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Pro- and anti-convulsant properties of PK 11195, a ligand for benzodiazepine binding sites: development of tolerance

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1 Ro 5-4864 is a benzodiazepine that differs from diazepam only in a *p*-chloro substituent and yet is inactive at the classical CNS binding sites. However it is a potent ligand for the peripheral type of benzodiazepine binding sites. PK 11195 is an isoquinoline carboxamide derivative that potently displaces [^{3}H]-Ro 5-4864 from its binding sites.

2 PK 11195 (30–60 mg kg^{-1}) significantly reduced the incidence of convulsions caused by Ro 5-4864 (30 mg kg^{-1}). PK 11195 (up to 120 mg kg^{-1}) was ineffective at counteracting seizures caused by the convulsant benzodiazepine Ro 5-3663, although this dose did increase the latency to seize after injection with pentylenetetrazole. PK 11195 had no anticonvulsant actions against picrotoxin, and at 60 mg kg^{-1} reduced the latency to seize. This possible proconvulsant property of the isoquinoline was further explored.

3 PK 11195 (30–90 mg kg^{-1}) had proconvulsant actions when combined with subconvulsant doses of strychnine and picrotoxin, but had none when combined with pentylenetetrazole.

4 No significant tolerance developed to the anticonvulsant action of PK 11195 (30 mg kg^{-1}) even after 25 days of dosing daily. In contrast, there was rapid tolerance (within 5 days) to the proconvulsant action of PK 11195 (60 mg kg^{-1}) with picrotoxin (3 mg kg^{-1}).

5 There was no cross-tolerance between the anticonvulsant actions of diazepam and PK 11195, which suggests that these two drugs act at different sites, as would be predicted from the results of the binding studies.

6 The possible sites of action and clinical relevance of these effects are discussed.

Introduction

Ro 5-4864 is a benzodiazepine differing from diazepam in only a *p*-chloro substituent, yet it is inactive at classical benzodiazepine binding sites. Instead, it is a potent ligand for the peripheral type of binding sites (Braestrup & Squires, 1977), that have now been found in the brain as well as in the periphery (Shoemaker *et al.*, 1981).

PK 11195, an isoquinoline carboxamide derivative, potently displaces [^{3}H]-Ro 5-4864 binding in peripheral organs and in the brain *in vitro* and *in vivo* (Le Fur *et al.*, 1983a,b), but is inactive at the classical CNS benzodiazepine binding sites (Le Fur *et al.*, 1983c). Thermodynamic analysis of [^{3}H]-PK 11195 binding has revealed that it is entropy driven, whereas [^{3}H]-Ro 5-4864 binding is enthalpy driven and therefore, by analogy with β-adrenoceptors, the suggestion was made that PK 11195 might be an antagonist at the peripheral type of benzodiazepine binding sites (Le Fur *et al.*, 1983d). An analysis of the behavioural effects of PK 11195 should thus shed some light on the function of Ro 5-4864 binding sites.

In a preliminary study we observed that PK 11195 (30 and 60 mg kg^{-1}) acted as an anticonvulsant against seizures induced by Ro 5-4864 (File & Mabbutt, 1983). The purpose of the present experiments was to explore the specificity of its anticonvulsant actions by testing PK 11195 against other convulsants. In some of these experiments, PK 11195 proved to be proconvulsant, and this action was therefore explored further. Experiment 2 investigated the effects of 5–25 days of treatment with PK 11195, in order to determine whether tolerance develops to its anti- and pro-convulsant effects. Experiment 3 explored whether there was cross tolerance between the anticonvulsant actions of diazepam and PK 11195.

