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Action of agonists and antagonists at muscarinic receptors present on ileum and atria *in vitro*

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1 The action of 'selective' agonists and antagonists at muscarinic receptors mediating ileal contractions, and the rate and force of atrial contractions has been assessed.

2 The effect of nicotinic receptor stimulation, catecholamine release and acetylcholinesterase (AChE) action on muscarinic activity has also been assessed.

3 The nicotinic actions of carbachol did not affect its agonist potency nor the antagonist affinity data obtained when this agonist was used in atrial and ileal preparations.

4 Antagonist data indicated that muscarinic receptors mediating the rate and force of atrial contractions did not differ. Differences in agonist potencies at these two muscarinic receptors were attributable to either differences in intrinsic efficacy or susceptibility to the action of acetylcholinesterase. The small differences in agonist potency observed between atrial and ileal muscarinic receptors were considered not sufficient to indicate receptor heterogeneity.

5 The pirenzepine affinity data indicated that all three receptors are of the M_2 type. Affinity data using secoverine and 4-diphenyl-acetoxy-*N*-methyl piperidine methiodide indicated that ileal and atrial muscarinic receptors differ. Data obtained using gallamine, pancuronium and stercuronium cannot be regarded as indicative of receptor affinity since the antagonism is not competitive; it did nonetheless corroborate the conclusion that ileal and atrial muscarinic receptors are different.

Introduction

Muscarinic receptors are currently classified with respect to their affinity for pirenzepine (Hammer & Giachetti, 1982). Those muscarinic receptors exhibiting a high affinity towards pirenzepine are denoted M_1, whilst those exhibiting a low affinity towards pirenzepine are denoted M_2 (Hirschowitz *et al.*, 1984). M_1-receptors are considered to be located almost exclusively on neural tissue, whilst M_2-receptors exist on both neural tissues, such as the cerebellum, and peripheral effector organs (Hammer *et al.*, 1980). However, it has also been proposed that muscarinic receptors present in the periphery do not form a heterogeneous population.

Barlow *et al.* (1976) have proposed that ileal and atrial muscarinic receptors differ, since antagonists such as 4-diphenyl-acetoxy-*N*-methyl piperidine methiodine (4-DAMP) are more selective for ileal muscarinic receptors. Mutschler & Lambrecht (1984) have also identified a series of antagonists derived from procyclidine and difenidol which are ileoselective. Antagonists such as gallamine, pancuronium and stercuronium exhibit the converse selectivity in that they are more selective for atrial muscarinic receptors (Mitchelson, 1984). However, the antagonist properties of these compounds are complicated by allosteric interactions and are, therefore, non-competitive in nature (Birdsall *et al.*, 1984).

Barlow *et al.* (1980), using data derived from agonist studies, have proposed that atrial muscarinic receptors mediating negative inotropic and chronotropic effects differ. This has been supported by Chaissing *et al.* (1984) and Mutschler & Lambrecht (1984).

However, these functional differences in atrial and ileal muscarinic receptors have not been confirmed by binding studies using pirenzepine (Hammer *et al.*, 1980), 4-DAMP, gallamine or pancuronium (Choo & Mitchelson, 1984).

The aim of this study was to assess, under standard conditions, the affinity and potency of a wide range of 'selective' and 'non-selective' muscarinic agonists and antagonists at muscarinic receptors mediating ileal contractions, and the rate and force of atrial contractions.

Abstracts of this work have previously been presented to the British Pharmacological Society (Clague *et al.*, 1985; Eglen *et al.*, 1985).

Methods

To reduce variation, all experiments were conducted at pH 7.4, 30°C. This temperature was chosen since it was observed that at 37°C atrial responses became erratic after 1–2 h. Similar observations have been made by Barlow *et al.* (1976). It was found that it was not possible to use identical physiological salt solutions for all experiments and the composition of those used are given below.

