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Modification of seizures elicited by the benzodiazepine Ro 5-3663—a comparison with picrotoxin

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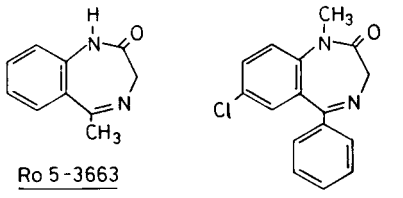
Ro 5-3663 is a convulsant 1,4-benzodiazepine that does not act at the benzodiazepine, but at the picrotoxin, site. To characterize the behavioural actions of Ro 5-3663, a comparison was made between its effects and those of picrotoxin, when combined with several compounds that act at the GABA-benzodiazepine receptor complex. The quinolines, PK 8165, PK 9084 and CGS 8216 caused myoclonic jerks when combined with subconvulsant doses of Ro 5-3663 or picrotoxin; in combination with picrotoxin they also caused full tonic-clonic convulsions. Ro 15-1788 (1, 10 mg kg^{-1}) caused myoclonic jerks when it was given 10 min before, or at the same time as, subconvulsant doses of either compound. Diazepam (2, 4 mg kg^{-1}) was anticonvulsant against both compounds. However, Ro 15-1788 (10, 20 mg kg^{-1}, 20 min before), PK 8165 (80 mg kg^{-1}) and PK 9084 (60 mg kg^{-1}) were effective only against the convulsions induced by Ro 5-3663. It is not possible to determine whether these differences between Ro 5-3663 and picrotoxin are quantitative or qualitative.

Ro 5-3663 is a 1,4-benzodiazepine (I), yet it is unable to displace [^{3}H]benzodiazepines from their binding sites (Leeb-Lundberg et al 1981). It does, however, inhibit the GABA-stimulation of benzodiazepine binding (O'Brien & Spirt 1980) and competitively and potently displaces [^{3}H]dihydropicrotoxinin binding to rat brain membranes (Leeb-Lundberg et al 1981). The picrotoxinin binding site is on a supramolecular complex with a benzodiazepine binding site, a GABA receptor and a chloride ionophore (Ticku & Olsen 1978).



Ro 5-3663 I DIAZEPAM

FIG. 1. The chemical structures of Ro 5-3663 and diazepam.

There is electrophysiological evidence that Ro 5-3663 has GABA antagonist actions in-vivo (Schlosser & Franco 1979), and in-vitro it has a picrotoxin-like action (Harrison & Simmonds 1983). Behaviourally, it has been reported to cause convulsions with a potency on i.v. administration just slightly less than that of picrotoxin (Schlosser & Franco 1979). However, the seizures caused by Ro 5-3663 have been little studied and the present experiment sought to examine the effects on these seizures of several drugs that have been reported to be proconvulsant and/or anticonvulsant in experiments with picrotoxin. Although the compounds used are not benzodiazepines, they are all potent displacers of benzodiazepines from their binding sites.

CGS 8216, a pyrazoloquinoline, is a benzodiazepine antagonist (Czernik et al 1982) that has clear proconvulsant actions with picrotoxin and pentetrazol (leptazol, metrazol, pentylenetetrazole) (File 1983). Two phenylquinolines, PK 8165 and PK 9084, with in vitro affinities for the benzodiazepine site in the high nanomolar range (LeFur et al 1981) are also proconvulsant in combination with picrotoxin and pentetrazol (File & Simmonds 1984). The purpose of experiment 1 was therefore to compare the proconvulsant effects of these three compounds when given in combination with Ro 5-3663 or with picrotoxin.

The imidazodiazepine, Ro 15-1788 is also a benzodiazepine antagonist, but in contrast to CGS 8216, it has some anticonvulsant actions, expecially at high doses (Nutt et al 1982; Vellucci & Webster 1983). However, in previous studies we have found that in low doses Ro 15-1788 can have proconvulsant actions with pentetrazol and picrotoxin (File 1983; File et al 1984), and that these seem to depend crucially on the timing of the two injections. Experiment 2 therefore investigated the effects of low doses of Ro 15-1788 given 10 min before, at the same time as, or 10 min after a subconvulsant dose of picrotoxin or Ro 5-3663.

