Br. J. Pharmacol. (1988), **95**, 1031–1038

The interaction of methoctramine and himbacine at atrial, smooth muscle and endothelial muscarinic receptors *in vitro*

R.M. Eglen, W.W. Montgomery, *I.A. Dainty, L.K. Dubuque & R.L. Whiting

Institute of Pharmacology, Syntex Research, 3401 Hillview Ave., Palo Alto, CA 94304, U.S.A. & *Autonomic Physiology Unit, Institute of Physiology, University of Glasgow, Glasgow G12 8QQ, Scotland

1 The action of methoctramine and himbacine at muscarinic receptors has been studied using guinea-pig isolated trachea, oesophageal muscularis mucosae, paced left atria, and rat aortic preparations.

2 Methoctramine (1×10^{-6}–3.2×10^{-4} M), but not himbacine, elicited positive inotropic responses. These responses were enhanced by pretreating the animals with reserpine. The responses in reserpine-treated animals were not antagonized by phentolamine (1×10^{-6} M) but were antagonized by propranolol (1×10^{-6} M).

3 Methoctramine, but not himbacine, exhibited allosteric inhibitory effects at cardiac muscarinic receptors, resulting in a curvilinear Schild plot. Deviations from competitive antagonism were also observed in combination dose-ratio experiments using atropine and methoctramine. At 1×10^{-6} M, the pK_B value for methoctramine was 7.88 ± 0.15 (mean ± s.e.mean, $n = 5$). The pA_2 value for himbacine at cardiac muscarinic receptors was 8.52 ± 0.06 ($n = 3$).

4 At tracheal and oesophageal muscularis mucosal smooth muscle receptors, the Schild plots for both antagonists were linear. The pA_2 values for methoctramine at receptors in these two preparations were similar (6.08 ± 0.05 and 6.03 ± 0.09 respectively, $n = 4$) and were approximately 60 fold less than those values observed at atrial receptors. Himbacine, also exhibited similar values at muscarinic receptors in the trachea and oesophageal muscularis mucosae (7.61 ± 0.05 and 7.57 ± 0.04 respectively, $n = 4$).

5 Muscarinic receptors mediating relaxation of the rat aortic endothelium exhibited pA_2 values for methoctramine (5.87 ± 0.12, $n = 6$) which were similar to those observed in the smooth muscle, but not the atria. The pA_2 values for himbacine at endothelial muscarinic receptors were approximately 0.5 pA_2 units lower than those observed at muscarinic receptors in smooth muscle (6.92 ± 0.80, $n = 6$). In addition, the Schild slopes for methoctramine and himbacine at these receptors were significantly ($P < 0.05$) less than unity.

6 Methoctramine, and to a lesser extent himbacine, are potent and selective antagonists for cardiac muscarinic receptors. However, caution should be used in interpretation of the data with methoctramine in view of the inhibitory allosteric properties and direct inotropic actions of this compound.

Introduction

The alkaloid himbacine (Ritchie & Taylor, 1967) has been shown to exhibit approximately a 10 fold degree of selectivity for cardiac muscarinic receptors, in comparison to those present on the ileum (Gilani & Cobbin, 1986; atrial $pA_2 = 8.2$, ileal $pA_2 = 7.2$). Methoctramine has also been shown to be a selective antagonist for muscarinic receptors present in atria, in comparison to those present in smooth muscle

(Melchiorre et al., 1987). These authors found an order of selectivity between atrial and smooth muscle receptors of approximately 270 fold which was larger than has been previously obtained for other cardioselective antagonists including AF-DX 116 (Micheletti et al., 1987), pancuronium or gallamine (see Eglen & Whiting, 1986 for review). These differential affinities were considered to provide further evidence for differences in muscarinic receptors in the atria and smooth muscle.

It has been shown (see Mitchelson, 1984 for review) that other cardioselective muscarinic antagonists such as pancuronium, gallamine and stercuronium also exhibit effects in cardiac tissue which include allosteric inhibitory interactions, inhibition of adrenaline uptake and direct positive inotropic effects. These properties complicate their use in muscarinic receptor classification. Melchiorre et al. (1987) have also shown that methoctramine in addition to muscarinic receptor antagonism acted as a weak α_1-adrenoceptor antagonist ($pK_B = 5.2$) and a non-competitive antagonist at nicotinic receptors. Himbacine, although less selective than methoctramine, also exhibited a slight positive inotropic response at concentrations of 1 μM and above (Gilani & Cobbin, 1986). This action has not been observed using the cardioselective muscarinic antagonist, AF-DX 116 (Eglen & Whiting, 1987a; Micheletti et al., 1987).

