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Binding and functional profiles of the selective M_1 muscarinic receptor antagonists trihexyphenidyl and dicyclomine

A. Giachetti, E. Giraldo, H. Ladinsky & E. Montagna

Departments of Pharmacology and Biochemistry, Istituto De Angeli, Via Serio 15 - 20139 Milan, Italy

1 The selectivity profiles of the muscarinic receptor antagonists dicyclomine and trihexyphenidyl have been examined in binding and functional studies and compared with those of pirenzepine and atropine.

2 Dicyclomine, trihexyphenidyl and pirenzepine demonstrated the highest affinity for the M_1 muscarinic receptor subtype as revealed in competition experiments against [^{3}H]-pirenzepine labelling of cortical membranes. Their affinity values lay in a narrow range (3.7–14 nM) approaching that of atropine (1.6 nM).

3 Competition experiments against [^{3}H]-N-methylscopolamine in cardiac and glandular (salivary) membranes revealed differences between the drugs examined. Dicyclomine, trihexyphenidyl and pirenzepine displayed low affinity for the cardiac and intermediate affinity for the glandular receptors. Thus, the drugs appeared to discriminate between the M_1 (cortical) and the peripheral muscarinic subtypes (cardiac and glandular). However, atropine displayed similar affinities for either subtype with IC_{50}s varying only slightly (1.6–4.6 nM). The rank order of selectivity was: pirenzepine > dicyclomine > trihexyphenidyl > atropine.

4 Mirroring the binding data, pirenzepine, dicyclomine and trihexyphenidyl showed a tenfold greater ability at inhibiting M_1-receptor mediated ganglionic responses (McN A-343 pressor effect in pithed rats and nictitating membrane contraction in cats) than at inhibiting peripheral muscarinic responses in the heart and cardiovascular smooth muscle (vagal bradycardia in rats and cats and vagally-induced vasodilatation in cats).

5 The muscarinic antagonists so far examined can be categorized into two groups. Trihexyphenidyl, dicyclomine and pirenzepine, included in one group, are characterized by a higher affinity for the neuronal (M_1) muscarinic receptor, hence they antagonize functional responses mediated by the M_1 subtype. Atropine, a member of the other group, shows essentially no selectivity.

6 Differentiation of M_1 and peripheral muscarinic receptor subtypes appears to be a property not confined to tricyclics such as pirenzepine but shared by diverse chemical structures. Both trihexyphenidyl and dicyclomine appear to be useful pharmacological tools in the classification of muscarinic receptor subtypes.

Introduction

Heterogeneity among muscarinic receptors was first noted by Goyal & Rattan (1978) in the lower oesophageal sphincter, a preparation in which vagal stimulation and exogenous muscarinic agonists elicit opposite responses. Studies on agonist binding to membranes have confirmed the original observation demonstrating the presence of heterogeneous muscarinic receptor populations in discrete brain areas (Birdsall *et al.*, 1978) and in peripheral tissues. Perhaps the clearest evidence for subclassifying muscarinic receptors into subtypes, now termed M_1 and M_2, has been obtained with the use of the selective M_1 antagonist pirenzepine, a hydrophilic tricyclic compound which readily differentiates muscarinic subtypes owing to its markedly higher affinity for the M_1 receptor (Hammer *et al.*, 1980; Watson *et al.*, 1982).

Among the many muscarinic antagonists available, dicyclomine (Luber-Narod & Potter, 1983; Marchi & Raiteri, 1985), and trihexyphenidyl (Tien & Wallace, 1985), whose structures are unrelated to pirenzepine, have been shown to have features typical of M_1 antagonists, but a systematic investigation of their

biochemical and pharmacological properties has not been performed. It was of interest therefore to compare the *in vitro* binding characteristics of these muscarinic antagonists and to evaluate their properties in functional responses. Parallel experiments, performed with atropine and pirenzepine, have yielded a coherent picture on the selective profile of these known muscarinic antagonists.

