Br. J. Pharmacol. (1988), **93**, 210–214

Interactions of the imidazodiazepine Ro 15-4513 with chemical convulsants

Richard G. Lister & David J. Nutt

Laboratory of Clinical Studies, DICBR, NIAAA, Building 10, Room 3C218, 9000 Rockville Pike, Bethesda, MD 20892, USA

1 The proconvulsant effects of the imidazodiazepine Ro 15-4513, were investigated in mice by use of intravenous infusion of a variety of convulsant drugs.

2 Dose-response and time course studies of Ro 15-4513 against γ-aminobutyric acid (GABA) antagonists were performed. On the basis of these studies a maximally effective dose of 5 mg kg^{-1} was administered 5 min before the determination of seizure thresholds in subsequent experiments.

3 Ro 15-4513 (5 mg kg^{-1}) significantly lowered seizure thresholds to pentylenetetrazole, bicuculline and the convulsant benzodiazepine Ro 5-3663, but failed to alter seizure thresholds to picrotoxin, strychnine, caffeine and quipazine.

4 Ro 15-4513 significantly raised seizure threshold to the benzodiazepine receptor inverse agonist methyl 6,7-dimethoxy-4 ethyl-β-carboline-3-carboxylate (DMCM).

5 These results are discussed in relation to other studies investigating the proconvulsant and alcohol-antagonizing effects of Ro 15-4513.

Introduction

The imidazodiazepine Ro 15-4513 (ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4] benzodiazepine-3-carboxylate) a structural analogue of the benzodiazepine receptor antagonist Ro 15-1788 (ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4] benzodiazepine-3-carboxylate) has been reported to antagonize some of the behavioural effects of ethanol (Bonetti *et al.*, 1984; 1985; Polc 1985; Suzdak *et al.*, 1986; Lister & Nutt, 1987). In addition, there has also been a preliminary report that it possesses proconvulsant properties in animals treated with pentylenetetrazole (Bonetti *et al.*, 1984). The present experiments were designed to investigate further the proconvulsant effects of Ro 15-4513, since this latter property has not been much studied. All the drugs used were convulsants which are effective when infused intravenously, and can therefore be used to determine seizure thresholds (Nutt *et al.*, 1980; 1984; 1986). This method is reliable and sensitive to both pro- and anti-convulsant drugs and requires a smaller number of animals than other methods of seizure measurement. Drugs with different sites of pharmacological action were chosen, and included agents acting at the γ-aminobutyric acid (GABA)-benzodiazepine receptor complex (e.g. pentylenetetrazole, bicuculline), glycine receptors (strychnine), 5-hydroxytryptamine (5-HT) receptors (quipazine) and adenosine receptors (caffeine).

Methods

Animals

Male NIH Swiss mice, weighing approximately 24 g were housed in groups of 10 and maintained on a 12 h light : 12 h dark cycle.

Procedure

In all experiments, seizure thresholds were determined by an intravenous infusion method (Nutt *et al.*, 1986). Briefly, mice were restrained in a perspex container and the convulsant drugs were infused into the tail vein at a rate of 1.1 ml min^{-1} via a 25 g butterfly (Abbott labs.). The latency to the onset of repeated myoclonic jerking of head and forelimbs was used for determining thresholds, except for strychnine where the onset of tonic seizure activity was used since this compound does not induce myoclonic jerking. In all cases seizures were observed within 1 min of starting the infusion.

Experiment 1

The dose-response relationship for Ro 15-4513 against pentylenetetrazole (PTZ) was determined by administering Ro 15-4513 (0.0, 0.75, 1.5, 3.0 or 6.0 mg kg^{-1}) to animals 5 min before infusion of PTZ. A 5 min interval was chosen since pilot studies suggested that this imidazodiazepine, like its structural homologue Ro 15-1788 (Lister *et al.*, 1984), has a short duration of action.

Experiment 2

In experiment 2 a time course of the effects of Ro 15-4513 was investigated by administering Ro 15-4513 (5 mg kg^{-1}) or its vehicle 5, 15, 30, 60 or 90 min before infusion of bicuculline. The dose of 5 mg kg^{-1} was chosen on the basis of the results of experiment 1, which showed that this was a maximally effective dose.

Experiment 3

In this experiment, animals were treated with either Ro 15-4513 (5 mg kg^{-1}) or its vehicle 5 min before infusing the various convulsants (PTZ, bicuculline, caffeine, quipazine, strychnine or picrotoxin). The 5 min pretreatment period was chosen on the basis of the results of experiment 2 which showed the maximal effect of Ro 15-4513 at this time.

