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Pro- and anti-convulsant drug effects in combination with the convulsant benzodiazepine Ro 5-4864

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The effects of several compounds believed to act at the GABA-benzodiazepine receptor complex and which have anticonvulsant or proconvulsant properties when administered in combination with picrotoxin and pentetrazol (leptazol, pentylenetetrazole) were investigated in combination with the convulsant benzodiazepine Ro 5-4864. Tracazolate (25–100 mg kg^{-1}) failed to affect convulsions induced by Ro 5-4864; however, they were prevented by treatment with CL 218, 872 (20 mg kg^{-1}). Compounds having proconvulsant activity in combination with a subthreshold dose of Ro 5-4864 were: CL 218,872 (5 mg kg^{-1}), and CGS 8216 (20 mg kg^{-1}) and FG 7142 (40 mg kg^{-1}), two compounds characterized as 'inverse agonists' at benzodiazepine receptors. The phenylquinolines PK 8165 and PK 9084, originally believed to have anxiolytic properties, had no significant effect in combination with Ro 5-4864 (25–100 mg kg^{-1}). The convulsant profile of Ro 5-4864 is compared with that of picrotoxin and pentetrazol.

(Ro 5-4864 (7 chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepin-2-one) differs from diazepam only by a *p*-chloro substituent; however, unlike other benzodiazepines it does not displace the binding of [^{3}H]diazepam from classical CNS benzodiazepine receptors. In contrast, Ro 5-4864 has high affinity for the 'peripheral-type' of benzodiazepine binding site found both in the periphery and in the brain, the functional relevance of which is unknown (Braestrup & Squires 1977; Schoemaker et al 1981). Similarly, behavioural studies have shown that Ro 5-4864 has properties different from those of other 1,4-benzodiazepines, being anxiogenic (File & Lister 1983; Pellow & File 1984b), sedative (File & Pellow 1983) and convulsant (File & Mabbutt 1983; Pieri et al 1983; Weissman et al 1983; File et al 1984). Recent biochemical and electrophysiological evidence, however, suggests that Ro 5-4864 also interacts with the GABA-benzodiazepine receptor complex in the CNS (see Pellow & File 1984a, b for review), possibly through its ability to bind at high concentrations to the picrotoxinin domain on this complex (Ticku & Ramanjaneyulu 1984).

If the picrotoxinin site is the one at which Ro 5-4864 produces its behavioural activity, it may be expected that its behavioural profile would be similar to that of picrotoxin and pentetrazol (leptazol, pentylenetetrazole) (see Olsen 1982). All three compounds have convulsant and anxiogenic properties (File & Lister 1984). The purpose of the present study was to further characterize the convulsant profile of Ro 5-4864 and to compare it with the profile of picrotoxin and pentetrazol. We have therefore examined its effects in combination with several compounds that have anticonvulsant or proconvulsant properties in combination with these two drugs. The compounds selected were:

1. Tracazolate is a pyrazolopyridine that, unlike the other compounds chosen for this study, does not displace benzodiazepine binding from classical benzodiazepine CNS sites, but enhances the affinity of benzodiazepine binding (Meiners & Salama 1982) and displaces binding from the picrotoxinin site (Leeb-Lundberg, cited in Goldberg et al 1983). This compound has weak anticonvulsant activity against pentetrazol (Patel & Malick 1982) and so its effects were examined with a convulsant dose of Ro 5-4864. The effects of tracazolate were also examined in combination with picrotoxin.

2. CL 218,872 (3-methyl-6-[3-(trifluoromethyl)-phenyl]-1,2,4-triazolo[4,3*b*]pyridazine) is a triazolopyridazine that displaces benzodiazepine binding from benzodiazepine receptors and that has been claimed to distinguish different receptor subtypes (Lippa et al 1979). At high doses (10–20 mg kg^{-1}) CL 218,872 is anticonvulsant against picrotoxin and pentetrazol, but at lower doses (0·5–2 mg kg^{-1}) it has proconvulsant effects with subthreshold doses of picrotoxin but not those of pentetrazol (Melchior et al 1984). High doses of CL 218,872 were therefore investigated in combination with a convulsant dose

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of Ro 5-4864, and low doses with a subthreshold dose.

