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Inhibition of non-adrenergic transmission in the rat vas deferens by prazosin

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Summary

Isolated preparations of rat vas deferens were induced to contract by neurogenic and non-neurogenic stimuli, and the effect of prazosin on these contractions was investigated. Prazosin (24 μM) exerted a profound inhibitory effect on neurogenic contractions and had no significant effect on non-neurogenic contractions. These experiments indicate that prazosin is capable of interacting with the neurotransmission processes in the vas deferens to produce inhibition of transmission.

Résumé

Des préparations isolées de vas deferens du rat ont été induites pour contracter par des stimuli neurogéniques et non-neurogéniques, et l'effet de la prazosine sur ces contractions a été étudié. La prazosine (24 μM) exerce un effet profond d'inhibition sur les contractions neurogéniques. En revanche elle n'a aucun effet significatif sur les contractions non-neurogéniques. Ces études montrent que la prazosine est capable d'interréagir avec les procédés de neurotransmission dans le vas deferens pour produire une inhibition de transmission.

Introduction

Prazosin is a newly developed anti-hypertensive agent (Constantine *et al.*, 1973; Wood, Phelan & Simpson, 1975) with a low affinity for presynaptic α_2-adrenoceptors Cambridge, Davey & Massingham, 1977; Doxey, Smith & Walker, 1977; Cavero, Lefevre & Roach, 1977). Low concentrations of prazosin will abolish the post-synaptic effects of α-adrenoceptor agonists on the vas deferens without inhibiting electrically evoked contractions (Brown, Doxey & Handley, 1980).

The efficacy of prazosin in anti-hypertensive therapy is believed to be due to its selectivity for blocking α_1-adrenoceptors (Davey, 1980). Because of its selective effect on the post-synaptic α_1-adrenoceptors, prazosin blocks the vasoconstriction produced by noradrenaline released from sympathetic nerves but allows noradrenaline acting on pre-synaptic, α_2-adrenoceptors to exert a negative feedback inhibition of its own release. However, this mechanism of action of prazosin does not fully explain all its cardiovascular effects (Blaschke & Melmon, 1981).

Investigations have been carried out to examine the effects of low and high concentrations of prazosin on the contractile responses to field stimulation in the isolated rat vas deferens. A preliminary account of this investigation was presented to the West African Society for Pharmacology in April 1985.

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Materials and methods

Male albino Wistar rats, 250–350 g, were stunned by a blow on the back of the head and killed by exsanguination. The vasa deferentia were dissected free from their mesenteric attachments. Each vas deferens was cut from its urethral and epididymal connections, placed in a petri-dish containing Krebs–Henseleit

solution and was carefully cleaned of its mesenteric covering. A length from the urethral end of the vas deferens, approximately 1 cm long, was cut and mounted between platinum electrodes in a 5 ml organ-bath in Krebs–Henseleit solution of the following composition (mM/l) NaCl, 113; KCl, 4.7; $CaCl_2 \cdot 2H_2O$, 2.53; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11.5; containing phentolamine (5 μM) and propranolol (2 μM) and bubbled with 5% CO_2 in O_2 at 37°C.

After allowing 30 min for equilibration, the tissues were subjected to electrical field stimulation with trains of five pulses (1 msec pulse duration, 10 Hz) at 1-min intervals.

Longitudinal tension of the vas deferens was monitored through an isometric transducer and a recorder. When the responses to electrical stimulation were stable, the preparation was exposed to freshly prepared solution of prazosin hydrochloride (0.24, 2.4 or 24 μM) for 1 h.

For studying the effect of prazosin on the excitability of the smooth muscle, two series of experiments were conducted. In one series (six experiments) the vas deferens was induced to contract by exposure to 37.5 mM KCl (15 sec) before exposure to prazosin and after 1-h treatment with prazosin (24 μM). Field stimulation was interrupted for 2 min to allow response to KCl to be monitored. In a second series of experiments ($n = 4$), tetrodotoxin (TTX) 0.6 μM was employed to abolish the electrically evoked neurogenic twitches of the vas. The TTX-treated preparation was thereafter stimulated directly at 10-min intervals (trains of twenty pulses; 10 msec pulse duration, 10 Hz). Once the contration had stabilized the preparation was treated with prazosin (24 μM) for 1 h and its effet on the myogenic contractions was recorded.

