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Interaction of some muscarinic agonists and antagonists at the prejunctional muscarinic receptor in the rabbit ear artery preparation

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1 The inhibitory effect of several muscarinic agonists on responses to sympathetic nerve stimulation of the isolated perfused ear artery of the rabbit was compared to that of acetylcholine in preparations pretreated with dyflos, cocaine and yohimbine.

2 In general the potency of the agonists was similar to that observed at peripheral muscarinic sites except for arecaidine propargyl ester and 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyl trimethylammonium chloride (McN-A-343). The inhibitory effect observed with N-benzyl-3-pyrrolidyl acetate methobromide (AHR-602) was not exerted via muscarinic receptors.

3 With carbachol (CCh) as an agonist, the antagonist 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP) was found to have a pK_B value of 7.74 and thus was 19 fold less active as an antagonist of responses to the agonist, than previously reported for guinea-pig ileum. When McN-A-343 was used as the agonist, the slope of the Schild plot with the antagonist was significantly less than unity. It is suggested that an allosteric interaction of 4-DAMP may be involved with this agonist. By use of an allosteric model, a pK_B of 8.56 for 4-DAMP was obtained.

4 Secoverine produced similar pK_B values with either CCh (8.19) or McN-A-343 (8.13) as the agonist.

Introduction

The perfused rabbit ear artery preparation is a convenient tissue for the study of prejunctional muscarinic receptors on sympathetic nerves innervating vascular tissue. Other possible muscarinic receptor sites within the tissue do not appear to complicate the interaction of agonists with the prejunctional site. For example, endothelial cells, which on muscarinic receptor activation lead to vasodilatation (Furchgott, 1984), have been reported in the ear artery, but they appear to be readily lost in the initial preparation of the tissue for perfusion (Steinsland, quoted in Furchgott & Cherry, 1984). Furthermore, responses to exogenous noradrenaline are unaffected by cholinomimetics such as carbachol (Rand & Varma, 1970; Steinsland *et al.*, 1973; Leung & Mitchelson, 1983) or McN-A-343 (Rand & Varma, 1971; Vong, unpublished observations).

Vasoconstriction from activation of muscarinic receptors on the smooth muscle (Kuriyama *et al.*, 1982) or nicotinic receptors on sympathetic nerve endings (Steinsland & Furchgott, 1975) does not occur with the concentrations of agonists used to inhibit responses to sympathetic nerve stimulation, although such vasoconstrictor responses may occur with high concentrations of agonists.

Previous work has suggested that the prejunctional muscarinic receptor on the sympathetic nerve endings in the rabbit ear artery may be different from those on intestinal smooth muscle, as some selective muscarinic receptor antagonists such as pancuronium, have a higher affinity for these muscarinic receptors in the ear artery (Leung & Mitchelson, 1983).

Other studies have indicated that there may be differences in the responsiveness of this prejunctional receptor to agonists compared to prejunctional muscarinic receptors on other sympathetic nerves in the rabbit, as McN-A-343 is a potent agonist on the receptors in the ear artery (Rand & Varma, 1971) but not in the heart (Fozard & Muscholl, 1972) or pulmonary artery (Nedergaard, 1980; 1981). Furthermore, the muscarinic receptor antagonist, pirenzepine, was a more potent inhibitor of responses to McN-A-343 in the ear artery than of those to carbachol (Choo *et al.*, 1985). In view of these findings, a detailed comparison of the relative potencies of a range of muscarinic receptor agonists was undertaken to characterize further the receptor. Additionally, the effectiveness of two selective muscarinic antagonists, 4-diphenylacetoxy-N-methylpiperidine (4-DAMP) and

and secoverine were determined. The former has been reported to exhibit selectivity for muscarinic receptors in ileum over those in atrial tissue (Barlow *et al.*, 1976; 1980) while the latter has selectivity for muscarinic receptors in intestinal smooth muscle compared to those in glandular tissue (Zwagemakers & Claassen, 1980; 1981).

