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Muscarinic activity of McN-A-343 and its value in muscarinic receptor classification

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1 The affinity and potency of McN-A-343 (4-(*m*-chlorophenyl-carbamoyloxy) -2-butynyltrimethylammonium chloride) has been assessed at a range of M_1 and M_2 muscarinic receptors. McN-A-343 was shown to act as a full agonist at M_2 receptors present in the guinea-pig isolated taenia caeci ($-\log EC_{50} = 5.14$). McN-A-343 exhibited no agonist action in the guinea-pig ileum, atria, bladder or trachea.

2 McN-A-343 was not selective in terms of affinity since its dissociation constants at M_1 and M_2 binding sites in the rat cerebral cortex and myocardium respectively, were very similar (cortical pPKi = 5.05; myocardial pKi = 5.22). The selectivity previously reported for the compound may be due to differences in intrinsic efficacy and/or tissue receptor reserve.

3 Based on differential antagonist affinities, the muscarinic receptor profile of the taenia caeci, trachea and bladder was similar to that observed in the ileum, but dissimiliar to that observed in the atria.

Introduction

Muscarinic receptors are currently classified into two subtypes: M_1-receptors are characterized by a high affinity for the antagonist pirenzepine and are present on sympathetic ganglia and in CNS areas such as the cerebral cortex (Birdsall *et al.*, 1984); M_2-receptors, which exhibit a low affinity towards pirenzepine, are present on peripheral effector organs, including the ileum and atria (Birdsall *et al.*, 1984). In addition, it has been proposed that muscarinic receptors present in the ileum may differ from those present in the atria, since antagonists such as 4-diphenylacetoxy-N-methyl piperidine methiodide (4-DAMP) and hexahydrosiladiphenidol exhibit a higher affinity for ileal receptors than atrial receptors (Barlow *et al.*, 1980; Barlow & Shepherd, 1985; Fuder *et al.*, 1985; Clague *et al.*, 1985).

McN-A-343, [4-(*m*-chlorophenylcarbamoyloxy) -2-butynyltrimethylammonium chloride] has been proposed as a selective M_1-agonist since it is a potent agonist at receptors in the sympathetic ganglia and has little or no activity at ileal or atrial receptors (Hammer & Giachetti, 1982). However, it is possible that differences in agonist potency may be the result of differences in intrinsic efficacy and/or receptor reserve (Kenakin, 1984) and this could account for the apparent selectivity observed with McN-A-343. The compound has been reported to act as a weak partial agonist at guinea-pig ileal and atrial receptors (van Rossum, 1962; Pappano & Rembish, 1971), and a full agonist at receptors on the taenia caeci (Hobbiger *et al.*, 1969). In the rat ileum, the compound acts as a competitive antagonist (van Rossum, 1962). Such behaviour is typical of an agonist of low intrinsic efficacy and this may reflect differences in the tissue receptor reserves (Kenakin, 1984; Mitchelson, 1984). Brown *et al.* (1980a) have reported that McN-A-343 is more efficacious at the muscarinic receptors in the superior cervical ganglia than at ileal muscarinic receptors. The receptor reserves, at least for muscarine, were very similar in the two preparations (Brown *et al.*, 1980a). However, it was unlikely that the affinity of McN-A-343 differed in the two tissues since the potency at receptors in the superior cervical ganglia (Brown *et al.*, 1980a) was very similar to the affinity at ileal receptors (van Rossum, 1962). The affinity of McN-A-343 for ganglionic muscarinic receptors was therefore, probably not greater than the affinity for ileal receptors. The reason for the higher efficacy was unknown. Hammer & Giachetti (1982) suggested that McN-A-343 was a selective M_1-agonist

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since the ganglionic tissues exhibited a high affinity towards pirenzepine (Brown et al., 1980b). However, it is unlikely that McN-A-343 selectively stimulates only M_1-receptors since the contractile response of the taenia caeci to McN-A-343 appears to be mediated by M_2-receptors (Mitchelson, 1984).

The aim of the present study was to examine the potency and affinity of McN-A-343 at a range of muscarinic receptors in order to determine the selectivity of the compound at muscarinic receptor subtypes.

