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Antagonizing the Anticonvulsant Effect of Ethanol Using Drugs Acting at the Benzodiazepine/GABA Receptor Complex

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NUTT, D. J. AND R. G. LISTER. *Antagonizing the anticonvulsant effect of ethanol using drugs acting at the benzodiazepine/GABA receptor complex.* PHARMACOL BIOCHEM BEHAV 31(3) 751–755, 1988.—The ability of various benzodiazepine receptor ligands to antagonize the anticonvulsant action of ethanol was investigated using intravenous infusion of the GABA antagonist bicuculline. The partial inverse agonists FG 7142, RO 15-4513 and RO 15-3505 produced dose-related reductions in seizure threshold. These compounds also partially reversed the anticonvulsant action of ethanol. However, the magnitude of the effects in each case was only equivalent to the reduction in seizure threshold caused by each compound when administered alone. That is the proconvulsant effect of each compound merely subtracted from the anticonvulsant effect of ethanol. ZK 93426, a benzodiazepine receptor antagonist which alone failed to alter seizure threshold, did not affect the anticonvulsant action of ethanol. Both RO 15-4513 and RO 15-3505 also lowered the seizure threshold of barbiturate-treated mice, again in a subtractive fashion. The ability of RO 15-4513 and other inverse agonists to antagonize the anticonvulsant effect of ethanol appears to result from their intrinsic proconvulsant properties.

Alcohol Barbiturate GABA RO 15-4513 Convulsion Benzodiazepines Pentylenetetrazole Bicuculline Mice

ETHANOL possesses marked anticonvulsant properties in a number of seizure paradigms which can be convenient indices of its central effects. In the present study we have used the measurement of seizure threshold to examine the ability of a variety of drugs to antagonize the threshold elevations produced by ethanol. We review some of our earlier studies which examine the intrinsic activity in seizure paradigms of RO 15-4513, a compound reported to antagonize some effects of ethanol (2, 9, 11, 17, 25, 27). Since our data suggested that the ability of RO 15-4513 to antagonize the anticonvulsant effect of ethanol resulted from its intrinsic proconvulsant properties (20), we then examined the proconvulsant actions of several other benzodiazepine receptor inverse agonists and their interactions with ethanol.

The pharmacology of the benzodiazepine/GABA receptor in complicated and is illustrated in Fig. 1. The site of action of ethanol is presently unclear, although some evidence suggests interaction at the picrotoxinin/barbiturate site (28).

It can be seen that RO 15-4513 is classified as an inverse agonist at the benzodiazepine/β-carboline site. The evidence for this comes from a number of sources: receptor binding studies (26), electrophysiological studies (18,25), and behavioral experiments [(1, 8, 12, 15, 16, 19, 20), see also articles by Lister, Koob *et al.*, Bonetti *et al.* and Glowa *et al.*, this volume]. These effects of RO 15-4513 are reversed by the benzodiazepine receptor antagonist flumazenil. In our own studies (16) we have reported that RO 15-4513 lowers seizure threshold to a variety of convulsant drugs (see Table 1). It should also be noted that RO 15-4513 elevates seizure threshold to the β-carboline convulsant DMCM (a full inverse agonist), which is consistent with it acting as a partial, rather than a full inverse agonist.

In view of the reliability and sensitivity of bicuculline infusion for detecting pro- and anticonvulsant drug effects we used this method in the following studies (21). Initially dose-response curves were determined for three different partial inverse agonists: RO 15-4513; FG 7142, a β-carboline derivative that has been shown to be anxiogenic [4]; and RO 15-3505, a potent benzodiazepine receptor ligand, structurally similar to RO 15-4513, which has been used to antagonize the effects of benzodiazepines in humans, but which possesses proconvulsant activity (5, 6, 24).

METHOD

Animals

Male NIH Swiss mice were used in all experiments. They were housed in groups of 10, allowed ad lib access to food and water, and maintained on a 12 hr light/12 hr dark cycle. They weighed approximately 24 g at the time of testing.

