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EFFECT OF ANTIMUSCARINIC AGENTS ON THE CONTRACTILE RESPONSES TO CHOLINOMIMETICS IN THE RAT ANOCOCCYGEUS MUSCLE

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1 The effects of antimuscarinic agents alone and in the presence of neostigmine on the contractile responses to exogenously applied cholinomimetics or (−)-noradrenaline were studied in the rat anococcygeus muscle.

2 Atropine (1×10^{-9}–1×10^{-6}M) alone, in the presence of hexamethonium (1×10^{-4}M), or phentolamine (1×10^{-6}M), inhibited responses to acetylcholine but not to (−)-noradrenaline. The inhibitory effect with the higher concentrations of atropine (1×10^{-8}–$\times 10^{-6}$M), was seen as an increase in the slopes of the concentration-response curves. Atropine (1×10^{-8}M) alone inhibited the responses to methacholine and carbachol without altering the slopes of the concentration-response curves.

3 Homatropine (1×10^{-6}M) alone had no effect on responses to (−)-noradrenaline and inhibited responses to acetylcholine and methacholine. The inhibitory effect on responses to acetylcholine but not to methacholine, included an increase in the slopes of the concentration-response curves.

4 Neostigmine (1×10^{-6}M) alone had no effect on responses to (−)-noradrenaline and potentiated responses to acetylcholine and methacholine. The potentiating effect included an increase in the slopes of the concentration-response curves.

5 In the presence of neostigmine (1×10^{-6}M), atropine (1×10^{-9}M–1×10^{-6}M) caused a parallel concentration-dependent shift of the concentration-response curves to acetylcholine. The pA_2 values, in the presence of neostigmine, were independent of the concentration of atropine and of the agonist (acetylcholine, methacholine, or carbachol) used. In the presence of neostigmine (1×10^{-6}M), homatropine (1×10^{-6}M) also failed to alter the slopes of the concentration-response curves to acetylcholine and was approximately 100 times less potent than atropine as an antimuscarinic agent.

6 These results illustrate that, in the rat anococcygeus muscle, it is necessary to inhibit acetylcholinesterase before determining the relative potencies of antagonists at muscarinic receptors.

Introduction

In his original description of the isolated anococcygeus muscle of the rat, Gillespie (1972) described an excitatory, noradrenergic and an inhibitory, as yet unknown transmitter, but apparently no cholinergic transmitter. Thus the excitatory and inhibitory responses to field stimulation were insensitive to both hexamethonium and atropine. In consequence, investigations which have used the rat anococcygeus muscle subsequently have been concerned either with aspects of noradrenergic transmission (e.g., Gibson & Gillespie, 1973; Gibson & Pollock, 1973; Gillespie & McGrath, 1974; Doggrell & Woodruff, 1977) or the nature of the inhibitory process (Gillespie & McGrath, 1973; Burnstock, Cocks & Crowe, 1978).

Recently, the role of ganglia in the innervation of the rat anococcygeus has been reinvestigated (McKirdy & Muir, 1978) and a ganglionated nerve plexus, lying on the surface of the muscle and through which some cholinergic synaptic transmission occurs, described. Cholinesterase activity has also been detected in the tissue (Gibson & Pollock, 1975; Smith & Spriggs, 1979). However, although Gillespie's original paper (1972) demonstrated atropine-sensitive, acetylcholine-induced contractions and a subsequent study (Gillespie & McGrath, 1974) illustrated the ability of neostigmine to potentiate responses to acetylcholine, no further detailed studies on the muscarinic antagonist/agonist interaction have been undertaken. The present paper describes

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such a study and shows the effects of atropine and homatropine, alone and in the presence of neostigmine, on the contractile responses produced by exogenously applied cholinomimetics or (−)-noradrenaline.

Methods

Anococcygeus muscles were dissected from mature male Wistar rats as described by Gillespie (1972). All experiments were performed in the presence of a modified Krebs solution of the following composition (mM): NaCl 116, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 22.0, D-glucose 11.2 and Na_2EDTA 0.04, equilibrated with 5% CO_2 in O_2 at 37°C.

Individual muscles were mounted under 0.5 g tension in 5 ml organ baths containing Krebs solution. The tissues were allowed to equilibrate for 30 min, the resting tension being maintained throughout. Concentration-response curves for agonists were determined non-cumulatively. Exposure to an agonist was continued for 30 s or until a maximum response was obtained. Tissues were then allowed to recover for a minimum period of 5 min before further addition of agonist. An interval of 30 min was allowed between the construction of a response curve to a muscarinic agonist and one to (−)-noradrenaline. Contractile responses were recorded isometrically with force displacement transducers (Grass model FT 03.C) and displayed on a polygraph (Grass model 79B).

When experiments were carried out in the presence of hexamethonium, phentolamine, or neostigmine, these drugs were present in the Krebs solution from the beginning of the equilibration period. When studying their effect on the responses to agonists, one concentration of the drug was present in the solution bathing one of the anococcygeus muscles from the beginning of the equilibration period while the other muscle of the pair received no drug. When their effect on the response to muscarinic agonists or to noradrenaline was tested, only one concentration of drug was used for each pair of muscles.