Abstracts of this work have previously been communicated to the British Pharmacological Society (Eglen et al., 1985 a,b).

Methods

The composition of the physiological salt solution was as follows (mM): guinea-pig ileum, bladder and taenia caeci; 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, $CaCl_2 \cdot 6H_2O$ 1.8. The solution was gassed with O_2.

Guinea-pig atria and trachea; NaCl 118.4, KCl 4.7, $MgSO_4 \cdot 7H_20$ 1.2, KH_2PO_4 1.2, glucose 11.1, $NaHCO_3$ 25.0, $CaCl_2 \cdot 6H_2O$ 2.5. The solution was gassed with 5% CO_2/95% O_2.

All isolated tissue experiments were conducted at pH 7.4, 30°C. This temperature was chosen, since at 37°C it was observed that atrial responses to muscarinic agonists became erratic (Clague et al., 1985).

Isolated tissue experiments: agonist studies

Paired atria, proximal ilea, trachea, bladder and taenia caeci were removed from Dunkin-Hartley guinea-pigs (200–250 g body wt) and were suspended under 1.0 g tension. The tissues, with the exception of the taenia caeci, were place in 30 ml organ baths. Two cm segments of taenia caeci were placed in perspex chambers and superfused at a rate of 6 ml min^{-1}.

All tissues were allowed 60 min equilibration, before construction of a concentration-response curve to carbachol (1×10^{-8} to $1 - 10^{-4}$M). At least two such curves were undertaken before exposure of the tissues to McN-A-343 (1×10^{-8} to 1×10^{-4}M). Non-cumulative concentration-response curves were constructed for atrial, ileal and taenia caeci preparations, whilst cumulative agonist additions were used for the bladder and tracheal tissues. The two different methods were used because of the slow response time of the latter two tissues. In experiments on the ilea and taenia caeci, agonists were added for a period of 30 s on a 5 min dose cycle. In experiments on the atria, agonists were added for 3 min, also using a 5 min dose cycle. The negative chronotropic response in the atria was measured over the last 15 s of the agonist exposure period. In the bladder and trachea preparations, the responses were allowed to stabilize before addition of the next agonist concentration.

Isolated tissue experiments: antagonist studies

In experiments in which the affinities of the antagonists atropine, pirenzepine, 4-DAMP, silaprocyclidine and gallamine, were measured, carbachol was used as the agonist. In some experiments on ilea and atria, McN-A-343 was employed as an antagonist and again carbachol was used as the agonist.

In experiments on the taenia caeci, both carbachol and McN-A-343 were used as agonists and the affinity of pirenzepine was measured. In all experiments each concentration of antagonist was allowed to equilibrate for 45 min before repeating the concentration-response curve. The affinity of McN-A-343 for muscarinic receptors in this tissue was assessed by the method of Furchgott & Bursztyn (1967). The tissues were incubated with phenoxybenzamine (3×10^{-6}M, 20 min) to reduce the free receptor concentration. Preliminary experiments (not shown) showed that the concentration-response curve to carbachol was shifted (50 fold) to the right and the maximum reduced by approximately 15%. Phenoxybenzamine only inactivated muscarinic receptors under our experimental conditions, since incubation of the tissues with atropine (0.1 μM) completely protected against inactivation. The responses to McN-A-343 were abolished. The concentration-response curve to carbachol was then repeated after an equilibration period of 45 min with McN-A-343 (3×10^{-4}M). The concentration-response curve to carbachol was shifted further to the right in a parallel fashion. The dose-ratio was calculated, and the affinity for McN-A-343 was then derived (Gaddum, 1943).