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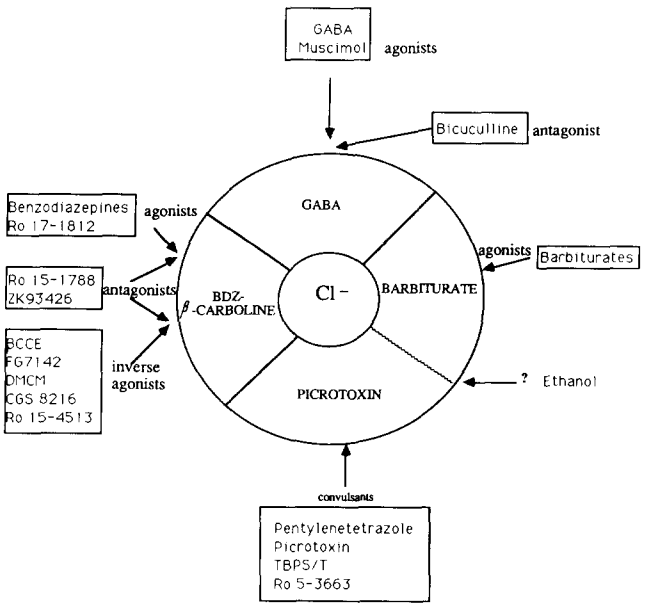

FIG. 1. A schematic representation of the benzodiazepine/GABA receptor complex. Reproduced from (17) with permission.

Drugs

RO 15-4513, RO 15-3505, FG 7142 and ZK 93426 were all suspended in a solution of 0.3% Tween 20. Sodium pentobarbital was dissolved in distilled water. Ethanol was diluted with distilled water. For all solutions, concentrations were calculated to give an injection volume of 10 ml/kg. All these drugs were administered IP.

Bicuculline (Sigma, St. Louis) was dissolved in dilute hydrochloric acid and the pH of the solution was then adjusted to 3 using dilute sodium hydroxide. The final concentration was 0.05 mg/ml.

Infusion

Seizure threshold was determined to bicuculline using an intravenous infusion method (21). Briefly, mice were restrained in a perspex container and bicuculline was infused into the tail vein at a rate of 1.1 ml/min via a 25 gauge butterfly. The latency to the onset of repeated myoclonic jerking of the head and forelimbs was used for determining seizure thresholds.

Experiment 1

In the first experiment we obtained dose-response curves to RO 15-4513, RO 15-3505 and FG 7142 using the bicuculline infusion.

Fifty-five mice received injections of RO 15-4513 (0, 0.03, 0.1, 0.3, 1.0, 3.0 or 10 mg/kg). Five minutes after injection seizure threshold to bicuculline was determined.

Fifty-five mice received injections of RO 15-3505 (0, 0.19, 0.38, 0.75, 1.5, 3 or 6 mg/kg). Five minutes later seizure threshold to bicuculline was determined.

Forty-one mice received injections of FG 7142 (0, 5, 10, 20, 40 or 80 mg/kg). They were watched for 15 min for any signs of seizure activity and then seizure threshold to bicuculline was determined.

TABLE 1

THE EFFECT OF RO 15-4513 ON SEIZURE THRESHOLD TO VARIOUS CONVULSANTS INFUSED INTO THE TAIL VEIN OF MICE

Bicuculline	↓↓
Pentylenetetrazole	↓↓
RO 5-3663	↓↓
Caffeine	—
Quipazine	—
DMCM	↑↑
Strychnine	—

Experiment 2

On the basis of the results of Experiment 1, doses of RO 15-4513, RO 15-3505 and FG 7142 were selected to antagonize the anticonvulsant effect of 2.4 g/kg ethanol. In addition, we also looked at the effect of ZK 93426, a β-carboline which is a potent ligand for benzodiazepine receptors and which antagonizes the behavioral effects of both benzodiazepines and β-carbolines, but has little intrinsic behavioral activity (10).

Forty-one mice were divided into two approximately equal groups receiving either ethanol (2.4 g/kg) or its vehicle. Five minutes later approximately half the mice in each group received RO 15-4513 (5 mg/kg) and the remaining mice received the vehicle. Five minutes later seizure threshold to bicuculline was determined.

Forty-five mice were divided into two approximately equal groups receiving either FG 7142 (40 mg/kg) or the vehicle. Five minutes later half the mice in each group received ethanol (2.4 g/kg) and the rest received the vehicle. Ten minutes later seizure threshold to bicuculline was determined.

Thirty-seven mice were divided into two approximately equal groups receiving either ethanol (2.4 g/kg) or its vehicle. Approximately half the mice in each group received RO 15-3505 (0.1 mg/kg) and the remaining half received the vehicle 5 min before seizure threshold to bicuculline was determined.

Forty-three mice were divided into four approximately equal groups receiving ethanol (2 g/kg), ZK 93426 (5 mg/kg), ethanol (2 g/kg) + ZK 93426 (5 mg/kg) or the drug vehicles. Thirty minutes later seizure threshold to bicuculline was determined.