Tissue preparation

All tissues were prepared according to methods previously described (Edinburgh Staff, 1974). Paired atria and proximal portions of ileum were removed following cervical dislocation from Dunkin-Hartley guinea-pigs (female, 200–300 g bodyweight). The rectus abdominis muscle was removed from frogs (male, *Rana pipiens*, 20 g bodyweight), previously stunned and pithed.

Ileum

Portions of ileum (1.5 cm) were washed and suspended under 1 g tension in the modified Krebs bicarbonate buffer. One hour was allowed for equilibration and non-cumulative concentration-response curves to carbachol were then constructed. Carbachol was added for 30 s in a 5 min time cycle, during which time the tissues were washed twice. The responsiveness of each piece of tissue was assessed by constructing concentration-response curves until reproducible responses were obtained, allowing 45 min between each curve.

Agonist potency ($-\log EC_{50}$) and maximum response obtained were determined using one agonist, in addition to carbachol, on each piece of tissue. Antagonist affinities were determined by constructing concentration-response curves to carbachol at 3–4 concentrations of antagonist, allowing 45 min equilibration at each concentration.

Electrically paced atria

Paired atria were suspended under 1 g tension in the modified Krebs bicarbonate buffer. Tissues were electrically paced, using platinum punctate electrodes (4 Hz, 2–4 ms duration, supramaximal voltage). The electrical pacing conditions were chosen to override any spontaneous rhythm due to pacemaker activity and only changes in contractile force were apparent. One hour equilibration was allowed before the construction of non-cumulative concentration-response curves to carbachol. This agonist was added for 3 min using a 5 min time cycle. Tissue responses were measured during the last 15 s of the carbachol exposure period. In a similar manner to the ileum, the tissue responsiveness was checked by constructing concentration-response curves until reproducible responses were obtained. An interval of 45 min was allowed between each curve. Agonist potencies, maximum response and antagonist affinities were determined as described for the ileum.

In both the ileal and atrial preparations responses were measured isometrically.

Spontaneously beating atria

The procedure used was identical to that described using electrically paced atria, except that tissues were allowed to beat spontaneously. Only changes in rate of contraction were measured, except in those experiments using physostigmine, where both rate and force (change in isometric tension) were determined simultaneously.

Rectus abdominis muscle

The rectus abdominis muscle was bisected and placed under 1 g tension in frog Ringer solution. Cumulative concentration-response curves to carbachol were constructed following an equilibration period of 1 h. Cumulative concentration-response curves were used since the response time of this tissue was slow (>5 min). Once reproducible curves to carbachol were attained, the potency and maximum response of other muscarinic agonists were measured. An interval of 45 min was allowed between each concentration-response curve during which time the resting tension was increased to 1.5 g to facilitate relaxation. This was restored to 1 g before the construction of each curve. Responses were measured isometrically.

Effect of hexamethonium on carbachol potency

The potencies of carbachol and nicotine were assessed at ileal muscarinic receptors before and after exposure to hexamethonium (1×10^{-4} mol l^{-1}) for 45 min.

Effect of reserpine treatment on carbachol potency

Guinea-pigs were depleted of catecholamines by pretreatment with reserpine (2 mg kg^{-1} twice daily, s.c.) and ileum and atria were removed 12 h after the final dose. The extent of the effectiveness of the reserpine treatment was assessed with tyramine. The potency of carbachol was assessed by comparing concentration-response curves at ileal and atrial muscarinic receptors in tissues from control and reserpine-treated animals.

Effect of acetylcholinesterase inhibition on muscarinic agonist potency at atrial muscarinic receptors

The potencies of the agonists were determined in the absence and presence of physostigmine

(5×10^{-7} mol l^{-1}). Rate and force of atrial contractions were determined simultaneously in each preparation.

Measurement of responses

Tissue responses were measured as changes in isometric tension in the case of the ileum, electrically paced atria and rectus abdominis, or as changes in rate in the case of spontaneously beating atria. The responses were then calculated as a percentage of the maximum response obtained to carbachol and plotted against the logarithm of the agonist concentration.

