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Determination of the muscarinic receptor subtype mediating vasodilatation

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The muscarinic receptor mediating vasodilatation of the rabbit aorta and dog femoral artery has been assessed using muscarinic antagonists. With the exception of pirenzepine, the antagonist affinities were similar to those reported for the ileal receptors and dissimilar to those reported for the atrial receptors. Pirenzepine exhibited an affinity (7.54) intermediate between that reported for the CNS receptors (8.4) and that reported for the ileal receptors (6.77). This value for pirenzepine was confirmed using acetylcholine as the agonist and using the dog femoral artery as the vascular tissue. It is concluded that the muscarinic receptor profile mediating vasodilatation is not easily accommodated into the current receptor classification.

Introduction Stimulation of the muscarinic receptor (mAChR) present on the vascular endothelium produces vasodilatation (Furchgott & Zawadzki, 1980). It has been proposed (Hirschowitz *et al.*, 1984) that mAChRs exist in multiple subclasses. Two subclasses, M_1 and M_2, have been proposed on the basis of pirenzepine binding affinities (Hammer & Giachetti, 1982). In addition, the M_2 subtype may be subdivided on the basis of functional antagonist affinities into two further subtypes (Mitchelson, 1984). Thus mAChRs may exist in three subclasses (Birdsall & Hulme, 1983): receptors predominantly present in the CNS, in the myocardium, and on gastrointestinal smooth muscle. This paper presents data using muscarinic antagonists, which may classify the mAChR subtype present on the vascular endothelium.

Methods Descending thoracic aortae or femoral arteries were removed from female New Zealand white rabbits (3 kg bodyweight) and male Beagle dogs (12 kg bodyweight) respectively and cut into rings (2–3 mm thick). These tissues were mounted on pins, avoiding damage to the endothelium, and placed in a perfusion chamber (Patmore & Whiting, 1982) under 2 g tension. The tissues were perfused at a rate of 5 ml min^{-1} with Krebs-bicarbonate solution (pH 7.4, 30 °C, gassed with 5% CO_2 in O_2). This was composed as follows (mmol $litre^{-1}$): NaCl 118.41, KCl 4.69, $MgSO_4.7H_2O$ 1.18, KH_2PO_4 1.18, glucose 11.1, $NaHCO_3$ 24.99, $CaCl_2.6H_2O$ 2.52 and ascorbate 0.09). After a 2 h equilibration period, the tissues were brought to a moderate level of tonic contraction (Furchgott & Zwadski, 1980) by perfusion with noradrenaline 1×10^{-6} mol $litre^{-1}$. Concentration-response curves (CRCs) to carbachol were then constructed until reproducible concentration-dependent relaxations were obtained. Antagonists were preincubated with the tissues for 30 min prior to repeating the CRCs. Antagonist affinities were assessed from 3 concentrations of each antagonist, by measurement of the pA_2 (Arunlakshana & Schild, 1959). Each tissue was exposed to only one antagonist.

Results The antagonist affinities (pA_2) and slopes from Arunlakshana & Schild plots are shown in Table 1. The pA_2 values for atropine, secoverine and 4-DAMP (4-diphenyl acetoxy-N-methylpiperidine methiodide) were 8.59, 7.39 and 9.02 respectively. Pirenzepine, when used to antagonize carbachol-induced vasodilatation of the rabbit aorta exhibited a pA_2 value of 7.92. When the Schild slope was constrained to unity this value was 7.54. Similar affinities for pirenzepine were observed when acetylcholine was used to induce vasodilatation of the rabbit aorta (7.57) and also when carbachol was used to induce vasodilatation of the dog femoral artery (7.64). Gallamine and pancuronium acted as non-competitive antagonists, and exhibited very low antagonist affinities.

Discussion There is evidence to suggest that the mAChR exists in two subtypes, M_1 and M_2 (Hammer & Giachetti, 1982). The work presented in this paper has used the affinities of antagonists to assess the mAChR subtype responsible for vasodilatation. Atropine has been reported to exhibit little or no selectivity for mAChR subtypes (Mitchelson, 1984). The value obtained in this study is similar to that obtained by our group for mAChR present in the ileum (8.69) or atria (8.76) (Eglen *et al.*, 1984). Secoverine has been reported to exhibit selectivity toward mAChRs present in the gastrointestinal tract (Zwagemakers &

Table 1 Antagonist affinities for mAChR mediating vasodilatation

Antagonist	*Tissue*	*Agonist*	*pA_2*	*Slope*
Atropine	RAR	CCh	8.59 (8.41–8.77)	1.03 (0.96–1.10)
Secoverine	RAR	CCh	7.39 (7.15–7.74)	0.99 (0.93–1.05)
4-DAMP	RAR	CCh	9.02 (8.85–9.19)	0.98 (0.92–1.04)
Gallamine	RAR	CCh	>3.0	0.53 (0.48–0.58)
Pancuronium	RAR	CCh	>3.0	0.64 (0.58–0.70)
Pirenzepine	RAR	CCh	7.92 (7.72–7.74)	0.84 (0.79–0.89)
			7.54† (7.40–7.68)	
Pirenzepine	RAR	ACh	7.57 (7.45–7.69)	1.04 (0.95–1.13)
Pirenzepine	DFAR	CCh	7.64 (7.31–7.97)	0.97 (0.91–1.03)

Values are mean with 95% confidence limits derived from 4 preparations.
† Values is that obtained after imposing the unity constraint.
Abbreviations: RAR–rabbit aortic rings; DFAR–dog femoral artery rings; CCh–carbachol; ACh–acetylcholine.

Claassen, 1980). The pA_2 value shown in Table 1 is similar to that observed for mAChRs present in the ileum (7.68) but less than that observed for the atrial mAChR (8.76) (Clague *et al.*, 1984). Conversely 4-DAMP exhibited an affinity similar to that found for the ileal mAChR (9.04), but greater than that for the atrial mAChR (7.90) (Eglen *et al.*, 1984). Barlow *et al.* (1980) has reported that 4-DAMP exhibits a 10 fold selectivity for ileal mAChRs. Pancuronium and gallamine have been reported to exhibit selectivity for the atrial mAChR (Marshall & Ojewole, 1979). These agents exhibited a very low affinity for the rabbit aorta which suggests that the mAChR present on this tissue differs from those present on the atria.

Pirenzepine is a selective muscarinic antagonist with approximately a 100 fold greater affinity for the M_1 subtype (Brown *et al.*, 1980). In the present study the pA_2 value was higher than that found for either the ileum (6.77) (Eglen *et al.*, 1984) or for the atria (5.47) (Eglen *et al.*, 1984; Mitchelson, 1984) but less than that for the CNS (8.4) (Brown *et al.*, 1980). The pA_2 value obtained for pirenzepine at mAChRs present on the rabbit aorta was confirmed using another agonist, acetylcholine, and also another vascular tissue from another species, the dog femoral artery. This suggests that the mAChR present on vascular tissue, mediating vasodilatation, differs from those present on ileal or atrial tissue.

Furchgott & Cherry (1984) have studied the action of the agonists, acetylcholine, methacholine, *n*-butyryl trimethylammonium and pilocarpine, in inducing vasodilatation. They considered that the mAChR mediating this action was not of a novel subtype, but noted in addition that the results were too limited to make a final judgement.

In conclusion, the receptor profile described for vascular smooth muscle mAChR mediating vasodilatation appears to differ from that profile described for the CNS, myocardium and gastrointestinal smooth muscle.

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