This paper is an extension of results which have been communicated to the British Pharmacological Society (Giachetti *et al.*, 1982).

Methods

Male rats of the Sprague-Dawley strain (200–300 g; Charles River, Italy) and cats (3–4 kg; Al Serio Breeding, Italy) of either sex were used for these experiments.

In vitro *radioligand binding studies*

Rats were killed by cervical dislocation. Tissues were removed, cleaned and homogenized (w/v: cerebral cortex 1:200, submandibular gland 1:150, total heart 1:300) with an Ultra-Turrax at maximal speed for 30 s followed by a Potter-Elvehjem (15 strokes) in Na^+-Mg^{2+}-HEPES buffer pH 7.4 (100 mM NaCl, 10 mM $MgCl_2$, 20 mM HEPES) and filtered through two layers of cheesecloth.

Binding curves for the different compounds were derived indirectly from competition experiments against 0.5 nM [^{3}H]-pirenzepine ([^{3}H]-PZ) for the cerebral cortex and 0.3 nM [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) for the submandibular gland and heart. One millilitre of homogenate was incubated for 45 min at 30°C (conditions in which equilibrium was achieved, as determined by association experiments) in the presence of the marker ligand and different concentrations of the cold ligand. The incubation was terminated by centrifugation (12,000 r.p.m. for 3 min) at room temperature in an Eppendorf microcentrifuge. The resultant pellet was washed with 2 × 1.5 ml of saline to remove the free radioactivity and the final pellet was allowed to drain. The tips of the tubes containing the pellet were cut off, 200 µl of tissue solubilizer (Lumasolve, Lumac) added and the mixture left to stand overnight. Radioactivity was then counted after the addition of 4 ml of liquid scintillation mixture (Dimilume/Toluene 1:10 v/v, Packard).

Assays were carried out in triplicate and the non-specific binding was defined as the radioactivity bound or entrapped in the pellet when the incubation medium contained 1 µM 3-quinuclidinyl-benzilate (QNB) in [^{3}H]-NMS experiments and 1 µM atropine in [^{3}H]-PZ experiments.

Corrected IC_{50} values were obtained by non-linear least squares regression analysis on the basis of a one binding site model with a TOPFIT-pharmacokinetic programme package (Heinzel, 1982) and corrected to allow for competition with the radioligand using the equation: corr. $IC_{50} = IC_{50}/(1 + [*C]/*K_D)$ where [*C] and $*K_D$ represent the concentration and the dissociation constant of the radioligand used, respectively (Cheng & Prusoff, 1973). The K_Ds for NMS were 0.25 nM for the submandibular gland (Hammer *et al.*, 1980) and 0.6 nM for the heart (data from this laboratory). For PZ, the K_D value was found to be 14 nM for labelling of the M_1-receptor subtype in the cerebral cortex under our experimental conditions.

Hill coefficients (n_H) were calculated by linear regression analysis and analysed for statistical deviation from unity.

Pharmacological experiments

Rats were lightly anaesthetized with ether; after cannulation of the trachea they were pithed by introducing a sharp-pointed rod from the left orbit through the atlas. The rod penetrated about 3 cm into the spinal cord. Artificial respiration was provided immediately by means of a Bird respirator (80 strokes min^{-1}). A femoral vein and the left carotid artery were cannulated for the administration of drugs and the recording of arterial pressure, respectively. Heart rate was calculated from ECG recordings. The right vagus nerve was stimulated by placing bipolar platinum electrodes around the peripheral stump. The characteristics of stimulation were: 10 V (supramaximal), 10 Hz, 2 ms duration, for 30 s. Antagonists were given intravenously 2 min before vagal or McN-A-343-induced stimulation. In experiments investigating displacement of McN-A-343 responses, a full agonist pressor-response curve was first established (5 geometrically-spaced doses) and repeated 5 min after antagonist administration. Three to four doses of each antagonist were examined.