Experiment 4

In view of the problems in interpreting the results of experiments using picrotoxin infusions (Nutt *et al.*, 1980) a further experiment was performed using the convulsant benzodiazepine Ro 5-3663 (1,3-dihydro-5-methyl 2H-1,4-benzodiazepine-2-one) which also acts at the picrotoxin receptor (Leeb-Lundberg *et al.*, 1981; Harrison & Simmonds, 1983). Ro 15-4513 (0, 1.5, 3 or 5 mg kg^{-1}) was administered 5 min before infusion of Ro 5-3663 (Green *et al.*, 1982).

Experiment 5

Ro 15-4513 (5 mg kg^{-1}) or its vehicle was injected 5 min before infusion of DMCM (see Nutt *et al.*, 1984).

Statistics

The results of experiments 1 and 4 were analysed by analysis of variance with dose of Ro 15-4513 as the independent measure. The data from experiment 2 were analysed by analysis of variance with time of drug administration as the independent measure. Between group comparisons were made using Dunn's test. The results of the other experiments were analysed using Student's *t* tests.

Drugs

Ro 15-4513 (Hoffmann-La Roche, Basel) was suspended in distilled water to which a drop of Tween 20/10 ml had been added and in all experiments was administered intraperitoneally in a volume of 10 ml kg^{-1}. Bicuculline (Sigma, St Louis) was dissolved in dilute HCl and the pH of the solution was adjusted to 3 with dilute NaOH. The final concentration was 0.05 mg ml^{-1}. Pentylenetetrazole (PTZ), strychnine, caffeine and picrotoxin were all obtained from Sigma (St Louis) and were dissolved in distilled water to concentrations of 10 mg ml^{-1}, 0.1 mg ml^{-1}, 20 mg ml^{-1} and 1.0 mg ml^{-1} respectively. Quipazine maleate (Research biochemicals, Natick, MA) was dissolved in distilled water to a concentration of 10 mg ml^{-1}. Ro 5-3663 (Hoffmann-La Roche, Basel) was dissolved in distilled water to a concentration of 0.5 mg ml^{-1}. DMCM (methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate, Research Biochemicals, Natick, MA) was dissolved in 1 ml 0.1 M HCl and diluted with distilled water to a concentration of 0.5 mg ml^{-1}.

Results

Experiment 1

The results of experiment 1 are shown in Figure 1 in which it can be seen that Ro 15-4513 significantly reduced seizure threshold to PTZ ($F(4,34) = 453.1$, $P < 0.001$). Even the lowest dose (0.75 mg kg^{-1}) significantly reduced seizure threshold ($P < 0.01$, Dunn). At no dose were spontaneous seizures observed.

Experiment 2

The time course of the proconvulsant effect of Ro 15-4513 against bicuculline is shown on Figure 2. The control values did not differ across time and have been pooled for clarity. There was a significant Ro 15-4513 × time interaction in the analysis of variance ($F(4,30) = 2.8$, $P < 0.05$). In Figure 2 it can be seen that Ro 15-4513 significantly reduced seizure threshold to bicuculline 5 min after its administration and also reduced seizure threshold 15 min after its administration, but at the later time points, animals treated with Ro 15-4513 did not differ from vehicle-treated controls.

Experiment 3

The results of experiment 3 are shown in Table 1. A significant reduction in seizure threshold was observed

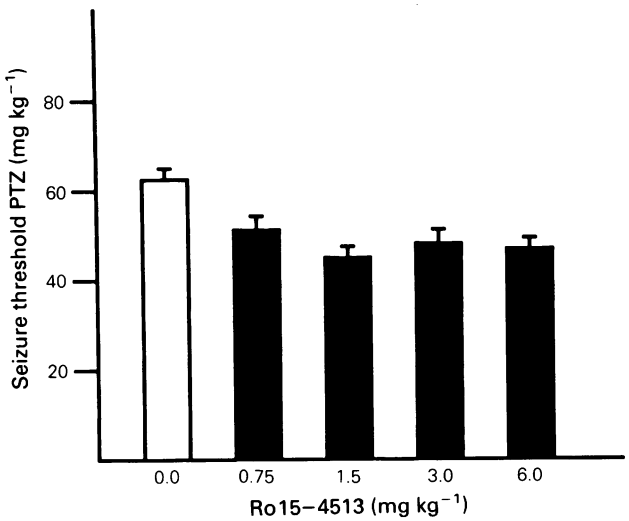

Figure 1 The effect of administering Ro 15-4513 (0–6 mg kg^{-1}) 5 min before measuring seizure threshold to intravenous pentylenetetrazole (PTZ). Values are means of $n = 6–9$ per group; s.e.means shown by vertical lines. See text for statistics.