Analysis of results

Agonists

The agonist potency ($-\log EC_{50}$) was determined by a non-linear iterative curve fitting procedure (Michel & Whiting, 1984).

Antagonists

The antagonist affinity (pA_2) was calculated according to the method of Arunlakshana & Schild (1959).

Three concentrations of antagonist were tested on each piece of tissue. The dose-ratios were calculated for each concentration and for each tissue. All the data were then pooled for each antagonist. The intercept on the abscissae and slope of the Arunlakshana and Schild plots were determined using linear regression by the method of least squares.

Statistical analysis

Statistical differences were determined by use of Student's *t* test adapted for a microcomputer (Barlow, 1983).

Physiological salt solutions

The composition of the physiological salt solutions was as follows (mmol l^{-1}): guinea-pig ileum; NaCl 136.89, KCl 2.68, $MgCl_2.6H_2O$ 1.05, $NaH_2PO_4.2H_2O$ 0.42, glucose 5.55, $NaHCO_3$ 11.90, $CaCl_2.6H_2O$ 1.80. Guinea-pig atria; NaCl 118.41, KCl 4.69, $MgSO_4.7H_2O$ 1.18, KH_2PO_4 1.18, glucose 11.10, $NaHCO_3$ 24.99, $CaCl_2.6H_2O$ 2.52. Frog rectus abdominis muscle; NaCl 111.23, KCl 1.88, NaH_2PO_4 0.06, $NaHCO_3$ 2.38, glucose 11.10, $CaCl_2.6H_2O$ 1.08.

Drugs used

The following were used: pancuronium (Organon), pirenzepine (Boots), secoverine (Duphar) and stercuronium (Gist-Brocades). 4 DAMP was kindly donated by Dr R.B. Barlow. 3-Acetoxy-*N*-methyl piperidine methiodide (AMP) and arecaidine propargyl ester (APE) were synthesized by Dr R. Clark (Syntex, Palo Alto). Suberyldicholine was obtained from Aldrich Chemical Co. Ltd. All remaining compounds were obtained from the Sigma Chemical Co. Ltd.

Table 1 Antagonist affinities for muscarinic receptors mediating ileal contraction and atrial chronotropic and inotropic effects

Antagonist	*Ileum*		*Atria (rate)*		*Atria (force)*	
	pA_2	*Slope*	pA_2	*Slope*	pA_2	*Slope*
Atropine	9.10 ± 0.09	1.0 ± 0.03	8.87 ± 0.05	1.1 ± 0.13	8.76 ± 0.06	0.97 ± 0.02
Pirenzepine	6.77 ± 0.05	0.90 ± 0.02	6.60 ± 0.15	0.97 ± 0.07	6.81 ± 0.03	0.93 ± 0.03
Secoverine	7.90 ± 0.07	1.00 ± 0.02	8.30 ± 0.08	1.1 ± 0.04	8.76 ± 0.10	0.97 ± 0.04
4-DAMP	9.04 ± 0.14	0.94 ± 0.05	7.90 ± 0.12	1.1 ± 0.07	7.83 ± 0.09	1.0 ± 0.03
Gallamine	4.84 ± 0.20	0.63 ± 0.05	5.84 ± 0.22	0.7 ± 0.10	4.92 ± 0.04	0.96 ± 0.03
Pancuronium	5.81 ± 0.13	0.77 ± 0.03	6.31 ± 0.13	1.2 ± 0.19	6.12 ± 0.10	0.92 ± 0.07
Stercuronium	6.59 ± 0.15	0.80 ± 0.04	6.92 ± 0.07	0.93 ± 0.04	6.45 ± 0.17	0.91 ± 0.11

Antagonist affinities (pA_2) and slopes from Arunlakshana-Schild plots at ileal and atrial muscarinic receptors. Values are mean ± s.e.mean; $n = 4-6$ preparations.
4-DAMP = 4-diphenyl-acetoxy-*N*-methyl piperidine methiodide.