Cats were anaesthetized with chloralose (80 mg kg^{-1} i.v.). Cannulae were placed in the trachea (spontaneous respiration), in a femoral vein for the administration of drugs and in the contralateral femoral artery for measuring arterial blood pressure by means of a pressure transducer (Bell & Howell). Mean blood pressure was recorded on a Devices polygraph. Heart rate was calculated from the ECG recording. The contractions of the nictitating membrane (usually left) were measured isometrically by a force displacement transducer (Devices UF1) and recorded on a Devices polygraph. Initial tension was usually 5 g. Contractions were elicited either through preganglionic sympathetic trunk stimulation (5 V, 2 Hz, 0.7 ms duration for 15 s) or by intra-arterial injection of McN-A-343 delivered to the superior cervical ganglion via a cannulated lingual artery.

During the injection of the agonist (50 µl), both the common carotid and the external carotid arteries were temporarily clamped. The severed right vagus was stimulated with platinum hook electrodes placed around the peripheral stump. Pulses of 10 V, 10 Hz, 2 ms duration for 30 s were delivered from a Grass S48 stimulator. Antagonists were administered intravenously 2 min before applying agonist or nerve stimulation.

ID_{50}s were calculated by linear regression analysis of responses expressed as percentage inhibition.

DR_2 values, i.e. the dose of antagonist causing a two fold shift of the agonist dose-response curve, were obtained from the intercept on the abscissa scale of the regression line relating the log dose of antagonist to the log of the dose-ratio -1.

Drugs

[^{3}H]-PZ (84 Ci mmol^{-1}) and [^{3}H]-NMS (84.8 Ci-mmol^{-1}) were purchased from New England Nuclear, Boston, MA, U.S.A. The sources of other compounds were: HEPES (Sigma, U.S.A.), atropine sulphate (BDH, U.K.), trihexyphenidyl hydrochloride (Serva, W. Germany), (±)-dicyclomine hydrochloride (MAG, Italy), pirenzepine dihydrochloride and (±)-QNB (Thomae, W. Germany), chloralose (Merck, W. Germany). McN-A-343 (4-[*m*-chlorophenylcarbamoyloxy]-2-butynyltrimethyl ammonium chloride) was synthesized by Dr M. Gil, Dept. of Chemistry, Istituto De Angeli, Italy. Istituto De Angeli, Italy.

Results

In vitro *radioligand binding studies*

The binding curves of pirenzepine, trihexyphenidyl, dicyclomine and atropine to the M_1 muscarinic subtype of the cerebral cortex were obtained in competition experiments against a concentration of [^{3}H]-PZ (0.5 nM) far below the K_D value (Watson *et al.*, 1982).