with PTZ ($t(11) = 2.42$, $P < 0.025$) and bicuculline ($t(10) = 4.30$, $P < 0.005$). Ro 15-4513 failed to alter significantly the seizure thresholds to picrotoxin, strychnine, caffeine or quipazine ($P > 0.1$).

Experiment 4

Seizure threshold to Ro 5-3663 was lowered by pretreatment with Ro 15-4513 ($F(3,19) = 7.1$, $P < 0.005$). In Figure 3 it can be seen that all 3 doses significantly reduced seizure threshold.

Experiment 5

Ro 15-4513 significantly increased seizure threshold to DMCM ($t(9) = 4.68$, $P < 0.005$), see Table 1.

Discussion

The present experiments demonstrated that Ro 15-4513 was proconvulsant but did not cause spontaneous seizures. In this regard it resembles another partial inverse agonist for central benzodiazepine receptors, FG 7142 (Little *et al.*, 1984).

In experiment 2, the proconvulsant effect of Ro 15-4513 was found to have a rapid onset and had disappeared 30 min after the drug's administration. In this regard Ro 15-4513 seems to resemble the structurally related analogue Ro 15-1788 (Lister *et al.*, 1984).

In experiment 3 the proconvulsant effects against PTZ and bicuculline were replicated. In contrast, seizure thresholds were not lowered to strychnine, caffeine, quipazine or picrotoxin. These results are consistent with the previously reported phar-

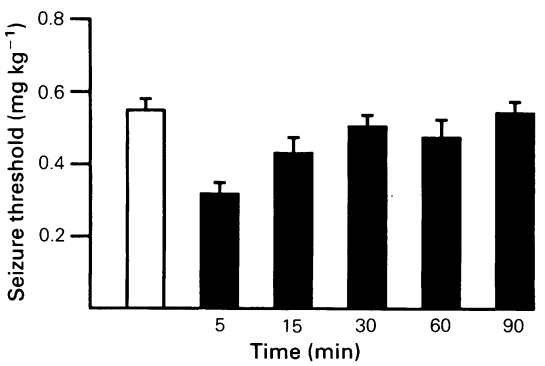

Figure 2 The effect of Ro 15-4513 (5 mg kg^{-1}, solid columns) 5, 15, 30, 60 and 90 min before measuring seizure threshold to bicuculline. Control values (open column) at the various time points have been pooled for clarity. Values are means of, $n = 5$ or 6 per group; s.e.means shown by vertical lines. See text for statistics.

Table 1 Seizure thresholds (mg kg^{-1}) to pentylenetetrazole, bicuculline, picrotoxin, caffeine, strychnine, quipazine and methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) 5 min after i.p injection of Ro 15-4513 (5 mg kg^{-1})

	Vehicle	*Ro 15-4513* (5 mg kg^{-1})
Pentylenetetrazole	90 ± 9	65 ± 6*
Bicuculline	0.66 ± 0.04	0.49 ± 0.04**
Picrotoxin	28.0 ± 1.2	26.3 ± 1.4
Caffeine	296 ± 26	266 ± 21
Strychnine	1.22 ± 0.12	1.14 ± 0.07
Quipazine	113 ± 6	125 ± 9
DMCM	6.9 ± 0.3	14.4 ± 1.7**

Values are means ± s.e.mean; $n = 5-9$ per group.
Significantly different from vehicle-treated animals: *$P < 0.025$; **$P < 0.005$.

macological actions of these agents. It is generally agreed that strychnine acts as a potent antagonist at glycine receptors. Although it has been suggested that benzodiazepines might act at this site (Young *et al.*, 1974) this now appears not to be the case.