Methods

A proximal section (approx. 1 cm long) of the central ear artery was removed from the ear of a rabbit and perfused as previously described by Choo *et al.* (1985). Briefly, the isolated artery was cannulated at both ends and perfused at 1–3 ml min^{-1} with McEwen's solution (McEwen, 1956) containing cocaine (10 μM) and yohimbine (1 μM) while mounted on a plastic holder in a 10 ml organ bath filled with the same solution. The perfusate passed out of the artery and did not enter the organ bath fluid. Both solutions were gassed with a mixture of 95% O_2: 5% CO_2 and maintained at 37°C. Perfusion pressure was recorded on a polygraph via a Statham pressure transducer P23DC. The periarterial sympathetic nerves were stimulated via co-axial platinum wire electrodes for 5 s every 2–3 min with monophasic pulses of 30–60 volts and 1 ms duration at a frequency of 3 Hz. In some experiments with pilocarpine, frequencies up to 20 Hz were used.

Concentration-response curves to agonists

Agonists were added to the extraluminal fluid between 1 and 2 min before a stimulation period and allowed to remain in contact with the tissue until the full inhibitory effect of the agonist had developed. The preparation was then washed twice and control responses to sympathetic nerve stimulation were re-established before addition of another concentration of agonist. The procedure was repeated to obtain responses with four to six concentrations of agonist; each concentration of agonist usually being tested twice. In preparations pretreated with dyflos (5.4 μM) the anticholinesterase was added to the extraluminal fluid for 20 min. Following this period, the preparation was washed and no further addition of dyflos was made.

Effects of antagonists

To test the effects of an antagonist, the drug was added to both the perfusate and extraluminal bath fluid and allowed to equilibrate with the tissue for 30–40 min before responses to the agonists were re-determined. A new concentration-response curve to the agonist was established as described above. The same procedure was then repeated when several concentrations of an antagonist were investigated in one preparation.

Evaluation of data

For assessing the response to an agonist, the percentage inhibition produced by the agonist was obtained by comparing the vasoconstriction obtained on stimulation in the presence of the agonist with the mean of the two vasoconstrictor responses to stimulation obtained immediately before the addition of the agonist.

The EC_{50} value and slope of the concentration-response curve was determined on a computer by fitting a logistic function of the following form to the data (Parker & Waud, 1971):

$$E = \frac{E_{max}[A]^n}{K^n + [A]^n}$$

where E = percentage inhibition; E_{max} = maximal percentage inhibition; [A] = concentration of agonist; K = EC_{50} value; n = slope factor.

The data points were not weighted, but E_{max} was set at 100% as all the agonists, with the exception of AHR-602, were capable of producing complete inhibition.

Concentration-ratios (dose-ratios) were calculated as the EC_{50} value in the presence of the antagonist/ EC_{50} value in the absence of antagonist. To determine if antagonists produced a competitive inhibition, Schild plots were determined from experiments in which usually three concentrations of the antagonist were tested in the one preparation against a single agonist.

Statistical comparisons were made using Student's *t* test. The slope of the Schild plot was compared to the theoretical value of 1.0 expected for a competitive antagonist by estimating the standard error of the slope and performing a *t* test on the data (Moore *et al.*, 1972). Estimates of a pK_B value were obtained by extrapolation of the Schild plot and when the slope of the Schild plot did not differ significantly from unity ($P > 0.05$), by fitting a regression with the slope constrained to unity, to obtain a better estimate of the pK_B value (Mackay, 1978).

Drugs

Drugs used were: acetylcholine chloride (Sigma), acetyl-β-methylcholine chloride (Sigma), AHR-602 (N-benzyl-3-pyrrolidyl acetate methobromide, Robins), arecaidine propargyl ester (gift, courtesy Dr G. Lambrecht), atropine sulphate (Sigma), bethanechol (Sigma), carbachol (Sigma), cocaine hydrochloride (MacFarlan-Smith), 4-diphenylacetoxy-*N*-methylpiperidine methiodide (gift, courtesy Dr R.B. Barlow), dyflos (di*iso*propylfluorophosphate, Sigma), gallamine triethiodide (May & Baker), McN-A-343 (4-

(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride, McNeil), oxotremorine (ICN Pharmaceuticals), pilocarpine hydrochloride (Calbiochem), pirenzepine hydrochloride (Boehringer Ingelheim), secoverine hydrochloride (Philips-Duphar), tetramethylammonium bromide (BDH Chemicals) and yohimbine hydrochloride (Sigma).