Table 1 Incidence of myoclonic jerks and full tonic-clonic convulsions following various doses of 4 convulsant compounds

	Ro 5-4864			
	5 mg kg^{-1}	*10 mg kg^{-1}*	*20 mg kg^{-1}*	*30 mg kg^{-1}*
Myoclonic jerks	0/8	0/8	3/8	8/8
Convulsions	0/8	0/8	2/8	6/8
	Ro 5-3663			
	5 mg kg^{-1}	*7.5 mg kg^{-1}*	*10 mg kg^{-1}*	
Myoclonic jerks	0/8	3/8	8/8	
Convulsions	0/8	3/8	6/8	
	Pentylenetetrazole (PTZ)			
	30 mg kg^{-1}	*40 mg kg^{-1}*	*60 mg kg^{-1}*	*80 mg kg^{-1}*
Myoclonic jerks	0/8	1/8	4/8	8/8
Convulsions	0/8	1/8	8/8	8/8
	Picrotoxin			
	3 mg kg^{-1}	*6 mg kg^{-1}*	*8 mg kg^{-1}*	
Myoclonic jerks	0/8	5/8	8/8	
Convulsions	0/8	4/8	8/8	

Methods

Animals

Male mice (Tuck No. 1 strain) 30–40 g were housed in groups of 8 with food and water freely available.

Expt 1: acute administration of PK 11195

(a) *Anticonvulsant actions* The doses of each convulsant compound to be used in this experiment were established in an initial study. In each case a dose was selected that was the lowest dose to cause myoclonic jerks in all the mice tested and full tonic-clonic convulsions in at least 75% of mice (see Table 1). A myoclonic jerk was defined as a sudden extension of the forelimbs, and full convulsions as tonic-clonic extensions and contractions of both fore- and hindlimbs. The seizures caused by Ro 5-4864 were different in nature from those caused by the other compounds in that the mice also showed rotational movements and loss of righting reflex (see also File *et al.*, 1984).

Mice were randomly allocated ($n = 8$ per group) to the various drug groups. PK 11195 (30–120 mg kg^{-1}) was injected intraperitoneally 30 min before the convulsant (Ro 5-4864, Ro 5-3663, PTZ or picrotoxin). Observations commenced immediately after i.p. injection of the convulsant and continued for 15 min. Each mouse was observed singly and the latencies to the first myoclonic jerk and to the first full tonic-clonic convulsion were recorded. All testing took place between 12 h 00 min and 15 h 00 min.

(b) *Proconvulsant action* The doses of picrotoxin and PTZ used in this study were chosen as the highest dose that did *not* cause myoclonic jerks or convulsions in any of the mice tested (see Table 1). Mice were randomly allocated ($n = 8$ per group) to be tested with subconvulsant doses of picrotoxin or PTZ alone, or in combination with PK 11195 (60–90 mg kg^{-1}), injected intraperitoneally 30 min before the convulsant. Other groups were tested with strychnine (0.75–1.5 mg kg^{-1}) alone or in combination with PK 11195 (30 mg kg^{-1}). All testing took place between 12 h 00 min and 15 h 00 min.

Expt 2: effects of chronic administration of PK 11195

(a) *Anticonvulsant effects* In order to equate handling in the groups of mice tested in this experiment all mice received 25 daily injections of water until the appropriate time for the drug treatment to start. Daily injections were given between 12 h 00 min and 14 h 00 min. Mice were randomly allocated ($n = 8$ per group) among the following groups: Ro 5-4864 (90 mg kg^{-1}) alone or plus PK 11195 (30 mg kg^{-1}) acutely or after 5, 10 or 25 pretreatment days. On the test day, PK 11195 was given 30 min before Ro 5-4864 and the mice then observed for the next 15 min. All testing took place between 12 h 00 min and

Table 2 Effects of PK 11195 (30–120 mg kg^{-1}) against seizures induced by convulsant doses of Ro 5-4864, Ro 5-3663, pentylenetetrazole (PTZ) and picrotoxin