In the present study, we have also attempted to characterize the interaction of methoctramine and himbacine at atrial and smooth muscle muscarinic receptors of the guinea-pig. The data obtained are consistent with previous findings (Gilani & Cobbin, 1986; Melchiorre et al., 1987) showing that methoctramine and himbacine are cardioselective muscarinic antagonists, although methoctramine appears to possess other properties in cardiac tissue.

We have employed these antagonists to characterize further the muscarinic receptors present on the rat aortic endothelium. The endothelial muscarinic receptor mediating vascular relaxation remains to be definitively characterized. Eglen & Whiting (1985) using the superfused rabbit aortic ring found a pA_2 value for pirenzepine of 7.6, which was dissimilar to those values generally considered to indicate M_1 (8.4) or M_2 (6.8) receptor function. However, Choo et al. (1986) obtained a pA_2 value for pirenzepine of 6.8 using both rat and rabbit aortic ring preparations in the organ bath. Rubanyi et al. (1986) have shown that in a superfused system EDRF is released in a biphasic manner and pirenzepine exhibits different potencies at muscarinic receptors mediating the two phases. A further complicating factor in the use of pirenzepine to characterize endothelial muscarinic receptors is that there is some species variation, in that high pK_i values for pirenzepine were observed at binding sites in the bovine endothelium but low pK_i values were obtained in the rabbit endothelium (Yamanaka et al., 1986). Similar pA_2 values to those obtained by Choo et al. (1986) were observed in the rabbit ear artery using functional and radioligand binding studies (Hynes et al., 1986). The action of muscarinic antagonists which discriminate between M_2-receptors in the atria and smooth muscle have, however, not been extensively studied at endothelial muscarinic receptors. Studies using pancuronium, stercuronium, gallamine and 4-diphenylacetoxy N methyl piperidine methiodide (4-DAMP; Barlow et al., 1980) have shown similar pA_2 values at the endothelial and ileal but not atrial muscarinic receptors (Eglen & Whiting, 1985; Choo et al., 1986). The high selectivity of methoctramine (Melchiorre et al., 1987) and to a lesser extent himbacine (Gilani & Cobbin, 1986), therefore, may allow a more definitive classification of the endothelial muscarinic receptor to be made.

Abstracts of this work were presented at the Third International Symposium on Subtypes of Muscarinic Receptors, Sydney, August 29–31, 1987 (Whiting et al., 1987) and at the British Pharmacological Society, London, January 1988 (Dainty et al., 1988).

Methods

Left atria, trachea and oesophageal muscularis mucosae were isolated from male Dunkin-Hartley guinea-pigs (300–350 g) and thoracic aortae were isolated from male Sprague-Dawley rats (250–320 g). The experiments were undertaken at 30°C, in either Krebs physiological salt solution (left atria, aorta and trachea) or Tyrode physiological salt solution (oesophageal muscularis mucosae); the composition of these solutions is given below.

Hexamethonium (3×10^{-4} M) was initially added routinely to the Krebs or Tyrode physiological salt solutions. However, preliminary experiments showed that the pK_B values for methoctramine at atrial muscarinic receptors were reduced by approximately 8 fold in the presence of hexamethonium. In contrast, no effect was observed on the smooth muscle or aortic preparations. Similar effects using hexamethonium on the pK_B values for pancuronium have been observed by Leung & Mitchelson (1982). Hexamethonium may therefore act as a weak cardioselective muscarinic antagonist, since it has been shown that pK_B values of less than 2.8 and 4.0 are observed as ileal and atrial muscarinic receptors, respectively (Leung & Mitchelson, 1982; Zonta et al., 1987). Consequently, all further experiments using left atria were conducted in the absence of hexamethonium.

Guinea-pig left atria were electrically paced using parallel platinum electrodes (Clague *et al.*, 1985). Guinea-pig trachea were cut in a zig-zag fashion, as described by Emmerson & MacKay (1979). Guinea-pig oseophageal muscularis mucosae (OMM) were prepared according to the method of Kamikawa *et al.* (1985). Aortic rings (2 mm) were prepared from male Sprague-Dawley rats (250–350 g). All tissues were suspended under 1.0 g tension and allowed 60 min to equilibrate during which time the bathing fluid was replaced every 15 min.

