Presence of postsynaptic $\alpha_2$-adrenoreceptors of predominantly extrasynaptic location in the vascular smooth muscle of the dog hind limb

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Summary

1. In the autoperfused hind limb of the dog prazosin (10$\mu$g/kg intravenously) markedly antagonized responses to lumbar sympathetic stimulation, whereas responses to injected noradrenaline were largely unaffected.

2. In $\beta$-adrenoreceptor- and ganglion-blocked animals, the hind limb pressor responses to phenylephrine were antagonized to a greater degree by prazosin than responses to injected noradrenaline.

3. Rauwolscine, a selective $\alpha_2$-adrenoreceptor-blocking agent, antagonized responses to the $\alpha_2$-adrenoreceptor agonist guanabenz, but not those to phenylephrine.

4. Hind limb pressor responses to noradrenaline were significantly inhibited by rauwolscine and further reduced by prazosin.

5. These results demonstrate that in this vascular bed $\alpha_1$- and $\alpha_2$-adrenoreceptors are located postsynaptically. Furthermore the results suggest that neuronally released noradrenaline acts mainly upon $\alpha_1$-adrenoreceptors, whereas exogenous noradrenaline acts upon $\alpha_1$- and $\alpha_2$-adrenoreceptors.

6. It is suggested that this selectivity of prazosin in blocking the vasoconstriction to neuronally-released noradrenaline may in part explain the effectiveness of this drug as an antihypertensive agent.

Key words: $\alpha_1$- and $\alpha_2$-adrenoreceptors, autoperfused dog hind limb, neuronally released and exogenous noradrenaline, prazosin, rauwolscine, vascular smooth muscle.

Introduction

Considerable evidence has accumulated during the last few years in support of the view that $\alpha$-adrenoreceptors can be subclassified into $\alpha_1$ and $\alpha_2$ categories (Langer, 1974, 1977; Berthelsen & Pettinger, 1977). The subclassification of $\alpha$-adrenoreceptors is based on pharmacological differences and is independent of the pre- or post-synaptic localization of these receptors (Starke & Langer, 1979; Langer, 1980).

Prazosin is the first member of a new class of antihypertensive agents (see Brogden, Heel, Speight & Avery, 1977, for review). Pharmacological and receptor-binding studies confirm that prazosin is a potent, highly selective, postsynaptic $\alpha_1$-adrenoreceptor blocking agent and that it is this action which is probably responsible for its hypotensive effects in several animal models of hypertension (see Langer, Cavero & Massingham, 1980, for review).

Several reports have shown that diastolic blood pressure responses to injected noradrenaline in rats and cats are resistant to blockade by prazosin (Drew & Whiting, 1979; Docherty, MacDonald & McGrath, 1979). Since these results are inconsistent with the earlier findings that prazosin lowers blood pressure by $\alpha$-adrenoceptor blockade, we decided to investigate whether prazosin selectively antagonized noradrenaline released from sympathetic neurons and whether $\alpha_2$-adrenoreceptors are present postsynthetically in vascular smooth muscle, as one preliminary report has suggested (Docherty et al., 1979).

Methods

Mongrel dogs (10–20 kg body weight) were anaesthetized with pentobarbitone (35 mg/kg and 6 mg h$^{-1}$ kg$^{-1}$ intravenously). The animals were
respired artificially and blood pressure was monitored from the left carotid artery. The right jugular vein and left brachial vein were cannulated and used for drug infusions or injections respectively. After injecting heparin into the animals, blood was taken, via a roller pump, from the right femoral artery and used to perfuse the left hind limb via the left femoral artery. Resting hind limb perfusion pressure was measured with a Statham P23D pressure transducer and was set to mean arterial pressure at the start of the experiment by adjusting the perfusion rate of the pump. In some animals the left lumbar sympathetic chain was stimulated (supramaximal voltage, 1 ms for 1 min) every 3 min at various frequencies (0.1–2 Hz), and between each series of stimulations dose–response curves were constructed to noradrenaline (0.3–3 μg/kg intravenously). After stable control responses to nerve stimulation and injected noradrenaline, prazosin (10 μg/kg intravenously) a selective α1-adrenoreceptor antagonist (Cambridge, Davey & Massingham, 1977) or rauwolscine, a preferential α2-adrenoreceptor antagonist (Tanaka, Weitzell & Starke, 1978), was injected and the series of nerve stimulations and injections of noradrenaline were repeated. Finally, the effect of the combination of the two antagonists was assessed on endogenously released and exogenously injected noradrenaline. Similar experiments were conducted in β-adrenoreceptor-blocked animals propranolol 0.5 mg/kg intravenously and 0.25 mg h⁻¹ kg⁻¹ intravenously) and in animals receiving atropine (1 mg/kg intravenously) to block all known hind limb dilator mechanisms (sympathetic, cholinergic muscarinic and β-adrenoreceptor) which may possibly be activated when the lumbar sympathetic chain is stimulated.