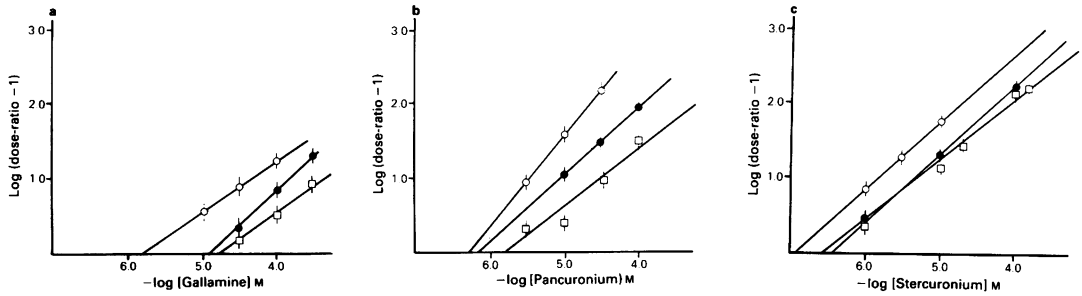

Figure 1 Arunlakshana-Schild plots of log (dose-ratio − 1) versus − log concentration of antagonist for the effects of (a) gallamine (b) pancuronium and (c) stercuronium on responses to carbachol at muscarinic receptors mediating ileal contractions (□) and the rate (○) and force (●) of atrial contractions. The symbols shown are mean values and the vertical lines represent s.e.mean. $n = 4$ preparations at each point.

Results

Antagonists – muscarinic receptors

The results are shown in Table 1. In all three preparations atropine, pirenzepine, secoverine and 4-DAMP exhibited Arunlakshana-Schild slopes not significantly different from unity indicating competitive antagonism. Gallamine, pancuronium and stercuronium exhibited Arunlakshana-Schild slopes not significantly different from unity at muscarinic receptors mediating negative inotropic effects. However, deviations from unity were observed at muscarinic receptors mediating negative chronotropic effects with gallamine and pancuronium and at muscarinic receptors mediating ileal contractions with all three neuromuscular blockers (Figures 1a, b and c).

Atropine exhibited similar affinities for muscarinic receptors present in all three preparations, but the value at muscarinic receptors mediating the force of atrial contractions achieved statistical significance ($P < 0.05$) compared with the value at ileal muscarinic receptors. Pirenzepine exhibited pA_2 values at all three muscarinic receptors which were not significantly different from each other.

Statistically significant differences between the pA_2 values were observed with secoverine. The pA_2 value observed at ileal muscarinic receptors was significantly different ($P < 0.01$) from that at both types of atrial receptors. In addition, the affinities observed at atrial

Table 2 Potencies of muscarinic agonists at muscarinic receptors mediating ileal contractions and atrial chronotropic and inotropic effects

	Potency (− log EC_{50})		
Agonist	*Ileum*	*Atria (rate)*	*Atria (force)*
Acetylcholine	6.15 ± 0.03	5.95 ± 0.08	6.70 ± 0.05
Methacholine	6.22 ± 0.05	6.42 ± 0.06	6.32 ± 0.03
Carbachol	6.74 ± 0.08	6.92 ± 0.03	7.04 ± 0.08
Bethanechol	5.30 ± 0.12	4.62 ± 0.09	4.71 ± 0.05
Oxotremorine	7.22 ± 0.03	7.65 ± 0.05	7.76 ± 0.07
Muscarine	5.52 ± 0.02	6.13 ± 0.03	6.11 ± 008
Arecoline	6.70 ± 0.13	6.61 ± 0.09	6.79 ± 0.05
APE	6.98 ± 0.08	7.33 ± 0.03	7.26 ± 0.07
AMP	3.52 ± 0.07	No effect	2.82 ± 0.08
Pilocarpine	5.30 ± 0.13‡	5.02 ± 0.12‡	5.13 ± 0.09‡
McN-A-343	No effect	No effect	No effect
Suberyldicholine	5.52 + 0.05	No effect	5.31 + 0.06‡