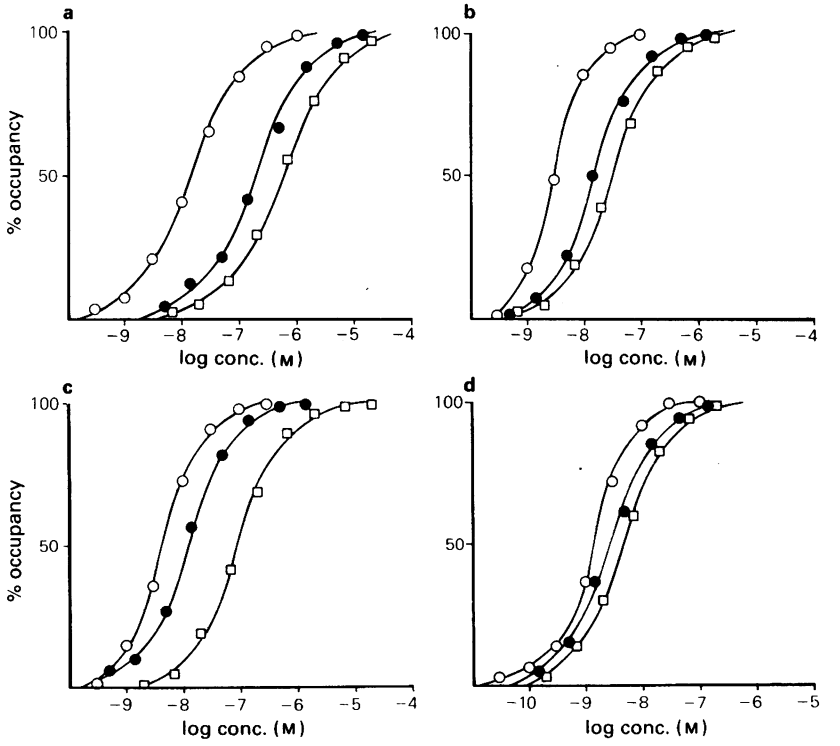

Figure 1 Concentration-occupancy curves of the binding of selective muscarinic antagonists, (a) pirenzepine, (b) trihexyphenidyl, (c) dicyclomine, and (d) atropine in different tissues (○, cerebral cortex; ●, submandibular gland; □, whole heart). The free concentrations of the drugs are shown on the abscissa scale. For the cerebral cortex, data were obtained in competition experiments against 0.5 nM [^{3}H]-pirenzepine which specifically labels the M_1 subtype. For the submandibular gland and heart, 0.3 nM [^{3}H]-N-methylscopolamine was used. The data are the means of two independent experiments, each performed in triplicate, which differed from each other in IC_{50} values by less than 20%.

The binding curves of the same drugs for the muscarinic receptors of submandibular gland and heart were obtained using [^{3}H]-NMS (Figure 1). The affinity values and Hill coefficients calculated from the binding data are given in Table 1. Pirenzepine, trihexyphenidyl and dicyclomine discriminated between muscarinic receptors in the different organs and showed similar selectivity patterns. The compounds displayed the highest affinity for the muscarinic receptors in the cerebral cortex with their affinity values varying only narrowly (3.7–14 nM). Although they exhibited intermediate affinity for the submandibular gland, there was greater disparity in their corr. IC_{50} values (17, 13 and 210 nM for trihexyphenidyl, dicyclomine and pirenzepine, respectively). The compounds displayed the lowest affinity for the muscarinic receptor of the heart with large differences in their corr. IC_{50} values (31 and 95 nM for trihexyphenidyl and dicyclomine, respectively; 500 nM for pirenzepine). Dicyclomine was unique in showing an almost 8 fold difference in selectivity between the glandular and heart subtypes. As is now well known, atropine presented essentially no selectivity for the different muscarinic receptors. This is illustrated by the close apposition of its binding curves in the three tissues (Figure 1) and the narrow, albeit potent, affinity range (1.6–4.6 nM). The affinity ratios between heart and cerebral cortex, shown in Table 1, give an estimate of the selectivity of the drugs. The rank order of selectivity was: pirenzepine>dicyclomine > trihexyphenidyl > atropine.

In the submandibular gland and heart, linear regression analysis of the concentration-occupancy curves for antagonists indicated that the experimental data followed the law of mass action ($n_H \simeq 1$; Table 1). In contrast, in the cerebral cortex, except for pirenzepine ($n_H \simeq 1$), the other three compounds had n_H values ranging from 1.16 to 1.37 which were significantly different from unity. This behaviour might be related to the high affinity that these compounds show for the M_1 muscarinic receptor in the cerebral cortex, i.e. appreciable amounts are bound at low concentrations so that the free concentration values given on the abscissa scale are distorted by compound depletion.