It is believed that the primary effect of caffeine is at adenosine receptors (Snyder *et al.*, 1981); however, some studies have suggested that caffeine's convulsant effects are mediated through benzodiazepine receptors (Vellucci & Webster, 1984). More recent work has suggested that there may be no direct receptor interaction in that although diazepam antagonized caffeine-induced seizures, this effect was reversed by Ro 15-1788, and the latter drug was only a weak caffeine antagonist (Chweh *et al.*, 1986). Our study supports this conclusion in that the dose of Ro 15-4513 used might be expected to occupy the majority of benzodiazepine receptors, yet it showed no indication of elevating the caffeine seizure threshold. In contrast it clearly elevated the seizure threshold to DMCM as discussed below. Quipazine is a 5-HT agonist (Green *et al.*, 1976) which has a convulsant action (Nutt *et al.*, 1981). Our finding that Ro 15-4513 failed to lower seizure threshold to quipazine suggests that the imidazodiazepine does not interact directly with 5-HT receptors.

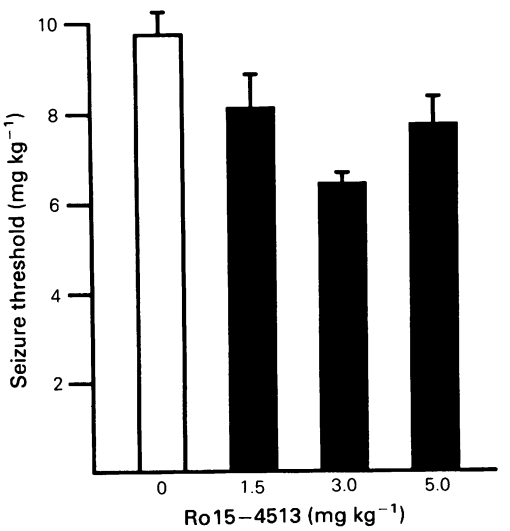

Figure 3 The effect of Ro 15-4513 (0, 1.5, 3.0 and 5.0 mg kg^{-1}) 5 min before measuring seizure threshold to Ro 5-3663. Values are means, $n = 5-7$ per group; s.e.means shown by vertical lines. See text for statistics.

That Ro 15-4513 did not lower seizure threshold to picrotoxin should be noted with caution, since this agent shows a delayed onset of action which complicates its use via the infusion method (Nutt *et al.*, 1980) and Ro 15-4513 did lower seizure thresholds to both Ro 5-3663 and PTZ which are both believed to act through the picrotoxin site (Leeb-Lundberg *et al.*, 1981; Simmonds, 1982; Harrison & Simmonds, 1983; Ramanjaneyulu & Ticku, 1984).

The lowering of seizure thresholds to bicuculline, PTZ and Ro 5-3663 and the lack of interactions with the other convulsants is consistent with the suggestion that Ro 15-4513 is a partial inverse agonist at the benzodiazepine receptor (Bonetti *et al.*, 1984). This hypothesis is further supported by our observation that Ro 15-4513 raised seizure threshold to the benzodiazepine receptor inverse agonist, DMCM. A competitive interaction at benzodiazepine receptors is the most parsimonious explanation of this latter finding. Thus, the partial inverse agonist FG 7142 behaves similarly against these convulsants (Little *et al.*, 1984; Nutt *et al.*,1984) which act at the γ-aminobutyric acid-benzodiazepine receptor complex (Olsen, 1981).

We are grateful to Dr W. Haefely (Hoffmann La-Roche, Basel) for the gift of Ro 15-4513 and to Dr P. Sorter for the gift of Ro 5-3663.

References

BONETTI, E.P., BURKARD, W.P., GABL, M. & MOHLER, H. (1985). The partial inverse benzodiazepine agonist RO 15-4513 antagonises acute ethanol effects in mice and rats. *Br. J. Pharmacol.*, **86**, 463P.

BONETTI, E.P., POLC, P. & PIERI, L. (1984). An azido analogue of the benzodiazepine antagonist RO 15-1788 (RO 15-4513) behaves as a partial inverse benzodiazepine agonist. *Neurosci. Lett.*, Suppl., **18**, S30.

CHWEH, A.Y., ULLOQUE, R.A. & SWINYARD, E.A. (1986). Benzodiazepine inhibition of [^{3}H]-flunitrazepam binding and caffeine-induced seizures. *Eur. J. Pharmacol.*, **122**, 161–165.

GREEN, A.R., YOUDIM, M.V.H., GRAHAME-SMITH, D.G. (1976). Quipazine: its effect on rat brain 5-hydroxytryptamine metabolism, monoamine oxidase activity and behavior. *Neuropharmacology*, **15**, 173–179.

