Pharmacology Biochemistry & Behavior, Vol. 39, pp. 803–807. Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

BRIEF COMMUNICATION

RO15-4513 Antagonizes the Anxiolytic Effects of Ethanol in a Nonshock Conflict Task at Doses Devoid of Anxiogenic Activity

HOWARD C. BECKER[1] AND ROBERT L. HALE

Veterans Administration Medical Center and Department of Psychiatry and Behavioral Sciences
Medical University of South Carolina, Charleston, SC 29403

Received 24 August 1990

BECKER, H. C. AND R. L. HALE. *RO15-4513 antagonizes the anxiolytic effects of ethanol in a nonshock conflict task at doses devoid of anxiogenic activity.* PHARMACOL BIOCHEM BEHAV **39**(3) 803–807, 1991.—RO15-4513 is a partial benzodiazepine inverse agonist that has been reported to antagonize some of the biochemical and neurobehavioral actions of ethanol. However, whether this antagonistic action of RO15-4513 is dependent on the drug exerting its intrinsic (inverse agonist) properties is unclear at present. The purpose of the present study was to examine whether RO15-4513 was capable of antagonizing the anxiolytic effects of ethanol in a nonshock conflict task at doses that, by themselves, do not reveal the compound's intrinsic anxiogenic properties. The consummatory conflict task employed (negative contrast) involves quantifying how animals respond to an abrupt, unexpected reduction in reward (sucrose solution), and is particularly sensitive to the effects of anxiolytic agents, including ethanol. As previously demonstrated, depressed consummatory behavior engendered by reward reduction was significantly alleviated by ethanol (0.75 g/kg). This anxiolytic effect of ethanol, however, was antagonized dose dependently by RO15-4513 (0.1875–3.0 mg/kg). Only the highest dose of RO15-4513 (3.0 mg/kg) showed evidence of further response suppression. Lower doses of RO15-4513 tested did not exert an anxiogenic effect when given alone. Thus the antagonism of EtOH's anxiolytic (contrast-reducing) effects occurred at doses of RO15-4513 (0.375–1.5 mg/kg) that did not exhibit any intrinsic anxiogenic activity. As such, these results suggest that RO15-4513 interacts with the anxiolytic effects of ethanol in a nonadditive fashion in this test situation.

Ethanol RO15-4513 Benzodiazepine inverse agonist Anxiety Consummatory negative contrast Rats

RO15-4513 (ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5a][1,4] benzodiazepine-3 carboxylate) binds with high affinity to central benzodiazepine (BDZ) recognition sites and possesses a pharmacological profile characteristic of a partial inverse agonist [e.g., (10)]. In recent years, this compound has received a great deal of attention due to its reported ability to antagonize several (but not all) biochemical and neurobehavioral actions of ethanol [for reviews, see (29,37)]. While these studies have served to substantiate an involvement of the GABA/benzodiazepine/chloride channel receptor complex in mediating some of the behavioral effects of ethanol (EtOH), the mechanism by which RO15-4513 antagonizes these EtOH actions remains unclear. Moreover, the specificity of this effect is controversial as well. For example, some studies have shown RO15-4513 to be capable of antagonizing barbiturate effects (9, 11, 12, 23, 25, 27, 31), while others have found the antagonizing effects of RO15-4513 to be relatively selective for EtOH (24, 33–36). In some cases, higher doses of RO15-4513 were required to antagonize the effects of barbiturates than that for ethanol (21,24). Likewise, conflicting results have been generated from studies examining whether other partial BDZ inverse agonists share this property of antagonizing EtOH actions [e.g., (2, 6, 11, 20, 22, 26–28, 32, 33, 35)].