Ligand binding studies

Membrane preparation EDTA washed (Cheung et al., 1982) membranes were used in all studies. Briefly, male Sprague-Dawley rats (150–300 g body wt) were stunned and whole hearts and cerebral cortices were removed and dissected free of vasculature and connective tissue over ice. Tissues were separately homogenized in 30 volumes (w/v) of ice cold Tris-EDTA buffer (5×10^{-2}M Tris; 5×10^{-3}M EDTA: pH 7.4 at 4°C), in a Polytron P10 tissue disrupter (setting 8, 2×10 s bursts). After filtration through a single layer of cheesecloth to remove tissue debris, the homogenates were centrifuged at 30,000 g_{av} for 15 min. The crude membrane pellets obtained were washed by resuspension (Polytron P10; setting 5, 1×10 s burst) in 30 volumes of ice-cold Tris-EDTA buffer, and centrifugation at 30,000 g_{av} for 15 min. After a further two such washes in Tris-EDTA assay buffer, the crude

membrane pellets were resuspended in Tris-EDTA assay buffer (pH 7.4 at 32°C) and used immediately in the binding studies or stored under liquid N_2.

Muscarinic binding assay In competition binding experiments, [^{3}H]-N methyl scopolamine ([^{3}H]-NMS) (K_d values for [^{3}H]-NMS in cardiac and cerebrocortical membranes were 1.67×10^{-10}M and 8.5×10^{-11}M respectively) was used as the ligand at 5×10^{-11}M. Under the experimental conditions employed, less than 10% of the radioligand was bound at equilibrium. All assays were conducted at 32°C in a fixed volume of 3 ml containing 5×10^{-2}M Tris, 5×10^{-4}M EDTA assay buffer (pH 7.4). Specific binding was defined using 1×10^{-6}M atropine. This concentration of atropine was sufficient to displace all specific binding of [^{3}H]-NMS even when high concentrations of this radioactive ligand (4×10^{-9}M) were used. Incubations were for 3 h after which the samples were filtered through Whatman GF/B glass fibre filters under 2.93 kPa vacuum. Filters were washed 3 times with 5 ml aliquots of assay buffer and the radioactivity retained on the filters was determined by liquid scintillation spectrophotometry. With the experimental procedures outlined above, saturation analysis revealed that [^{3}H]-NMS bound to apparently homogeneous populations of muscarinic binding sites in cardiac and cerebrocortical membranes. No evidence for a low affinity rapidly dissociating component of ligand binding was observed.

Measurement and analysis of results

Responses of the ileum, taenia caeci, bladder and trachea were measured as increases in baseline isometric tension. The taenia caeci preparations exhibited a level baseline upon which contractions to agonists were clearly distinguishable. The use of the superfusion method obviated the need to correct for artefactual changes in baseline activity due to washout procedures as previously reported by Hobbiger *et al.* (1969). Atrial responses were measured as changes in the rate of contraction.

The data were fitted to a logistic function by a non-linear iterative curve fitting procedure (Michel & Whiting, 1985) to calculate the EC_{50} and maximum response. The antagonist affinities ($-\log K_d$) were calculated using 3 to 4 antagonist concentrations by the method of Arunlakshana & Schild (1959) except in experiments with the taenia caeci when McN-A-343 was used as the agonist. In these studies, the concentration-response curve to McN-A-343 was repeated in the presence of one concentration of the antagonist pirenzepine. The dose-ratio was calculated and the affinity estimated by the Gaddum-Schild relationship (Gaddum, 1943).

The ligand binding data were analysed by iterative curve fitting techniques (Munson & Rodbard, 1980; Michel & Whiting, 1984).

Statistical differences were determined using Student's t test.

The following drugs were used: atropine, carbachol and gallamine (Sigma Chemical Co. Ltd.) Pirenzepine was obtained from Boots; phenoxybenzamine from S.K. and F.; 4-DAMP (4 diphenylacetoxy N methylpiperidine methiodide), silabenzhexol and McN-A-343 were synthesized by Dr R. Clark, Syntex Research, Palo Alto. [^{3}H]-N methyl scopolamine specific activity 72 Ci mmol^{-1}, was obtained from Amersham plc.

Results

Isolated tissue experiments: agonists

The potencies of carbachol and McN-A-343 at receptors present in the ilea, atria, bladder, trachea and taenia caeci are shown in Table 1. Carbachol exhibited similar potencies in the ilea, atria, and taenia caeci which were significantly ($P < 0.05$) higher than the potencies observed for this agonist at receptors in the trachea and bladder. In contrast, McN-A-343 (1×10^{-8} to 1×10^{-4}M) exhibited no agonist activity in the ileal, atrial, tracheal or bladder preparations. However, a response was observed in the taenia caeci where McN-A-343 acted as a full agonist in relation to carbachol (Figure 1).