	Alone	*+PK 11195 (30 mg kg^{-1})*	*+PK 11195 (60 mg kg^{-1})*	*+PK 11195 (120 mg kg^{-1})*
(a) *Ro 5-4864 (30 mg kg^{-1})*				
Myoclonic jerks	8/8 497.9 ± 42.9	6/8 598.2 ± 87.4	5/8 766.6 ± 48.8	—
Convulsions	7/8 802.6 ± 149.9	2/8*	2/8*	—
(b) *Ro 5-3663 (10 mg kg^{-1})*				
Myoclonic jerks	7/8 136.6 ± 15.8	—	7/8 207.1 ± 28.4	8/8 189.1 ± 25.6
Convulsions	6/8 171.2 ± 47.9	—	6/8 345.4 ± 94.5	8/8 338.0 ± 84.6
(c) *PTZ (80 mg kg^{-1})*				
Myoclonic jerks	8/8 48.8 ± 3.6	8/8 43.8 ± 1.8	8/8 48.8 ± 3.6	8/8 56.9 ± 6.1
Convulsions	8/8 81.3 ± 10.1	8/8 76.9 ± 4.3	8/8 92.5 ± 13.9	8/8 134.4 ± 22.1
(d) *Picrotoxin (8 mg kg^{-1})*				
Myoclonic jerks	8/8 328.1 ± 41.6	8/8 340.6 ± 14.5	8/8 263.8 ± 14.7	
Convulsions	8/8 611.3 ± 71.3	8/8 504.4 ± 59.4	8/8 299.4 ± 12.9**	

The incidence of myoclonic jerks and full tonic-clonic convulsions are shown, as well as the mean ± s.e.mean latencies (s) of these responses if > 50% of the group responded.
*$P < 0.05$ compared to PTZ alone; **$P < 0.001$ compared to picrotoxin alone (Student's t test).

15 h 00 min.

(b) *Proconvulsant effects* Once again, all mice received 25 days of water or drug injections, between 1200 and 1400 h. They were randomly allocated ($n = 8$ per group) among the following groups: picrotoxin (3 mg kg^{-1}) alone or plus PK 11195 (60 mg kg^{-1}) acutely or after 5, 10 or 25 days of pretreatment. On the test day PK 11195 was given 30 min before picrotoxin and the mice were then observed for 30 min, between 12 h 00 min and 15 h 00 min.

Expt 3: effects of chronic administration of diazepam on the anticonvulsant action of PK 11195

Twenty-four mice received 10 daily injections of diazepam (4 mg kg^{-1}) and on the test day were randomly allocated ($n = 8$ per group) to: diazepam (4 mg kg^{-1}) plus PTZ (80 mg kg^{-1}); diazepam (4 mg kg^{-1}) plus Ro 5-4864 (90 mg kg^{-1}) or PK 11195 (30 mg kg^{-1}) plus Ro 5-4864 (90 mg kg^{-1}). Forty mice received 10 days pretreatment with water and thus served as control test animals for the following acute drug groups: RO 5-

Table 3 Effects of PK 11195 (60 and 90 mg kg^{-1}) in combination with subconvulsant doses of picrotoxin and pentylenetetrazole (PTZ) on the incidence and latencies (s) of myoclonic jerks and full tonic-clonic convulsions

(a) *Picrotoxin (3 mg kg^{-1})*

	Alone	*+PK 11195 (60 mg kg^{-1})*	*+PK 11195 (90 mg kg^{-1})*
Myoclonic jerks	0/8	6/8** 564.2 ± 50.3	5/8** 567.0 ± 85.5
Convulsions	0/8	4/8* 910.0 ± 115.2	3/8 1080 ± 130.4

(b) *PTZ (30 mg kg^{-1})*

	Alone	*+PK 11195 (60 mg kg^{-1})*	*+PK 11195 (90 mg kg^{-1})*
Myoclonic jerks	0/8	0/8	0/8
Convulsions	0/8	0/8	0/8

Scores significantly different from picrotoxin alone; *$P < 0.01$, **$P < 0.002$ (Fisher exact probability test). The latencies shown are mean ± s.e.mean.

Table 4 Effects of PK 11195 (30 mg kg^{-1}) in combination with strychnine (0.75–1.5 mg kg^{-1}) on the incidence of and latencies (s) to myoclonic jerks and full tonic-clonic convulsions

	Strychnine alone	*Strychnine + PK 11195*
(a) *Strychnine (0.75 mg kg^{-1})*		
Myoclonic jerks	0/8	0/8
Convulsions	0/8	0/8
(b) *Strychnine (1.0 mg kg^{-1})*		
Myoclonic jerks	2/8	7/8* 230.0 ± 15.9
Convulsions	2/8	6/8 347.5 ± 16.6†
(c) *Strychnine (1.5 mg kg^{-1})*		
Myoclonic jerks	8/8 216.9 ± 7.20	8/8 187.5 ± 6.0**
Convulsions	8/8 317.5 ± 18.9	8/8 230.6 ± 16.9**

Significantly different from strychnine alone: *$P < 0.02$, †$P = 0.06$ (Fisher exact probability test); **$P < 0.01$ (two-tailed t test). The latencies shown are mean ± s.e.mean.