Another unresolved issue pertains to whether RO15-4513 antagonizes EtOH effects in an interactive or additive fashion. That is, it is unclear whether RO15-4513 antagonizes EtOH effects only at doses that behaviorally express the intrinsic (inverse agonist) properties of the compound. This is particularly evident in studies examining the ability of RO15-4513 to antagonize the anxiolytic actions of EtOH. For example, some studies have demonstrated an antagonism of EtOH's anxiolytic effects at doses of RO15-4513 which were behaviorally devoid of inverse agonist (anxiogenic) activity (20,35). In contrast, other studies have indicated that RO15-4513 antagonizes the anxiolytic effects of EtOH only at doses that, when given alone, reveal the compound's intrinsic anxiogenic properties (7, 23, 27, 30). Similar

[1]Requests for reprints should be addressed to Howard C. Becker, VAMC-Research Service, 109 Bee Street, Charleston, SC 29403.

mixed results have been obtained with other related BDZ inverse agonists [e.g., (8, 22, 27, 28)]. At present, an explanation for these discrepant results is not apparent, although several procedural differences including species, doses of RO15-4513 and EtOH tested as well as the tests of anxiety employed remain possibilities [e.g., (7)].

The purpose of the present study was to examine whether RO15-4513 could antagonize the anxiolytic actions of EtOH in a nonshock conflict task at doses that did not, themselves, produce an anxiogenic effect. Toward this end, a wide range of RO15-4513 doses were tested against an effective anxiolytic dose of EtOH in a consummatory conflict task, referred to as negative contrast. This task involves the quantification of how animals respond to an abrupt, unexpected reduction in reward (sucrose solution). More specifically, animals downshifted from a preferred (32%) to a less preferred (4%) sucrose solution consume substantially less of the latter than unshifted controls that have only experienced the less preferred 4% solution. Animals experiencing this negative contrast effect may be considered to be in an approach-avoidance conflict situation [e.g., (3,13)]. That is, while the 4% sucrose solution is relatively not as rewarding as that previously experienced by shifted animals, it does, itself, have some intrinsic rewarding property since unshifted animals readily consume it. The fact that depressed consummatory behavior engendered by reward reduction is accompanied by elevated plasma levels of corticosterone provides physiological support for the notion that the negative contrast task is aversive or anxiogenic (16). Moreover, the suppressed behavior exhibited by downshifted subjects is selectively alleviated by anxiolytic drugs including benzodiazepines (1, 5, 19), barbiturates (15), as well as EtOH (3,4). Appropriate control groups indicated that the contrast-reducing or anxiolytic action of these compounds could not be attributed to a generalized appetite-stimulating effect or to rate-dependency and state-dependency drug effects (19). Other drugs such as neuroleptics, antidepressants, anticholinergics, and antihistaminergics do not produce similar effects (1,14). Clonidine and serotonergic agents have generally produced negative results as well (17,18). Recently, the GABA/BDZ/chloride channel receptor complex has been implicated, at least in part, in mediating the anxiolytic effects of EtOH in this task since EtOH's actions have been shown to be potentiated by the indirect GABA agonist valproate and antagonized by the chloride channel blocker picrotoxin (3).

METHOD

Subjects

Drug-naive male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) were individually housed under a 12-hour light/dark cycle for a minimum of two weeks prior to being used. Following this acclimation period, the rats (approximately 75 days of age) were maintained at 82% of their normal free-feeding body weight for the remainder of the experiment by single daily feeding; water was continuously available in the home cage.

Apparatus

Subjects were tested in five identical metal cages (24.5 × 17.5 × 18 cm) configured to receive a drinking spout 7 cm above the floor on one side of each of the cages. A graduated cylinder was placed outside the chamber such that the orifice of the drinking spout was centered in the hole and flush with the outside wall of the cage. The testing cages and drinking tubes were individually housed in sound-attenuating chambers equipped with dim red light and speakers delivering white noise. Licking responses were monitored and recorded by programmed solid-state circuitry (Coulbourn Instruments, Lehigh Valley, PA).