Isolated tissue experiments: antagonists

McN-A-343 acted as a competitive antagonist over the concentration-range tested (1×10^{-4}–1×10^{-3}M) at muscarinic receptors present in the ileum and atria. The $-\log K_d$ values and the Arunlakshana-Schild slopes are shown in Table 2. The $-\log K_d$ values were similar and the Arunlakshana-Schild slopes did not differ significantly from unity ($P > 0.05$). These values are very similar to those calculated from the affinity of

Table 1 Potencies ($-\log EC_{50}$) of carbachol and McN-A-343 at muscarinic receptors in isolated preparations

Tissue	*Carbachol*	*McN-A-343*
Ileum	6.77 ± 0.08	No response
Atria	6.72 ± 0.11	No response
Bladder	5.89 ± 0.03	No response
Trachea	5.62 ± 0.05	No response
Taenia caecum	6.61 ± 0.08	5.14 ± 0.07

Values are mean ± s.e.mean, $n = 4$–6.

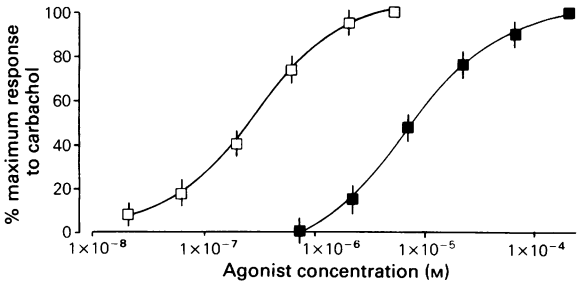

Figure 1 Concentration-response curves to carbachol (□) and McN-A-343 (■) at muscarinic receptors in the taenia caeci. Each point is the mean from 4–6 determinations, and the vertical bars represent s.e.mean.

Table 2 pK_d values for McN-A-343 at muscarinic receptors present in ileum, atria and taenia caeci

Tissue	*pK_d*	*Slope*
Ileum	4.46	0.98
Atria	4.75	1.00
Taenia caeci	4.42[a]	—

Values are mean, s.e.mean less than 5% in each case, $n = 4$.
[a]pK_d value ($-\log K_d$) calculated according to Furchgott & Bursztyn (1967), see methods.

McN-A-343 for muscarinic receptors present in the taenia caeci. (Table 2).

The $-\log K_d$ values obtained for the antagonists at muscarinic receptors present in ileum, atria, taenia caeci, bladder and trachea are shown in Table 3. Some of the values are taken from work previously published (Clague *et al.*, 1985), and this is indicated. Atropine exhibited similar $-\log K_d$ values in all preparations. The affinities obtained for 4-DAMP and silabenzhexol were significantly greater at receptors present on the ileum, taenia caeci, trachea and bladder than at receptors in the atria. In contrast, the $-\log K_d$ values obtained for gallamine were lower in the other tissues compared to the affinity obtained in the atria. Pirenzepine exhibited similar affinities at all tissues examined. The $-\log K_d$ value obtained in taenia caeci for pirenzepine when McN-A-343 was used as the agonist (6.86 ± 0.12, mean ± s.e., $n = 4$) was not significantly different ($P > 0.05$) from the value obtained when carbachol was used as the agonist (see Table 3).

