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Phentolamine lacks α_2-adrenoceptor agonist activity in anaesthetized dogs

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1 This study was performed in order to determine whether the blockade of sympathetic vasoconstriction in anaesthetized dogs by phentolamine is due to the antagonist action of the drug at postjunctional adrenoceptors, or is due to depression of neurotransmitter release by an agonist action at prejunctional adrenoceptors.

2 In dogs made areflexic by ganglion blockade with hexamethonium, phentolamine (0.5 mg i.a. or 0.5 mg kg^{-1} i.v.) elevated or did not affect femoral blood flow. By contrast, clonidine (0.5–2.5 nmol, i.a.) produced femoral vasoconstriction, which was attenuated by prior administration of phentolamine.

3 Prior blockade of prejunctional α_2-adrenoceptors with yohimbine (30 μg kg^{-1}, i.v.) did not reduce the blocking effect of phentolamine (0.5 mg kg^{-1}, i.v.) on neurogenic vasoconstriction.

4 The results indicate that, in anaesthetized dogs, phentolamine lacks appreciable agonist activity at either prejunctional or postjunctional α_2-adrenoceptors. The blockade of neurogenic responses by phentolamine is therefore likely to be due to postjunctional adrenoceptor blockade.

Introduction

The traditional view of sympathetically-mediated vasoconstriction involves the interaction of noradrenaline (NA) with subjunctional α-adrenoceptors. Recent electrophysiological data, by contrast, have shown that the primary electrical events associated with transmission from sympathetic nerves to single vascular muscle cells are resistant to α-adrenoceptor antagonists. This has led to suggestions that neurally-released NA may interact with another receptor type (Hirst & Neild, 1980; Holman & Surprenant, 1980; Makita, 1983; Hirst *et al.*, 1985) or that the main vasoconstrictor transmitter released at low (physiological) frequencies of nerve stimulation may not be NA (Sneddon & Burnstock, 1984; Burnstock & Kennedy, 1986).

In anaesthetized dogs, graded vasoconstrictor responses to low frequency sympathetic stimulation and to exogenous NA are depressed in parallel by phentolamine, indicating that the primary subsynaptic event does involve interaction of neurogenic NA with α-adrenoceptors (Bell, 1985a). By contrast, Hirst & Lew (1987) have now reported that, in rabbits, the α-adrenoceptor antagonist benextramine is not an effective antagonist of neurogenic vasoconstriction. As previous experiments in rabbits had shown that phentolamine has a partial agonist action on both pre- and postsynaptic α_2-adrenoceptors (Angus & Lew, 1984), Hirst & Lew (1987) suggested that the sympathetic blocking action of phentolamine observed by Bell (1985a) may be due to depression of NA release by presynaptic adrenoceptor activation. The present experiments were designed to assess the viability of this proposition.

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Methods

Adult mongrel dogs of either sex (12–16 kg) were anaesthetized with α-chloralose (70 mg kg^{-1}, i.v. after thiopentone induction) and artificially ventilated under positive pressure. Circulation to the left paw was occluded with a ligature in order to make the vascular bed more homogeneous (Bell, 1985b), and blood flow through the left femoral artery was recorded in the thigh with a cuff-type electromagnetic flow probe (Devices). Systemic arterial blood pressure and heart rate were recorded from the contralateral femoral artery. A catheter was passed from a branch of the right artery into the lower abdominal aorta, for injection of vasoactive substances into the left femoral circulation.

In some animals, the left lumbar sympathetic trunk

was exposed via a retroperitoneal flank incision between L_4 and L_5 ganglia, and crushed rostrally. Silver stimulating electrodes were placed on the interganglionic ramus and the site was insulated and immobilised with a pool of paraffin wax and vaseline (1:1, m.p. 43°C). The trunk was stimulated with single or 10 pulse trains of 0.2 ms biphasic pulses, delivered at 4 Hz from a Grass SD9 stimulator. In these animals, atropine sulphate (0.4 mg kg^{-1}, i.v.) was administered at the beginning of the experiment in order to prevent activation of cholinergic vasodilator fibres (Bell, 1985a).

Drugs used were clonidine hydrochloride (Catapres; Boehringer-Ingelheim), hexamethonium chloride (Sigma), noradrenaline bitartrate (Levophed, Winthrop), phentolamine mesylate (Regitine; Ciba-Geigy) and yohimbine hydrochloride (Sigma).

Results

Initial baseline values for eight animals used were: blood pressure 113 ± 7 mmHg, heart rate 105 ± 9 beats min^{-1} and femoral blood flow 12 ± 2 ml min^{-1} kg^{-1}.

Postjunctional adrenoceptors

In order to study the activation of postjunctional vascular α_2-adrenoceptors without interference by reflex adjustments in vasomotor tone, total ganglionic blockade was produced in six dogs by administration of hexamethonium (10–20 mg kg^{-1}, i.v.) (Bell, 1985b). This produced a sustained fall in blood pressure to 79 ± 11 mmHg, and stabilized heart rate at 115 ± 14 beats min^{-1}. Femoral flow was transiently elevated, but returned to 12 ± 4 ml min^{-1} kg^{-1} within 5 min.