4864 (90 mg kg^{-1}) alone or plus diazepam (4 mg kg^{-1}) or plus PK 11195 (30 mg kg^{-1}); PTZ (80 mg kg^{-1}) alone or plus diazepam (4 mg kg^{-1}). All chronic treatment was given between 1200 and 1400 h and testing took place between 1200 and 1500 h.

Drugs

Strychnine, picrotoxin and pentylenetetrazole (PTZ) (Sigma) were dissolved in water. Diazepam, Ro 5-4864 (chlordiazepam), Ro 5-3663 (1,3-dihydro-5-methyl-2H-1,4 benzodiazepine-2-one) (Roche Products Ltd.) and PK 11195 (1-(2-chlorophenyl)-N-methyl-N (1-methylpropyl)-3-isoquinoline carboxamide) (Pharmuka) were suspended in water to which a drop of Tween-20 had been added. Concentrations were adjusted to give injection volumes of 4 mg ml^{-1}.

Results

Expt 1

(a) *Anticonvulsant effects* Table 2 shows the number of mice in each group showing myoclonic jerks or full convulsions, and, where more than 50% did so, the mean latency for each response. It can be seen that

Table 5 Effects of chronic (5–25 days) treatment with PK 11195 on its anticonvulsant action against Ro 5-4864 and on its proconvulsant action when combined with subconvulsant doses of picrotoxin

	Ro 5-4864 (90 mg kg^{-1}) Alone	*+ PK 11195 (30 mg kg^{-1})*			
		Acute	*Chronic* (days)		
			5	*10*	*25*
Myoclonic jerks	8/8* 229.4 ± 10.8	0/8	0/8	2/8	3/8
Convulsions	6/8* 1275.8 ± 24.2	0/8	0/8	0/8	0/8
	Picrotoxin (3 mg kg^{-1}) Alone	*+ PK 11195 (60 mg kg^{-1})*			
		Acute	*Chronic* (days)		
			5	*10*	*25*
Myoclonic jerks	0/8*	8/8 387.5 ± 12.9	0/8*[a]	0/8*[a]	0/8*
Convulsions	0/8*	7/8 905.7 ± 34.4	0/8*	0/8*	0/8*

Scores are numbers of mice showing myoclonic jerks or full convulsions, and, if more than 50% did so, the mean ± s.e.mean latencies (s) of these responses.
*Significantly different from acute treatment $P < 0.01$ (Fisher exact probability test).
[a]Four of these mice showed the fine shivering response that usually precedes myoclonus, but none had a myoclonic jerk.

Table 6 Effects of chronic (10 days) treatment with diazepam (DZ, 4 mg kg^{-1}) on its anticonvulsant action and on the anticonvulsant action of PK 11195 (PK, 30 mg kg^{-1})

	Ro 5-4864 (90 mg kg^{-1})			*Pentylenetetrazole (80 mg kg^{-1})*	
	Alone	*+ DZ (4 mg kg^{-1})*	*+ PK (30 mg kg^{-1})*	*Alone*	*+ DZ (4 mg kg^{-1})*
(a)	*Acute effects of drugs (i.e. mice pretreated with water)*				
Myoclonic jerks	8/8 228.1 ± 11.1	0/8*	0/8*	8/8 56.3 ± 3.9	0/8*
Convulsions	6/8 1279.2 ± 24.7	0/8*	0/8*	8/8 83.8 ± 9.9	0/8*
(b)	*After 10 days of pretreatment with diazepam*				
Myoclonic jerks		8/8 262.5 ± 10.0	2/8*		8/8 81.3 ± 6.9
Convulsions		5/8 1304 ± 19.6	0/8*		7/8 103.6 ± 4.7

Scores are numbers of mice showing myoclonic jerks or full convulsions, and, if more than 50% did so, the mean ± s.e.mean latencies (s) of these responses.
*Significantly different from convulsant alone, $P < 0.01$ (Fisher exact probability test).

whereas PK 11195 (30 and 60 mg kg^{-1}) significantly reduced the number of convulsions caused by Ro 5-4864, it was unable to reduce the incidence of either myoclonic jerks or full convulsions induced by Ro 5-3663, picrotoxin or pentylenetetrazole (PTZ).