McN-A-343 pressor action in the pithed rat

Intravenous administration of the muscarinic ganglionic stimulant McN-A-343 in the pithed rat caused a small decrease of mean blood pressure followed by a sharp pressor response. This latter effect, which is thought to be mediated through the M_1 subtype (Hammer & Giachetti, 1982), was dose-related within the range 30 to 350 $\mu g\,kg^{-1}$ (Figure 2). The muscarinic antagonists were tested for their ability to inhibit the pressor response of a dose of McN-A-343 (300 $\mu g\,kg^{-1}$ i.v.) which increased blood pressure by 90% of maximal (Δ 95 ± 6 mmHg). All caused a dose-related reduction of the vasoconstrictor action of the agonist. Their potencies, expressed as ID_{50}s, are shown in Table 2. The ID_{50}s of pirenzepine and atropine were similar (12 and 8.7 $\mu g\,kg^{-1}$, respectively) while those of trihexyphenidyl and dicyclomine (54 and 418 $\mu g\,kg^{-1}$, respectively) indicated a 5–35 fold weaker inhibitory action. The nature of the antagonism exerted by the muscarinic antagonists was defined by the shifts in the dose-response curves to McN-A-343 in the presence of various doses of each antagonist. An experiment depicting the shifts produced by graded doses of pirenzepine is seen in Figure 2. This compound, as well as the other antagonists, produced parallel rightward shifts without depressing the maximum. Schild plots (Arunlakshana & Schild, 1959) of these data are shown in Figure 3. With the limitations inherent in *in vivo* assays, in which equilibrium conditions may not be reached, all the muscarinic antagonists examined acted as competitive antagonists. Calculations of DR_2, that is, the dose required to produce a two fold rightward shift in the agonist dose-response curve,

Table 1 Binding profiles of selective muscarinic antagonists and atropine in the cerebral cortex, submandibular gland and whole heart of the rat

	Cerebral cortex		*Submandibular gland*		*Heart*		
	IC_{50} (nM)	n_H	IC_{50} (nM)	n_H	IC_{50} (nM)	n_H	*Ratio (heart/cortex)*
Pirenzepine	14	0.95	210	0.91	500	0.91	36
Trihexyphenidyl	3.7	1.37*	17	1.08	31	1.02	8
Dicyclomine	5.1	1.22*	13	1.09	95	1.02	19
Atropine	1.6	1.16*	2.8	1.01	4.6	0.99	3

The corrected IC_{50}s shown, and n_H values were calculated as described in Methods from the data points of Figure 1. The values in the last column show the ratios between the corrected IC_{50} values of the heart and cortex. Standard errors for IC_{50} and n_H values were less than 10% of the mean values shown. The tissue equivalents of the membrane preparations were (in mg wet weight per tube): heart, 3.3; cerebral cortex, 5; submandibular gland, 6.7.
*Significantly different from 1 ($P < 0.05$).

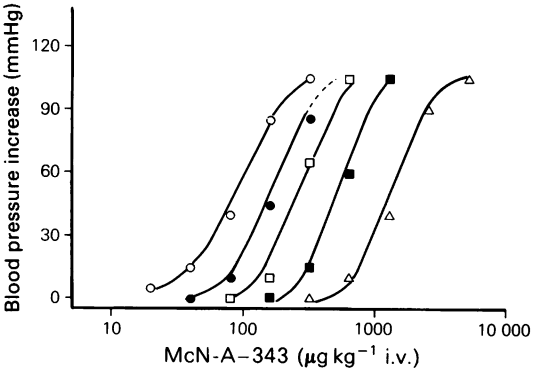

Figure 2 Displacement of the pressor dose-response curve to McN-A-343 by pirenzepine in the pithed rat. Pirenzepine was administered 5 min before McN-A-343 at the following doses (μg kg^{-1} i.v.): (○), 0; (●), 3; (□), 10; (■), 30; (△), 100.