Femoral responses to intra-arterial clonidine (0.5–2.5 nmol) and phentolamine (0.5 mg) were examined in three animals. In each case, clonidine elicited dose-dependent vasoconstriction (mean flow reductions: 0.5 nmol, 17 ± 6 ml min^{-1}; 2.5 nmol, 27 ± 8 ml min^{-1}), while phentolamine produced vasodilatation in one experiment and had no effect on flow in the others (mean flow increase: 9 ± 7 ml min^{-1}). Following intra-arterial phentolamine, responses to clonidine were attenuated considerably (more than 50%) or abolished, recovering over about 20 min.

In all six dogs, phentolamine was also administered systemically at the dose of 0.5 mg kg^{-1} used by Bell (1985a). This produced no effect on blood pressure, and a transient (less than 2 min duration) and slight (less than 5% change) elevation of femoral flow. In all cases, flow returned to pre-phentolamine values within 2 min.

Prejunctional adrenoceptors

As reported previously (Bell, 1985a), lumbar sympathetic trunk stimulation with either single pulses or with trains of 10 pulses produced short-lived femoral vasoconstrictor responses which were not accompanied by any evidence of reflex activation. Constrictor responses to 10 pulse trains could be matched in amplitude by intra-arterial injection of NA (0.5 μg).

In four animals, yohimbine (30 μg kg^{-1}, i.v.) was administered between 10 and 20 min before phentolamine (0.5 mg kg^{-1}, i.v.). Yohimbine reduced somewhat the vasoconstrictor responses to both NA (before yohimbine: 3.9 ± 0.9 ml min^{-1} kg^{-1}; after yohimbine 3.2 ± 0.5 ml min^{-1} kg^{-1}) and single pulse sympathetic stimulation (before yohimbine: 1.3 ± 0.2 ml min^{-1} kg^{-1}; after yohimbine: 0.8 ± 0.1 ml min^{-1} kg^{-1}), but did not consistently affect responses to train stimulation (before yohimbine: 4.1 ± 0.7 ml min^{-1} kg^{-1}; after yohimbine: 4.0 ± 0.5 ml min^{-1} kg^{-1}).

In the presence of yohimbine, phentolamine reduced responses to both sympathetic trunk stimulation and NA to similar extents (Figure 1). The mean percentage reductions were: NA 66 ± 5%, 1 pulse stimulation 75 ± 5%, train stimulation 63 ± 4%. These results were closely similar to those obtained

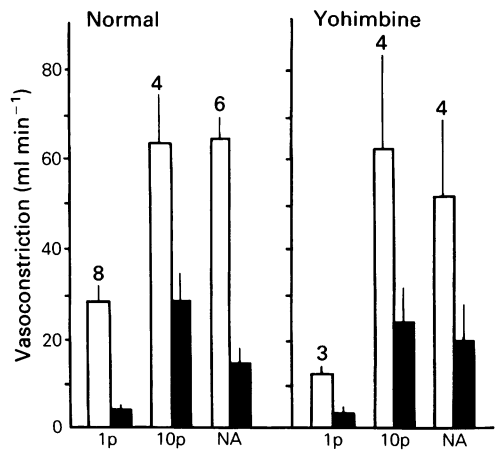

Figure 1 Attenuation by phentolamine (0.5 mg kg^{-1}, i.v.) of femoral vasoconstrictor responses to sympathetic nerve stimulation (1 pulse (1p) and 10 pulses (10p) at 4 Hz) and exogenous noradrenaline (0.5 μg) in normal dogs (data redrawn from Bell, 1985a), and in dogs pretreated with yohimbine (30 μg kg^{-1}, i.v.). The open columns represent control data, and the filled columns those obtained after phentolamine. The vertical bars represent one s.e.mean, and the numbers above each pair of columns indicate the number of experiments performed.

when phentolamine was administered in the absence of yohimbine (Figure 1). Responses to both NA and sympathetic stimulation recovered in parallel over 30–60 min.

Discussion

Postjunctional α_2-adrenoceptors are known to be present in the dog femoral vasculature (Horn *et al.*, 1982), and this was confirmed in the present study by the fact that, in dogs made areflexic by ganglionic blockade, intra-arterial injections of the selective α_2-adrenoceptor agonist clonidine (Langer, 1980) evoked vasoconstriction. By contrast, phentolamine did not cause femoral vasoconstriction when administered intra-arterially, nor when administered systemically in the larger doses used previously to produce postjunctional adrenoceptor blockade. The drug therefore appears, in the dog, to be devoid of agonist activity at postjunctional α_2-adrenoceptors. This contrasts with the observations of Angus & Lew (1984) in rabbits, but is similar to results obtained in guinea-pigs and rats by them and by other workers (Drew, 1976; Timmermans & Van Zwieten, 1980).