Ligand binding studies

Under our experimental conditions, muscarinic receptors of cardiac membranes were exclusively of the M_2-subtype, as adjudged by the low affinity of pirenzepine ($K_I = 3.30 \times 10^{-7}$M, nH = 0.94) at the sites (Table 4). In contrast, muscarinic receptors of cerebral cortex appear heterogeneous since the displacement isotherms for pirenzepine were shallow (nH = 0.78) and could be resolved into two components (Table 4). From such an analysis (Table 4) approximately 74% of receptors in cerebral cortex were of the M_1-subtype (high affinity [$K_I = 1.28 \times 10^{-8}$M] for pirenzepine) whereas the remaining 26% of muscarinic receptors were of the M_2-subtype (low affinity [$K_I = 4.40 \times 10^{-7}$M] for pirenzepine). In rat cerebral cortical membranes, McN-A-343 (1×10^{-8}–1×10^{-2}M; 19 data points) displaced [^{3}H]-NMS binding with a pK_i value of 5.05 ± 0.11 (mean ± s.e.mean, $n = 4$). The Hill coefficient for this inhibition was close to unity

Table 3 Affinities ($-\log K_d$) at muscarinic receptors in isolated tissue preparations

Antagonist	*Ileum*	*Atria*	*Bladder*	*Trachea*	*Taenia caeci*
4-DAMP	9.04[b]	7.90[b]	8.92	9.10	8.73
	(8.59–9.49)	(7.51–8.30)	(8.47–9.37)	(8.64–9.56)	(8.29–9.17)
Silabenzhexol	8.90	7.52	8.51	8.63	8.55
	(8.46–9.36)	(7.14–7.90)	(8.08–8.94)	(8.20–9.06)	(8.12–8.98)
Gallamine	4.84[a,b]	5.84[a,b]	4.52[a]	4.41[a]	4.12[a]
	(4.60–5.08)	(5.60–6.08)	(4.29–4.75)	(4.19–4.63)	(3.91–4.33)
Pirenzepine	6.77[b]	6.60[b]	6.76	7.07	6.34
	(6.43–7.11)	(6.27–6.93)	(6.42–7.10)	(6.72–7.42)	(6.02–6.66)
Atropine	9.10[b]	8.87[b]	8.90	9.12	8.69
	(8.65–9.56)	(8.43–9.31)	(8.46–9.36)	(8.66–9.58)	(8.26–9.13)

Carbachol was used as the agonist in all cases. [a]Arunlakshana-Schild slope significantly ($P < 0.05$) less than unity. [b]Data previously published, Clague *et al.* (1985). Values are mean $-\log K_d$, (95% confidence limits) $n = 4$–6 preparations. 4-DAMP = 4-diphenylacetoxy-N-methylpiperidine methiodide.

(0.95) suggesting that McN-A-343 in contrast to pirenzepine, did not discriminate between M_1 and M_2 muscarinic receptors present in this preparation (Table 4). The inability of McN-A-343 to discriminate between M_1 and M_2 muscarinic receptors was also indicated by the close agreement between the affinity estimates for the compound in cardiac (predominantly M_2 muscarinic receptors) and cortical (predominantly M_1 muscarinic receptor) membranes (Table 4).

At the low concentrations of [^{3}H]-NMS (5×10^{-11}M) used in the present study, the Hill coefficients of McN-A-343 displacement isotherms at both cerebral cortical and cardiac muscarinic receptors were close to unity (Table 4).

When higher concentrations of radioligand (2×10^{-10}–1.8×10^{-9}M) were employed in an attempt to determine whether McN-A-343 was competitively inhibiting [^{3}H]-NMS binding in rat cardiac membranes, it was apparent that the compound was displacing non-specifically bound radioligand (Table 5). This phenomenon prohibited quantitative experimentation with radioligand concentrations greater than 1×10^{-10}M (data not shown).

Table 4 Binding of McN-A-343 and pirenzepine to membranes from cerebral cortex and myocardium

	Cortex		*Myocardium*	
	pK_i	nH*	pK_i	nH*
McN-A-343	5.05	0.95	5.22	0.89
Pirenzepine	7.36	0.78[a]	6.48	0.99

The values were determined from inhibition of binding of [^{3}H]-methyl scopolamine.
[a]Binding isotherm could be better described by a two-site model. 74% of receptors displayed high affinity ($pK_i = 7.64$) for pirenzepine, while the remaining 24% of muscarinic receptors exhibited low pirenzepine affinity ($pK_i = 6.36$).
*nH is the Hill coefficient of the displacement isotherm.
Values are mean, s.e.mean less than 10% in each case, $n = 4$.