Procedure

Subjects were randomly assigned to two groups: shifted and unshifted. Shifted rats received brief daily access to a 32% sucrose solution for 10 days, and then a 4% solution for four postshift days. The remaining animals served as unshifted controls, receiving 4% sucrose on all 14 days of the experiment. Thus, during the postshift period, all animals received the same reward (4% sucrose). The difference between shifted and unshifted groups was that only the former group had prior experience with a more preferred reward (32% sucrose). The 14 daily sessions were 5 minutes in duration, timed from the first lick. Following the first postshift day session (day 11 of testing), shifted and unshifted rats were matched on the basis of their first postshift day lick rates and then further separated on the basis of drug treatment. On the second postshift day, subjects were injected with RO15-4513 (0–3.0 mg/kg) followed by an injection of either saline or 0.75 g/kg EtOH. The RO15-4513 and EtOH injections were administered IP 6 and 5 minutes prior to testing, respectively.

Drugs

RO15-4513, a generous gift from Dr. W. E. Haefely (Hoffmann-La Roche; Basel), was suspended in distilled water to which 1 drop of Tween-20 per 10 ml was added and injected in a volume of 1 ml/kg. EtOH (95%) was mixed with saline to yield a 15% (w/v) solution and administered in a volume of 5.5 ml/kg. Sucrose solutions (w/v) were presented at room temperature and prepared from commercial grade cane sugar and tap water, 24 hours prior to each session.

RESULTS

As illustrated in Fig. 1, response (lick) rates on the second postshift day for rats shifted from a more preferred (32%) to a less preferred (4%) sucrose solution were significantly depressed in comparison to unshifted controls that only experienced the smaller 4% sucrose reward, $F(1,142) = 33.23$, $p < 0.0001$. This negative contrast effect, however, varied as a function of drug treatment, $F(6,142) = 2.17$, $p < 0.05$. Subsequent analysis with Fisher's Least Significant Difference test indicated that EtOH (0.75 g/kg) reliably increased responding in shifted rats ($p < 0.01$) without significantly influencing the unshifted (control) response rate. The net effect was an elimination of the differential response rate between shifted and unshifted groups. Moreover, this anxiolytic (contrast-reducing) effect of EtOH was antagonized by pretreatment with RO15-4513 in a dose-related fashion. That is, while the lowest dose of RO15-4513 (0.1875 mg/kg) was without effect, higher doses (0.375–3.0 mg/kg) significantly antagonized the contrast-ameliorating action of EtOH ($p < 0.01$). Importantly, RO15-4513 significantly reversed the effects of EtOH on shifted rats without altering the response rates of unshifted controls.

Similar results were obtained when data from shifted and unshifted groups were expressed as percent of the control (vehicle + saline) response rate. As can be seen in Fig. 2 (right panel), EtOH (0.75 g/kg) increased responding in shifted rats (approximately 45% above the control response rate), while having a minimal effect on unshifted responding. A significant interaction between shift and drug conditions indicated that the

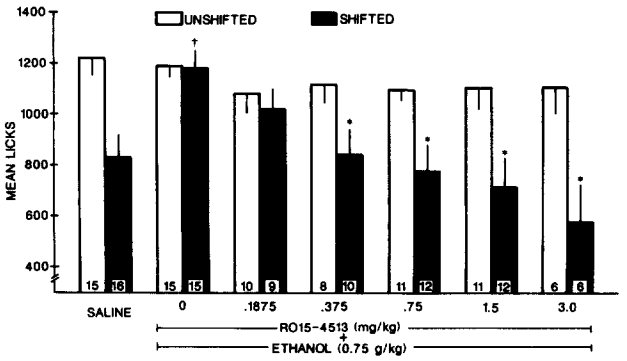

FIG. 1. Mean ± SE lick (response) rate in 5-minute test session for shifted and unshifted groups as a function of drug condition. Numbers in bars represent group size. †$p<0.01$: significantly differ from saline-shifted group; *$p<0.01$: significantly differ from vehicle + EtOH-shifted group.

anxiolytic effect of ETOH was antagonized dose dependently by RO15-4513, $F(6,142)=2.71$, $p<0.02$. In fact, the contrast-ameliorating effects of EtOH were completely reversed by doses of 0.375–3.0 mg/kg RO15-4513. It is important to note that the antagonizing effects of RO15-4513 against EtOH were produced at doses that did not significantly alter unshifted control responding. Hence, the observed antagonism cannot be attributed to a general depressant effect of the drug.