3. PK 8165 (2-phenyl-4-[2-(4-piperidinyl)ethyl]-quinoline) and PK 9084 (2 phenyl-4-[2-(3-piperidinyl)ethyl]quinoline), two phenylquinolines that in-vitro displace benzodiazepine binding from their receptors (LeFur et al 1981), a claim that has recently been questioned by in-vivo studies (Keane et al 1984; (File & Pellow 1984), given 1–50 mg kg^{-1}, had proconvulsant activity with picrotoxin and pentetrazol (File & Simmonds 1984), but at higher doses (40–80 mg kg^{-1}) had some anticonvulsant activity against the convulsant benzodiazepine Ro 5-3663 (1,3-dihydro-5-methyl-2*H*-1,4-benzodiazepin-2-one), that also acts at the picrotoxin site (File 1984b). The effects of these compounds were therefore investigated against both a convulsant and a subconvulsant dose of Ro 5-4864.

4. CGS 8216 (2-phenylpyrazolo[f,3*c*]quinolin-3(5*H*)-one) is a pyrazoloquinoline that non-competitively displaces benzodiazepines from their receptors (Yokoyama et al 1982) and has been characterized as an inverse agonist (i.e. it has activity generally in the opposite direction to that of benzodiazepines, Jensen et al 1983). This compound (5–20 mg kg^{-1}) has proconvulsant activity in combination with picrotoxin and pentetrazol (File 1983; Jensen & Petersen 1983), and so its effects were examined in combination with a subthreshold dose of Ro 5-4864.

5. FG 7142 (β-carboline-3-carboxylic acid methyl amide), is a β-carboline that displaces benzodiazepine binding in-vivo and has been characterized as an inverse agonist (Jensen et al 1983). It has proconvulsant activity (30–40 mg kg^{-1}) in combination with picrotoxin and pentetrazol (Jensen & Petersen 1983; Little & Nutt 1984) and so its effects were examined in combination with a subthreshold dose of Ro 5-4864.

MATERIALS AND METHODS

Animals

Male albino mice (Tuck no. 1 strain), 30–40 g, were housed in groups of 30 with free access to food and water in a room with an 11 h light : 13 h dark cycle.

Procedure

Pilot experiments established a convulsant dose of Ro 5-4864 (the lowest dose causing myoclonic spasms and convulsions in all mice) and a subconvulsant dose (the highest dose to cause no myoclonic spasms or convulsions in mice). Mice were tested between 14.00 and 18.00 h. After injection of the convulsant each mouse was placed in an individual box and observed for 30 min. The latency to the first myoclonic jerk (sudden extension of the forelimbs) and the latency to the first full convulsion (extension and contraction of the fore- and hind-limbs) were recorded by an observer. Mice were randomly allocated to the following treatment groups:

Experiment 1: in combination with a convulsant dose of Ro 5-4864 (60 mg kg^{-1}): vehicle control, CL 218,872 (20 and 50 mg kg^{-1}), tracazolate (25, 50 and 10 mg kg^{-1}), PK 8165 or PK 9084 (50 and 100 mg kg^{-1}). In combination with a convulsant dose of picrotoxin (6 mg kg^{-1}): vehicle control, tracazolate (50 and 100 mg kg^{-1}), n = 8 per group.

Experiment 2: in combination with a subconvulsant dose of Ro 5-4864 (30 mg kg^{-1}): vehicle control, CL 218,872 (5 mg kg^{-1}), PK 8165 or PK 9084 (25, 50 and 100 mg kg^{-1}), CGS 8216 (5 and 20 mg kg^{-1}), FG 7142 (40 mg kg^{-1}), n = 8 per group.

Drugs

All drugs were injected intraperitoneally, in concentrations to give an injection volume of 4 ml kg^{-1}, 30 min before Ro 5-4864. All compounds were suspended in distilled water with a drop of polysorbate 20 (Tween 20).

Statistics

Latency data were analysed by Student's *t*-test, and frequency data by the Fisher exact probability test.

RESULTS

Experiment 1: Anticonvulsant effects with Ro 5-4864

Ro 5-4864 (60 mg kg^{-1}) alone caused convulsions in 7/8 mice. CL 218, 872 (20 mg kg^{-1}) had no significant effect on the number of mice showing myoclonus or convulsions with Ro 5-4864 (Table 1), but significantly increased the latency to the first myoclonic spasm (Table 1). At 50 mg kg^{-1}, CL 218,872 significantly reduced the number of mice having convulsions, but not myoclonus, after Ro 5-4864 treatment, but significantly increased the latency to myoclonus (Table 1).

Tracazolate (50–100 mg kg^{-1}) significantly reduced the number of mice having myoclonic spasms or convulsions with picrotoxin (6 mg kg^{-1}) (Table 1). Tracazolate (25–100 mg kg^{-1}) had no significant effect on the number of mice having myoclonic jerks or convulsions after Ro 5-4864 treatment, and similarly did not affect the latency of either of these responses (Table 1).