Results

Effects of agonists

All the agonists investigated produced a concentration-dependent inhibition of the responses to sympathetic nerve stimulation at 3 Hz (Figure 1). Comparisons of the potency of agonists with that of acetylcholine were performed following dyflos pretreatment (5.4 μM for 20 min) of the tissue. As reported previously (Choo *et al.*, 1985) the mean EC_{50} for acetylcholine and slope of the concentration-response relationship were not significantly affected by pretreatment with the anticholinesterase.

With the exception of AHR-602 and tetramethylammonium, all agonists produced concentration-response curves with slope factors not differing from that of acetylcholine (Table 1). For AHR-602 and tetramethylammonium, the slope factors were lower ($P < 0.05$) and similar to that obtained previously with McN-A-343 (Table 1).

Pilocarpine has been reported to act as a partial agonist in this tissue (Steinsland *et al.*, 1973) but no evidence of this was found in our experiments. Although less potent than carbachol, pilocarpine produced comparable degrees of inhibition with that produced by carbachol at all frequencies investigated (3–20 Hz). The ability of the agonists to produce 100% inhibition in each experiment was not rigorously established, but an inhibition greater than 90% was frequently obtained at 3 Hz with all the agonists investigated except AHR-602. The low potency of this compound necessitated the use of concentrations up to 20 mM and

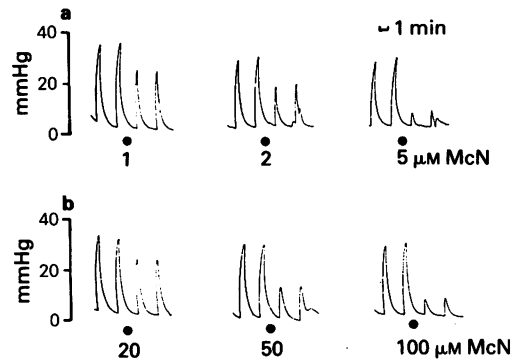

Figure 1 An illustration of the concentration-dependent inhibition of the responses to sympathetic nerve stimulation of the rabbit ear artery preparation by McN-A-343 (McN) in the absence (a, upper trace) and presence of 4-DAMP (30 nM) (b, lower trace).

Table 1 Concentrations of agonists required to produce 50% inhibition of the response to sympathetic nerve stimulation at 3 Hz in preparations pretreated with dyflos (5.4 μM, 20 min): effective concentrations (EC_{50}) and slope factors of the concentration-response relationships determined by fitting logistic function (see Methods) to experimental data

Agonist	n#	*Geometric mean EC_{50}(M) (95% confidence limits)*	*−log EC_{50}*	**Relative potency* ± *s.e.mean*	*e.p.m.r.*§	*Slope (± s.e.mean)*
Acetylcholine	13	2.11×10^{-8} (1.20–3.63)	7.68	1	1	1.62 ± 0.12
Oxytremorine	4	4.52×10^{-8} (2.75–7.44)	7.34	$(4.65 \pm 1.32) \times 10^{-1}$	2	1.52 ± 0.12
Arecaidine propar gylester	4	9.46×10^{-8} (2.59–34.6)	7.02	$(4.79 \pm 0.68) \times 10^{-1}$	2	1.37 ± 0.13
Methacholine	3	1.73×10^{-7} (1.19–2.52)	6.76	$(1.23 \pm 0.07) \times 10^{-1}$	8	2.03 ± 0.19
Carbachol**	3	2.13×10^{-7} (0.87–5.24)	6.67	$(9.85 \pm 0.57) \times 10^{-2}$	10	1.62 ± 0.15
McN-A-343**	4	2.17×10^{-6} (1.33–3.55)	5.66	$(6.08 \pm 2.39) \times 10^{-3}$	160	1.06 ± 0.06†
Bethanechol	3	4.92×10^{-6} (4.16–5.82)	5.31	$(3.63 \pm 0.21) \times 10^{-3}$	280	1.40 ± 0.13
Pilocarpine	3	7.28×10^{-6} (2.20–24.1)	5.14	$(1.68 \pm 0.53) \times 10^{-3}$	600	1.47 ± 0.21
Tetramethylammonium	8	1.58×10^{-4} (0.95–2.60)	3.80	$(1.08 \pm 0.13) \times 10^{-4}$	9300	1.19 ± 0.16†
AHR-602	3	6.00×10^{-3} (1.00–24.0)	2.22	$(3.90 \pm 1.46) \times 10^{-6}$	56000	0.92 ± 0.06†