In order to study aortic relaxant responses the tone was increased by the addition of U46619 (3×10^{-8} M) in studies using methoctramine or phenylephrine (1×10^{-5} M) in studies using himbacine. U46619, a thromboxane A_2 mimetic, was employed since methoctramine has been shown to possess α_1-adrenoceptor antagonism (Melchiorre *et al.*, 1987), and consequently inhibited the contractile responses to phenylephrine (Dainty, unpublished observations). The rat thoracic aorta was used to study endothelial-dependent relaxations, since preliminary experiments had shown that relaxations of the guinea-pig thoracic aorta were either small or non-existent and only contractions to acetylcholine were seen (Montgomery, unpublished observations). The rat thoracic aorta, in contrast, exhibited reproducible and measurable endothelial-dependent relaxations.

All responses were determined as changes in isometric tension, in mg. Concentration-response curves to carbachol or acetylcholine (aorta) were constructed in a cumulative fashion using concentrations of agonist spaced at 0.5 log intervals after the method described by van Rossum (1963).

In experiments in which the effect of antagonists were studied, the tissues were allowed 60 min equilibration period at each antagonist concentration before construction of a concentration-response curve to the agonist. Each preparation was exposed to only one antagonist concentration and the method of Arunlakshana & Schild (1959) was used to estimate the equilibrium dissociation constant, by determining the pA_2 value. In order to obtain an accurate estimation of the dissociation constant (K_B), the Schild slopes were also constrained to unity. The intercept on the abscissae and the slope of the Schild plot were determined by linear regression. In atrial studies, the equilibrium dissociation constant for methoctramine was estimated using individual concentrations of antagonist by the method of Furchgott (1972).

In a separate series of experiments, the effect of methoctramine was studied using atrial tissue isolated from animals pretreated with reserpine. The regimen of pretreatment with reserpine has been described previously by Micheletti *et al.* (1987); reserpine was injected i.p., at a single dose of 5 mg kg^{-1} and the animals were killed 18 h following the last injection. The extent of catecholamine depletion was assessed by determining the response of the isolated tissues to tyramine (1×10^{-4} M).

Statistical analysis

Significant differences were assessed by use of Student's t test, with a value of $P < 0.05$ being considered significant.

Drugs used

Acetylcholine, atropine, carbachol, hexamethonium, isoprenaline, reserpine and tyramine were obtained from Sigma Chemical Co. Ltd. Methoctramine was synthesized by Dr R. Clark, Syntex, Palo Alto. Himbacine was generously donated by Prof W.C. Taylor, University of Sydney, NSW.

Krebs solution (mM): NaCl 118.4, KCl 4.9, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, glucose 11.1, $NaHCO_3$ 25.0 and $CaCl_2 \cdot 6H_2O$ 2.5.

Tyrode solution (mM): NaCl 136.9, KCl 2.7, $MgCl_2 \cdot 6H_2O$ 1.1, $NaH_2PO_4 \cdot 2H_2O$ 0.4, glucose 5.6, $NaHCO_3$ 11.9 and $CaCl_2 \cdot 6H_2O$ 1.8.

Results

Electrically stimulated left atrium

Methoctramine Methoctramine (1×10^{-6} M–3.2×10^{-4} M) elicited a concentration-dependent positive inotropic response. At the maximum concentration employed (3.2×10^{-4} M), an increase of 1000 ± 65 mg (mean ± s.e.mean, $n = 5$) in developed tension was observed. The $-\log EC_{50}$ was not calculated in these experiments since no maximum response was attained (Figure 1).

In tissues isolated from animals pretreated with reserpine, responses to tyramine were abolished. In these tissues, the response to methoctramine was enhanced with respect to tissues from untreated animals, both in terms of potency and maximal response. Positive inotropic responses were now observed over the concentration range 1×10^{-8} to 1×10^{-5} M and the maximal response observed to 1×10^{-5} M methoctramine was 1800 ± 73 mg ($n = 5$)

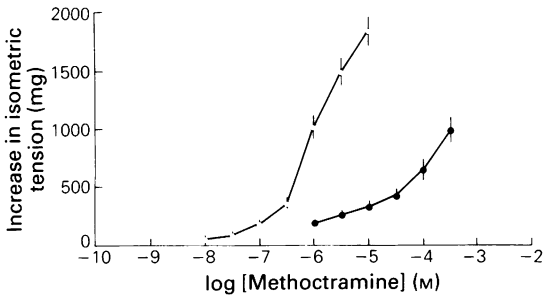

Figure 1 Increases in isometric tension elicited by methoctramine in electrically paced guinea-pig left atria. Data are shown from both untreated (●) and reserpine-pretreated animals (○). Values shown are mean with vertical lines indicating s.e.mean.