All agonists tested between 1×10^{-8} and 3×10^{-2} mol l^{-1}.
Values are mean ± s.e.mean; $n = 4-6$ preparations.
‡Denotes partial agonist. Pilocarpine exhibited the following maximum responses relative to the carbachol maximum: ileum 0.42; atria (rate) 0.38; atria (force) 0.45. Suberyldicholine exhibited a maximum response of 0.32 at atrial (force) muscarinic receptors.
APE = arecaidine propargyl ester; AMP = 3-acetoxy-*N*-methyl piperidine methiodide.

muscarinic receptors mediating changes in the rate of contraction were significantly ($P<0.05$) lower than those mediating changes in force. 4-DAMP exhibited a significantly ($P<0.01$) greater affinity for ileal muscarinic receptors compared with those observed at atrial muscarinic receptors. The pA_2 values observed at either type of atrial muscarinic receptor were not significantly different from each other.

Gallamine exhibited a significantly ($P<0.05$) greater pA_2 value for atrial muscarinic receptors mediating the rate of contraction compared with those obtained for the other two types of muscarinic receptors studied. Pancuronium exhibited a significantly ($P<0.05$) greater pA_2 value for atrial muscarinic receptors mediating changes in rate compared with that for ileal muscarinic receptors. The pA_2 value of stercuronium for ileal muscarinic receptors was not significantly different from that for atrial muscarinic receptors mediating changes in force but both were significantly different ($P<0.05$) from those mediating changes in rate.

Agonists – muscarinic receptors

The results are shown in Table 2. All agonists with the exception of McN-A-343, pilocarpine and suberyldicholine exhibited full agonism. Bethanechol and AMP exhibited significantly higher ($P<0.05$) potencies at ileal muscarinic receptors in comparison to those at both atrial muscarinic receptors. Conversely, oxotremorine, muscarine and APE exhibited significantly ($P<0.05$) higher values at atrial muscarinic receptors in comparison to ileal receptors. McN-A-343 was inactive at muscarinic receptors present in all three preparations.

Table 3 Potency of muscarinic agonists at nicotinic receptors mediating contraction of frog rectus abdominis muscle

Agonist	*Potency ($-\log EC_{50}$)*
Acetylcholine	5.32 ± 0.08
Carbachol	5.03 ± 0.05
Bethanechol	No response
Methacholine	No response
Oxotremorine	No response
APE	No response
Arecoline	4.05 ± 0.03
Pilocarpine	No response
Suberyldicholine	5.30 ± 0.07
Muscarine	No response
McN-A-343	No response

All agonists tested between 1×10^{-8} and 1×10^{-3} mol l^{-1}.
Values are mean ± s.e.mean; $n = 4$ preparations.
APE = arecaidine propargyl ester.

Acetylcholine, AMP and suberyldicholine exhibited significantly ($P<0.05$) lower $-\log EC_{50}$ values at muscarinic receptors mediating the rate of atrial contraction in comparison to those mediating the force of atrial contraction. The remaining agonists did not discriminate between the two types of atrial muscarinic receptors.

Agonists – nicotinic receptors

The results are shown in Table 3. Only acetylcholine, carbachol, arecoline and suberyldicholine produced a contractile response in the frog rectus abdominis muscle. No response was observed with bethanechol, methacholine, oxotremorine, APE, pilocarpine, muscarine or McN-A-343.

Effect of hexamethonium on carbachol potency at ileal muscarinic receptors

The potencies of carbachol and nicotine at ileal muscarinic receptors before and after equilibration with hexamethonium are shown in Table 4. The presence of hexamethonium did not affect the response to carbachol but significantly abolished that to nicotine.

Effect of reserpine treatment on carbachol potency

The potencies of carbachol at muscarinic receptors located on tissues from control and reserpine-treated animals are shown in Table 5. Reserpine did not significantly alter the potency of carbachol but abolished the responses to tyramine.