Langer *et al.* (1980) reported that, in anaesthetized dogs, activation of prejunctional α_2-adrenoceptors by clonidine was prevented by administration of the α_2-adrenoceptor antagonist yohimbine in doses as low as $1\,\mu g\,kg^{-1}$. In the present study, a dose of $30\,\mu g\,kg^{-1}$ yohimbine was given, in order to ensure complete blockade of the prejunctional α_2-adrenoceptors. Following yohimbine, there was attenuation of constrictor responses to injected NA and to sympathetic stimulation with single pulses, presumably due to blockade of postjunctional α_2-adrenoceptors, but no effect on responses to sympathetic stimulation with trains of pulses, presumably due to concomitant enhancement of neural NA release.

In untreated dogs, phentolamine reduces femoral vasoconstrictor responses to exogenous noradrenaline and to sympathetic nerve stimulation by closely similar extents, and has a greater effect on nerve-mediated responses to single pulses than on those to trains of stimuli (Bell, 1985a). The present experiments have shown that phentolamine has identical effects after yohimbine pretreatment. Thus, neither the depressant action of phentolamine on responses to sympathetic stimulation, nor its relatively greater attenuation of responses to single stimulating pulses, can be attributed to a prejunctional clonidine-like activity.

The results of this study provide no support for the suggestion made by Hirst & Lew (1987), that the depression of neurogenic vasoconstriction by phentolamine is due to inhibition of NA release by presynaptic α_2-adrenoceptor activation. Consequently, they favour the alternative explanation, that phentolamine blocks sympathetic neurotransmission by a postjunctional antagonist action. This supports the proposal (Bell, 1985a) that, in the anaesthetized dog, vasoconstriction elicited by physiological frequencies of sympathetic nerve activation is mediated by the action of NA on subjunctional α-adrenoceptors.

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References

ANGUS, J.A. & LEW M.J. (1984). Phentolamine – an unexpected agonist in the rabbit. *Br. J. Pharmacol.*, **81**, 423–425.

BELL, C. (1985a). Comparison of the antagonistic effects of phentolamine on vasoconstrictor responses to exogenous and neurally released noradrenaline *in vivo*. *Br. J. Pharmacol.*, **85**, 249–253.

BELL, C. (1985b). Differentiation of neurogenic vasoconstrictor responses in skin and skeletal muscle of dog hindlimb. *J. Autonom. Nerv. Syst.*, **14**, 13–21.

BURNSTOCK, G. & KENNEDY, C. (1986). A dual function for adenosine 5′-triphosphate in the regulation of cardiovascular tone. Excitatory cotransmitter with noradrenaline from perivascular nerves and locally released intravascular agent. *Circ. Res.*, **58**, 319–330.

DREW, G.M. (1976). Effects of α-adrenoceptor agonists and antagonists on pre- and postsynaptically located α-adrenoceptors. *Eur. J. Pharmacol.*, **36**, 313–320.

HIRST, G.D.S. & LEW, M.J. (1987). Lack of involvement of α-adrenoceptors in sympathetic neural vasoconstriction in the hindquarters of the rabbit. *Br. J. Pharmacol.*, **90**, 51–60.

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.

HIRST, G.D.S., DE GLERIA, S. & VAN HELDEN, D.F. (1985). Neuromuscular transmission in arterioles. *Experientia*, **41**, 874–879.

HOLMAN, M.E. & SURPRENANT, A.M. (1980). An electrophysiological analysis of the effects of noradrenaline and α-receptor antagonists on neuromuscular transmission in mammalian muscular arteries. *Br. J. Pharmacol.*, **71**, 651–662.

HORN, P.T.,KOHLI, J.D., LISTINSKY, J.J. & GOLDBERG, L.I. (1982). Regional variation in the alpha-adrenergic recep-

tors in the canine resistance vessels. *Naunyn Schmiedebergs Arch. Pharmacol.*, **318,** 166–172.

LANGER, S.Z. (1980). Presynaptic regulation of the release of catecholamines. *Pharmacol. Rev.*, **32,** 337–362.

LANGER, S.Z., MASSINGHAM, R. & SHEPPERSON, N.B. (1980). *In vivo* α-adrenoceptor selectivity of WB 4101: a widely used α_1-adrenoceptor ligand. *Br. J. Pharmacol.*, **70,** 60P.

MAKITA, Y. (1983). Effects of adrenoceptor agonists and antagonists on smooth muscle cells and neuromuscular transmission in the guinea-pig renal artery and vein. *Br. J. Pharmacol.*, **80,** 671–679.

SNEDDON, P. & BURNSTOCK, G. (1984). ATP as a co-transmitter in rat tail artery. *Eur. J. Pharmac.*, **106,** 149–152.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1980). Postsynaptic α_1- and α_2-adrenoceptors in the circulatory system of the pithed rat: selective stimulation of the α_2-type by B-HT 933. *Eur. J. Pharmacol.*, **63,** 199–202.

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