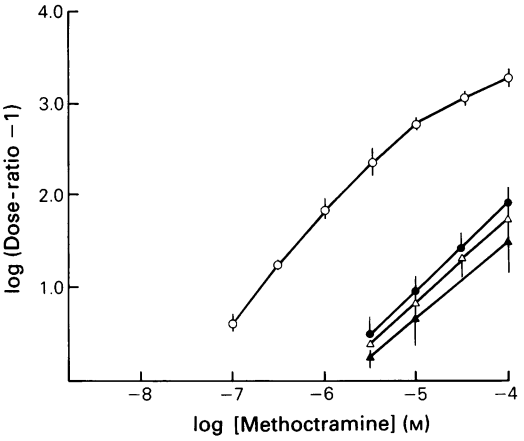

Figure 2 Schild analysis of antagonism of muscarinic responses by methoctramine in guinea-pig electrically paced left atria (○), trachea (●), oesophageal muscularis mucosae (△) and rat thoractic aorta (▲). Values are mean (n = 4–8) and vertical lines indicate s.e.mean.

developed tension (Figure 1). In tissues isolated from animals pretreated with reserpine the positive inotropic responses to methoctramine were unaffected by the presence of phentolamine (3×10^{-6} M). However, they were completely abolished in the presence of propranolol (1×10^{-6} M).

In tissues isolated from untreated animals, methoctramine (3.2×10^{-8}–1×10^{-4} M) produced parallel rightward shifts in the concentration-response curve to carbachol with no change in the maximal response. The slope of the Schild plot, over the entire range of concentrations used, was 0.87 ± 0.05 ($n = 5$), which was significantly ($P < 0.05$) different from unity. Preliminary experiments, in which the equilibration period was extended to 120 min showed no additional effect on the slope of the Schild plot. The Schild plot appeared to be curvilinear over the entire range of antagonist concentrations, although deviation from linearity was particularly pronounced at concentrations of 1×10^{-6} M and above. These data are shown in Figure 2. At each concentration of methoctramine the pK_B ($-\log K_B$) value was calculated according to the method of Furchgott (1972) and it was observed that this value varied with the concentration of methoctramine studied. The dose-ratio obtained in the presence of 3.2×10^{-8} M methoctramine was not significantly ($P < 0.05$) different from the changed sensitivity to the control. These data are shown in Table 1. The dose-ratios obtained between 1×10^{-7} and 1×10^{-5} M were used in a Schild plot in which the slope was constrained to unity. In this case, the pK_B value for methoctramine was 7.8.

Combination dose-ratio experiments with atropine (5×10^{-7} M) and methoctramine (1×10^{-5} M), using the method of Paton & Rang (1965), showed that the observed combined dose-ratios were significantly different ($P < 0.05$) from the predicted combined dose-ratios (Table 2). These statistical differences were assessed by use of Student's t test. It should be noted that at the concentration of methoctramine (1×10^{-5} M) used in these experiments deviations from linearity of the Schild plot were marked. In these experiments, the effect of atropine was first ascertained and the effect of methoctramine was then determined in the presence of atropine. The total shift in the concentration-response curve for the

Table 1 Dose-ratios and pK_B values (negative log of the dissociation constants) obtained with methoctramine in guinea-pig left atria

Methoctramine concentration (M)	*Dose-ratio*	pK_B[a]	n
1×10^{-7}	5.8 ± 0.7	7.68 ± 0.08	5
3.2×10^{-7}	19 ± 2	7.76 ± 0.05	5
1×10^{-6}	94 ± 27	7.97 ± 0.15	5
3.2×10^{-6}	280 ± 60	7.94 ± 0.14	5
1×10^{-5}	680 ± 90	7.83 ± 0.05	5
3.2×10^{-5}	1200 ± 180	7.59 ± 0.06	5
1×10^{-4}	2200 ± 330	7.34 ± 0.07	4

Values shown are means ± s.e.mean.
[a] Estimated from dose-ratios (DR) obtained using the relationship $K_B = B/(DR - 1)$ where B is the concentration of methoctramine (Furchgott, 1972). The pK_B value is the negative logarithm of the K_B.

Table 2 Combination dose-ratio experiments using methoctramine and atropine in guinea-pig left atria

[a]*Dose-ratio (DR_1)* *atropine* (5×10^{-7} M)	*Dose-ratio (DR_2)* *methoctramine* (1×10^{-5} M)	[a]*Experimental combined dose-ratio*	[b]*Expected combination dose-ratio* ($DR_1 + DR_2 - 1$
1030 ± 190	680 ± 90	1230 ± 70	1710 ± 210

Values are mean ± s.e.mean, $n = 5$–8.
[a] Determined in separate experiments.
[b] Dose-ratio expected for combination of 2 competitive antagonists (Paton & Rang, 1965). The expected value differs significantly ($P < 0.05$) from the experimental combined dose-ratio (Student's t test).

agonist from the combination of the two antagonists was estimated and compared to that predicted for a combination of two competitive antagonists (Paton & Rang, 1965).