Effect of acetylcholinesterase inhibition on agonist potencies at atrial muscarinic receptors

The presence of physostigmine abolished any differences in potency previously observed with acetylcholine and AMP (see Agonists section). In the presence of physostigmine all the agonists tested exhibited poten-

Table 4 Action of hexamethonium on the contractile actions of carbachol and nicotine in the guinea-pig ileum

	Control	*+ Hexamethonium*
Carbachol	6.52 ± 0.03	6.54 ± 0.08
Nicotine	4.83 ± 0.02	No response

Values show potencies ($-\log EC_{50}$) of carbachol and nicotine at muscarinic receptors mediating contraction in the guinea-pig ileum before and after treatment with hexamethonium (1×10^{-4} mol l^{-1}) and are mean ± s.e.mean; $n = 4$ preparations.

Table 5 Effect of reserpine on the potency of carbachol at muscarinic receptors mediating ileal contractions and the rate and force of atrial contractions

	Carbachol $-\log EC_{50}$		
	Ileum	*Atria (rate)*	*Atria (force)*
Control	6.61 ± 0.03	6.58 ± 0.05	6.67 ± 0.07
Reserpine-treated	6.54 ± 0.05	6.63 ± 0.03	6.73 ± 0.08

Values are mean ± s.e.mean; $n = 4$ preparations. (No response was observed with tyramine (1×10^{-4} mol l^{-1}) following reserpine treatment.

Table 6 Potencies of muscarinic agonists at muscarinic receptors mediating rate and force of atrial contractions in the absence and presence of physostigmine

	$-\log EC_{50}$			
	Control		*+ Physostigmine*	
Agonist	*Rate*	*Force*	*Rate*	*Force*
Acetylcholine	5.95	6.70*	7.15	7.07
Methacholine	6.42	6.32	6.30	6.31
Carbachol	6.92	7.04	6.51	6.48
Bethanechol	4.62	4.71	4.95	4.95
Oxotremorine	7.65	7.76	7.60	7.65
Muscarine	6.13	6.11	6.23	6.23
Arecoline	6.61	6.79	6.81	6.91
APE	7.33	7.26	7.58	7.49
AMP	No effect	2.82*	No effect	No effect

Values are mean, s.e.mean $< 5\%$ in each case; $n = 4-6$ preparations. (All agonists tested between 1×10^{-8} and 3×10^{-2} mol l^{-1}.)
APE = arecaidine propargyl ester; AMP = 3-acetoxy-*N*-methylpiperidine methiodide.
*$P < 0.005$.

cies at muscarinic receptors mediating the rate of atrial contraction which were not significantly different from those at receptors mediating the force of atrial contraction. These results are shown in Table 6.

Discussion

This study has investigated the action of muscarinic agonists and antagonists at muscarinic receptors present in the ileum and atria. The use of data from isolated tissue responses in receptor classification requires the application of strict criteria, previously described by Furchgott (1972) and Kenakin (1984). These criteria have been employed in the interpretation of the data obtained in this study. The experiments have been conducted, as far as possible, under identical conditions, in order to attain uniformity and stability. In addition, other factors which may give rise to apparent selectivity such as nicotinic receptor activity and acetylcholinesterase (AChE) activity have been studied.

The pA_2 values obtained in this study using 4-DAMP and secoverine indicate that ileal and atrial muscarinic receptors differ. The differences are greater than the 0.5 pA_2 units suggested by Furchgott (1972) to represent receptor heterogeneity. The results obtained in this study with 4-DAMP are similar to those obtained by Barlow *et al.* (1980). The selectivity observed using secoverine has not been described previously. The pA_2 value obtained at ileal muscarinic receptors is lower than that found by Zwagemakers & Claasen (1980), although their value was assessed under non-equilibrium conditions. Lobbezzoo *et al.* (1984) observed no difference in the binding affinity of secoverine at ileal or atrial muscarinic receptors, although binding studies have not confirmed other functional differences between these receptors (Choo & Mitchelson, 1984).