In experiment 3, two compounds were selected that were expected to be anticonvulsant against Ro 5-3663 seizures. One was diazepam, which is an effective anticonvulsant against a wide range of chemically induced convulsions, including those caused by Ro 5-3663 (Green et al 1982). The other was Ro 15-1788, which when it was given 20 min before Ro 5-3663 in doses of 25 mg kg^{-1} and above has been found to be anticonvulsant (Vellucci & Webster 1983). Since Experiment 1 indicated that at high doses the phenylquinolines were losing their proconvulsant actions, possible anticonvulsant actions of these compounds were also investigated.

Methods

Animals. Male mice (Tuck No. 1 strain), 30 g, were housed in groups of 8 with food and water freely available. They were housed in a room with light on from 1700 to 1800 h.

Drugs. Diazepam, Ro 5-3663, Ro 15-1788 (Roche) and CGS 8216 (Ciba-Geigy) were suspended in distilled water to which a drop of Tween-20 was added and dispersed by ultrasound until immediately before injec-

tion. Picrotoxin (Sigma), PK 8165 and PK 9084 (Pharmuka) were dissolved in distilled water. All drugs were injected intraperitoneally in a volume of 4 ml kg^{-1}. All the drugs, except Ro 15-1788, were injected 30 min before Ro 5-3663 or picrotoxin. Control animals received the appropriate vehicle injection.

Procedure. For both experiments 1 and 2, pilot experiments established the subconvulsant doses of Ro 5-3663 and picrotoxin. These were taken as the highest dose that failed to cause myoclonic jerks or convulsions in any of the mice. It was generally 50% of the CD100, i.e. the lowest dose that caused seizures in 100% of the mice. For Experiment 3 the CD100 for the two drugs were also established first in a pilot study.

In each experiment mice were randomly allocated 8 to each of the drug groups. They were tested between 1400 and 1600 h, in an order randomized for drug treatment. After injection with Ro 5-3663, each mouse was placed in an individual box and observed for 15 min.

Table 1. Number of mice showing myoclonic jerks and number showing full tonic-clonic convulsions after (A) Ro 5-3663 (5 mg kg^{-1}) alone or (B) Picrotoxin (4 mg kg^{-1}) alone or each drug in combination with PK 8165 (25–50 mg kg^{-1}), PK 9084 (25–50 mg kg^{-1}) or CGS 8216 (5–20 mg kg^{-1}). Where >50% of a group responded the mean (±s.e.m.) latencies (s) are given. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, Fisher exact probability test compared with Ro 5-3663 or picrotoxin alone.

	Myoclonic jerks		Convulsions	
(A) Ro 5-3663 (5 mg kg^{-1})				
Alone	0/16		0/16	
+PK8165 (25)	8/8***	316·9 ± 50·5	3/8*	
+PK8165 (50)	8/8***	435·8 ± 39·6	2/8	
+PK9084 (25)	8/8***	267·5 ± 25·1	4/8**	
+PK9084 (50)	8/8***	476·2 ± 35·3	0/8	
+CGS8216 (5)	8/8***	164·4 ± 21·2	5/8**	295·0 ± 51·7
+CGS8216 (10)	8/8***	158·6 ± 23·2	6/8***	302·4 ± 38·4
(B) Picrotoxin (4 mg kg^{-1})				
Alone	0/8		0/8	
+PK8165 (25)	8/8***	623·8 ± 37·6	8/8***	782·1 ± 83·7
+PK8165 (50)	8/8***	785·4 ± 42·6	8/8***	1364·4 ± 97·6
+PK9084 (25)	8/8***	18·2 ± 42·6	8/8***	976·7 ± 95·6
+PK9084 (50)	8/8***	1276·8 ± 101·1	4/8*	
+CGS8216 (5)	8/8***	612·9 ± 63·6	7/8***	1062·8 ± 103·6
+CGS8216 (10)	8/8***	621·4 ± 54·3	7/8***	887·6 ± 89·4

The latency to the first myoclonic jerk (sudden extension of the forelimbs) and the latency to the first full tonic-clonic convulsion (extension and contraction of fore- and hind-limbs) were recorded.