Himbacine No positive inotropic responses to himbacine were observed at the concentrations studied (1×10^{-9}–1×10^{-4} M). Himbacine acted as a competitive antagonist at atrial muscarinic receptors, since parallel rightward shifts in the concentration-response curve which attained the same maximum were observed. The slope of the Schild plot was 1.00 ± 0.01, which was not significantly different from unity and the pA_2 value was 8.52 ± 0.06 ($n = 3$). Since the slope of the Schild plot was exactly unity, then the pK_B value was also 8.52. These data are shown in Figure 3.

Smooth muscle

Methoctramine Parallel rightward shifts in the concentration-response curves to carbachol were observed in the presence of methoctramine (1×10^{-7} M–1×10^{-4} M), but these were smaller dextral shifts in comparison to those observed in the atria. The slopes of Schild plots in the OMM and trachea were not significantly ($P < 0.05$) different from unity. The calculated pA_2 values obtained at receptors in the trachea did not differ significantly ($P < 0.05$) from values obtained in the OMM. However, both pA_2 values were approximately 60 fold less than the pK_B value calculated at atrial receptors. These data are shown in Table 3 and Figure 2. The slopes of the Schild plots were also constrained to unity and these are shown in Table 3.

Methoctramine antagonized the relaxant responses to acetylcholine in aortic rings resulting in parallel rightward shifts in the concentration-

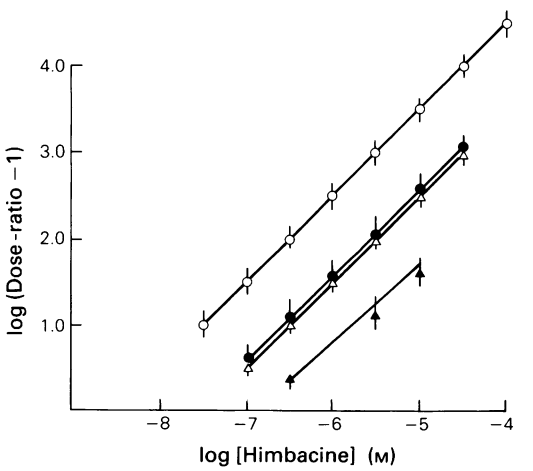

Figure 3 Schild analysis of antagonism of muscarinic responses by himbacine in guinea-pig electrically paced left atria (○), trachea (●), oesophageal muscularis mucosae (△) and rat thoracic aorta (▲). Values are mean ($n = 4$–8) and vertical lines indicate s.e.mean.

Table 3 pA_2 values and Schild slopes of methoctramine and himbacine at receptors in guinea-pig smooth muscle and rat aortic endothelium

Preparation	pA_2	*Slope*	n
	Methoctramine		
Trachea	6.08 ± 0.08 (5.97)	0.92 ± 0.07	4
OMM	6.03 ± 0.09 (5.82)	0.95 ± 0.05	4
Aorta	5.87 ± 0.12 (5.57)	0.74 ± 0.15	4
	Himbacine		
Trachea	7.61 ± 0.05 (7.47)	0.95 ± 0.02	4
OMM	7.57 ± 0.04 (7.44)	0.91 ± 0.08	4
Aorta	6.92 ± 0.08 (6.60)	0.80 ± 0.12	4

Values are mean ± s.e.mean.
The values in parentheses are the pA_2 values obtained after imposing the unity constraint.
OMM = oesophageal muscularis mucosae.

response curve. However, the slope of the Schild plot was significantly ($P < 0.05$) less than unity (Table 3 and Figure 2). The pK_B value obtained when the Schild slope was constrained to unity is shown in Table 3. The pA_2 values calculated at the aortic endothelial receptors were similar to those pA_2 values calculated at receptors in the trachea and OMM but different from those observed in the atria.

Himbacine Himbacine antagonized responses to carbachol in the trachea and oesophageal muscularis mucosae in a competitive fashion which resulted in parallel rightward shifts in the concentration-response curve, and no change in the maximum response. The Schild plots calculated in these two preparations were not significantly ($P < 0.05$) different from unity. As was observed using methoctramine, the pA_2 values were similar at muscarinic receptors in these two preparations. However, the pA_2 values were approximately 8 fold less than those observed at atrial muscarinic receptors. These data are shown in Table 3 and Figure 3. In addition, the pK_B values calculated when the Schild slopes were constrained to unity are shown in Table 3.