The pA_2 values for gallamine, pancuronium and stercuronium are, in general, in good agreement with the literature (Mitchelson, 1984). However, it should be noted that the pA_2 values for gallamine and pancuronium at atrial (force) receptors differ by almost an order of magnitude from those found by Mitchelson (1984). In addition, the pA_2 value for stercuronium at ileal receptors was higher than that previously published (Mitchelson, 1984). These values

cannot be regarded as an indication of their receptor affinities since the slope of the Arunlakshana-Schild plot deviates from unity (Kenakin, 1984). This behaviour of these compounds has been described by other workers (Mitchelson, 1984) and has been attributed to the ability of these agents to act as allosteric regulators (Birdsall *et al.*, 1984). However, the difference in potency observed using gallamine may be indicative of differences in the muscarinic receptor present in the ileum and atria. These agents are, to date, the only antagonists which exhibit selectivity towards atrial muscarinic receptors.

The results obtained with atropine and pirenzepine are similar to those found by other workers (Szelenyi, 1982; Barlow *et al.*, 1981). The pirenzepine data indicate that ileal muscarinic receptors and both types of atrial muscarinic receptors fall within the M_2 subclass, as proposed by Hammer & Giachetti (1982).

The antagonist data do not indicate differences between atrial muscarinic receptors mediating the rate and force of contraction. The results are therefore in contrast to those of Chaissing *et al.* (1984), who found a larger than 3 fold difference in pA_2 values for pirenzepine. A difference was observed in this study for secoverine, but this does not meet the criteria defined by Furchgott (1972) for receptor hetereogeneity. The pA_2 values observed at atrial muscarinic receptors for gallamine, pancuronium and stercuronium do not reflect different subtypes for the reasons previously outlined.

The differences in agonist potencies between ileal and atrial muscarinic receptors were small and are not clear evidence of receptor subtypes. The values are similar to those observed by other workers (Barlow *et al.*, 1980; Mutschler & Lambrecht, 1984). APE has been found to exhibit 5 fold selectivity for atrial muscarinic receptors (Mutschler & Lambrecht, 1984) and similar results were obtained in this study. However, equivalent selectivity was observed with muscarine and bethanechol, agents which have been previously thought to be non-selective. Agonist selectivity, particularly of partial agonists, varies from tissue to tissue because of variations in receptor number and the relative efficiency of stimulus-response relationships (Kenakin, 1984). The apparent selectivity observed in this study using suberyldicholine, for example, is probably a result of its low intrinsic efficacy (Clague *et al.*, 1984). Therefore, it is considered that a greater than 5 fold difference in potency with agonists is required to indicate receptor heterogeneity.

The selectivity of muscarinic agonists, such as AMP and acetylcholine, for atrial muscarinic receptors mediating rate and force effects described by Barlow *et al.* (1980) and Chaissing *et al.* (1984) was also observed in this study. However, our results are at variance with those of Chaissing *et al.* (1984) in that no selectivity was seen using carbachol or arecoline. In the present study the majority of agonists studied showed no discrimination. The selectivity observed with acetylcholine is probably attributable to the action of AChE, since such selectivity was abolished in the presence of physostigmine. Webb (1950) found high concentrations of AChE in mycardial nodal tissue. The 40–60 fold difference in affinity of a bridged analogue of arecoline reported by Mutschler & Lambrecht (1984) may also be attributable to the action of AChE.

The use of carbachol as an agonist at muscarinic receptors has been considered to be complicated by its nicotinic action in certain tissues. However, this appears unlikely since its potency at nicotinic receptors is approximately 100 fold less than its potency at muscarinic receptors. This is confirmed by the fact that the pA_2 values derived in this study are similar to those obtained in studies in which hexamethonium was routinely used (Barlow *et al.*, 1972; 1976).

In conclusion, it appears likely that M_2-receptors differ in the heart and ileum. However, those muscarinic receptors mediating negative inotropic and chronotropic effects do not appear to differ. This conclusion is in agreement with that of Barlow *et al.* (1976), and confirms the conclusion of Hirschowitz *et al.* (1984) that the M_2 subclass may be composed of two further subtypes.

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