The effects of RO15-4513 given alone are presented in the left panel of Fig. 2. As can be seen, only the highest dose of RO15-4513 (3.0 mg/kg) showed any evidence of response suppression. Although this effect was only marginally significant, $F(5,115)=2.05$, $p<0.08$, separate analyses of shifted and unshifted subjects that received vehicle or 3.0 mg/kg RO15-4513 revealed that this dose did produce an anxiogenic effect. That is, 3.0 mg/kg RO15-4513 reliably decreased the response rate of shifted and unshifted rats, $F(1,39)=7.38$, $p<0.01$. While the interaction term was not significant, the suppression of responding was greater in shifted animals than unshifted subjects. This is supported by separate analyses of shifted, $t(20)=2.11$, $p<0.05$, and unshifted groups, $t(19)=1.78$, $p=0.1$. Lower doses of RO15-4513 did not even marginally influence responding in either shifted or unshifted groups. Thus, the antagonism of EtOH's anxiolytic (contrast-reducing) effects occurred at doses of

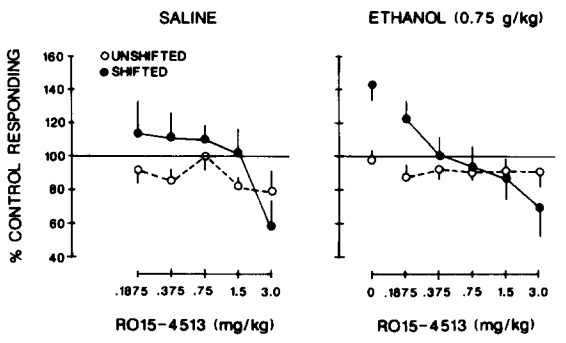

FIG. 2. Mean ± SE percent of control (saline) response rate for shifted and unshifted groups as a function of RO15-4513 dose. Left panel: saline-injected groups (RO15-4513 treatment alone); right panel: EtOH-injected groups (EtOH + RO15-4513 treatments).

RO15-4513 (0.375–1.5 mg/kg) that did not exhibit any intrinsic anxiogenic activity when given alone in this test.

DISCUSSION

As previously indicated, an issue under continued debate is whether antagonism of EtOH's anxiolytic actions by RO15-4513 represents an interactive or additive mechanism. In the former situation, RO15-4513 would be predicted to antagonize EtOH effects at doses devoid of its intrinic inverse agonist properties, while in the latter situation, antagonism of EtOH would be expected to occur at doses of RO15-4513 which produce its intrinsic pharmacological profile (the anxiogenic effects of the drug merely subtracting from the anxiolytic action of EtOH). Results from the present study appear to support the former interpretation. This is in agreement with studies employing other tests of anxiety such as the staircase task (7) and operant conflict procedures (20,35). In addition, a related partial BDZ inverse agonist (RO15-3505) has been shown to reverse the anxiolytic effects of EtOH at doses that were devoid of intrinsic activity in the staircase and two chambered light/dark choice procedures [(8); but cf. (28)]. Further, anxiogenic agents other than BDZ inverse agonists (e.g., picrotoxin, pentylenetetrazol) were found to antagonize the anxiolytic action of chlordiazepoxide in the defensive burying task without exhibiting intrinsic activity (38). However, these findings contrast with those from several studies indicating that the ability of RO15-4513 and related compounds to counteract the anxiolytic effects of EtOH is related to their intrinsic anxiogenic properties exhibited in various tests of anxiety, including the plus-maze (27,28), the two chambered light/dark choice (7, 8, 30), and operant conflict procedures (12,22). At present, there is no apparent explanation for these discrepant results.