Himbacine also antagonized relaxant responses of the rat aortic endothelium to acetycholine, resulting in parallel rightward shifts in the concentration-response curve, although the slope of the Schild plot was significantly different from unity. The pA_2 value was significantly less ($P < 0.05$) than the values obtained at muscarinic receptors in the trachea and OMM. These data are shown in Table 3 and Figure 3. The pK_B value calculated by imposing the unity constraint are shown in Table 3.

Discussion

In the present study we have examined the effects of methoctramine and himbacine; muscarinic antagonists which have been found (Gilani & Cobbin, 1986; Melchiorre *et al.*, 1987) to exhibit selectivity for atrial muscarinic receptors.

Methoctramine produced a positive inotropic response and similar effects have been demonstrated for gallamine and pancuronium (see Mitchelson, 1984 for review). However, unlike these antagonists in which a large component of the response was abolished by pretreatment of the animals with reserpine (Mitchelson, 1984), the response to methoctramine was enhanced. This probably indicates that the response was direct and not due to release of catecholamines. The positive inotropic responses to methoctramine were unaffected by phentolamine, indicating a lack of effect at α-adrenoceptors, but were abolished by propranolol, probably implicating β-adrenoceptor agonism. This finding is consistent with the increased sensitivity observed to methoctramine after reserpine treatment; a procedure which also increases sensitivity to β-adrenoceptor agonists (Chess-Williams *et al.*, 1986).

Himbacine, unlike methoctramine, did not elicit a positive inotropic response in the present studies and in this respect resembles the action of AF-DX 116, another cardioselective muscarinic antagonist (Eglen & Whiting, 1987a; Micheletti *et al.*, 1987). Gilani & Cobbin (1986) showed that a slight positive inotropic response to himbacine occurred at concentrations of 10^{-6} M and above and this was unaffected by reserpine pretreatment or propranolol.

Methoctramine appeared to act as a competitive antagonist at receptors in the tracheal and OMM preparations because parallel shifts in the concentration-response curves were observed and the Schild plots were linear with unity slopes. The pK_B value at atrial receptors was approximately 2 fold less than the pA_2 value obtained by Melchiorre *et al.* (1987), whilst the pA_2 values at smooth muscle receptors were approximately 2 fold greater than the values obtained by this group at ileal and bladder receptors. The Schild plot constructed from atrial data in the present study was curvilinear, particularly at high concentrations. This feature is not consistent with competitive antagonism, but it is similar to that observed with other cardioselective antagonists such as pancuronium, gallamine or stercuronium (Mitchelson, 1984; Eglen & Whiting, 1986 for review). One explanation for this effect is an allosteric inhibitory interaction with the receptor. This hypothesis was in agreement with the data showing that the observed dose-ratios deviated from the predicted dose-ratios in the combination experiments with atropine. Preliminary radioligand binding experiments have also shown methoctramine to possess inhibitory allosteric properties at concentrations of 1×10^{-6} M and above (A.D. Michel, unpublished observations).

Methoctramine exhibited lower pA_2 values at smooth muscle receptors in comparison to those observed in the atria. This indicates that the compound acts as a selective antagonist for cardiac muscarinic receptors. It was not possible to determine if the compound acted allosterically at receptors in smooth muscle, in view of the relatively high concentrations required to antagonize the contractile responses to carbachol.

Himbacine exhibited a 10 fold degree of selectivity for atrial muscarinic receptors, in comparison to those present on smooth muscle. The order of selectivity for himbacine was much less than was observed using methoctramine (60 fold) but was similar to that obtained for AF-DX 116 (6 fold,

Eglen & Whiting, 1987a; 10 fold Micheletti *et al.*, 1987). Whilst the order of selectivity observed in the present study using himbacine is similar to that previously obtained (Gilani & Cobbin, 1986), the absolute pA_2 values at atrial and smooth muscle preparations are approximately 2.5 fold greater than those previously found (Gilani & Cobbin, 1986). The reason for this disparity is unclear, although a possible explanation could be that differing equilibration periods were used in the present study in comparison to that of Gilani & Cobbin (1986), i.e., 60 and 15–30 min, respectively.

The pA_2 values obtained using methoctramine at muscarinic receptors mediating aortic relaxation were similar to those obtained at receptors in the trachea and oesophageal muscularis mucosae, but dissimilar to those obtained in the atria. The absolute pA_2 values for himbacine obtained at the endothelial receptors were slightly lower than those pA_2 values obtained at receptors in the trachea or oesophageal muscularis mucosae.