Results

Experiment 1 Proconvulsant actions. No mice showed myoclonic jerks or convulsions to the doses of Ro 5-3663 or picrotoxin when given alone (Table 1). However, in combination with PK 8165 and PK 9084 (10 and 25 mg kg^{-1}) or with CGS 8216 (5 mg kg^{-1}) all the mice showed myoclonic jerks and some showed full seizures. Higher doses of CGS 8216 did not alter the % of mice convulsing or the latencies to myoclonus or full convulsion. Higher doses of the phenylquinolines (especially PK 9084) resulted in *fewer* convulsions and the latency to myoclonus with the 50 mg kg^{-1} dose was significantly increased, compared with the 25 mg kg^{-1} dose.

Experiment 2 proconvulsant actions of low doses of Ro 15-1788. Ro 15-1788 (1, 10 mg kg^{-1}) had proconvulsant actions in causing myoclonic jerks, when it was given either 10 min before, or at the same time as, subconvulsant doses of Ro 5-3663 or picrotoxin (Table 2). There were no significant differences between the two doses of Ro 15-1788. However, when it was given 10 min *after* the convulsants no proconvulsant effects were seen.

Experiment 3. All the mice convulsed following Ro 5-3663 (10 mg kg^{-1}), and diazepam (2, 4 mg kg^{-1}) was completely effective at counteracting both the myoclonic jerks and the full convulsions (Table 3A). Ro 15-1788 (10, 20 mg kg^{-1}, given 20 min before) significantly reduced the number of full convulsions, and whilst it had only a small effect on the incidence of myoclonic jerks, it did significantly increase the latency to the first jerk. PK 8165 (40–80 mg kg^{-1}) was without significant effect against myoclonic jerks caused by Ro 5-3663, but did slightly reduce the number of full seizures. PK 9084 (40, 60 mg kg^{-1}) reduced the incidence of myoclonic jerks and the higher dose also significantly reduced the incidence of full convulsions.

By comparing parts A and B of Table 3 the effects of

Table 2. Number of mice showing myoclonic jerks and full convulsions after subconvulsant doses of Ro 5-3663 (5 mg kg^{-1}) or picrotoxin (3 mg kg^{-1}) in combination with Ro 15-1788 (1, 10 mg kg^{-1}) given 10 min before (−10), at the same time, or 10 min after (+10 min) the convulsants. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, Fisher exact probability test, compared with Ro 5-3663 or picrotoxin alone. Where >50% of a group responded the mean (±s.e.m.) latencies (s) are given.

		+Ro 15-1788					
	Alone	−10 min		0 min		+10 min	
		1	10 mg kg^{-1}	1	10 mg kg^{-1}	1	10 mg kg^{-1}
(A) Ro 5-3663 (5 mg kg^{-1})							
Myoclonic jerks	0/8	6/8**	7/8***	5/8*	5/8*	0/8	0/8
		111·6 ± 8·9	121·2 ± 11·3	108·0 ± 13·5	130·0 ± 20·2		
Convulsions	0/8	0/8	0/8	0/8	0/8	0/8	0/8
(B) Picrotoxin (3 mg kg^{-1})							
Myoclonic jerks	0/8	4/8*	8/8***	4/8*	6/8**	0/8	0/8
			665·0 ± 28·3		491·0 ± 76·4		
Convulsions	0/8	0/8	2/8	0/8	2/8	0/8	0/8

these anticonvulsants against picrotoxin-induced seizures can be compared with those described above against Ro 5-3663 seizures. Diazepam (2, 4 mg kg^{-1}) significantly reduced both full convulsions and myoclonic jerks caused by picrotoxin (8 mg kg^{-1}). However, Ro 15-1788 (10, 20 mg kg^{-1}) was ineffective against these convulsions. PK 8165 and PK 9084 did not reduce the incidence of myoclonic jerks or full convulsions caused by picrotoxin, but they did prolong the latencies of these responses.