Table 5 Inhibition of non-specifically bound [^{3}H]-N methyl scopolamine ([^{3}H]-NMS) by McN-A-343

Concentration of McN-A-343 (M)	*% inhibition of non-specifically bound [^{3}H]-NMS* *Concentration of [^{3}H]-NMS* (M)			
	3.3×10^{-11} (92%)†	2×10^{-10} (81%)†	5×10^{-10} (72%)†	1.8×10^{-9} (46%)†
1×10^{-2}	4 ± 1.9	30 ± 3.9*	50 ± 4.7*	52 ± 6.3*
1×10^{-3}	1 ± 2.2	10 ± 4.2*	31 ± 6.3*	25 ± 3.2*
1×10^{-4}	0	8 ± 3.1*	14 ± 3.1*	11 ± 2.1*

In these experiments non-specific binding of [^{3}H]-NMS to rat cardiac membranes (determined in the presence of 10^{-6}M atropine) was measured in the presence and absence of the indicated concentrations of McN-A-343.
*Significant ($P < 0.05$) inhibition of non-specific [^{3}H]-NMS binding.
†The values in parentheses represent specific binding as a percentage of total binding.
All concentrations are in mol litre^{-1}.

Discussion

McN-A-343 has been shown by a number of workers (see Introduction) to stimulate excitatory muscarinic receptors present on sympathetic ganglia and exert little or no agonist action at muscarinic receptors present in the ileum or atria. The muscarinic receptors present on the sympathetic ganglia have been shown to be more sensitive to pirenzepine ($-\log K_d = 8.4$) in comparison to ileal or atrial ($-\log K_d = 6.8$) receptors (Brown *et al.*, 1980b). These differences have been postulated to represent M_1- and M_2- receptor subtypes (Hammer & Giachetti, 1982) and McN-A-343 has been proposed as an M_1- selective agonist (Hammer & Giachetti, 1982; Wess *et al.*, 1984). The results obtained in this study, however, indicate that McN-A-343 is not a selective M_1-agonist either in terms of affinity using ligand binding, or potency, as determined by contractions of the taenia caeci.

Previous work (Roszkowski, 1961; Smith, 1966; Hammer & Giachetti, 1982; Wess *et al.*, 1984) has shown that a pressor response is observed in the pithed rat to McN-A-343. This action has been shown to be antagonized by low doses of pirenzepine, thus suggesting the presence of M_1-receptors (Hammer & Giachetti, 1982; Wess *et al.*, 1984). This conclusion was

supported by the lack of agonist action by McN-A-343 in the present study on the isolated ileum, atria, trachea or bladder. This was also in accordance with previous work using the rat superior cervical ganglion (Brown *et al.*, 1980a), although some authors have reported very weak partial agonist actions of McN-A-343 in the guinea-pig ileum and atria (van Rossum, 1962; Pappano & Rembish, 1971). The $-\log K_d$ values obtained with pirenzepine on the ileum, atria, trachea and bladder were consistent with previous work (see Mitchelson, 1984, for review) and suggested that the receptors may be denoted as M_2. However, a full agonist action of McN-A-343 was observed in the taenia caeci in the present study. Previously, Hobbiger *et al.* (1969) showed that McN-A-343 was an agonist in this tissue. The affinity of pirenzepine at muscarinic receptors in this tissue showed them also to be of the M_2-subtype and this was observed when either McN-A-343 or carbachol was used as the agonist. This is also in agreement with previous work (Mitchelson, 1984). Thus, McN-A-343 can stimulate both M_1- and M_2-receptors depending upon the preparation used, as previously reported by Gilbert *et al.* (1984).