In all experiments, the contralateral was served as the control. The control vasa were exposed to equivalent concentrations of the solvent, ethyl alcohol (see below), as the prazosin-treated vasa.

Drugs used were phentolamine methanesulphonate (Ciba-Geigy, Basle, Switzerland), prazosin hydrochloride (Pfizer, Connecticut, U.S.A.), DL-propranolol hydrochloride (Sigma, Poole, U.K.) and tetrodotoxin (Sankyo, Japan). Prazosin was dissolved in 30% ethyl alcohol to make 2.4 mM stock solution, from which further dilutions were made in distilled water. All other drugs were dissolved in distilled water.

Results

Effect of prazosin on electrically evoked neurogenic contractions

The neurogenic twitches in control preparations showed some decline after 1-h exposure to the appropriate concentrations of prazosin-solvent: ethanol (Table 1). Prazosin exerted superficially opposite effects on the transmission: in low concentrations (0.24 μM) it caused a small but significant potentiation of the transmission, but in higher concentrations (2.4 and 24 μM) it produced inhibition of the transmission (Table 1).

With 24-μM prazosin, the mean inhibition in ten experiments was 81%, whereas the corresponding control value in the presence of equivalent concentrations of ethanol was only 20.59% (Table 2). The inhibition caused by prazosin was gradual in onset and was still progressing at the end of a 60-min period of observation (Fig. 1).

Effect of prazosin on non-neurogenic contractions

(i) *KCl-induced contractions.* Fifteen seconds' exposures to a submaximal dose of KCl (37.5 mM) produced fairly reproducible contractions if repeated at intervals in excess of 10 min. Prazosin (24 μM) did not exert any significant inhibitory effect on KCl-induced contractions (Table 1, Fig. 1).

(ii) *Electrically evoked myogenic contractions.* In preparations treated with TTX, stimulation with trains of twenty pulses of prolonged duration (10 msec) produced contractions presumably due to a direct depolarization of the smooth muscle cells. Exposure to prazosin (24 μM for 1 h) did not have any appreciable effect on the electrically evoked myogenic contractions (Table 1, Fig. 2).

Discussion

The results of the experiments reported here show that prazosin (24 μM) exerts a significant inhibitory effect on the motor transmission in

Table 1. Effect of 1-h exposure to prazosin on indirectly evoked (neurogenic) and directly evoked (non-neurogenic) contractions of rat vas deferens

Concentration of prazosin (μM)	% inhibition of contractions (mean ± s.e.m.)					
	Electrically evoked (neurogenic)		Electrically evoked (non-neurogenic)		KCl-induced (non-neurogenic)	
	Control	Prazosin treated	Control	Prazosin treated	Control	Prazosin treated
0.24	7.10 ± 1.77 (n = 4)	−8.44 ± 2.82* (n = 4)				
2.4	18.86 ± 4.28 (n = 4)	8.73 ± 1.80* (n = 4)				
24	20.59 ± 3.42 (n = 10)	81.16 ± 2.73** (n = 10)	8.43 ± 6.79 (n = 4)	7.94 ± 5.53 (n = 4)	4.40 ± 1.90 (n = 6)	5.60 ± 4.24 (n = 6)

n = Number of experiments.
* = $P < 0.05$, ** = $P < 0.01$.

Table 2. Time-course of the effect of prazosin (24 μM) on electrically evoked contractions of the rat vas deferens

Duration of exposure to prazosin (min)	% inhibition of electrically evoked contractions	
	Prazosin treated* (n = 10)	Control† (n = 10)
0	0	0
5	19.89 ± 2.14	5.59 ± 1.48
10	33.98 ± 3.18	6.81 ± 1.13
15	39.76 ± 3.70	8.30 ± 2.48
20	45.29 ± 4.84	11.01 ± 2.13
25	49.82 ± 4.14	11.11 ± 1.34
30	57.35 ± 4.05	14.75 ± 1.02
35	62.23 ± 3.80	15.01 ± 1.36
40	66.45 ± 3.69	16.48 ± 2.62
45	70.33 ± 3.50	18.86 ± 1.89
50	73.61 ± 3.32	19.05 ± 1.36
55	76.67 ± 3.15	19.56 ± 1.15
60	81.16 ± 2.73	20.59 ± 3.42

*The values for prazosin-treated preparations are significantly different from those for controls ($P < 0.01$).