Lister (27) has suggested that some testing conditions are not sensitive to anxiogenic drug action. Thus a drug may antagonize the anxiolytic effects of EtOH by virtue of its BDZ inverse agonist properties even though it may not exhibit anxiogenic activity in a particular task. In the present study, however, the negative contrast procedure does appear to be sensitive to the anxiogenic effects of RO15-4513. While lower doses of RO15-4513 did not significantly influence responding in shifted and unshifted groups, the highest dose tested (3.0 mg/kg) resulted in further response suppression in shifted subjects. This dose also decreased responding (to a lesser extent) in unshifted animals, an effect that may be, at least in part, attributable to the drug's anxiogenic properties [e.g., (22)]. In addition, another related BDZ inverse agonist, FG 7142, has been found to dose-dependently produce an anxiogenic profile in the negative contrast task (unpublished data). More specifically, relative to controls, doses of 5 and 10 mg/kg FG 7142 produced a 16% and 28% decrease in shifted responding while only reducing unshifted response rates by 6% and 13%, respectively. Therefore, the fact that RO15-4513 was capable of antagonizing the anxiolytic effects of EtOH at doses that did not produce an anxiogenic response when administered alone is not likely related to the test conditions being insensitive to such an effect.

This is not to suggest, however, that the antagonism of EtOH's anxiolytic effects was unrelated to the intrinsic or pharmacological effects of RO15-4513. Rather, the results indicate that, under the present testing conditions, antagonism of EtOH can occur at doses of RO15-4513 that do not produce an observable anxiogenic effect when the drug is administered alone. Indeed, it may be that under some conditions lower doses or a smaller fractional receptor occupancy is required for BDZ inverse agonists to antagonize EtOH actions than that required to

produce an overt pharmacologic effect related to its intrinsic properties. The results of the present study would appear to be congruent with such an interpretation. It should also be noted that, in most published studies on the interaction of RO15-4513 and the anxiolytic effects of EtOH, doses below 1.5 mg/kg RO15-4513 were not examined. Therefore, it is not possible to compare the results of this study with those of previously published studies.

It has also been suggested that the ability of RO15-4513 to reverse some of EtOH's actions may be related to its proconvulsant and associated depressive properties (7,30). In the present study, however, RO15-4513 did not produce a general depressant effect. That is, the drug reversed the effects of EtOH on shifted animals, but did not significantly influence the response rate of unshifted controls. Thus, while the proconvulsant action of RO15-4513 has been clearly established [e.g., (10, 31, 32)], depressant effects associated with this intrinsic property would not appear to explain the ability of the drug to reverse the anxiolytic effects of EtOH in the present study.

In summary, results from this study have demonstrated that RO15-4513 is capable of antagonizing the anxiolytic effects of EtOH in a nonshock conflict task (negative contrast) in a dose-dependent fashion. Moreover, the antagonism of EtOH's anxiolytic action in this consummatory conflict test was apparent at doses of RO15-4513 that did not reveal its inverse agonist (anxiogenic) properties at the BDZ receptor complex. While these data are suggestive of a nonadditive interaction between the drugs, an explanation for the above cited discrepant results related to this issue remains unclear.

ACKNOWLEDGEMENTS

This research was supported in part by awards from the Department of Veterans Affairs and the National Institute on Alcohol Abuse and Alcoholism.