In a third group of animals, with ganglia blocked with chlorisondamine (1 mg/kg intravenously) and atropine (1 mg/kg intravenously), and β-adrenoreceptors blocked with propranolol (0.5 mg/kg intravenously and 0.25 mg h⁻¹ kg⁻¹ intravenously), dose–response curves on hind limb perfusion pressure were constructed to noradrenaline (0–1–1 μg/kg intravenously), guanabenz (1–10 μg/kg intravenously) and to phenylephrine (1–10 μg/kg intravenously). Animals were then given prazosin (10 μg/kg intravenously) and responses to noradrenaline, phenylephrine and guanabenz re-examined in the presence of each antagonist given separately and finally in the presence of both antagonists in combination.

Results are expressed as changes in hind limb perfusion pressure or diastolic blood pressure since administration of the blocking agents did not markedly affect basal pressure levels in most experiments. Statistical significance was assessed by analysing differences in area under the dose–response curve; *P* < 0.05 was taken as significant.

Results

Prazosin markedly inhibited the hind limb responses to all frequencies of nerve stimulation used (Fig. 1a) but did not significantly affect either the peak hind limb perfusion pressure responses to injected noradrenaline or the maximum diastolic blood pressure responses to injected noradrenaline (1.0 μg of noradrenaline/kg produced an increase in arterial blood pressure of 41 ± 6 mmHg and an increase of hind limb perfusion pressure of 50 ± 8 mmHg). After 10 μg of prazosin/kg these responses were 41 ± 5 mmHg and 40 ± 5 mmHg respectively. Once established, the prazosin blockade was not reversed by atropine or propranolol or a combination of these drugs. Analysis of the area under the frequency–response curve showed that after the administration of prazosin the hind limb response to lumbar sympathetic stimulation was inhibited by 45 ± 5.9% ([P] < 0.05) and the hind limb response to injected noradrenaline was reduced by only 12.8 ± 4.4% (not significant compared with control: *P* > 0.05).

In ganglion- and β-adrenoreceptor-blocked dogs, prazosin antagonized hind limb responses to all doses of phenylephrine by 40–60% (Fig. 1d) but did not affect responses to injected guanabenz (3 μg/kg produced an increase in hind limb perfusion pressure of 42.5 ± 2.9 mmHg before prazosin and 44.2 ± 8.9 mmHg after). Furthermore prazosin did not significantly affect responses to 0.1 and 0.3 μg of noradrenaline/kg intravenously although the 1 μg/kg intravenous dose was slightly but significantly reduced (*P* < 0.05; Fig 1b). In contrast, rauwolscine antagonized significantly all doses of injected guanabenz (Fig. 1c) and noradrenaline (results not shown). The same dose of rauwolscine did not block the responses to phenylephrine except for the highest dose (10 μg/kg intravenously; 3 μg/kg produced an increase in hind limb perfusion pressure of 42.0 ± 3.4 mmHg before rauwolscine and 33.3 ± 3.1 mmHg after). Treatment with rauwolscine of the dogs which had already received prazosin did not further affect the hind limb responses to phenylephrine (Fig. 1d) but prazosin pretreatment rendered the hind limb responses to noradrenaline very susceptible to rauwolscine blockade (Fig. 1b). As shown in Fig. 1(b), the combination
**Postsynaptic $\alpha_2$-adrenoreceptors**