*Relative potency assessed in each experiment using the EC_{50} for ACh in that experiment as unity.
**Choo *et al.* (1985).
†Slope is significantly different ($P < 0.05$) from slope of dose-response curve to ACh.
Number of experiments.
§ Equipotent molar ratios.

at this concentration the maximal inhibition observed was between 60 and 70%.

Effects of antagonists

Responses to all agonists with the exceptions of AHR-602 were inhibited by atropine (10 nM). Responses to AHR-602 were unaffected by atropine (10–100 nM) or by hexamethonium (2.8 μM).

The effect of the muscarinic receptor antagonists 4-DAMP (30 nM–1 μM) and secoverine (5 nM–1 μM) were investigated with carbachol as the agonist. Both antagonists produced a competitive inhibition of responses to carbachol, causing parallel shifts of the concentration-response relationship for the agonist leading to linear Schild plots with slopes not significantly different from unity ($P > 0.05$) (Table 2, Figure 2).

Responses to McN-A-343 were also inhibited by 4-DAMP (10 nM–1 μM) (Figure 1b) and secoverine (3 nM–0.3 μM). Unlike secoverine which produced a similar degree of inhibition of both carbachol and McN-A-343 (Table 2), low concentrations of 4-DAMP produced greater inhibition of responses to McN-A-343 than of carbachol. Using a concentration of 30 nM 4-DAMP in six preparations where both agonists were employed, the dose-ratio obtained with carbachol as agonist [4.90 (3.19–7.53) (geometric mean dose-ratio, 95% confidence limits)] was significantly different ($P < 0.05$) from that using McN-A-343 [10.95 (5.71–21.01)].

The Schild plot for 4-DAMP with McN-A-343 had a slope significantly less than unity ($P < 0.001$) suggesting the interaction was not competitive (Figure 2). When the data were fitted to the equation derived for an allosteric interaction by Van den Brink (1977) (see legend Figure 2), a pK_B value of 8.56 was obtained. This closely approximated the value of 8.50 ± 0.21 obtained using the lower concentrations of 4-DAMP (10–100 nM) where the Schild plot had a slope of 0.98 ± 0.9 (27 data points).

Gallamine has been reported previously to inhibit responses to carbachol in this preparation (Li & Mitchelson, 1980). In view of the suggestion that gallamine and McN-A-343 may interact at an allosteric site on muscarinic receptors in atria (Birdsall *et al.*, 1983) the interaction between gallamine and McN-A-343 in the ear artery was investigated. Gallamine (3–100 μM) produced parallel shifts of the concentration-response relationships for McN-A-343 and the interaction appeared competitive (Table 2).

It was previously reported (Choo *et al.*, 1985) that pirenzepine is a more selective antagonist of responses to McN-A-343 than of responses to carbachol or acetylcholine. As tetramethylammonium, like McN-A-343, exhibited a concentration-response relationship that was less steep than for other agonists, the effect of antagonists on responses to tetramethylammonium was investigated further.