Neither PK 8165 nor PK 9084 (50–100 mg kg^{-1}) significantly affected the number of mice convulsing

Table 1. Mean (± s.e.m.) latencies (s) to the first myoclonic jerk and to the full convulsions, and number of mice having jerks and convulsions, in mice given a convulsant dose of Ro 5-4864 in combination with tracazolate, CL 218,872, PK 8165 and PK 9084, or of tracazolate in combination with picrotoxin, n = 8. Latencies are given only when > 50% animals showed the behaviour in question. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$, Fisher exact probability test (number) or *t*-test (latency).

Drug (mg kg^{-1})	Latency (s) Myoclonus	Latency (s) Convulsion	Number Myo.	Number Conv.
Ro 5-4864	400·6± 70·80	846·0± 41·74	7/8	7/8
+ CL 20	700·6± 60·44**	1020·0±100·91	7/8	7/8
+ CL 50	1187·5± 74·19***/*	—	6/8	1/8**
+ Trac 25	552·8± 41·21	874·0± 69·17	7/8	5/8
+ Trac 50	444·3± 44·77	787·8± 30·72	8/8	7/8
+ Trac 100	574·8± 63·53	828·0± 55·16	8/8	6/8
+ 8165 50	669·4± 57·69**	1017·0± 80·14	7/8	5/8
+ 8165 100	636·1± 71·65*	1094·1± 75·75**	8/8	7/8
+ 9084 50	563·7± 39·90*	987·8±126·36	8/8	7/8
+ 9084 100	582·8± 65·80	1003·7± 45·78*	7/8	7/8
Picrotoxin 6	265·1± 21·25	536·8± 34·09	8/8	8/8
+ Trac 50	437·5±141·45	621·2±164·75	4/4*	4/4*
+ Trac 100	—	—	0/8**	0/8**

or having myoclonic jerks; however, at 50 mg kg^{-1} both compounds significantly increased the latency to the first myoclonic spasm (Table 1). At 50 mg kg^{-1}, PK 8165 increased the latency to the first myoclonic spasm and at 100 mg kg^{-1} also to the first convulsion; PK 9084 (50 mg kg^{-1}) increased the latency to the first myoclonic spasm and (100 mg kg^{-1}) to the first convulsion (Table 1).

Experiment 2: Proconvulsant effects with Ro 5-4864

Ro 5-4864 (30 mg kg^{-1}) produced myoclonus in 1/8 animals and no convulsions. CL 218,872 (5 mg kg^{-1}) significantly increased the number of mice having myoclonic jerks and convulsions (Table 2).

Table 2. Mean (±s.e.m.) latency (s) to first myoclonic jerk and convulsion, and number of mice jerking and convulsing, in mice treated with a subconvulsant dose of Ro 5-4864, in combination with CL 218,872, FG 7142, CGS 8216, PK 8165 and PK 9084, n = 8. Latencies are shown only when > 50% of the mice showed the behaviour in question. $^{*}P < 0.05$, $^{**}P < 0.01$, Fisher exact probability test (number) or *t*-test (latency).

Drug (mg kg^{-1})	Latency (s) Myoclonus	Latency (s) Convulsion	Number Myo.	Number Conv.
Ro 5-4864 30	—	—	1/8	0/8
+ CL 5	543·4 ± 47·17	—	5/8*	4/8*
+ FG 40	267·8 ± 26·50	540·0 ± 51·16	7/8**	6/8**
+ CGS 5	—	—	3/8	0/8
+ CGS 20	294·4 ± 14·40	636·2 ± 24·38	8/8**	8/8**
+ 8165 25	—	—	2/8	0/8
+ 8165 50	—	—	4/8	3/8
+ 8165 100	—	—	2/8	1/8
+ 9084 25	—	—	3/8	0/8
+ 9084 50	—	—	3/8	2/8
+ 9084 100	—	—	1/8	0/8

Neither PK 8165 nor PK 9084 significantly affected the number of mice having myoclonic jerks or convulsions (Table 2).

FG 7142 (40 mg kg^{-1}) significantly increased the number of mice having myoclonic jerks and convulsions (Table 2).

CGS 8216 (5 mg kg^{-1}) had no significant effect on either measure, but at 20 mg kg^{-1} there was an increase both in the number of mice having myoclonic jerks and those having convulsions (Table 2).

