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ROLE OF CENTRAL β-ADRENOCEPTORS IN THE CONTROL OF PENTYLENETETRAZOL-INDUCED CONVULSIONS IN RATS

W.J. LOUIS, JENNY PAPANICOLAOU, R.J. SUMMERS & F.J.E. VAJDA

University of Melbourne, Clinical Pharmacology and Therapeutics Unit, Department of Medicine, Austin Hospital, Heidelberg, Victoria, 3084, Australia

1 The role of central β-adrenoceptors in the anticonvulsant effect of β-adrenoceptor antagonists has been examined.

2 Oral administration of (−)- and (+)-propranolol (0.05–1 mg/kg) and (±)-pindolol (0.025–0.5 mg/kg) produced a dose-dependent decrease in duration of convulsions produced by pentylenetetrazol (PTZ 50 mg/kg, i.p.) in rats.

3 At the EC_{50} level, (−)-propranolol is seven times more effective than the (+)-isomer.

4 Oral administration of (−)-, (+)- or (±)-practolol (1–10 mg/kg) or (−)- or (+)-timolol (1–10 mg/kg), two β-adrenoceptor antagonists that do not penetrate the blood brain barrier, had no significant effect on the duration of PTZ-induced convulsions.

5 Intracerebroventricular administration of (−)-propranolol (0.5 μg/kg) or (−)-timolol (0.25 μg/kg) produced highly significant anticonvulsant effects whereas the (+)-isomers at the same dose level were ineffective. (±)-Pindolol (0.25 μg/kg) was also much more effective given by this route than when given orally. The (+)- and (−)-isomers of the β_1-adrenoceptor selective antagonist practolol (10 μg/kg) exerted only weak anticonvulsant effects.

6 This study provides evidence that β-adrenoceptor antagonists exert an anticonvulsant effect through central β_2-adrenoceptors. At high dose levels, additional anticonvulsant activity is associated with membrane stabilization in those antagonists which possess this property.

Introduction

Central noradrenaline is believed to play a role in the control of seizures produced by either drugs or electroconvulsive shock (ECS). Lowering of central monoamine levels by reserpine or α-methyl-*p*-tyrosine lowers the convulsive threshold (Chen, Ensor & Bohner, 1954; Yeoh & Wolf, 1968; Kilian & Frey, 1973; Jobe, Stull & Geiger, 1974). Central noradrenaline appears to be important since selective lesions of the dorsal forebrain bundle by 6-hydroxydopamine injection enhances pentylenetetrazol (PTZ)-induced convulsions in rats (Mason & Corcoran, 1979). Other experiments suggest a role for β-adrenoceptors since central administration of noradrenaline or isoprenaline can produce seizures (Walker, Lewin & Moffitt, 1974). High cyclic adenosine 3′,5′-monophosphate (cyclic AMP) levels in brain are associated with seizures in animals (Sattin, 1971; Ferrendelli & Kinscherf, 1977; Clarenbach, Wenzel & Cramer, 1978) or in the CSF of epileptic patients following a fit (Myllyla, Heikkinen, Vapaatalo & Hokkanen, 1975). This rise in cyclic AMP is antagonized by the β-adrenoceptor antagonist, propranolol (Gross & Ferrendelli, 1979), which has significant anticonvulsant activity in animals (Murmann, Almirante & Saccani-Guelfi, 1966; Leszkovszky & Tardos, 1966; Yeoh & Wolf, 1968; Madan & Barar, 1974; Anlezark, Horton & Meldrum, 1979). At high dose levels (> 2 mg/kg) both (+)- and (−)-propranolol have anticonvulsant activity which has been attributed to membrane stabilization (Anlezark *et al.*, 1979) but at lower doses the (−)-isomer of propranolol is seven times more effective than the (+)-isomer, indicating that at these doses the effect may be mediated through β-adrenoceptors (Papanicolaou, Vajda, Summers & Louis, 1981).

In this study a comparison has been made of oral and intracerebroventicular (i.c.v.) administration of β-adrenoceptor antagonists to determine whether an anticonvulsant effect can be produced by central β-adrenoceptor blockade.

Methods

Convulsions were produced in fed Sprague-Dawley rats (♀ or ♂, 150–200 g) by injection of PTZ (50 mg/kg, i.p.). β-Adrenoceptor antagonists were dissolved in sterile pyrogen-free 0.9% w/v NaCl solution (saline) and administered by gavage

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(1 ml/100 g) 40 min before PTZ challenge. Maximum anticonvulsant activity is correlated with maximum brain levels of propranolol which occur 40 min after oral administration (Papanicolaou *et al.*, 1981). Examination of the time course of development of anticonvulsant activity after oral administration for the other β-adrenoceptor antagonists with this property also showed a maximum effect at 40 min. Controls received the same volume of saline.