GREEN, A.R., NUTT, D.J. & COWEN, P.J. (1982). Using RO 15-1788 to investigate the benzodiazepine receptor in vivo: studies on the anticonvulsant and sedative effect of melatonin and the convulsant effect of the benzodiazepine Ro 05-3663. *Psychopharmacology*, **78**, 293–295.

HARRISON, N.L. & SIMMONDS, M.A. (1983). The picrotoxin-like action of a convulsant benzodiazepine, Ro 5-3663. *Eur. J. Pharmacol.*, **87**, 155–158.

LEEB-LUNDBERG, F., NAPIAS, C. & OLSEN, R.W. (1981). Dihydropicrotoxinin binding sites in mammalian brain: interactions with convulsant and depressant benzodiazepines. *Brain Res.*, **216**, 199–408.

LISTER, R.G. & NUTT, D.J. (1987). Is Ro 15-4513 a specific alcohol antagonist? *Trends Neurosci.*, **10**, 223–225.

LISTER, R.G., GREENBLATT, D.J., ABERNETHY, D.R. & FILE, S.E. (1984). Pharmacokinetic studies on RO 15-1788, a benzodiazepine receptor ligand, in the brain of the rat. *Brain Res.*, **290**, 183–186.

LITTLE, H.J., NUTT, D.J. & TAYLOR, S.C. (1984). Acute and chronic effects of the benzodiazepine receptor ligand FG 7142: Proconvulsant properties and kindling. *Br. J. Pharmacol.*, **83**, 951–958.

NUTT, D.J., COWEN, P.J. & GREEN, A.R. (1980). On the measurement in rats of the convulsant effect of drugs and the changes which follow electroconvulsive shock. *Neuropharmacology*, **19**, 1017–1023.

NUTT, D.J., COWEN, P.J. & GREEN, A.R. (1981). Studies on the post-ictal rise in seizure threshold. *Eur. J. Pharmacol.*, **71**, 287–295.

NUTT, D.J., LITTLE, H.J., TAYLOR, S.C. & MINCHIN, M.C.W. (1984). Investigating benzodiazepine receptor function in vivo using an intravenous infusion of DMCM. *Eur. J. Pharmacol.*, **103**, 359–362.

NUTT, D.J., TAYLOR, S.C. & LITTLE, H.J. (1986). Optimising the pentylenetetrazol infusion test for seizure threshold measurement. *J. Pharm. Pharmacol.*, **38**, 697–698.

OLSEN, R.W. (1981). Gaba-benzodiazepine-barbiturate receptor interactions. *J. Neurochem.*, **37**, 1–13.

POLC, P. (1985). Interactions of partial inverse benzodiazepine agonists Ro 15-4513 and FG 7142 with ethanol in rats and cats. *Br. J. Pharmacol.*, **86**, 465P.

RAMANJANEYULU, R. & TICKU, M.K. (1984). Binding characteristics and interactions of depressant drugs with [35S]t-butylbicyclophosphorothionate, a ligand that binds to the picrotoxinin site. *J. Neurochem.*, **42**, 221–229.

SIMMONDS, M.A. (1982). Classification of some GABA antagonists with regard to site of action and potency in slices of rat cuneate nucleus. *Eur. J. Pharmacol.*, **80**, 347–358.

SNYDER, S.H., KATIMS, J.J., ANNAU, Z., BRUNS, R.F. & DALY, J.W. (1981). Adenosine receptors and behavioral actions of methylxanthines. *Proc. Natl. Acad. Sci., U.S.A.*, **78**, 3260–3264.

SUZDAK, P., GLOWA, J.R., CRAWLEY, J.N., SCHWARTZ, R.D., SKOLNICK, P. & PAUL, S.M. (1986). A selective imidazodiazepine antagonist of ethanol in the rat. *Science*, **234**, 1243–1247.

VELLUCCI, S.V. & WEBSTER, R.A. (1984). Antagonism of caffeine-induced seizures in mice by Ro 15-1788. *Eur. J. Pharmacol.*, **97**, 289–293.

YOUNG, A.B., ZUKIN, S.R. & SNYDER, S.H. (1974). Interaction of benzodiazepines with central nervous glycine receptors: possible mechanism of action. *Proc. Natl. Acad. Sci., U.S.A.*, **71**, 2246–2250.

(Received May 19, 1987.
Revised July 27, 1987.
Accepted September 19, 1987.)