The differences in potency observed with McN-A-343 may, in addition to receptor heterogeneity, reflect differences in either intrinsic efficacy and/or receptor reserve. Brown *et al.* (1980a) concluded that when muscarine was used as the agonist, the receptor reserve in the rat sympathetic ganglion was similar to that in the ileum. It is possible that the selective action of McN-A-343 at the former may be due to differences in intrinsic efficacy or receptor number and not the presence of M_1-receptors. The ability of McN-A-343 to stimulate the taenia caeci, in contrast to the ileum, atria, trachea or bladder also does not appear to be due to the presence of M_1-receptors. It is likely that differences in receptor reserve may account for this apparent selective action. It is well known that the potency of agonists, particularly agonists of low efficacy, is critically dependent upon the tissue receptor reserve (Kenakin, 1984). Similar conclusions have been drawn with regard to the ability of McN-A-343 to stimulate polyphosphoinositide hydrolysis (Brown *et al.*, 1985) and to induce gastric secretion *in vitro* (Black *et al.*, 1985).

Definitive evidence of receptor heterogeneity relies upon differences in either agonist or antagonist affinities (Kenakin, 1984). Thus, differences in the bindng affinities of pirenzepine to cerebral cortical and myocardial membranes have been reported (Birdsall *et al.*, 1984) to be consistent with the presence of M_1- and M_2-subtypes respectively, although this has been questioned (Roeske & Venter, 1984). Results supporting differential binding affinities for pirenzepine were obtained in this ligand binding study. However, McN-A-343 did not discriminate between the M_1- and M_2-receptor binding sites, since the dissociation constants were very similar. Birdsall *et al.* (1983) have indicated that McN-A-343 may be an allosteric modulator of the M_2-receptor, which may invalidate dissociation constants obtained in this study. However, attempts to examine the interaction of McN-A-343 with muscarinic receptors in more detail were complicated by the ability of McN-A-343 to displace non-specifically bound [^{3}H]-NMS. This reduction in non-specific binding (NSB) may have resulted from potential membrane pertubing properties of McN-A-343 which could have enhanced the dissociation of NSB during the filtration period. The finding that affinity estimates at the myocardial M_2-receptor were in reasonable agreement in functional studies where McN-A-343 was a competitive antagonist and in binding studies, would indicate that possible allosteric interactions of McN-A-343 with the muscarinic receptor did not influence the affinity estimates obtained in binding studies.

Assuming that McN-A-343 equilibrated with the receptor within the contact time of the agonist, then estimations of the affinity by functional Arunlakshana-Schild analysis showed that McN-A-343 acted as a competitive antagonist at atrial muscarinic receptors. Similar results were also observed at ileal muscarinic receptors and the $-\log K_d$ values were in good agreement with previous reports (van Rossum, 1962). These values were also similar to the $-\log K_d$ value of McN-A-343 for muscarinic receptors present in the taenia caeci, indicating further that the selective action in this tissue was not due to receptor heterogeneity. Thus, McN-A-343 appears not to be M_1-selective in terms of affinity, as determined by ligand binding, and as determined by contractions of the taenia caeci.

In vivo studies with McN-A-343 have also shown non-selective actions. Thus, the pressor action of McN-A-343 (Roszkowski, 1961), although marked in the rat, cat and dog, is virtually absent in the guinea-pig (Smith, 1966). However, this probably does not indicate a lack of M_1-receptors in the ganglia. McN-A-343, in contrast to *in vitro* data, inhibits intestinal motility in the cat and dog (Roszkowski, 1961; Smith, 1966) and contracts the urinary bladder (Saxena, 1972), indicating that its action is unrelated to the presence or absence of M_1/M_2-receptors. These *in vivo* effects support the contention of this study that the action of McN-A-343 is not due to selective stimulation of M_1-receptors. These data, plus the other actions of McN-A-343 such as nicotinic receptor stimulation (Roszkowski, 1961) and inhibition of noradrenaline uptake (Allen *et al.*, 1972), appear to limit the use of McN-A-343 in muscarinic receptor classification.

The antagonist affinity data obtained in this study further support the proposal that the M_2-subtype is heterogeneous (Barlow & Shepherd, 1985; Fuder *et al.*, 1985). These data indicate that smooth muscle

from the ileum, taenia caeci, trachea, bladder, anococcygeus muscle (Oriowo, 1983), and rectum (Akah & Oriowo, 1985) contain the same subpopulation of muscarinic receptors.

These data obtained in the present study indicate that McN-A-343 is not an M_1-selective agonist, and thus its use in the classification of muscarinic receptors is limited.

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