Intracerebroventricular injections were made through indwelling i.c.v. cannulae (Yeda R. & D. Ltd, type 92-103A) implanted at least 3 days before the experiment. For implantation, rats were anaesthetized with Althesin (Glaxo) (3 ml/kg, i.p.), placed in a head holder and the cannula placed 1.5 mm lateral and caudal to the coronal and sagittal sutures (Noble, Wurtman & Axelrod, 1967). Cannulae were supported and the wound closed with dental cement. Drugs or vehicle were injected in a volume of 25 μl/kg in saline using a 5 μl syringe (Scientific Glass Engineering, Melbourne) with the needle cut to protrude 2–2.5 mm beyond the tip of the cannula. The position of each cannula was checked after experiment by dye injection. The following parameters were noted; time of onset of myoclonic jerk and generalized seizure, duration of seizure and subsequent period of unconsciousness, characteristics of seizure (tonic/clonic) and the recovery time for each rat. All time measurements were made with a stop watch. Results are expressed as means ± s.e.mean and significance of differences between means was assessed using Student's *t* test.

Drugs

The following drugs were used: (−)-, (+)-propranolol, (−)-, (+)-, (±)-practolol (ICI Ltd); (−)-, (+)-timolol maleate (Merck, Sharp & Dohme); (±)-pindolol (Sandoz), pentylenetetrazol (Sigma).

Results

Oral administration of β-adrenoceptor antagonists

Administration of (−)-propranolol (0.05–1 mg/kg) produced a dose-dependent decrease in the duration of seizures after PTZ (50 mg/kg i.p.) as shown in Figure 1. In contrast, (+)-propranolol produced significant anticonvulsant effects only at the highest dose level (1 mg/kg). A comparison of the anticonvulsant effects of (−)- and (+)-propranolol at the EC_{50} levels showed that (−)-propranolol was seven times more effective than the (+)-isomer. These results indicated that both β-blockade and another

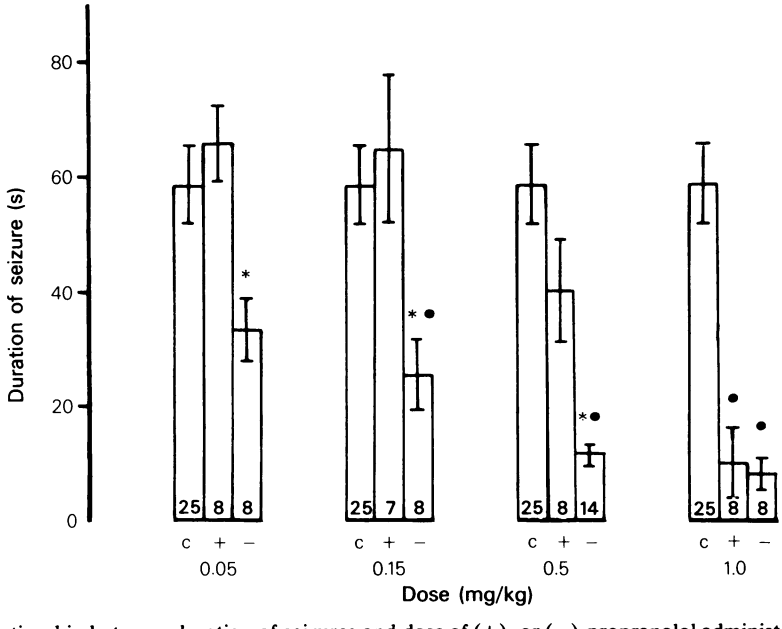

Figure 1 Relationship between duration of seizures and dose of (+)- or (−)-propranolol administered by gavage 40 min before pentylenetetrazol (PTZ) challenge (50 mg/kg, i.p.). Circles (●) indicate significant differences between treated groups and control ($P < 0.001$) and asterisks (*) significant differences between the two isomers ($P < 0.001$). Vertical bars indicate the s.e.mean and numbers at the base of each histogram refer to the number of rats used.

