g/kg ethanol was similarly reported in the shuttlebox performance of overtrained rats (28).

Furthermore, the present study shows that Ro 15-4513 antagonized the enhancement induced by 2 g/kg ethanol on all the parameters of the elevated plus-maze test and also diminished the increase of %TOA produced by 4 g/kg ethanol. Overall, such results agree with previous studies using the elevated plus-maze and other animal tests of anxiety, but they are not entirely consistent with the contention that the ethanol-antagonist effects of Ro 15-4513 could be exclusively due to its partial inverse agonist (i.e., anxiogenic) profile (5,21), since:

1. Although Ro 15-4513 showed an overall significant effect on the %EOA and %TOA in the elevated plus-maze, the observation of Figs. 1b and 1c indicates that this effect was produced by the important increase observed in the groups treated with ethanol alone. Thus, Ro 15-4513 did not show any anxiogenic effect on its own. According to this, the groups treated with Ro 15-4513 (VEH-RO5, ETOH2-RO5, and ETOH4-RO5) did not show lower scores than the VEH-VEH group, in either %EOA or %TOA.
2. In spite of that, VEH-RO5 and ETOH2-RO5 groups showed a decrease in the total arm entries (TE, Fig. 1a). Thus, interestingly, besides antagonizing the anxiolytic action of ethanol in the elevated plus-maze, Ro 15-4513 reduced locomotor activity and reversed the ethanol-induced increase on that measure (i.e., the locomotor stimulating action of 2 g/kg ethanol, as indicated by the increase in "total arm entries"). Such a result is in contradiction with other reports using mice instead of rats (21,22), but it is consistent with our (unpublished) results showing that Ro 15-4513 blocks the stimulating effects of ethanol (2 g/kg) on locomotor activity of rats.

Thus, the fact that the increase in locomotor activity (i.e., increase of "total arm entries") induced by ethanol was reversed by Ro 15-4513 in the elevated plus-maze does not appear to agree with the suggestion that the stimulant effects of ethanol (when used at relatively low doses) would be mainly due to its interaction with non-GABAergic neurotransmitter systems, whereas only its depressant effects (when high doses are used) have been related, at least partly, to an enhancement of GABAergic function (8,17,26,27).

In some respects the data from the elevated plus-maze test strongly contrast with those from shuttlebox avoidance acquisition. Thus, 1) 4 g/kg ethanol had a disruptive action upon avoidance acquisition while it increased behavior in the open arms (%TOA) of the elevated plus-maze, and 2) Ro 15-4513 did not block the improvement of shuttlebox avoidance acquisition induced by 2 g/kg ethanol, whereas it clearly reversed the anxiolytic action of that dose in the elevated plus-maze. On the other hand, the effects of Ro 15-4513 on shuttlebox avoidance (although nonsignificant) were in the same direction as those of 4 g/kg ethanol (i.e., a reduction of avoidances in both cases; see Fig. 2), and yet a clear mutual antagonism was found when the partial inverse agonist and 4 g/kg ethanol were combined.

In summary, Ro 15-4513 (5 mg/kg), without showing intrinsic effects on its own [as in our previous work (12)], was able to antagonize ethanol effects in both tests, although the pattern of interactions was somewhat different: In the elevated plus-maze, Ro 15-4513 reversed both the anxiolytic and the locomotor disinhibitory actions of ethanol, but in the avoidance acquisition model the anxiolytic effect of the low dose of ethanol remained intact, whereas the detrimental action of the high dose was completely blocked by Ro 15-4513. Probably this reflects the different behavioral requirements involved in each test, which contribute to the different actions of each drug on its own, and of the combination as well.

Such observations suggest that having an anxiogenic effect (typically observed after treatment with benzodiazepine-receptor partial inverse agonists) is not a necessary condition for antagonizing the anxiolytic activity of ethanol (at least in some tests, as the plus-maze) or for reversing its depressant or detrimental effects (as in the shuttlebox), which is in agreement with similar results obtained in other tasks (14).

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