The geometric mean EC_{50} for tetramethylammon-

Table 2 Effect of antagonists on responses to carbachol (CCh), McN-A-343 (McN) or tetramethylammonium (TMA) in rabbit ear artery

Antagonist	*Agonist*	n#	*Slope* of Schild plot*	*pK_B Mean ± s.e.mean*	*pK_B† (Slope of 1)*
Atropine[a]	CCh	4	—	8.95 ± 0.18	—
Atropine[b]	McN	3	0.88 ± 0.13 (9)	8.96 ± 0.13	8.89 ± 0.14
4-DAMP	CCh	17	0.90 ± 0.12 (30)	7.81 ± 0.15	7.74 ± 1.12
4-DAMP	McN	20	0.56 ± 0.10 (38)**	8.56††	ND
Secoverine	CCh	5	1.09 ± 0.08 (16)	8.05 ± 0.10	8.19 ± 0.15
Secoverine	McN	4	1.29 ± 0.34 (10)	7.86 ± 0.25	8.13 ± 0.20
Gallamine[c]	CCh	5	—	5.16 ± 0.08	—
Gallamine	McN	4	0.94 ± 0.09 (11)	4.96 ± 0.08	4.94 ± 0.18
Pirenzepine[b]	CCh	3	0.96 ± 0.10 (8)	6.19 ± 0.10	6.17 ± 0.16
Pirenzepine[b]	McN	12	0.68 ± 0.10 (40)**	7.57††	ND
Pirenzepine	TMA	3	0.96 ± 0.18 (9)	6.36 ± 0.13	6.34 ± 0.10

[a] *Kwok & Mitchelson (1982).*
[b] Choo *et al.* (1985).
[c] Li & Mitchelson (1980).
Number of experiments.
* Number of data points used in Schild regression.
† pK_B determined from line with unit slope (Mackay, 1978).
ND Not determined (see text).
** Significantly different from unity ($P < 0.01$).
†† Determined using equation of Van den Brink (1977) (see legend, Figure 2).

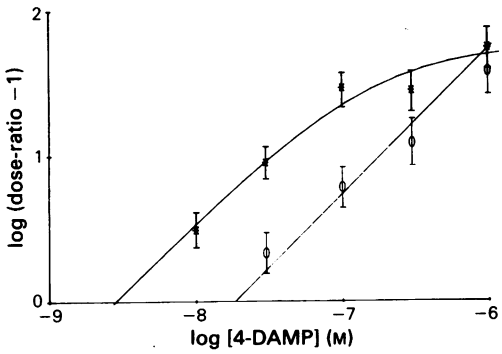

Figure 2 Schild plots for 4-DAMP using McN-A-343 (*) and carbachol (O) as agonists in the rabbit ear artery preparation. The standard error of the mean associated with each point is shown by the vertical line. The number of experiments associated with each point for McN-A-343 is 5 (10^{-8}), 13 (3×10^{-8}), 9 (10^{-7}), 7 (3×10^{-7}) and 4 (10^{-6}) and for carbachol, 11 (3×10^{-8}), 9 (10^{-7}), 3 (3×10^{-7}) and 5 (10^{-6}). The curve plotted through the data points obtained with McN-A-343 as agonist is based on the theoretical equation for an allosteric antagonist derived by Van den Brink (1977);

$$\text{Dose ratio} = \frac{1}{1 + (q-1)\beta/(1 + K_B/B)}$$

The curve is plotted with $\beta = 1$, $q = 0.017$ and $K_B = 2.74\,\mu M$. β is a constant, the intrinsic activity of the antagonist at its binding site; q is a constant characterizing the interrelation between the allosteric site and the agonist binding site such that the maximal dose-ratio obtained is given by 1/q. B is the concentration of antagonist. The regression line for CCh is drawn with the slope constrained to 1.0 and a K_B value of 18.4nM (see Table 2).

ium in the absence of dyflos 0.16 mM (0.09–0.27, 7) was similar to that in the presence of the anticholinesterase (Table 1). In half of the experiments, tetramethylammonium in concentrations greater than 0.3 mM produced a transient vasoconstriction, perfusion pressures returning to basal levels usually within 1 min. In agreement with Steinsland & Furchgott (1975), the concentration-response relationship appeared to be bell-shaped. The vasoconstriction was still observed in the presence of atropine (10–100 nM) or pirenzepine (0.3–3 μM) but was reduced or abolished in the presence of hexamethonium (2.8 μM). Treatment of the ear artery with hexamethonium (2.8 μM) did not alter the EC_{50} or slope of the concentration-response relationship for the inhibitory effect of tetramethylammonium on responses to nervous stimulation, the geometric mean EC_{50} before hexamethonium being 0.19 mM (0.02–1.53, 3) (95% confidence limits, *n*) and after the nicotinic receptor antagonist 0.19 mM (0.03–1.35, 3). The corresponding mean slope factors were 1.84 ± 0.29 and 1.88 ± 0.26.