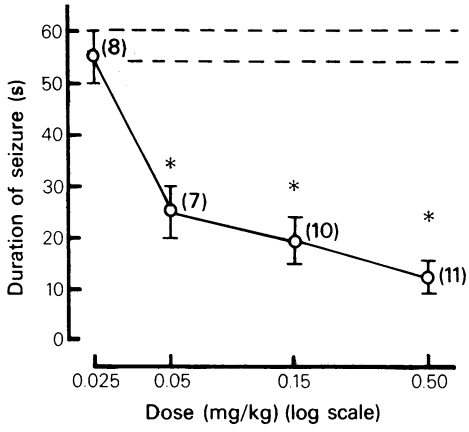

Figure 2 The effect of (±)-pindolol (0.025–0.5 mg/kg, orally) administered 40 min before pentylenetetrazol (PTZ) challenge on duration of seizures in rats. Asterisks indicate values significantly different from control ($P<0.001$) and figures in parentheses indicate the number of animals used. The upper and lower limits of the s.e.mean are shown by broken lines in the control group ($n=15$) and by the vertical-bars for the treated animals.

mechanism, possibly membrane stabilization, play a part in the anticonvulsant effect of propranolol.

In order to elucidate the relative importance of the two mechanisms, the effects of pindolol, a β-adrenoceptor antagonist that penetrates the blood brain barrier but has much less membrane stabilizing activity than propranolol (Scriabine, 1979) was studied. As shown in Figure 2, (±)-pindolol (0.025–0.5 mg/kg orally) produced a dose-dependent anticonvulsant effect, the duration of convulsions falling from 57±3 s (control) to 25±5 s (0.05 mg/kg, $P<0.001$), to 18±2 s (0.15 mg/kg, $P<0.001$) and 11±3 s (0.5 mg/kg, $P<0.001$). To establish the importance of the central action of these drugs, experiments were carried out using practolol and timolol, two β-adrenoceptor antagonists that do not penetrate the blood brain barrier (Street, Hemsworth, Roach & Day, 1979; Tocco, Clineschmidt, Duncan, de Luna & Baer, 1980). Neither (−)- nor (+)-timolol (1–10 mg/kg) (Figure 3a) nor (−)-, (+)- or (±)-practolol (1–10 mg/kg) (Figure 3b) had any significant effect on the duration of convulsions induced by PTZ.

Intraventricular injection of β-adrenoceptor antagonists

In order to obtain more information on the central mode of action of β-adrenoceptor antagonists in this model, the drugs were given i.c.v. at 1% of the dose level given orally 3 min before PTZ challenge. The (+)-isomers of propranolol (0.5 μg/kg) and timolol (0.25 μg/kg) were ineffective as shown in Figure 4 but the same dose levels of the (−)-isomers exerted highly significant anticonvulsant effects ($P<0.001$, $n=3$). The result with (−)-timolol was of particular interest since this compound does not penetrate the blood brain barrier and was ineffective when given orally. In addition, timolol apparently has no membrane stabilizing activity and no partial agonist activity (Scriabine, 1979), indicating that the potent anticonvulsant effects of this compound when given i.c.v. is due to β-adrenoceptor blockade. (±)-Pindolol (0.25 μg/kg) was also much more effective when given i.c.v. In contrast the β_1-selective antagonist, practolol, (Dunlop & Shanks, 1968) was relatively ineffective. At high dose levels both (+)-practolol and (−)-practolol (10 μg/kg) exert a weak anticonvulsant effect with the (−)-isomer being only slightly more effective. Thus it is likely that central β_2-adrenoceptors rather than β_1-adrenoceptors are im-