Discussion

Picrotoxin appeared to be a slightly more potent convulsant than Ro 5-3663, although it has a slower onset of action. The lowest dose of Ro 5-3663 to cause seizures in all mice was 10 mg kg^{-1}, compared with the dose of 8 mg kg^{-1} for picrotoxin. All four proconvulsant agents that were tested were equally effective at provoking myoclonic jerks whether they were combined with Ro 5-3663 or with picrotoxin, however the two phenylquinolines were more effective at causing full convulsions when combined with picrotoxin. It is thus slightly easier to see proconvulsant effects with picrotoxin than with Ro 5-3663. One interpretation of the differences between Ro 5-3663 and picrotoxin shown in Table 3 is that it is easier to see anticonvulsant effects against Ro 5-3663 than against picrotoxin. Thus, it was possible to see anticonvulsant effects of Ro 15-1788 and the phenylquinolines against the incidence of myoclonic jerks and full convulsions when these were caused by Ro 5-3663, but not when they were caused by picrotoxin. The phenylquinolines were able only to prolong the latency to convulse when picrotoxin was used. However, at this point it is not possible to determine whether the differences between Ro 5-3663 and picrotoxin are in degree of effect, or whether they are qualitative.

Table 3. Number of mice showing myoclonic jerks and full convulsions after Ro 5-3663 (10 mg kg^{-1}) alone or picrotoxin (8 mg kg^{-1}) alone; or after a combination of each convulsant with diazepam (2 & 4 mg kg^{-1}), Ro 15-1788 (10 & 20 mg kg^{-1}), PK 8165 (40–80 mg kg^{-1}), or PK 9084 (40–60 mg kg^{-1}). Ro 15-1788 was given 20 min before the convulsants, the other drugs were given 30 min before. $^{**}P < 0.01$, $^{*}P < 0.05$ Fisher exact probability test, compared with Ro 5-3663 or picrotoxin alone. Where > 50% of a group responded the mean (±s.e.m.) latencies (s) are given. $\dagger\dagger\dagger P < 0.001$, $\dagger P < 0.05$, Student's *t*-test.

	Myoclonus		Convulsions	
(A) Ro 5-3663 (10 mg kg^{-1})				
Alone	16/16	108·8 ±18·1	16/16	353·3 ±46·2
+diazepam (2)	0/8		0/8	
+diazepam (4)	0/8		0/8	
+Ro 15-1788 (10)	5/8*	166·0† ±22·3	3/8**	
+Ro 15-1788 (20)	6/8	190·0† ±40·9	1/8**	
Alone	16/16	151·2 ±27·9	14/16	271·2 ±47·9
+PK8165 (40)	8/8	170·5 ±32·6	5/8*	203·1 ±18·0
+PK8165 (60)	7/8	237·5 ±42·3	6/8	342·3 ±48·5
+PK8165 (80)	6/8	275·2† ±35·6	5/8*	282·6 ±75·5
+PK9084 (40)	5/8*	840·0††† ±113·6	7/8	889·7 ±145.1
+PK9084 (60)	5/8*	287·5	3/8*	
(B) Picrotoxin (8 mg kg^{-1})				
Alone	8/8	387·1 ±23·5	8/8	575·0 ±46·0
+diazepam (2)	3/8*		2/8**	
+diazepam (4)	0/8***		0/8***	
+Ro 15-1788 (10)	8/8	383·1 ±13·1	8/8	624·4 ±14·1
+Ro 15-1788 (20)	8/8	370·6 ±5·4	8/8	584·4 ±47·9
Alone	8/8	285·8 ±52·1	8/8	517·3 ±44·0
+PK8165 (40)	8/8	599·5††† ±46·7	8/8	681·0 ±22·0
+PK8165 (60)	8/8	441·3††† ±40·8	8/8	522·4 ±44·7
+PK8165 (80)	8/8	584·5††† ±39·3	7/8	686·9† ±60·6
+PK9084 (40)	8/8	446·8††† ±32·4	8/8	902·1† ±108·2
+PK9084 (60)	8/8	621·9††† ±93·2	6/8	856·0† ±124·2
+PK9084 (80)	8/8	654·3††† ±65·8	8/8	854·1† ±119·9

The results of this experiment provide further data on the rather complex profiles of Ro 15-1788 and PK 8165 and PK 9084. The phenylquinolines have clear proconvulsant actions from doses of 5 mg kg^{-1} (File & Simmonds 1984), whereas their relatively weak anticonvulsant actions are found above 40 mg kg^{-1}, with those of PK 9084 being more marked than those of PK 8165. In the terminology proposed by Braestrup et al (1983) they would therefore be classified as partial inverse agonists, at least as far as their effect on seizures is concerned.