Experiment 3

This experiment compared the abilities of RO 15-4513 and RO 15-3505 to antagonize the anticonvulsant effect of sodium pentobarbital. Sixty-one mice were divided into two approximately equal groups receiving either sodium pentobarbital (30 mg/kg) or the vehicle. Twenty-five minutes later, approximately one-third of the mice in each group received RO 15-4513 (3 mg/kg), one-third received RO 15-3505 (1.5 mg/kg) and the remaining mice received the water/Tween vehicle. Five minutes later seizure threshold to bicuculline was determined.

RESULTS

Experiment 1

Three of the mice that received the 80 mg/kg dose of FG 7142 had tonic/clonic seizures within 20 min. These mice

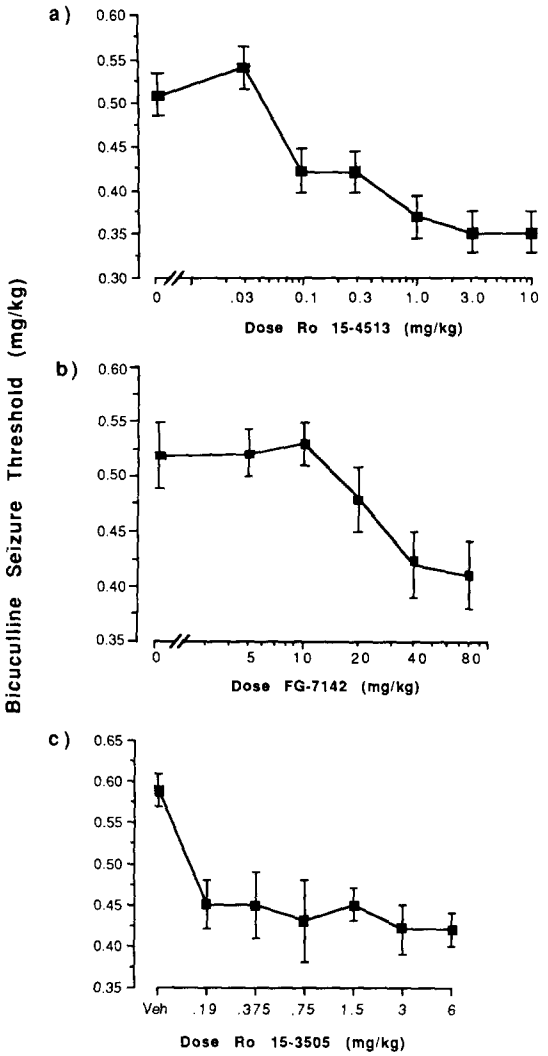

FIG. 2. The effects of (a) RO 15-4513, (b) FG 7142 and (c) RO 15-3505 on seizure threshold to bicuculline. Values are means±SEM. RO 15-4513 and RO 15-3505 were given 5 min before test, and FG 7142 was given 15 min before test.

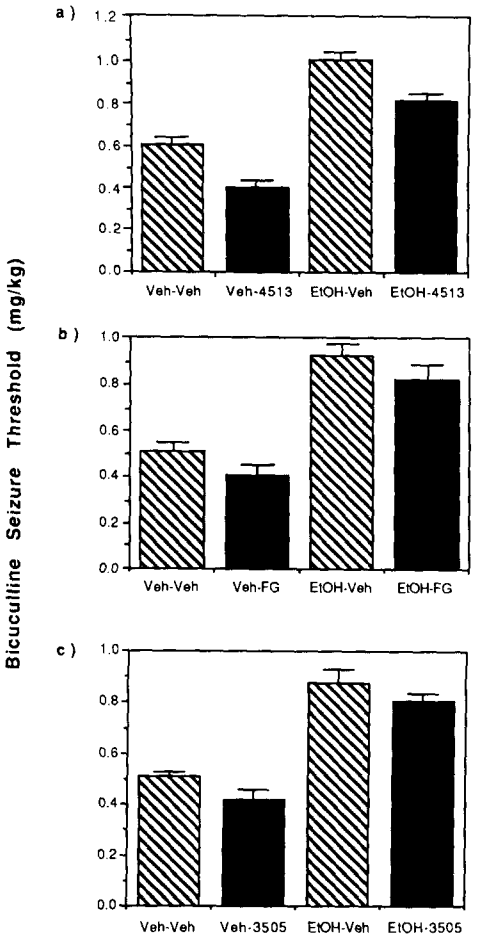

FIG. 3. The effects of (a) RO 15-4513 (5 mg/kg), (b) FG 7142 (40 mg/kg) and (c) RO 15-3505 (0.1 mg/kg) on the anticonvulsant effect of 2.4 g/kg ethanol. Values are means±SEM, n=8–12 per group.

were excluded from the analysis. RO 15-4513, $F(6,48)=8.7$, $p<0.0001$; FG 7142, $F(5,32)=3.0$, $p<0.03$; and RO 15-3505, $F(6,48)=5.2$, $p<0.001$, reduced seizure thresholds to bicuculline (see Fig. 2).