REFERENCES

1. Becker, H. C. Comparison of the effects of the benzodiazepine midazolam and three serotonin antagonists on a consummatory conflict paradigm. Pharmacol. Biochem. Behav. 24:1057–1064; 1986.
2. Becker, H. C. Effects of the imidazobenzodiazepine RO15-4513 on the stimulant and depressant actions of ethanol on spontaneous locomotor activity. Life Sci. 43:643–650; 1988.
3. Becker, H. C.; Anton, R. F. Valproate potentiates and picrotoxin antagonizes the anxiolytic action of ethanol in a nonshock conflict task. Neuropharmacology 29:837–843; 1990.
4. Becker, H. C.; Flaherty, C. F. Influence of ethanol on contrast in consummatory behavior. Psychopharmacology (Berlin) 77:253–258; 1982.
5. Becker, H. C.; Flaherty, C. F. Chlordiazepoxide and ethanol additively reduce gustatory negative contrast. Psychopharmacology (Berlin) 80:35–37; 1983.
6. Becker, H. C.; Hale, R. L. Ethanol-induced locomotor stimulation in C57BL/6 mice following RO15-4513 administration. Psychopharmacology (Berlin) 99:333–336; 1989.
7. Belzung, C.; Misslin, R.; Vogel, E. Does RO 15-4513 reverse the anxiolytic effects of ethanol by its intrinsic properties? Pharmacol. Biochem. Behav. 30:867–870; 1988.
8. Belzung, C.; Misslin, R.; Vogel, E. The benzodiazepine receptor inverse agonists b-CCM and RO 15-3505 both reverse the anxiolytic effects of ethanol in mice. Life Sci. 42:1765–1772; 1988.
9. Bonetti, E. P.; Polc, P.; Pieri, L. An azido analogue of the benzodiazepine antagonist RO15-1788 (RO15-4513) behaves as a partial inverse benzodiazepine agonist. Neurosci. Lett. Suppl. 18:530; 1984.
10. Bonetti, E. P.; Burkard, W. P.; Gabl, M.; Hunkeler, W.; Lorez, H-P.; Martin, J. R.; Moehler, H.; Osterrieder, W.; Pieri, L.; Polc, P.; Richards, J. G.; Schaffner, R.; Scherschlicht, R.; Schoch, P.; Haefely, W. E. Ro 15-4513: Partial inverse agonism at the BZR and interaction with ethanol. Pharmacol. Biochem. Behav. 31:733–749; 1988.
11. Bonetti, E. P.; Burkard, W. P.; Gabl, M.; Mohler, H. The partial inverse agonist RO15-4513 anatgonises acute ethanol effects in mice and rats. Br. J. Pharmacol. 86:463P; 1985.
12. Britton, K. T.; Ehlers, C. E.; Koob, G. F. Is ethanol antagonist Ro15-4513 selective for ethanol? Science 239:648–649; 1988.
13. Flaherty, C. F. Incentive contrast: A review of behavioral changes following shifts in reward. Anim. Learn. Behav. 10:409–440; 1982.
14. Flaherty, C. F. Effects of anxiolytics and antidepressants on extinction and negative contrast. Pharmacol. Ther. 46:309–320; 1990.
15. Flaherty, C. F.; Becker, H. C.; Driscoll, C. D. Conditions under which amobarbital sodium influences contrast in consummatory behavior. Physiol. Psychol. 10:122–128; 1982.
16. Flaherty, C. F.; Becker, H. C.; Poherecky, L. A. Correlation of corticosterone elevation and negative contrast varies as a function of postshift day. Anim. Learn. Behav. 13:309–314; 1985.
17. Flaherty, C. F.; Grigson, P. S.; Demetrikopoulos, M. K. Effect of clonidine on negative contrast and novelty-induced stress. Pharmacol. Biochem. Behav. 27:659–654; 1987.
18. Flaherty, C. F.; Grigson, P. S.; Demetrikopoulos, M. K.; Weaver, M. S.; Krauss, K. L.; Rowan, G. A. Effect of serotonergic drugs on negative contrast in consummatory behavior. Pharmacol. Biochem. Behav. 36:799–806; 1990.
19. Flaherty, C. F.; Lombardi, B. R.; Wrightson, J.; Deptula, D. Conditions under which chlordiazepoxide influences successive gustatory contrast. Psychopharmacology (Berlin) 67:269–277; 1980.
20. Glowa, J. R.; Crawley, J. N.; Suzdak, P. D.; Paul, S. M. Ethanol and the GABA receptor complex: Studies with the partial inverse benzodiazepine receptor agonist Ro 15-4513. Pharmacol. Biochem. Behav. 31:767–772; 1988.
21. Hoffman, P. L.; Tabakoff, B.; Szabo, G.; Suzdak, P. D.; Paul, S. M. Effect of an imidazobenzodiazepine, Ro15-4513, on the incoordination and hypothermia produced by ethanol and pentobarbital. Life Sci. 41:611–610; 1987.
22. Koob, G. F.; Braestrup, C.; Thatcher-Britton, K. 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.
23. Koob, G. F.; Percy, L.; Britton, K. T. The effects of Ro 15-4513 on the behavioral actions of ethanol in an operant reaction time task and a conflict test. Pharmacol. Biochem. Behav. 31:757–760; 1988.
24. Le, A. D.; Mana, M. J.; Pham, T.; Khanna, J. M.; Kalant, H. Effects of Ro 15-4513 on the motor impairment and hypnotic effects of ethanol and pentobarbital. Eur. J. Pharmacol. 159:25–31; 1989.
25. Lister, R. G. Interactions of Ro 15-4513 with diazepam, sodium pentobarbital and ethanol in a holeboard test. Pharmacol. Biochem. Behav. 28:75–79; 1987.
26. Lister, R. G. The benzodiazepine receptor inverse agonists FG 7142 and RO15-4513 both reverse some of the behavioral effects of ethanol in a holeboard test. Life Sci. 41:1481–1489; 1987.
27. Lister, R. G. Interactions of ethanol with benzodiazepine receptor ligands in tests of exploration, locomotion and anxiety. Pharmacol. Biochem. Behav. 31:761–765; 1988.
28. Lister, R. G. Interactions of three benzodiazepine receptor inverse agonists with ethanol in a plus-maze test of anxiety. Pharmacol. Biochem. Behav. 30:701–706; 1988.
29. Lister, R. G.; Nutt, D. J. Alcohol antagonists—the continuing quest. Alcohol.: Clin. Exp. Res. 12:566–569; 1988.
30. Misslin, R.; Belzung, C.; Vogel, E. Interaction of RO 15-4513 and ethanol on the behaviour of mice: Antagonistic or additive effects? Psychopharmacology (Berlin) 94:392–396; 1988.
31. 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.
32. 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.
33. Polc, P. Interactions of partial inverse benzodiazepine agonists RO15-