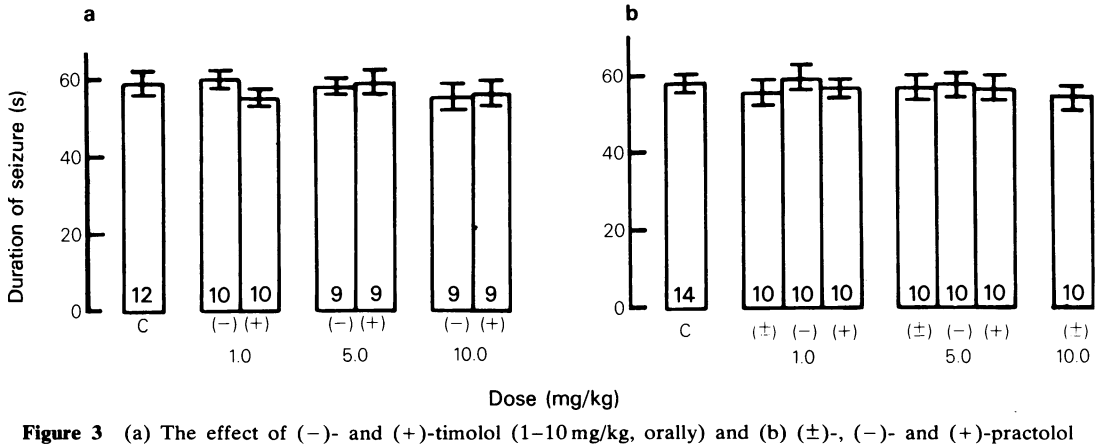

Figure 3 (a) The effect of (−)- and (+)-timolol (1–10 mg/kg, orally) and (b) (±)-, (−)- and (+)-practolol (1–10 mg/kg, orally) administered 40 min before pentylenetetrazol (PTZ) challenge on duration of seizures in rats. Vertical bars indicate the s.e.mean and numbers at the base of each histogram the numbers of animals used.

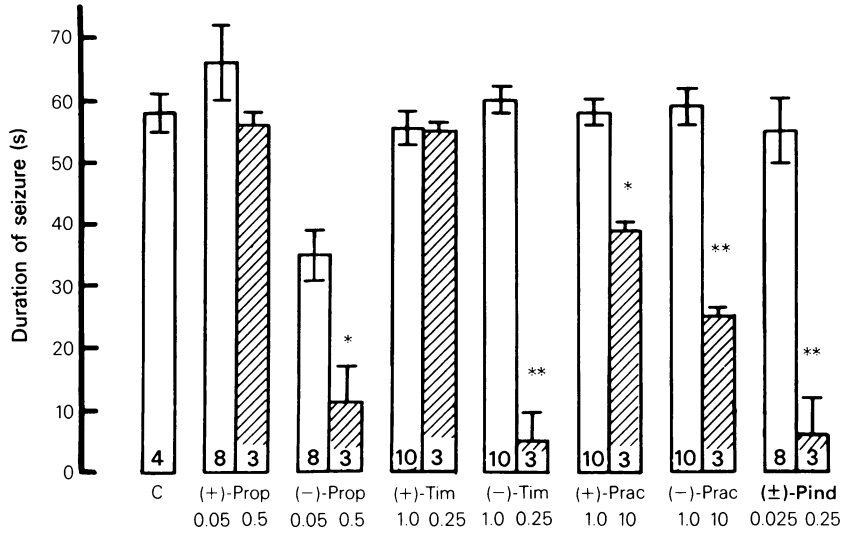

Figure 4 Comparison of the effects of β-adrenoceptor antagonists on duration of seizures produced by pentylenetetrazol (PTZ, 50 mg/kg i.p.). Open histograms, effect of drug given orally (mg/kg), shaded histograms drug given at 1/100th the dose level (propranolol, practolol, pindolol) or 1/4000th the dose level (timolol) intraventricularly (μg/kg). Vertical bars indicate the s.e.mean and numbers at the base of the histograms the number of animals used. Asterisks (*) refer to values significantly different from higher doses given orally ($*P < 0.05$, $**P < 0.001$).

portant in the mediation of the anticonvulsant effect of β-adrenoceptor antagonists.

Seizure characteristics

In every case the control animals exhibited tonic-clonic convulsions, with an associated loss of consciousness and balance. These characteristics were unaltered by either oral practolol or timolol, neither of which pass the blood-brain barrier. However, propranolol and pindolol altered the course and the characteristics of the seizures. In Table 1 the results obtained by pretreating the rats with pindolol (0.025–0.50 mg/kg orally) are shown. There was a dose-dependent increase in the number of animals in which no seizures were obtained and a dose-dependent decrease in the number of animals which exhibited loss of consciousness, tonic clonic convulsions, and loss of balance. Intracerebroventricular pindolol also exerted a powerful anticonvulsant effect with two of the three animals having no seizures and the remaining animal a much shorter duration of seizures than control.

Discussion

This study provides further evidence that β-adrenoceptor antagonists possess anticonvulsant activity against PTZ-induced seizures but in addition indicates that at least two mechanisms are involved, one of which is β-adrenoceptor blockade. The an-

Table 1 Effect of pindolol on the characteristics of pentylenetetrazol-induced seizures in rats

Characteristics	*Control*	*Oral dose* (mg/kg)				*i.c.v. Dose* (μg/kg)
		0.025	0.05	0.15	0.50	0.25
Animals with no seizures	0	0	1	3	6	2
Loss of consciousness	15	8	6	3	0	1
Tonic/clonic convulsions	15	8	4	0	0	1
Clonic convulsions	0	0	2	7	5	1
Loss of balance	15	8	6	4	0	1
n	15	8	7	10	11	3