Pirenzepine (0.3 to 3 μM) produced a competitive inhibition of responses to tetramethylammonium (Table 2), the pK_B value being similar to the value of 6.2 previously reported with carbachol as agonist (Choo *et al.*, 1985). Addition of hexamethonium (2.8 μM) did not alter the dose-ratios obtained with pirenzepine.

Discussion

The potency of agonists in the ear artery preparation was, in general, similar to that observed at peripheral muscarinic sites with the exception of arecaidine propargyl ester and McN-A-343. The former has been reported to have $-\log EC_{50}$ values of 8.07 and 7.51 in guinea-pig atria and ileum respectively (Mutschler & Lambrecht, 1984) whereas in the ear artery preparation the value was 7.02. In comparison, acetylcholine (in the presence of dyflos) has a similar potency in the three tissues with $-\log EC_{50}$ values of 7.7–8.0 (Mitchelson & O'Shea, 1977). The arecaidine ester was only 2 to 3 times more potent than carbachol in the ear artery preparation, whereas in atria and ileum of the guinea-pig it is 40 and 10 times more potent respectively (Mutschler & Lambrecht, 1984). On the other hand, McN-A-343 is more potent in the rabbit ear artery than on most peripheral muscarinic receptors, being only 164 times less potent than acetylcholine (Table 1). In guinea-pig ileum, it is approx. 1000 fold less potent than acetylcholine (in the presence of dyflos plus tetrodotoxin) (Vong, unpublished) and in guinea-pig atria, concentrations of greater than 0.1 mM are required to produce 50% inhibition of contractility or rate (Pappano & Rembish, 1971; Vong, unpublished).

AHR-602 produced some inhibition of responses to stimulation of the rabbit ear artery, but this was not exerted via activation of the prejunctional muscarinic receptor as the inhibition was unaffected by atropine in concentrations up to 100 nM. One possible explanation for the effect of AHR-602 is the local anaesthetic action observed with high concentrations of the agonist (Fozard & Muscholl, 1974). The failure of AHR-602 to activate muscarinic receptors in the ear artery was in agreement with findings for prejunctional muscarinic receptors on sympathetic nerves in the rabbit heart (Fozard & Muscholl, 1972) but was unexpected given that McN-A-343 was an agonist in the ear artery. In the superior cervical ganglion, McN-A-343 is 25 to 30 times more potent than acetylcholine, and AHR-602 is approx. 20–100 times less potent than McN-A-343 (Jones, 1963; Trendelenberg, 1966; Jaramillo & Volle, 1967a,b). In rabbit heart, the difference in potency between McN-A-343 and AHR-602 was approx. 25–80 fold for the negative inotropic

effect (Figure 2 of Fozard & Muscholl, 1972) and in the taenia of the guinea-pig caecum it was approx. 3 fold (Hobbiger *et al.*, 1969). In the ear artery the difference was over 2000 fold.

Taken overall, these findings suggest that while the rank order of potencies for acetylcholine, McN-A-343 and AHR-602 are identical in the various tissues apart from the superior cervical ganglion, there are nevertheless considerable differences in the magnitudes of their relative potencies.

The affinity of secoverine for muscarinic receptors in the ear artery was similar to that previously reported for post-junctional muscarinic receptors in the guinea-pig left atrium, rat bladder and guinea-pig or rat ileum (Choo & Mitchelson, 1985a).