Experiment 2

In Experiment 2, there were significant main effects of RO 15-4513, $F(1,37)=30.7$, $p<0.001$, and of ethanol, $F(1,37)=175.9$, $p<0.0001$. As can be seen in Fig. 3a, the proconvulsant action of RO 15-4513 appeared to subtract from the anticonvulsant effect of ethanol. In Fig. 3b and c it can be seen that the other inverse agonists interacted with ethanol in a similar manner. FG 7142 reduced seizure threshold, $F(1,41)=8.9$, $p<0.005$, and ethanol increased it, $F(1,41)=167.9$, $p<0.001$, but there was no interaction. RO 15-3505 reduced, $F(1,33)=4.7$, $p<0.05$, and ethanol increased, $F(1,33)=108.2$, $p<0.0001$, seizure threshold.

In the final part of this experiment ethanol again increased seizure threshold to bicuculline, $F(1,39)=37.5$, $p<0.0001$. ZK 93426 was without effect in either vehicle- or ethanol-treated mice (see Fig. 4).

Experiment 3

The results of this experiment are shown in Fig. 5. Two separate ANOVAs were run to examine the interactions of sodium pentobarbital with RO 15-4513 and with RO 15-3505. In the first, there were significant main effects of both sodium pentobarbital, $F(1,37)=54.3$, $p<0.0001$, and of RO 15-4513, $F(1,37)=12.8$, $p<0.002$. In Fig. 5 it can be seen that the proconvulsant effect of RO 15-4513 merely subtracted from the anticonvulsant effect of the barbiturate, and that mice that received the drug combination had seizure thresholds above those of the controls ($p<0.01$).

In the analysis of the effects of RO 15-3505, there was a significant main effect of the barbiturate, $F(1,36)=43.0$, $p<0.0001$. The effect of RO 15-3505 just failed to reach significance, $F(1,36)=3.3$, $p<0.08$. This reflected a tendency for RO 15-3505 to reduce seizure threshold in both vehicle- and barbiturate-treated mice (see Fig. 5).

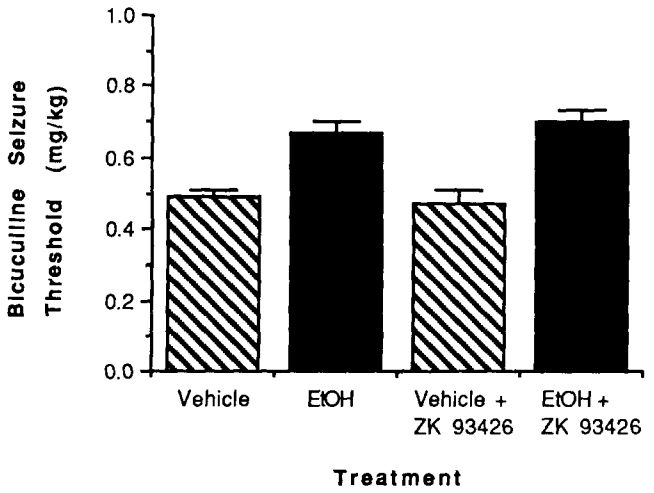

FIG. 4. The effect of ZK 93426 (5 mg/kg) on the anticonvulsant action of ethanol (2 g/kg). Values are means±SEM, n=10 or 11 per group.

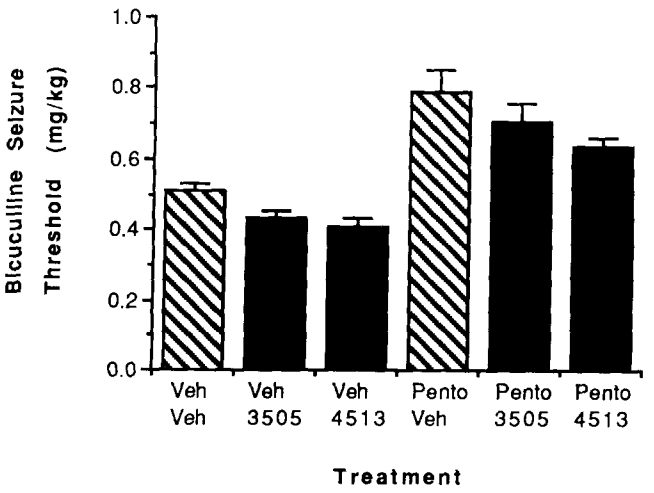

FIG. 5. The effects of RO 15-4513 (3 mg/kg) and RO 15-3505 (1.5 mg/kg) on the anticonvulsant action of sodium pentobarbital (30 mg/kg). Values are means±SEM, n=10 or 11 per group.