Pindolol was administered orally (0.025–0.5 mg/kg) 40 min or i.c.v. 3 min prior to PTZ (50 mg/kg i.p.). *n* indicates the number of rats used.

ticonvulsant activity of propranolol has been studied previously in a variety of animal models. In mice, propranolol antagonizes convulsions induced by PTZ (Murmann *et al.*, 1966), maximal electroshock (MES) (Leszkovszky & Tardos, 1965; Madan & Barar, 1974), loud auditory stimulation (Anlezark *et al.*, 1979) hyperbaric oxygen exposure (Levy, Ngai, Finck, Kawashima & Spector, 1976) and raises the threshold for electrically induced convulsions (Murmann *et al.*, 1966; Yeoh & Wolf, 1968). A similar protective effect against MES in rats has been described (Leszkovszky & Tardos, 1965; Jaeger, Esplin & Capek, 1979). However, it has also been reported that propranolol is ineffective in increasing the seizure threshold (Madan & Barar, 1974; Jaeger, *et al.*, 1979). In those studies in which anticonvulsant effects were observed it has been suggested that the main mechanism involved is membrane stabilization.

Although in normal animals, drugs that produce membrane stabilizing (local anaesthetic) effects tend to produce convulsions, this effect is believed to be due to depression of central inhibitory pathways (Frank & Sanders, 1963). However, in convulsions induced by either PTZ or electroshock, local anaesthetics have a protective effect (Tanaka, 1955). Membrane stabilization therefore is a likely explanation for the anticonvulsant effects of (+)-, (−)- or (±)- propranolol seen at dose levels greater than 1 mg/kg.

Recently, however, it has been shown that although in most models there is little difference in anticonvulsant activity at dose levels of (+)- and (−)-propranolol of 1 mg/kg or greater, stereoselectivity is observed at lower dose levels (0.15mg/kg) at which the (−)-isomer has markedly greater anticonvulsant activity measured against PTZ-induced convulsions in the rat (Papanicolaou *et al.*, 1981). These findings indicated that in this model β-adrenoceptors could play a role in the control of convulsions. In the present study this view was supported by the observation that (±)-pindolol, which lacks membrane stabilizing activity (Scriabine, 1979) also possessed marked anticonvulsant activity.

Both propranolol and pindolol readily enter the brain and are fairly evenly distributed throughout the brain regions (Garvey & Ram, 1975; Elghozi, Bianchetti, Morselli & Meyer, 1979). However, timolol and practolol do not cross the blood-brain-barrier in appreciable amounts (Street *et al.*, 1979; Tocco *et al.*, 1980) and neither of these β-antagonists had any significant effect on the duration of seizures when administered orally.

Evidence was also obtained in the present experiments that the β-adrenoceptor antagonists were acting centrally since when given i.c.v. they were effective at 1% of the dose level given orally. In addition (−)-timolol which does not pass the blood-brain barrier was highly effective when given i.c.v. at low dose levels. Since timolol has much less membrane stabilizing or partial agonist activity, it is likely that these effects are due to central β-adrenoceptor blockade. The selective β_1-antagonist, practolol, was not particularly effective even when given i.c.v. and did not display marked stereoselectivity. This may indicate that the receptors involved are of the β_2-type. In support of this view, recent observations using i.c.v. injections of the selective β_2-adrenoceptor antagonist, ICI 118,551 (Bilski, Dorries, Fitzgerald, Jessup, Tucker & Wale, 1980; O'Donnell & Wanstall, 1980) show that this compound has a powerful anticonvulsant effect in the PTZ model (Papanicolaou *et al.*, in preparation).

β-Adrenoceptor antagonists also interact with central 5-hydroxytryptamine (5-HT) receptors (Middlemiss, Blakeborough & Leather, 1977) and it is possible that this could play a part in the observed effect. However the relative anticonvulsant potency suggests that β-adrenoceptor antagonism is the most important factor since the order of potency was (±)-pindolol = (−)-timolol > (−)-propranolol >> (−)-practolol >> (+)-isomers which is identical with that observed against β-adrenoceptors (Frishman, 1979) and different from that observed against 5-HT receptors which is (−)-propranolol > (±)-propranolol > (±)-pindolol > (+)-propranolol >> (±)-practolol (Middlemiss *et al.*, 1977).

In conclusion this study provides evidence that β-adrenoceptor antagonists can protect against PTZ-induced convulsions in rats. At low doses the effect is mediated through centrally located β-adrenoceptors which appear to be of the β_2 type. At high dose levels, additional anticonvulsant activity is probably conferred by membrane stabilizing activity in those antagonists which possess this property.

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