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Reduction of the Intoxicating Effects of Ethanol by Drugs Acting at the Benzodiazepine-GABA Receptor Complex

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DURCAN, M. J. AND R. G. LISTER. *Reduction of the intoxicating effects of ethanol by drugs acting at the benzodiazepine-GABA receptor complex.* PHARMACOL BIOCHEM BEHAV **32**(3) 667–670, 1989.—The ability of the benzodiazepine receptor partial inverse agonists Ro 15-4513, Ro 15-3505 and FG 7142, and the picrotoxin site ligands pentylenetetrazole and Ro 5-3663 to reduce ethanol-induced intoxication were investigated. Ro 15-4513 (0.3–3 mg/kg), Ro 15-3505 (3 mg/kg), pentylenetetrazole (20 and 25 mg/kg) and Ro 5-3663 (4 mg/kg) all significantly attenuated the intoxicating effects of ethanol. In contrast, FG 7142 (20 and 40 mg/kg) failed to reduce ethanol intoxication, but reversed the effect of Ro 15-4513. This pattern of results differs from that obtained using other behavioral paradigms. Since drugs which reduce the effects of GABA generally reduce the intoxicating effects of ethanol, it is suggested that the β-carbolines may be unusual in their interaction with ethanol.

Alcohol Benzodiazepine receptor GABA Intoxication Mouse

THE ability of drugs such as pentylenetetrazole (PTZ) and picrotoxin, which we now believe act at the picrotoxin site on the benzodiazepine-GABA receptor complex (15,34), to reduce the intoxicating effects of ethanol has been established for many years (5, 8, 11, 16, 29, 35). Drugs that antagonize the effects of GABA via GABA-A receptors (e.g., bicuculline) also reduce the intoxicating effects of ethanol (7,16). More recently the benzodiazepine receptor partial inverse agonist Ro 15-4513 has also been reported to antagonize the intoxicating effects of ethanol (3,31). This may not be surprising in view of the fact that benzodiazepine receptor inverse agonists, like PTZ and bicuculline, reduce the effects of GABA, although through a different site on the complex—the benzodiazepine receptor. However, while other benzodiazepine receptor inverse agonists antagonise the effects of ethanol in some behavioral paradigms (1, 2, 12, 14, 17, 20, 26), in an observer-rated test of intoxication in rats, Ro 15-4513 appeared to differ from inverse agonists such as the β-carbolines β-CCE and FG 7142 (32). While one explanation of the apparent discrepancies between the findings was that the neurobiological mechanisms mediating ethanol's various behavioral effects might be different, another hypothesis was that there may be species differences. Many of the studies failing to find differences between the various inverse agonists have been performed in mice (1, 2, 12, 17, 26). The aim of the present study was to modify the test of intoxication used by Suzdak *et al.* (32) in rats for use in mice, and to compare the effects of various benzodiazepine receptor inverse agonists using this paradigm. The inverse agonists studied were the β-carboline FG 7142 (6), and the two imidazodiazepines Ro 15-4513 and Ro 15-3505. The latter drug has a high affinity for central benzodiazepine receptors but has a weaker inverse agonist action than Ro 15-4513 (19). It has been shown to reduce the effects of ethanol in several behavioral paradigms (2, 18, 27). The effects of the benzodiazepine receptor inverse agonists are also compared with those of PTZ and Ro 5-3663, two drugs which both act at the picrotoxin site of the benzodiazepine-GABA receptor complex (10, 15, 34). The doses of each drug were chosen on the basis of previous work in our laboratory (17,18).

METHOD

Animals

Male NIH Swiss mice weighing approximately 25 g were used in all experiments. They were housed in groups of 10, maintained on a 12-hr light:12-hr dark cycle and allowed ad lib access to food and water.

Drugs

Pentylenetetrazole (Sigma) and Ro 5-3663 (Hoffmann-La Roche) were dissolved in distilled water. Ro 15-4513, Ro 15-3505 (Hoffmann-La Roche) and FG 7142 (Research Biochemicals, Wayland, MA) were suspended in 0.3% Tween 20. These drugs were given IP in an injection volume of 10 ml/kg. Ethanol was

dissolved in distilled water (12% w/v) and given IP in an injection volume of 20 ml/kg.