Ro 15-1788 (1, 10 mg kg^{-1}), when it was administered at the same time as, or 10 min before, subconvulsant doses of Ro 5-3663 or picrotoxin, had significant proconvulsant actions that were restricted to the incidence of myoclonus. The proconvulsant actions of Ro 15-1788 (10 mg kg^{-1}) when administered simultaneously with picrotoxin has been found in previous experiments (File et al 1984), although if it is given 20 min before picrotoxin it does not produce myoclonus (File 1983). Ro 15-1788 has also been found to be proconvulsant in combination with isoniazid (Corda et al 1982).

In general, Ro 15-1788 has anticonvulsant actions in high doses (see Pellow 1985 for review) and it has therefore been proposed that it is a partial agonist acting at the benzodiazepine receptors (Vellucci & Webster 1983). In the present experiment, Ro 15-1788 (10, 20 mg kg^{-1}) was ineffective at blocking seizures caused by a convulsant dose of picrotoxin, which is in agreement with previous results (File 1983). However, it is likely that the dose of picrotoxin was too high and the dose of Ro 15-1788 too low to see any anticonvulsant effects, since a dose of 50 mg kg^{-1} of the latter is needed to block convulsions induced by threshold doses of pentetrazol, and it is ineffective against higher doses (see Vellucci & Webster 1983 for refs). In contrast to the results with picrotoxin, Ro 15-1788 (10, 20 mg kg^{-1}) was able to reduce the incidence of full tonic-clonic

convulsions and to prolong the latency to the first myoclonic jerk induced by Ro 5-3663 (10 mg kg^{-1}). Vellucci & Webster (1983) also found Ro 15-1788 (25, 50 mg kg^{-1}) effective against convulsions caused by Ro 5-3663 at 10 mg kg^{-1}, but not those caused by higher doses. Both of these findings contrast with the failure of Green et al (1982) to find any change in the seizure threshold to an intravenous infusion in rats of Ro 5-3663, following a 15 min pretreatment with Ro 15-1788 (10 mg kg^{-1} i.p.). This difference may be the result of different methodology or species used, but it is striking that the seizure threshold in rats, 10·7 mg kg^{-1} Ro 5-3663 is close to the one we found in our mice. The ability to see anticonvulsant effects against Ro 5-3663 more easily than picrotoxin is further evidence of the latter being a more powerful convulsant.

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Application of orthogonal functions to spectrophotometric analysis of the preservatives benzylalcohol, phenol and parabens in aqueous cyanocobalamin solutions

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The orthogonal polynomials P_3 and P_2, 12 and 8 points, at 2 nm intervals over certain wavelength ranges were used for the spectrophotometric analysis of benzylalcohol, phenol or parabens in aqueous cyanocobalamin solutions. The 12 point-methods proved to be more sensitive and direct and to have sufficient accuracy and precision.

Benzylalcohol, phenol and parabens are preservatives compatible with cyanocobalamin in aqueous solutions. They are currently determined by gas chromatography (USP XX; Hrivnak & Macak 1971; Johnson & Venturello 1971; Douglas 1972). Previous methods include spectrophotometric (Glenn 1960; Elvidge & Peutrell 1961) and colorimetric (Gibbs 1927; Emerson 1943; Johnson & Savidge 1958) methods, which have limitations in the presence of interfering substances. Glenn (1963) applied orthogonal functions to the spectrophotometric assay of phenol and adrenaline in a parenteral solution.

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We have applied orthogonal functions to the determinations of benzylalcohol, phenol and parabens in aqueous cyanocobalamin solutions since it has the advantage of being a direct fairly sensitive method that does not require expensive apparatus. Optimum conditions (Glenn 1963 Abdine et al 1971) were determined and computed from the absorption spectra of benzylalcohol, phenol, parabens and cyanocobalamin in 0·05 M sulphuric acid, scanned individually at 1 nm intervals (Fig. 1).

Materials and methods

Materials used were cyanocobalamin BP, phenol and benzylalcohol, both BDH laboratory reagents, the latter being further purified by shaking with sodium metabisulphite and filtering; methylparaben and propylparaben were of BP quality.

Solutions prepared: Aqueous cyanocobalamin solutions (0·01–0·1% w/v) were freshly prepared and contained