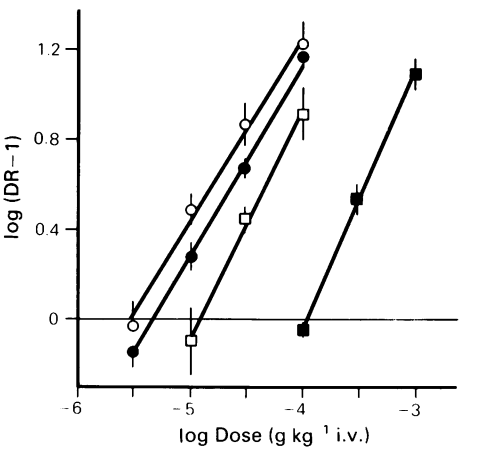

Figure 3 Schild plots of the antagonism exerted by the muscarinic antagonists on the McN-A-343-induced pressor responses in the pithed rat. Displacement of the dose-response curves to the agonist were obtained with 3–4 doses of the antagonists. Slopes (±95% confidence limits) of regression lines were: pirenzepine 0.86 (0.76–0.97) (●); trihexyphenidyl 1.02 (0.62–1.42) (□); dicyclomine 1.15 (0.97–1.33) (■); atropine 0.82 (0.63–1.09) (○). Each point is the mean of 5 animals and vertical lines show s.e.mean.

provided the following values (μg kg^{-1}, with 95% confidence limits): pirenzepine 4.7 (3.8–5.8); trihexyphenidyl 11.9 (7.0–20.2); dicyclomine 108 (86–135); atropine 2.9 (1.7–4.9).

The DR_2 estimates, particularly for trihexphenidyl and dicyclomine, were lower than the ID_{50} values, a finding which is not unexpected since determination of ID_{50} is not corrected for competition with the agonist. However, the order of potency of muscarinic antagonists was the same with either procedure employed.

Vagally-induced bradycardia in the pithed rat

Stimulation of the peripheral vagus in the pithed rat caused a marked bradycardia (from 250 ± 7 to 80 ± 13 beats min^{-1}, $n = 20$). All four muscarinic antagonists examined reduced this effect with their antagonistic potencies varying by as much as 3 orders of magnitude (Table 2). Whereas atropine was equipotent in reducing vagal bradycardia and McN-A-

Table 2 Inhibition by muscarinic antagonists of McN-A-343-induced pressor responses and vagally-induced bradycardia in the pithed rat

	ID_{50} (μg kg^{-1} i.v.)		
Antagonist	*Ganglionic stimulation* (McN-A-343 pressor effect)	*Vagal stimulation* (bradycardia)	*Ratio vagal/ganglionic*
Pirenzepine	12 (10–14)	475 (380–594)	40
Trihexyphenidyl	54 (40–74)	313 (184–533)	6
Dicyclomine	418 (321–542)	3089 (1952–4887)	7
Atropine	8.7 (7.6–9.9)	6.9 (6.1–7.7)	0.8

Values are means and 95% confidence limits (in parentheses) derived from 5 animals. The last column shows the ratios of the ED_{50}s between the vagal and ganglionic responses. ID_{50} values were obtained from the parallel shift of inhibition curves with 3–4 points on each line.

343 pressor activity (ID_{50}s of 6.9 and 8.7 $\mu g\,kg^{-1}$, respectively), the other antagonists weakly inhibited the atrial slowing with ID_{50}s ranging from 313 $\mu g\,kg^{-1}$ for trihexyphenidyl to 3089 $\mu g\,kg^{-1}$ for dicyclomine. The ratio between the ID_{50}s of the compounds in antagonizing the vagal bradycardia and the McN A-343 pressor increase, shown in Table 2, provided an estimate of their selectivity for the M_1 subtype. Pirenzepine showed the greatest discrimination between the muscarinic-mediated responses, its ratio being 40, while trihexyphenidyl and dicyclomine showed moderate discrimination and displayed equal but less selectivity. As expected, atropine did not discriminate between the two responses.

Ganglionic stimulation by McN-A-343 in cats

Small doses of McN-A-343 (10 μg) injected directly into the superior cervical ganglion of anaesthetized cats evoked submaximal contractions of the nictitating membrane. The responses to McN-A-343, which corresponded to approximately 50% of those elicited through stimulation of the preganglionic sympathetic nerves, resulted from muscarinic activation as denoted by their insensitivity to hexamethonium (data not shown).

The muscarinic antagonists inhibited the McN-A-343-induced responses in a dose-related fashion. Calculation of the ID_{50}s showed that atropine and pirenzepine were equipotent (1.9 vs 2.2 $\mu g\,kg^{-1}$ i.v.) whereas trihexyphenidyl and dicyclomine were 10 to 100 times weaker (Table 3) at inhibiting these responses.