The similarity between pA_2 values at receptors present in smooth muscle and the aortic endothelium are in agreement with previous work (Eglen & Whiting, 1985; Choo *et al.*, 1986) using other muscarinic antagonists such as 4-DAMP, pancuronium, stercuronium and gallamine. We therefore conclude that, despite the differences observed using himbacine, the muscarinic receptor present on the aortic endothelium is similar to that present in smooth muscle but dissimilar to that present in the atria. This hypothesis is in agreement with recent studies in our laboratory showing that the vascular relaxant response is unaffected by pretreatment with pertussis toxin; an agent which uncouples muscarinic receptors mediating negative inotropic responses to agonists acting through the Ni regulatory GTP binding protein, but is without effect on responses to muscarinic agonists in smooth muscle (Eglen & Whiting, 1987b).

Methoctramine, and to a lesser extent himbacine, therefore, act as selective muscarinic antagonists and are able to discriminate between cardiac and smooth muscle muscarinic receptors. These data, therefore, provide further evidence for differing muscarinic receptor subtypes in these tissues. However, radioligand binding studies using 4-DAMP or AF-DX 116 have shown little difference between binding sites in these tissues (Nedoma *et al.*, 1985; Choo *et al.*, 1986; Michel & Whiting, 1987). An explanation for this discrepancy is given by the recent finding in radioligand binding studies using methoctramine (Michel & Whiting, 1988), that only 20–30% of the total number of receptors in ileal longitudinal smooth muscle exhibit pK_i values similar to the pA_2 values obtained in smooth muscle in the present study. The remaining sites (approx. 80%) exhibit pK_i values which correspond to those observed in the atria. Whilst the function of these latter sites in smooth muscle remains speculative, these data may explain the previously observed disparity between functional and radioligand binding studies.

In conclusion, methoctramine and himbacine act as selective antagonists for atrial muscarinic receptors and could prove useful in muscarinic receptor classification. However, in a similar fashion to data from studies using gallamine, pancuronium and stercuronium (Eglen & Whiting, 1986), caution should be exercised in the use of methoctramine in view of its allosteric inhibitory properties and direct cardiac effects. Himbacine, in contrast, is less selective for atrial muscarinic receptors, but appears to act in a competitive fashion.

The authors wish to thank Margaret Huff for her assistance in the smooth muscle experiments.

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol.*, **14**, 48–58.

BARLOW, R.B., BURSTON, K.N. & VIS, A. (1980). Three types of muscarinic receptor? *Br. J. Pharmacol.*, **68**, 141–142P.

CHESS-WILLIAMS, R.G., BROADLEY, K.J. & SHERIDAN, D.J. (1986). Calculated and actual changes in β-adrenoceptor number associated with increases in rat and guinea pig cardiac sensitivity. *J. Pharm. Pharmacol.*, **38**, 902–906.

CHOO, L.K., MALTA, E. & MITCHELSON, F. (1986). The affinity of some selective muscarinic receptor antagonists for the muscarininc receptor mediating endothelial-dependent relaxation of the rabbit and rat thoracic aorta. *J. Pharm. Pharmacol.*, **38**, 843–845.

CLAGUE, R.U., EGLEN, R.M., STRACHAN, A.C. & WHITING, R.L. (1985). Action of agonists and antagonists at muscarinic receptors present on ileum and atria, *in vitro*. *Br. J. Pharmacol.*, **87**, 163–170.

DAINTY, I.A., EGLEN, R.M. McGRATH, J.C. & WHITING, R.L. (1988). Analysis of the muscarinic receptor on the rat aortic endothelium. *Br. J. Pharmacol. Proc. Suppl.*, **93**, 240P.

EGLEN, R.M. & WHITING, R.L. (1985). Determination of the muscarinic receptor subtype mediating vasodilation. *Br. J. Pharmacol.*, **84**, 3–5.

EGLEN, R.M. & WHITING, R.L. (1986). Muscarinic receptor subtypes: a critique of the current classification and a proposal for a working nomenclature. *J. Auton. Pharmacol.*, **5**, 323–346.

EGLEN, R.M. & WHITING, R.L. (1987a). Competitive and non-competitive antagonism exhibited by 'selective' antagonists at atrial and ileal muscarinic receptor subtypes. *Br. J. Pharmacol.*, **90**, 701–708.

EGLEN, R.M. & WHITING, R.L. (1987b). Effects of pertussis toxin and calcium on EDRF release. *Cardiovasc. Drug Ther.*, **1**, 237.