Experimental

In all studies mice received 2.4 g/kg ethanol. Five minutes later each mouse's intoxication was rated after placing it on a wire cage lid. Immediately after this they received their respective drug treatments, and their intoxication was rated every 5 min thereafter for a further 20 min by an observer ignorant of the treatment that each mouse had received. The rating scale was a modified version of that of Majchrowicz (23): 0 = no observable effect; 1 = mild ataxia; 2 = moderate ataxia; 3 = severe ataxia; 4 = very severe ataxia, only just able to recover righting reflex; 5 = loss of righting reflex.

Experiment 1

The first study examined the effects of Ro 15-4513. Sixty mice were divided into 4 equal groups receiving Ro 15-4513 (0, 0.3, 1.0 or 3.0 mg/kg) 5 minutes after ethanol (2.4 g/kg).

Experiment 2

In the second study the effect of the two picrotoxin site ligands was examined. Ninety-nine mice were divided into 5 approximately equal groups receiving PTZ (20 or 25 mg/kg), Ro 5-3663 (2 or 4 mg/kg) or the vehicle 5 min after ethanol (2.4 g/kg). Neither of these drugs induced observable seizures in undrugged mice in the doses used.

Experiment 3

In view of reports that the imidazodiazepine Ro 15-3505 could also reverse some of ethanol's effects, we examined the interaction of this compound with ethanol in the intoxication paradigm. Sixty mice were divided into 4 equal groups receiving Ro 15-3505 (0, 0.75, 1.5 or 3.0 mg/kg) 5 min after ethanol (2.4 g/kg).

Experiment 4

This experiment examined the ability of the benzodiazepine receptor inverse agonist FG 7142 to antagonize the intoxicating effect of ethanol. The interaction of FG 7142 with Ro 15-4513 was also investigated. Seventy-four mice were divided into two equal groups receiving Ro 15-4513 (3 mg/kg) or the water/Tween vehicle 5 min following treatment with ethanol (2.4 g/kg). Approximately one-third of the mice in each group received FG 7142 (either 0, 20 or 40 mg/kg) at the same time as the Ro 15-4513 (or vehicle).

RESULTS

The predrug intoxication scores obtained 5 min after treatment with ethanol did not differ across groups in any of the experiments.

Interaction of Ethanol With Ro 15-4513 (Experiment 1)

Ro 15-4513 (0.3–3.0 mg/kg) reduced the intoxicating effects of ethanol in a dose-related manner at all time points following its administration (see Fig. 1).

Interaction of Ethanol With Picrotoxin Site Ligands (Experiment 2)

In Fig. 2 it can be seen that both PTZ (20 and 25 mg/kg) and Ro 5-3663 (4 mg/kg) significantly reduced the intoxicating effects of ethanol.

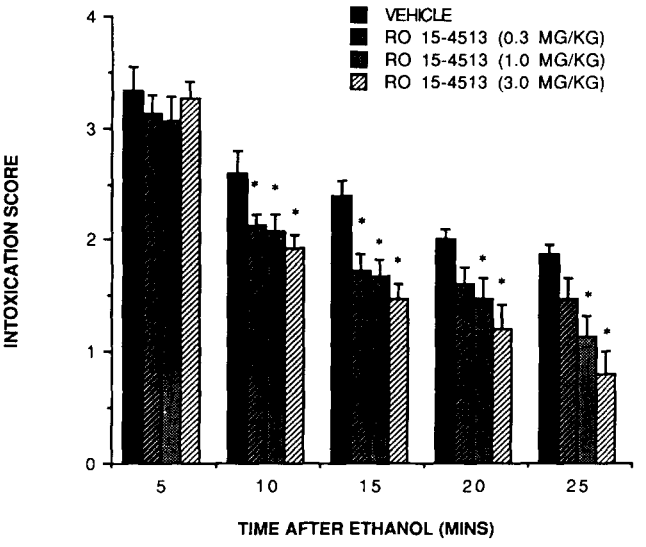

FIG. 1. The effect of Ro 15-4513 (0–3 mg/kg) on the intoxication of mice rated by an observer 5–25 min after treatment with ethanol (2.4 g/kg). Ro 15-4513 or its vehicle was given immediately after the first (5 min) rating. Values are mean ± SEM, n = 15 per group. *Significantly less intoxicated than vehicle-treated mice. $p<0.05$ Dunnett's test.