Vagally-induced bradycardia and vasodilatation in cats

The muscarinic antagonists showed a wide range of potencies in preventing the decrease of heart rate and hypotension caused by near maximal stimulation of the vagus in cats. Atropine antagonized both vagal effects in a dose-dependent manner with potencies (ID_{50}s 3.6 and 2.1 $\mu g\,kg^{-1}$) of the same order of magnitude as that for inhibiting ganglionic stimulation (Table 3). In contrast, the other antagonists inhibited either action of the vagus at markedly higher doses than those required to reduce the ganglionic effects of McN-A-343 (Table 3). The ratio of the ID_{50}s calculated for vagal bradycardia and ganglionic responses, an index of selectivity, was again highest for pirenzepine (90) and lowest for dicyclomine (6).

Discussion

The characterization of muscarinic receptor subtypes is currently the focus of renewed interest and lively debate (Eglen & Whiting, 1985; Birdsall & Hulme, 1986). As in other systems where potent selective antagonists helped to define the boundaries between subtypes, the classification of the muscarinic receptor has depended significantly on the discriminative properties of the tricyclic antagonist pirenzepine. High affinity for this ligand has identified the M_1 subtype, whereas low affinity has typified the M_2 subtype.

This classification, which has been valuable in understanding the heterogeneity of the receptor, is

Table 3 Inhibition by muscarinic antagonists of superior cervical ganglion stimulation by McN-A-343 and of vagal effects (bradycardia and vasodilation) in the anaesthetized cat

	ID_{50} ($\mu g\,kg^{-1}$ i.v.)			
	Ganglionic stimulation	*Vagal responses*		*Ratio*
Antagonist	(McN-A-343-evoked contraction of nictitating membrane)	Bradycardia	Vasodilatation	*vagal/ganglionic*
Pirenzepine	2.2 (1.7–3.0)	198 (147–267)	205 (155–271)	90
Trihexyphenidyl	27 (22–34)	257 (128–514)	226 (83–620)	9
Dicyclomine	284 (223–360)	1820 (1597–2073)	1655 (1379–1987)	6
Atropine	1.9 (1.6–2.3)	3.6 (3.0–4.3)	2.1 (1.5–2.8)	1

Values shown are means and 95% confidence limits (in parentheses) derived from 5 animals. The last column shows the approximate ratios of the ID_{50}s of the vagal and ganglionic responses.

at present inadequate to explain the accrued evidence. The M_2 subtype appears heterogeneous in both functional and biochemical studies. In fact, cardiac muscarinic receptors differ so extensively from those of smooth muscle and exocrine glands in their properties as to suggest the existence of distinct subtypes (Barlow *et al.*, 1976; Stockton *et al.*, 1983; Mutschler & Lambrecht, 1984; Anwar-ul *et al.*, 1986; Giachetti *et al.*, 1986; Hammer *et al.*, 1986). However, since our study is mainly concerned with drugs interacting with the M_1 subtype, the receptors which display low affinity for pirenzepine are generically termed peripheral muscarinic subtypes in the context of this paper.

The results demonstrate that trihexyphenidyl and dicyclomine discriminate between the M_1 and the peripheral muscarinic receptors. Both compounds displaced radioligands from membranes derived from various tissues in a manner similar to that of pirenzepine: i.e. high affinity for cortical M_1 muscarinic receptors and low affinity for the peripheral receptors of glands and heart. The ability of trihexyphenidyl and dicyclomine to discriminate between the M_1 and peripheral subtypes was less than pirenzepine, but distinctly greater than atropine.