FIG. 1. Effect of prazosin on the hind limb perfusion pressor responses to lumbar sympathetic stimulation, and of prazosin and rauwolscine on responses to guanabenz and exogenous noradrenaline, (a) Effect of prazosin on the response to lumbar sympathetic stimulation. (b) Effect of prazosin, and prazosin plus rauwolscine, on the response to noradrenaline. (c) Effect of rauwolscine on the response to guanabenz. (d) Effect of prazosin and prazosin plus rauwolscine on the response to phenylephrine. (a) was obtained in untreated animals and (b), (c) and (d) in ganglion- and $\beta$-adrenoreceptor-blocked animals.
of prazosin and rauwolscine reduced noradrenaline responses in the hind limb by at least 55% \((P < 0.05)\) at each dose.

**Discussion**

The high sensitivity of hind limb responses, elicited by lumbar sympathetic stimulation, to blockade by prazosin suggests that neurally released noradrenaline acts mainly at \(\alpha_1\)-adrenoceptors to cause constriction in this vascular bed. The sympathetic nerve supply to the hind limb also contains cholinergic dilator fibres (Cavero, Fenard, Gomeni, Lefevre & Roach, 1978) and it is possible that \(\beta_2\)-adrenoreceptors might be involved in the response. However, the prazosin blockade, once established, was not affected by atropine or propranolol, thus making it unlikely that these hind limb dilator mechanisms could contribute significantly to the differential effects of prazosin on neurally released and exogenously administered noradrenaline.

Whereas exogenous noradrenaline and guanabenz were resistant to prazosin, responses to phenylephrine, a selective \(\alpha_1\)-adrenoceptor agonist (Starke, Endo & Taube, 1975), were susceptible to blockade by prazosin. Furthermore, since rauwolscine preferentially inhibited the pressor effects of guanabenz and noradrenaline rather than phenylephrine these results suggest that injected noradrenaline, known to be equally effective as an agonist at \(\alpha_1\) and \(\alpha_2\)-adrenoreceptor sites (Starke et al., 1975), and guanabenz act mainly at postsynaptic vascular \(\alpha_2\)-adrenoreceptors to cause constriction.

These findings suggest that in this preparation, within the noradrenergic synapses, postsynaptic \(\alpha_1\)-adrenoreceptors are preferentially activated by the neurally released transmitter. The resistance of the pressor responses to injected noradrenaline to blockade by prazosin appears to be due to this agonist preferentially acting on postsynaptic \(\alpha_2\)-adrenoreceptors, since the response to noradrenaline was more susceptible to blockade by rauwolscine. Furthermore these results suggest that the postsynaptic vascular \(\alpha_1\)-adrenoreceptors may be predominantly located outside the synaptic regions, possibly together with an additional population of \(\alpha_2\)-adrenoreceptors, since prazosin induced a further blockade of the response to exogenous noradrenaline in rauwolscine pretreated animals.

Finally these results, if applicable to other vascular beds, could explain the effectiveness of prazosin as an antihypertensive drug since it would preferentially inhibit vasoconstriction mediated by neurally released noradrenaline.

This finding might also explain the better postural profile of this drug compared with other \(\alpha\)-adrenoceptor antagonists such as phentolamine and phenoxybenzamine, which would block all postsynaptic constrictor responses. Although the physiological role of these postsynaptic \(\alpha_2\)-adrenoceptors is not yet clear it is possible that the proportion of \(\alpha_1\) and \(\alpha_2\)-adrenoreceptors might vary between different vascular beds and between arteriolar and venous smooth muscle.

We would suggest therefore that whereas the absence of effect of prazosin at presynaptic release modulating \(\alpha_2\)-adrenoreceptors is important, a lack of effect at postsynaptic \(\alpha_1\)-adrenoceptors in vascular smooth muscle could significantly contribute to the beneficial haemodynamic changes induced by this drug in hypertension and heart failure.

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**References**