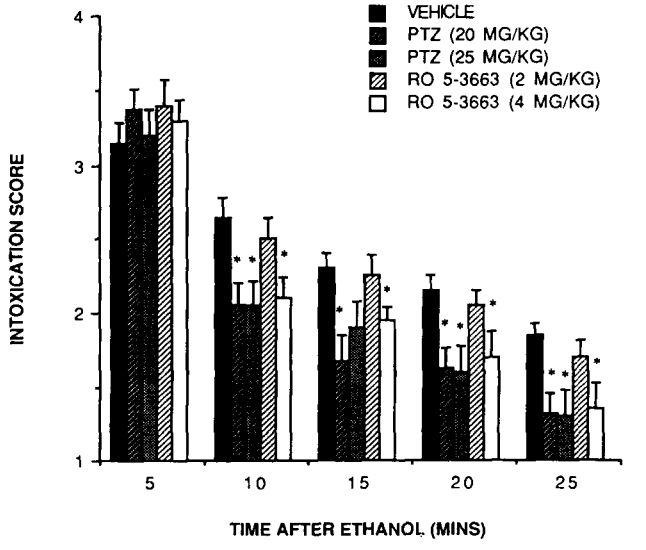

FIG. 2. The effect of pentylenetetrazole (PTZ 20 or 25 mg/kg), Ro 5-3663 (2 and 4 mg/kg) or the distilled water vehicle on the intoxication of mice rated by an observer 5–25 min after treatment with ethanol (2.4 g/kg). The drugs were given 5 min after administration of the ethanol. Values are means ± SEM, n = 19 or 20 per group. *Significantly less intoxicated than vehicle-treated mice. $p<0.05$ Dunnett's test.

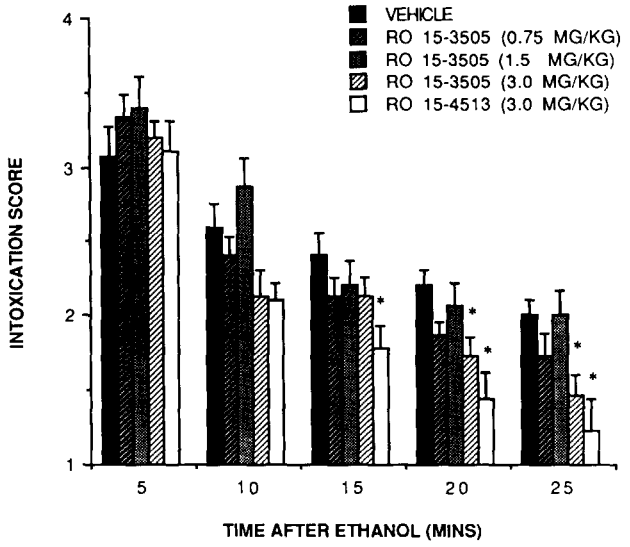

FIG. 3. The effect of Ro 15-3505 (0–3.0 mg/kg) or Ro 15-4513 (3 mg/kg) of the intoxication of mice rated by an observer 5–25 min after treatment with ethanol (2.4 g/kg). The drugs were given 5 min after administration of the ethanol. Values are means ± SEM 10–15 per group. *Significantly less intoxicated than vehicle-treated mice, $p<0.05$, Dunnett's test.