EMMERSON, J. & MACKAY, D. (1979). The zig-zag tracheal strip. *J. Pharm. Pharmacol.*, **31**, 798.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Handbook of Experimental Pharmacology, Catecholamines*, Vol. 33. ed. Blaschko, M. & Muscholl, E. pp. 283–335. New York: Springer-Verlag.

GILANI, S.A.H. & COBBIN, L.B. (1986). The cardio-selectivity of himbacine: a muscarinic receptor antagonist. *Naunyn-Schiemdebergs Arch. Pharmacol.*, **332**, 16–20.

HYNES, M.R., BANNER, W., YAMAMURA, H.I. & DUCKLES, S.P. (1986). Characterization of muscarinic receptors of the rabbit ear artery smooth muscle and endothelium. *J. Pharmacol. Exp. Ther.*, **238**, 100–105.

KAMIKAWA, Y., UCHIDA, K. & SHIMO, Y. (1985). Heterogeneity of muscarinic receptors in the guinea pig oesophageal muscularis mucosae and ileal longitudinal muscle. *Gastroenterol.*, **88**, 706–716.

LEUNG, E. & MITCHELSON, F. (1982). Modification by hexamethonium of the muscarinic receptor blocking activity of pancuronium and homatropine in isolated tissues of the guinea-pig. *Eur. J. Pharmacol.*, **80**, 11–17.

MELCHIORRE, C., CASSINELLI, A. & QUAGLIA, W. (1987). Differential blockade of muscarinic receptor subtypes by polymethylene tetraamines. Novel class of selective antagonists of cardiac M-2 muscarinic receptors. *J. Med. Chem.*, **30**, 201–204.

MICHEL, A.D. & WHITING, R.L. (1987). Direct binding studies on ileal and cardiac muscarinic receptors. *Br. J. Pharmacol.*, **92**, 755–767.

MICHEL, A.D. & WHITING, R.L. (1988). Methoctramine reveals heterogeneity of M_2 muscarinic receptors longitudinal ileal smooth muscle membranes. *Eur. J. Pharmacol.*, **145**, 305–311.

MICHELETTI, R., MONTAGNA, E. & GIACHETTI, A. (1987). AF-DX 116, a cardioselective muscarinic antagonist. *J. Pharmacol. Exp. Ther.*, **241**, 628–634.

MITCHELSON, F. (1984). Heterogeneity in muscarinic receptors: evidence from pharmacologic studies with antagonists. *Trends. Pharmacol. Sci.*, **5**, (Suppl.) 12–16.

NEDOMA, J., DOROFEEVA, N.A., TUCEK, S., SHELKOUNIKOV, S.A. & DANILOV, A.F. (1985). Interaction of the neuromuscular blocking drugs alcuronium decamethonium, gallamine, pancuronium, ritebronium, tecuronium and d-tubocurarine with muscarinic acetylcholine receptors in the heart and ileum. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **329**, 176–181.

PATON, W.D.M. & RANG, H.P. (1965). The uptake of atropine and related drugs by intestinal smooth muscle of the guinea pig in relation to acetylcholine receptors. *Proc. Roy. Soc. B.*, **163**, 1.

RITCHIE, E. & TAYLOR, W.C. (1967). The Galbulimma alkaloids. In *The Alkaloids*, Vol. 9, ed. Manske, R.H.F. pp. 529–543. New York: Academic Press.

RUBANYI, G.M., MCKINNEY, M. & VANHOUTE, P.M. (1986). Biphasic release of endothelium-derived relaxing factor(s) by acetylcholine from perfused canine femoral arteries. Characterizations of muscarinic receptors. *J. Pharmacol. Exp. Ther.*, **240**, 802–808.

VAN ROSSUM, J.M. (1963). Cumulative dose-response curves. Techniques for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn. Ther.*, **143**, 299–330.

WHITING, R.L., MICHEL, A.D. & EGLEN, R.M. (1987). Comparison of the selectivity of methoctramine, AF-DX 116 and pirenzepine at muscarinic receptors *in vitro*. Abstract no. 13, *Third International Symposium on Subtypes of Muscarinic Receptors*, Sydney, August 29–31.

YAMANAKA, K., MASHIMOTO, S. & MURAMATSU, I. (1986). Species differences in the muscarinic receptor of the thoracic aorta. *J. Pharmacol. Exp. Ther.*, **237**, 980–986.

ZONTA, F., DONDI, G., LUCCHELLI, A., SANTAGOSTINO-BARBONE, M.G. & GRANA, E. (1987). Antimuscarinic action of hexamethonium. *Med. Sci. Res.*, **15**, 513–514.

(Received January 5, 1988
Revised July 4, 1988
Accepted July 26, 1988)