4513 and FG 7142 with ethanol in rats and cats. Br. J. Pharmacol. 86:465P; 1985.
34. Rees, D. C.; Balster, R. L. Attenuation of the discriminative stimulus properties of ethanol and oxazepam, but not of pentobarbital, by Ro 15-4513 in mice. J. Pharmacol. Exp. Ther. 244:592–598; 1988.
35. Suzdak, P. D.; Glowa, J. R.; Crawley, J. N.; Schwartz, R. D.; Skolnick, P.; Paul, S. M. A selective imidazobenzodiazepine antagonist of ethanol in the rat. Science 234:1243–1247; 1986.
36. Suzdak, P. D.; Glowa, J. R.; Crawley, J. N.; Skolnick, P.; Paul, S. M. Is ethanol antagonist Ro15-4513 selective for ethanol? Science 239:649–650; 1988.
37. Ticku, M. K.; Kulkarni, S. K. Molecular interactions of ethanol with GABAergic system and potential of RO15-4513 as an ethanol antagonist. Pharmacol. Biochem. Behav. 30:501–510; 1988.
38. Treit, D. RO 15-1788, CGS 8216, picrotoxin and pentylenetetrazol: Do they antagonize anxiolytic drug effects through an anxiogenic action? Brain Res. Bull. 19:401–405; 1987.