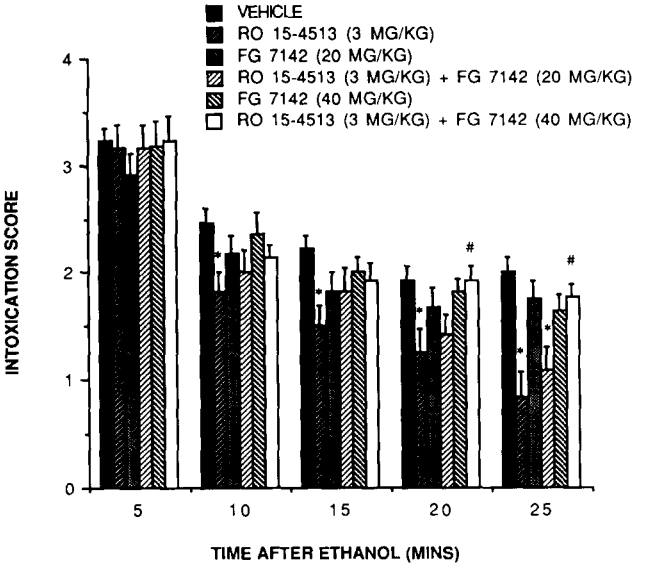

FIG. 4. The effect of Ro 15-4513 (0 or 3 mg/kg) in combination with FG 7142 (0, 20 or 40 mg/kg) on the intoxication of mice rated by an observer 5–25 min after treatment with ethanol (2.4 g/kg). The drugs were given 5 min after administration of the ethanol. Values are means ± SEM, n = 15 per group. *Significantly different from mice receiving the drug vehicles, $p<0.05$. #Significantly different from mice receiving Ro 15-4513 alone, $p<0.05$, Newman-Keuls.

Interaction of Ethanol With Ro 15-3505 (Experiment 3)

The highest dose of Ro 15-3505 significantly reduced the effect of ethanol 15 and 20 min after its administration (see Fig. 3). Although the mean intoxication scores of mice treated with the highest dose of Ro 15-3505 were above those of mice that received Ro 15-4513, this difference was not significant.

Interaction of Ethanol With FG 7142 and Ro 15-4513 (Experiment 4)

As in Experiments 1 and 3, Ro 15-4513 alone reduced the intoxicating effects of ethanol. In contrast, neither dose of FG 7142 alone reduced ethanol intoxication. Further, the reduction of ethanol's effect by Ro 15-4513 was reversed by FG 7142, i.e., animals that received Ro 15-4513 in combination with FG 7142 did not differ from vehicle-treated mice (see Fig. 4).

DISCUSSION

In the present study, the imidazodiazepine Ro 15-4513, which has been shown to reduce the effects of ethanol in a variety of behavioral paradigms (1, 3, 4, 13, 21, 22, 25, 27, 31–33, 35), also is able to reduce ethanol's intoxicating effects in mice. Ro 15-4513 is a partial inverse agonist at central benzodiazepine receptors (30), and reduces the effects of GABA via these sites. It might, therefore, be anticipated that drugs that reduce the effects of GABA via other sites on the benzodiazepine-GABA receptor complex would also reduce the intoxicating effects of ethanol. It was noted in the introduction that there is an old literature showing that this is indeed the case. In the current study PTZ and Ro 5-3663 both reduced the intoxicating effect of ethanol, presumably via an interaction at the picrotoxin site (10, 28, 34). In Experiment 3, Ro 15-3505, which is also a potent but weak partial inverse agonist at benzodiazepine receptors, also reduced ethanol intoxication. It appeared less effective than Ro 15-4513, but this might be anticipated based on the findings that Ro 15-4513's inverse agonist action is more marked than that of Ro 15-3505, e.g., Ro 15-3505 will antagonize some of Ro 15-4513's intrinsic effects (19). However, FG 7142, which is perhaps a more effective (but less potent) inverse agonist than Ro 15-4513, failed to reduce the intoxicating effects of ethanol. Furthermore, FG 7142 reversed the ethanol-antagonizing effects of Ro 15-4513, presumably by displacing Ro 15-4513 from benzodiazepine receptors. These data are consistent with those reported by others in rats (32).

Taken as a whole, what is surprising about the above data is not that Ro 15-4513 and Ro 15-3505 attenuate ethanol's intoxicating effects but rather that FG 7142 fails to reduce ethanol intoxication. It should be noted that in other behavioral paradigms (e.g., tests of anxiety, exploration and seizure threshold) FG 7142 is able to reduce the effects of ethanol in a manner similar to Ro 15-4513 (1, 4, 12, 14, 18, 26). Further, several in vitro studies also have found that FG 7142 and Ro 15-4513 interact with ethanol in a similar manner, with FG 7142 perhaps being more effective, but less potent than Ro 15-4513 [(9,24), and see work by Palmer in (22)]. The data from the present study, therefore, suggest that the neurobiological mechanisms mediating ethanol's intoxicating effects differ from those mediating some of ethanol's other behavioral effects such as its anxiolytic and anticonvulsant actions. FG 7142's failure to reduce ethanol intoxication suggests that β-carbolines interact with benzodiazepine receptors in a manner slightly different from other benzodiazepine receptor inverse agonists such as imidazodiazepines. The effects of inverse agonists on ethanol-stimulated chloride flux in the rat synaptoneurosomal preparation may reflect this different interaction (31). Data from other laboratories certainly suggest that β-carboline inverse agonists are less effective in reducing ethanol-intoxication than Ro 15-4513 (3,32). Work is currently in progress investigating whether other non-β-carboline inverse agonists can reduce ethanol's intoxicating effects.