DISCUSSION

Ro 5-4864 (60 mg kg^{-1}) produced myoclonus followed by convulsions. As has previously been described (Weissman et al 1984; File et al 1984) these convulsions are different from those observed with picrotoxin and pentetrazol; as well as producing tonic-clonic convulsions there were also rotational movements, loss of righting reflex and chewing of the forelimbs.

Table 3 summarizes the combinations of the compounds tested in the present study in combination with picrotoxin, pentetrazol and Ro 5-4864. This Table shows that anticonvulsant drug effects with Ro 5-4864 were not always identical to those observed with picrotoxin and pentetrazol. CL 218,872 at a high dose (50 mg kg^{-1}) had anticonvulsant activity against Ro 5-4864, as with picrotoxin and pentetrazol (Melchior et al 1984). A higher dose of CL 218,872 was necessary with Ro 5-4864 than with picrotoxin and pentetrazol; this may reflect differences in the relative potencies of doses of these compounds selected for study. PK 8165 and PK 9084 had no significant effects on the number of mice showing myoclonus or convulsions with Ro 5-4864, but a mild anticonvulsant activity at higher doses is suggested by the increases in latency to these measures, as shown in Table 1. Such an effect is observed when these two compounds are combined with the convulsant benzodiazepine Ro 5-3663 (File 1984a), but not with picrotoxin and pentetrazol (File & Simmonds 1984). Tracazolate was unable to prevent Ro 5-4864-

Table 3. Summary of the effective doses (mg kg^{-1}) of the compounds tested in the present study, on the convulsions induced by picrotoxin, pentetrazol and Ro 5-4864. For references see text. ↑ = proconvulsant, ↓ = anticonvulsant.

	Tracazolate	CL 218, 872	PK 8165	PK 9084	CGS 8216	FG 7142
Picrotoxin	↓ 50–100	↑ 0·5–2·0 ↓ 10–20	↑ 1–50	↑ 1–50	↑ 5–20	↑ 30–40
Pentetrazol	↓ ED50 = 27·7	↓ 10–20	↑ 1–50	↑ 1–50	↑ 5–20	↑ 30–40
Ro 5-4864	—	↑ 5 ↓ 50	—	—	↑ 20	↑ 40

induced seizures, at doses at which it was able to prevent seizures induced by picrotoxin (Table 1) and pentetrazol (Patel & Malick 1982). This was surprising, since Ro 5-4864-induced convulsions can be prevented by barbiturates (Weissman et al 1984), whose mechanisms of action appear to be similar to those of pyrazolopyridazines (see Olsen 1982).

Table 3 shows that the proconvulsant effects of low doses of CL 218,872, and of FG 7142 and CGS 8216 with Ro 5-4864 differed from their effects with picrotoxin and pentetrazol only in terms of the effective dose: as shown in Table 3, the doses required to potentiate the effects of Ro 5-4864 were higher than those required to potentiate picrotoxin and pentetrazol. The major difference in these studies between Ro 5-4864 and picrotoxin and pentetrazol was that neither PK 8165 nor PK 9084 had significant proconvulsant activity with Ro 5-4864 over a wide range of doses, although Table 2 shows a mild tendency to such activity that is clearest at the 50 mg kg^{-1} dose.

In conclusion, except for slight differences in the effective doses, the effects of CL 218,872, FG 7142 and CGS 8216 are similar with Ro 5-4864, picrotoxin and pentetrazol, but the studies with PK 8165, PK 9084 and tracazolate show that these compounds interact differently with Ro 5-4864 than with picrotoxin and pentetrazol. Other major differences in the profiles of these three convulsants have been described elsewhere; for example, PK 11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide—a ligand for peripheral-type benzodiazepine binding sites, LeFur et al 1983) and the clinically effective anticonvulsant phenytoin have anticonvulsant effects with Ro 5-4864 (File & Mabbut 1983), but potentiate convulsions induced by picrotoxin and pentetrazol (File 1984; File et al unpublished observations). These differences suggest that the mechanisms underlying the convulsions produced by these three compounds are not identical; similarly, Ro 5-4864 does not behave exactly like picrotoxin or pentetrazol in an in-vitro electrophysiological preparation reflecting activity at the GABA-benzodiazepine receptor complex (Simmonds unpublished observations). This study emphasizes that all convulsants acting at this domain do not have identical convulsant profiles. However, since the metabolism of many of these new compounds has not been fully described, we cannot exclude the possibility that pharmacokinetic factors may underlie some of the differences observed.

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