Two significant effects can be seen on the latency measures: a significant anticonvulsant effect of PK 11195 (120 mg kg^{-1}) to prolong the latency to convulse with PTZ (80 mg kg^{-1}); and a proconvulsant effect significantly shortening the latency to full convulsion with picrotoxin (8 mg kg^{-1}). Because of this latter effect with picrotoxin the possible proconvulsant effects of PK 11195 were further explored.

(b) *Proconvulsant effects* Table 3 shows that whereas PK 11195 (60 and 90 mg kg^{-1}) was proconvulsant when combined with picrotoxin, it did not have these effects when in combination with subconvulsant doses of PTZ.

Table 4 shows that PK 11195 (30 mg kg^{-1}) was proconvulsant when combined with strychnine. It significantly increased the incidence of myoclonus and increased the number of convulsions occurring to strychnine (1 mg kg^{-1}) and significantly reduced the latency to myoclonus and full convulsions with 1.5 mg kg^{-1} strychnine.

Expt 2

It can be seen from Table 5 that the protective effects of PK 11195 against seizures induced by Ro 5-4864 were retained even after 25 days of treatment. No mice showed full tonic-clonic convulsions, but there was an indication from the incidence of myoclonic jerks that PK 11195 was losing some of its protection. In contrast, its proconvulsant actions in combination with picrotoxin were lost by 5 days of chronic treatment.

Expt 3

Table 6 shows that both Ro 5-4864 and PTZ caused convulsions when given alone. Acute administration of diazepam was effective against both these convulsants, after 10 days of pretreatment diazepam had lost its anticonvulsant effect. In contrast, PK 11195 was effective against Ro 5-4864 seizures, regardless of whether the mice had been pretreated for 10 days with water or diazepam.

Discussion

The sites mediating the proconvulsant actions of PK 11195 are unknown, but the rapid development of tolerance to these effects suggests a site different from that mediating the anticonvulsant actions. A similar suggestion has recently been made for the β-carboline, FG 7142, which also manifests a profile of both pro- and anti-convulsant actions (Petersen *et al.*, 1983).

Since no mice had full convulsions even after 25 days of treatment with PK 11195 it is clear that tolerance develops much less rapidly, if at all, to the anticonvulsant actions of this compound than it does to the anticonvulsant actions of the benzodiazepines (File, 1983). This relative lack of tolerance development would potentially make PK 11195 an extremely useful anticonvulsant. However, its use may be limited, as so far it is only against seizures induced by Ro 5-4864 that it has proved successful, and we have no knowledge yet whether these seizures have any clinical relevance.

The different rates of development of tolerance for PK 11195 and diazepam suggests that different sites mediate the two anticonvulsant effects, and the lack of cross-tolerance between diazepam and PK 11195

(expt 3) is direct support for two distinct sites of action. This is consistent with binding data suggesting that PK 11195 acts on the peripheral type of benzodiazepine sites, but not on their classical CNS sites.

However, although from binding data one would not expect PK 11195 to be exerting its anticonvulsant action at the GABA-benzodiazepine receptor complex, recent electrophysiological evidence suggests that this possibility should not be dismissed. *In vitro* Ro 5-4864 has been found to antagonize flurazepam's potentiation of muscimol (at 0.1 μM) and at higher concentrations (30 μM) to directly antagonize muscimol, and to enhance the effects of picrotoxin (Simmonds, 1984). These actions could explain the reductions by Ro 5-4864 of evoked inhibition *in vivo* (Polc & Schaffner, 1983) and its convulsant actions. PK 11195 has also been found to have actions at a non-benzodiazepine site on the GABA-benzodiazepine receptor complex, and these would be compatible with an anticonvulsant profile (Simonds, unpublished data). The mixed pro- and anti-convulsant actions of PK 11195 may, therefore, be of more clinical relevance than might have been the case if only peripheral sites were concerned.

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