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REFERENCES

1. Becker, H. C. Effects of the imidazodiazepine Ro 15-4513 on the stimulant and depressant actions of ethanol on spontaneous locomotor activity. Life Sci. 43:643–650; 1988.
2. Belzung, C.; Misslin, R.; Vogel, E. The benzodiazepine receptor inverse agonists β-CCM and Ro 15-3505 both reverse the anxiolytic effects of ethanol in mice. Life Sci. 42:1765–1772; 1988.
3. Bonetti, E. P.; Burkard, W. P.; Gabl, M.; Mohler, H. The partial inverse benzodiazepine agonist RO 15-4513 antagonises acute ethanol effects in mice and rats. Br. J. Pharmacol. 86:463; 1985.
4. Britton, K. T.; Ehleres, C. L.; Koob, G. F. Ethanol antagonist Ro 15-4513 is not selective for ethanol. Science 239:648–649; 1988.
5. Cott, J.; Carlsson, A.; Engel, J.; Lindqvist, M. Suppression of ethanol-induced locomotor stimulation by GABA-like drugs. Naunyn Schmiedebergs Arch. Pharmacol. 295:203–209; 1976.
6. Dorow, R.; Horowski, R.; Paschelke, G.; Amin, M.; Braestrup, C. Severe anxiety induced by FG 7142, a β-carboline ligand for benzodiazepine receptors. Lancet II:98–99; 1983.
7. Frye, G. D.; Breese, G. R. GABAergic modulation of ethanol-induced motor impairment. J. Pharmacol. Exp. Ther. 223:750–756; 1982.
8. Hahn, F. Analeptics. Pharmacol. Rev. 12:447–530; 1960.
9. Harris, R. A.; Allan, A. M.; Daniell, L. C.; Nixon, C. Antagonism of ethanol and pentobarbital actions by benzodiazepine inverse agonists: neurochemical studies. J. Pharmacol. Exp. Ther. 247:1012–1017; 1988.
10. Harrison, N. L.; Simmonds, M. A. The picrotoxin-like action of a convulsant benzodiazepine Ro 5-3663. Eur. J. Pharmacol. 87:155–158; 1983.
11. Hess, J. Uber die Weckwirkung verschiedener Analeptica bei der Alkohol- und Chloralosenarkose. Arch. Exp. Pathol. Pharmakol. 197:204–209; 1941.
12. Hilakivi, L. A.; Lister, R. G. Ro 15-4513 and FG 7142 reverse the reduction in social behavior caused by ethanol in mice. Eur. J. Pharmacol. 154:109–113; 1988.
13. Hoffman, P. L.; Tabakoff, B.; Szabo, G.; Suzdak, P.; Paul, S. M. Effect of an imidazodiazepine, RO 15-4513, on the incoordination and hypothermia produced by ethanol and pentobarbital. Life Sci. 41: 611–619; 1987.
14. Koob, G. F.; Braestrup, C.; Britton, K. T. The effects of FG 7142 and Ro 15-1788 on the release of punished responding produced by chlordiazepoxide and ethanol in the rat. Psychopharmacology (Berlin) 90:173–178; 1986.
15. Leeb-Lundberg, F.; Napias, C.; Olsen, R. W. Dihydropicrotoxinin binding sites in mammalian brain: interactions with convulsant and depressant benzodiazepines. Brain Res. 216:399–408; 1981.
16. Liljequist, S.; Engel, J. Effects of GABAergic agonists and antagonists on various ethanol-induced behavioral changes. Psychopharmacology (Berlin) 78:71–75; 1982.
17. Lister, R. G. The benzodiazepine receptor inverse agonists FG 7142 and RO 15-4513 both reverse some of the behavioral effects of ethanol in a holeboard test. Life Sci. 41:1481–1489; 1987.
18. Lister, R. G. Behavioral interactions between ethanol and imidazodiazepines with high affinities for benzodiazepine receptors. Life Sci. 42:1385–1393; 1988.