†Controls were exposed to Krebs solution containing propranolol, phentolamine and ethanol in concentrations identical to those administered to prazosin-treated preparations.

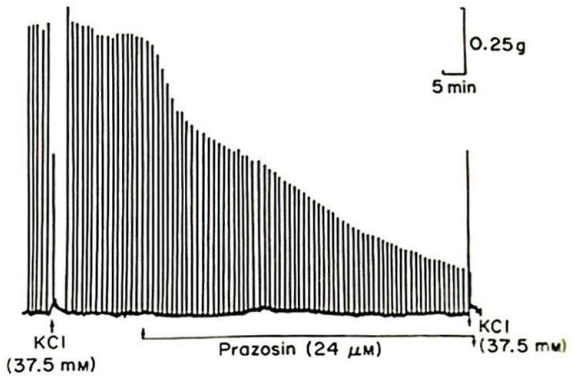

Fig. 1. Rat vas deferens. Effect of prazosin (24 μM) on electrically evoked neurogenic contractions and on KCl (37.5 mM) contractions. Prazosin inhibited electrically evoked contractions without inhibiting KCl contractions. The inhibitory effect of prazosin developed slowly and continued throughout the period of stimulation.

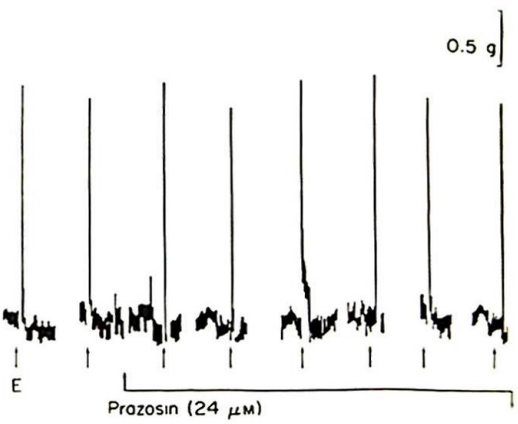

Fig. 2. Effect of prazosin (24 μM) on electrically evoked myogenic contractions. E (twenty pulses, 10 msec, 10 Hz) of the rat vas deferens. Preparations had been treated with tetrodotoxin (0.6 μM) and stimulation was at 10-min intervals.

the rat vas deferens. Prazosin has been reported to act as a direct smooth muscle relaxant in vascular tissues (Constantine *et al*, 1973; Hirst & Neild, 1980). The possibility of a direct smooth muscle relaxant action of prazosin being responsible for the observed inhibition of motor transmission in the vas deferens in the experiments described above is unlikely because electrically evoked myogenic contractions in TTX-treated preparations were not inhibited by prazosin. The failure of prazosin to inhibit submaximal KCl contraction also favours a similar conclusion. KCl contraction of the isolated guinea-pig vas deferens has been shown to be due to a direct depolarization of the smooth muscle cells (Westfall, 1970). It is likely that a similar mechanism is responsible for KCl-induced contractions of the rat vas deferens since TTX does not antagonize the action of KCl in this tissue.

In this study prazosin was used in preparations that had already been treated with the α-blocker, phentolamine. Phentolamine, in doses used, would be expected to block both α_1- and α_2-adrenoceptors (Pennefather, 1983). Resistance of motor transmission in rodent vas deferens to α-adrenoceptor antagonist is well documented and forms one of several independent lines of evidence for a non-adrenergic nature of the transmission (Ambache *et al.*, 1972; von Euler & Hedqvist, 1975; McGrath, 1978).