TABLE 2

THE INTRINSIC EFFECTS OF VARIOUS BENZODIAZEPINE RECEPTOR LIGANDS ON SEIZURE THRESHOLD AND THEIR INTERACTIONS WITH DOSES OF ETHANOL, A BARBITURATE, AND A BENZODIAZEPINE WHICH ELEVATE SEIZURE THRESHOLD WHEN ADMINISTERED ALONE

Intrinsic Effect		Effect on Action of: Ethanol	Barbiturates	Benzodiazepines
↓↓↓[3,23]	DMCM	[↓↓↓]	[↓↓↓]	↓↓↓
↓↓[*]	FG 7142	↓↓[*]	[↓↓]	[↓↓↓]
↓↓[1, 19, 20]	RO 15-4513	↓↓[20,*]	↓↓[20,*]	↓↓↓[20]
↓[24,*]	RO 15-3505	↓[*]	↓[*]	[↓↓↓]
—↑[22]	Flumazenil	—[†]	—[22]	↓↓↓[22]
—[10,*]	ZK 93426	—[*]	[—]	↓↓↓[10]
↑[7]	RO 17-1812	[↑]	[↑]	—[7]
↑↑↑[22]	Diazepam	[↑↑↑]	[↑↑↑]	↑↑↑

↑ Enhancement of effect; ↓ reduction of effect.

*This paper, †unpublished observations.

Where arrows are shown in brackets, these reflect predicted results rather than the results of experiments that have already been performed.

DISCUSSION

Several important points emerge from these studies. Firstly, RO 15-4513 is proconvulsant to bicuculline, at doses as low as 0.1 mg/kg. This information, together with previous results [(16), and see Table 1] confirms its action as a partial inverse agonist. Consistent with this is the observation that no overt seizures were observed in the NIH Swiss mice used in the present study. It should be noted, however, that a small proportion of DBA/2 mice have been found to seize following treatment with RO 15-4513 alone (15).

Secondly, RO 15-3505 was found to be potently proconvulsant in this paradigm. This is surprising in view of its reported weak activity in other seizure paradigms (24), and in other behavioral tests (13,14). It should be noted that this drug was designed as a benzodiazepine antagonist, and has been used in humans for this purpose (5). Our results clearly show that it is a partial inverse agonist, though some of our data (see below), together with those from other studies (14,24), suggest it may be less intrinsically active than RO 15-4513 and FG 7142.

Thirdly, the two imidazodiazepines were considerably more potent than FG 7142, although the maximal lowering of seizure threshold was approximately the same for all three drugs. The difference in potency presumably reflects the different affinities of these compounds for the benzodiazepine receptor.

Fourthly, the interaction of each inverse agonist with ethanol was subtractive. That is, the antagonism of ethanol's anticonvulsant action was due to the intrinsic proconvulsant action of each drug. In no case was the anticonvulant effect of ethanol completely reversed.

The interactions of RO 15-4513 and RO 15-3505 with the

sodium pentobarbital was also subtractive in nature (see Fig. 5). RO 15-3505 appeared less effective than RO 15-4513, and this is consistent with data showing it to be less intrinsically active. Indeed, in the holeboard test, RO 15-3505 will reverse the intrinsic effects of RO 15-4513 (14). It should be noted that the interactions of the inverse agonists with ethanol and sodium pentobarbital contrast with their interactions with benzodiazepines in which a complete antagonism of the anticonvulsant effect is observed (20). A summary of the effects of various benzodiazepine receptor ligands on the anticonvulsant actions of ethanol, barbiturates and benzodiazepines is given in Table 2. These interactions are mediated through benzodiazepine receptors since receptor antagonists such as RO 15-1788 and ZK 93426 block them.

In conclusion, we have examined the interactions of a number of benzodiazepine receptor ligands with ethanol in the seizure threshold paradigm. Only those with some partial inverse agonist activity (RO 15-4513, RO 15-3505 and FG 7142) were capable of reversing the anticonvulsant effect of ethanol. ZK 93426 and RO 15-1788, which have been classified as receptor antagonists, failed to alter ethanol's effects. It therefore appears that for a benzodiazepine receptor ligand to attenuate the anticonvulsant effect of ethanol it must possess inverse agonist activity.

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