Functional studies further emphasized the similarities between trihexyphenidyl, dicyclomine and pirenzepine. They showed that these drugs inhibit responses mediated by the M_1 subtype in sympathetic ganglia in preference to those mediated by peripheral subtypes in the heart and blood vessels. It appears that the muscarinic antagonists, so far examined, fall into two groups. One contains trihexyphenidyl, dicyclomine and pirenzepine, being characterized by a high affinity for the neuronal muscarinic receptor both in biochemical and functional assays. The other group, represented by atropine, and presumably by many of its congeners, displays an undifferentiated type of interaction, hence demonstrating similar affinities for muscarinic receptors of various organs.

Several findings of this study deserve further comment. The potency of trihexyphenidyl and dicyclomine in displacing [^{3}H]-pirenzepine from cortical binding sites was about 3 fold higher than that of pirenzepine, closely approaching that of atropine. A still higher potency relative to pirenzepine, 5 and 15 fold, respectively, was found for dicyclomine and trihexyphenidyl in displacing [^{3}H]-NMS from cardiac membranes. These differences reflect the lower discrimination between receptor subtypes exhibited by the two drugs. The ratio between the IC_{50}s in the heart and cortex indicates the ability of each compound to discriminate between the peripheral receptors and the M_1 subtype of the cortex, although analysis of Table 1 illustrates the previously mentioned differences existing within the peripheral subtypes. Among the M_1 receptor antagonists examined dicyclomine, for example, shows a lower affinity for the cardiac muscarinic receptor relative to the glandular one, thus revealing substantial (7–8 fold) selectivity between the two.

A comparison of the results obtained in the *in vitro* binding studies with those from the *in vivo* pharmacological assays reveals a very large discrepancy between the potency of trihexyphenidyl and dicyclomine in displacing radioligands and in inhibiting either of the muscarinic receptor-mediated responses. For example, both drugs were 1–2 orders of magnitude weaker than pirenzepine in suppressing McN-A-343-induced responses. These changes in potency may be explained by the different pharmacokinetic characteristics of the compounds investigated. Whereas pirenzepine is an exceptionally hydrophilic drug which is poorly metabolized (Eberlein *et al.*, 1977; Hammer & Koss, 1978), both trihexyphenidyl and dicyclomine are noted for their lipophilic character, possessing an oil/water partition coefficient several orders of magnitude higher than that of pirenzepine (Giachetti, unpublished). The reduced potency in whole animal experiments may be then attributable primary to a loss of drug from the receptor compartment in favour of lipid structures. These pharmacokinetic considerations do not seem to affect the ability of trihexyphenidyl and dicyclomine to recognize subtypes, since their discriminative property was quantitatively similar whether evaluated in binding or in functional studies.

Recently, the use of McN-A-343 as a selective M_1-receptor agonist has been questioned on the grounds that its selectivity may arise from differences in receptor reserve in various tissues (Eglen *et al.*, 1985). This agent may indeed possess low selectivity coupled with low intrinsic efficacy. However, whatever the mechanism of action of McN-A-343 may be, its utility as a tool to stimulate selectively sympathetic ganglia is sound, since there is evidence for a very high density of the M_1 subtype in ganglionic neurones as demonstrated by ligand binding studies (Hammer & Giachetti, 1982; Giraldo *et al.*, 1985) and autoradiography (Yamamura *et al.*, 1984).

An emerging issue in the classification of the muscarinic receptor system is whether the subtypes readily recognized by pirenzepine represent intrinsic differences in receptor proteins. An alternative hypothesis attributes to pirenzepine the ability, unique among antagonists, to induce conformational changes of the same basic protein. These constraining changes would be imposed by the microenvironment surrounding the receptor and vary in different tissues (Berrie *et al.*, 1986). The origin of subtypes would then be linked to membrane components accessory to the receptor protein which are peculiar for each tissue.

Our approach in trying to learn more about this phenomenon was to examine the behaviour of compounds chemically unrelated to pirenzepine. We re-

asoned that it would be difficult, although not impossible, for chemically diverse moieties to trigger in the receptor protein identical conformational changes. Although these studies do not allow an unequivocal answer to this problem, they nonetheless provide an interesting clue to the origin of subtypes. The evidence points to the existence of different proteins endowed with specific characteristics.

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