In an earlier publication, it was shown that pirenzepine was a more potent antagonist of responses to McN-A-343 than of other agonists (Table 2). As McN-A-343 produced a concentration-response relationship which was not as steep as that for other agonists such as acetylcholine or carbachol, one explanation was that a different receptor or a mix of receptors was involved. As tetramethylammonium also produced a concentration-response relationship with a similar slope to that of McN-A-343 in preparations pretreated with dyflos, the effect of pirenzepine on tetramethylammonium was investigated. However, the pK_B value obtained for pirenzepine was similar to that obtained using carbachol or acetylcholine. The small transient vasoconstrictor response to high concentrations of tetramethylammonium seen in some experiments was concluded to be due to activation of nicotinic receptors on sympathetic nerve endings (Steinsland & Furchgott, 1975) as the response was inhibited by hexamethonium. The action of tetramethylammonium on the prejunctional muscarinic receptors was not modified by any action on nicotinic receptors as responses to the agonist were unaffected by hexamethonium even with the higher concentrations required in the presence of pirenzepine.

Recently, Birdsall *et al.* (1983) suggested that in cardiac muscle McN-A-343 acted as an allosteric agonist and that gallamine may act as an antagonist at this allosteric site. In the ear artery, gallamine appeared to be a competitive antagonist of responses to McN-A-343 as the Schild plot had a slope of 0.94 and the pK_B value of 4.94 obtained was similar to that found previously using carbachol as agonist (Table 2).

Evidence that the prejunctional receptor site in the ear artery may differ from other muscarinic receptors in its interaction with antagonists comes from findings with 4-DAMP. This muscarinic receptor antagonist, has been reported previously to show selectivity for ileal muscarinic receptors over cardiac muscarinic receptors (Barlow *et al.*, 1976; 1980). In our hands using carbachol as the agonist, it exhibited an affinity in the ear artery significantly lower than that observed for either guinea-pig atria (pK_B, 8.45) or ileal longitudinal muscle (pK_B, 9.07) (Choo & Mitchelson, 1985b). The affinity is also lower than that reported in rabbit myometrium (pK_B 8.50; agonist, methacholine) (Crankshaw, 1984) or rat anococcygeus muscle (pK_B, 8.75; acetylcholine) (Oriowo, 1983). However, low values have been obtained for 4-DAMP in the atria in the absence (pK_B, 7.90, carbachol) (Eglen *et al.*, 1984, quoted in Eglen & Whiting, 1985) or presence of hexamethonium (pK_B, 7.79, carbachol) (Barlow *et al.*, 1976; 1980).

In the ear artery, responses to McN-A-343 were inhibited by 4-DAMP with a pK_B of 8.5, an affinity approx. 2 to 5 fold higher than with carbachol as the agonist. This suggests the presence of two binding sites for 4-DAMP at the muscarinic receptor in the ear artery. If McN-A-343 acts at a different site on the muscarinic receptor from carbachol as suggested for the heart (Birdsall *et al.*, 1983) then the interaction of McN-A-343 with 4-DAMP at the second site may yield a different pK_B value from that when carbachol is used as an agonist. This second site for 4-DAMP would involve an allosteric interaction with the site for McN-A-343. However, it is possible that the heterogeneity of sites for 4-DAMP would be induced by the allosteric interaction of McN-A-343 and 4-DAMP as suggested for a number of muscarinic receptor antagonists (Dunlap & Brown, 1983; Sokolovsky, 1984).

It is of interest that Gilbert *et al.* (1984) have recently reported a reverse selectivity in that contractions produced by McN-A-343 were inhibited by 4-DAMP to a lesser extent than bethanechol in the lower oesophageal sphincter of the opossum, although both agonists were considered to be acting on a peripheral muscarinic receptor on the smooth muscle.

In conclusion, the evidence presented shows that the prejunctional muscarinic receptor in the ear artery has relative potency differences for muscarinic agonists not observed for other muscarinic receptors such as those in the superior cervical ganglion, atria and ileum. The receptor also displays a different order of affinity towards 4-DAMP from that observed in other tissues and the apparent affinity is dependent on the agonist employed, at least for carbachol and McN-A-343.

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