19. Lister, R. G. Antagonism of the behavioral effects of ethanol, sodium pentobarbital and RO 15-4513 by the imidazodiazepine RO 15-3505. Neurosci. Res. Commun. 2:85–92; 1988.
20. Lister, R. G. Interactions of three benzodiazepine receptor inverse agonists with ethanol in the plus-maze test of anxiety. Pharmacol. Biochem. Behav. 30:701–706; 1988.
21. Lister, R. G.; Nutt, D. J. Is Ro 15-4513 a specific alcohol antagonist? Trends Neurosci. 10:223–225; 1987.
22. Lister, R. G.; Nutt, D. J. Alcohol antagonists—the continuing quest. Alcohol.: Clin. Exp. Res. 12:566–569; 1988.
23. Majchrowicz, E. Induction of physical dependence upon ethanol and the associated behavioral changes. Psychopharmacology (Berlin) 43:245–254; 1975.
24. Mehta, A. K.; Ticku, M. K. Ethanol potentiation of GABAergic transmission in cultured spinal cord neurons involves $GABA_A$-gated chloride channels. J. Pharmacol. Exp. Ther. 246:558–564; 1988.
25. Nutt, D. J.; Lister, R. G. The effect of the imidazodiazepine RO 15-4513 on the anticonvulsant effects of diazepam, sodium pentobarbital and ethanol. Brain Res. 413:193–196; 1987.
26. Nutt, D. J.; Lister, R. G. Antagonizing the anticonvulsant effect of ethanol using drugs acting at the benzodiazepine/GABA receptor complex. Pharmacol. Biochem. Behav. 31:751–755; 1988.
27. Polc, P. Interactions of partial inverse benzodiazepine agonists Ro 15-4513 and FG 7142 with ethanol in rats and cats. Br. J. Pharmacol. 86:465P; 1985.
28. Ramanjaneyulu, R.; Ticku, M. K. Binding characteristics and interactions of depressant drugs with [^{35}S]t-butylbicyclophosphorothionate, a ligand that binds to the picrotoxin site. J. Neurochem. 42:221–229; 1984.
29. Schoen, R. Beitrage zur Pharmakologie der Korperstellung und der Labyrinthereflexe. XXIII. Mitteilung: Die antagonistische Beeinflussung der Narkose durch Erregungsmittel. Arch. Exp. Pathol. Pharmakol. 113:275–304; 1926.
30. Sieghart, W.; Eichinger, A.; Richards, J. G.; Mohler, H. Photoaffinity labelling of benzodiazepine receptor proteins with the partial inverse agonist [^{3}H]Ro 15-4513: a biochemical and autoradiographic study. J. Neurochem. 48:46–52; 1987.
31. Suzdak, P.; Glowa, J. R.; Crawley, J. N.; Schwartz, R. D.; Skolnick, P.; Paul, S. M. A selective imidazodiazepine antagonist of ethanol in the rat. Science 234:1243–1247; 1986.
32. Suzdak, P. D.; Paul, S. M.; Crawley, J. N. Effects of Ro 15-4513 and other benzodiazepine receptor inverse agonists on alcohol-induced intoxication in the rat. J. Pharmacol. Exp. Ther. 245:880–886; 1988.
33. Syapin, P. J.; Gee, K. W.; Alkana, R. L. RO 15-4513 differentially affects ethanol-induced hypnosis and hypothermia. Brain Res. Bull. 19:603–605; 1987.
34. Ticku, M. K.; Maksay, G. Convulsant/depressant site of action at the allosteric benzodiazepine-GABA receptor-ionophore complex. Life Sci. 33:2363–2375; 1983.
35. Ticku, M. K.; Kulkarni, S. K. Molecular interactions of ethanol with GABAergic system and potential of Ro 15-4513 as an ethanol antagonist. Pharmacol. Biochem. Behav. 30:501–510; 1988.