Partial resistance of sympathetic nerve-

evoked motor responses in vascular tissues to α-adrenoceptor blockade has been attributed to the presence of two populations of receptors for noradrenaline; junctional receptors activated by neuronally released noradrenaline, and extra-junctional receptors, which are the conventional ones (Hirst & Neild, 1980; Illes, 1983). The possibility exists that the sympathetic nerves responsible for α-blocker-resistant reponses in vascular tissues are analogous to the sympathetic nerves in the vas deferens. If this is true, it could be that prazosin in higher concentrations is able to penetrate to the junctional receptors. Another explanation based on the uncertain nature of neurotransmission in the vas deferens is that there is an interaction of some sort between prazosin and a non-adrenergic transmission.

Whichever explanation, these results might provide one further reason for the observation that of all the α-adrenoceptor antagonists (including the irreversible types), prazosin has proved to be the most effective anti-hypertensive agent in clinical medicine. The possibility of prazosin, in higher concentrations used here, acting through a receptor mechanism still has to be eliminated. There is very little information on the full range of activity of prazosin in the body or the full spectrum of its toxicity. More investigations are, therefore, required to explore the full range of clinical usefulness of this drug.

References

Ambache, N., Dunk, L.P., Verney, J. & Zar, M.A. (1972) Inhibition of postganglionic motor transmission in vas deferens by indirectly acting sympathomimetic drugs. *J. Physiol.* **227**, 433–456.

Blaschke, T.P. & Melmon, K.L. (1981) Antihypertensive agents and the drug therapy of hypertension. In: *Pharmacological Basis of Therapeutics* (ed. by A. G. Gilman, L. S. Goodman and A. Gilman), pp. 793–818. Macmillan Publishing Company Inc., New York.

Brown, J., Doxey, J.C. & Handley, S. (1980) Effects of α-adrenoceptor agonists and antagonists and of anti-depressant drugs on pre- and post-synaptic α-adrenoceptors, *Eur. J. Pharmacol.* **67**, 33–40.

Cambridge, D., Davey, M.J. & Massingham, R. (1977) Prazosin, a selective antagonist of post-synaptic α-adrenoceptors. *Br. J. Pharmacol.* **59**, 514–515.

Cavero, I., Lefevre, F. & Roach, A.G. (1977) Differential effects of prazosin on the pre- and post-synaptic α-adrenoceptors in the rat and dog. *Br. J. Pharmacol.* **61**, 469.

Constantine, J.W., McShane, W.K., Scriabine, A. & Hess, H.J. (1973) In: *Hypertension: Mechanisms and Management* (ed. by G. Onesti, K. G. Kim and J. H. Moyer), pp. 429–444. Grune and Stratton, New York.

Davey, M.J. (1980) Relevant Features of the Pharmacology of Prazosin. *J. Cardiovasc. Pharmacol.* **2**, (suppl. 3), S287–301.

Doxey, J.C., Smith, C.F.C. & Walker, J.M. (1977) Selectivity of blocking agents for pre- and post-synaptic α-adrenoceptors. *Br. J. Pharmacol.* **60**, 91–96.

von Euler, U.S. & Hedqvist, P. (1975) A tentative transmission mechanism for the twitch elicited in guinea-pig vas deferens by field stimulation. *Med. Hypotheses* **1**, 214–216.

Hirst, G.D.S. & Neild, T.O. (1980) Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle. *Nature* **283**, 767–768.

Illes, P. (1983) Electrophysiology of noradrenaline release from smooth muscle organs. *T.I.P.S.* **4**, 335–338.

McGrath, J.C. (1978) Adrenergic and 'non-adrenergic' components in the contractile response of the vas deferens to a single indirect stimulus. *J. Physiol* **283**, 23–29.

Pennefather, J.N. (1983) A study of stimulation-evoked activation of α_2-adrenoceptors in the rat isolated vas deferens. *Clin. Exp. Pharmacol. Physiol.* **10**, 381–393.

Westfall, D.P. (1970) Non-specific supersensitivity of the guinea-pig vas deferens produced by decentralization and reserpine treatment. *Br. J. Pharmacol.* **39**, 110–120.

Wood, A.J. Phelan, E.L. & Simpson, F.O. (1975) Cardiovascular effects of prazosin in normotensive and genetically hypertensive rats. *Clin. Exp. Pharmacol. Physiol.* **2**, 294–304.

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