The maximum responses (g) obtained, under different conditions, were compared by Student's paired t test and were considered significantly different when $P < 0.05$. When the maximum responses, in the presence and absence of drugs, were not significantly different, responses were calculated as a percentage of the maximum response of the individual response curve (i.e. normalized). The slope (difference in percentage maximum of the response/unit of logarithm molar concentration of agonist) of each concentration-response curve was computed by regression line analysis (over the range 20–80% of the maximum response). When the maximum responses, in the presence and absence of drugs, were not significantly different, a pD_2 value (negative logarithm of molar concentration of agonist producing 50% of the maximum response) was also determined by regression line analysis. For each pair of tissues, the ability of a drug to potentiate or to inhibit responses was expressed as the dose-ratio (the antilogarithm of the difference between the pD_2 values in the presence and, from the other tissue of the pair, in the absence of drug). When the effects of antimuscarinics were compatible with a competitive antagonism (i.e. the antagonist had no effect on the slope of the concentration-response curve and reduced the pD_2 value) pA_2 values were determined. pA_2 values (the negative logarithm of the molar concentration of antagonists which causes a two fold shift of the concentration-response curve for agonist) were calculated for each pair of tissues from the formula $pA_2 = pA_x + \log(x-1)$, where pA_x is the negative logarithm of the molar concentration of the antagonist and x is the dose-ratio (inhibition). When the maximum responses (g), in the presence and absence of drugs, were significantly different, responses were calculated as a percentage of the maximum response of the control response curve. The individual values (percentage of the maximum response, slopes, pD_2 values, dose-ratio, and pA_2 values) obtained, under different conditions, were compared using Student's paired t test. Mean values±s.e.mean were also determined.

The drugs used were phentolamine mesylate (donated by Ciba-Geigy), acetylcholine chloride, atropine sulphate, carbachol chloride, hexamethonium bromide, DL-homatropine hydrobromide, methacholine chloride, neostigmine bromide and (−)-noradrenaline bitartrate (Sigma).

Results

Atropine (5×10^{-10}–1×10^{-6}M), homatropine (1×10^{-6}M), hexamethonium (1×10^{-4}M), phentolamine (1×10^{-6}M), and neostigmine (1×10^{-6}M) had no effect on the resting tone of the rat anococcygeus. Neither hexamethonium (1×10^{-4}M) nor phentolamine (1×10^{-6}M) affected the contractile responses to acetylcholine (i.e. the magnitude of the maximal responses to acetylcholine, the slopes of the concentration-response curves, or the pD_2 values).

The effects of antimuscarinic agents

Atropine The effects of atropine on the contractile responses to exogenously applied acetylcholine and (−)-noradrenaline were studied alone (Figure 1), in the presence of hexamethonium (1×10^{-4}M), and in

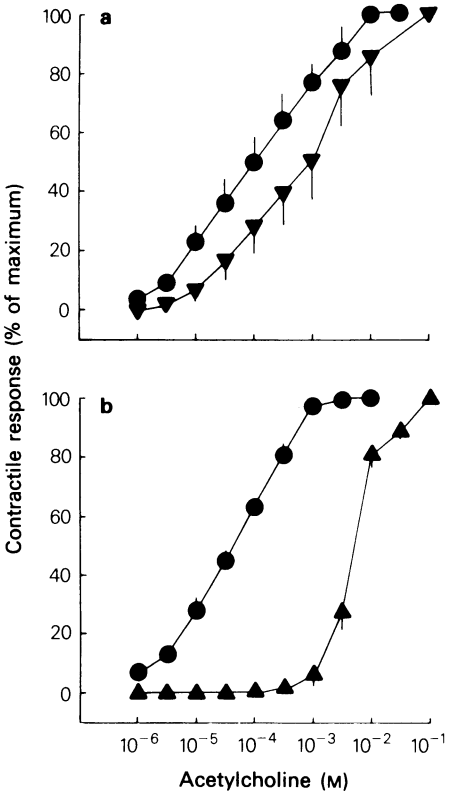

Figure 1 Acetylcholine concentration-response curves in the rat anococcygeus: (a) 5 tissues alone in Krebs solution (●) and paired tissues in 1×10^{-9}M atropine (▼); (b) 6 tissues alone in Krebs solution (●) and paired tissues in 1×10^{-6}M atropine (▲). All responses are expressed as a percentage of the maximum response of the individual concentration-response curve (ordinate scale) and plotted against the logarithm of the molar concentration of acetylcholine (abscissa scale). Each value is the mean; vertical lines show s.e.mean. Atropine, 1×10^{-9}M, inhibited responses to acetylcholine without altering the slope of the concentration-response curve; however, at 1×10^{-6}M the inhibitory effect of atropine included an increase in the slope of the concentration-response curve.

the presence of phentolamine (1×10^{-6}M). Under these conditions atropine (5×10^{-10}–1×10^{-6}M) had no effect on the responses to (−)-noradrenaline or on the magnitude of the maximal responses to acetylcholine.

Atropine at 5×10^{-10} and 1×10^{-9}M alone had no effect but in higher concentrations (1×10^{-8}–1×10^{-6}M) increased the slopes of the concentration-response curves to acetylcholine (Table 1). The pD_2 values for acetylcholine were not altered by 5×10^{-10}M but were reduced by 1×10^{-9}–1×10^{-6}M atropine (Table 1). Responses were inhibited ×8.9±4.9 (5) (mean dose-ratio± s.e.mean, $n = 5$), ×14.7±5.9 (7), ×148.1±64.4 (6) and ×170.1±50.3 (6) by 1×10^{-9}, 1×10^{-8}, 1×10^{-7}M, and 1×10^{-6}M atropine, respectively. At higher concentrations of atropine (1×10^{-8}–1×10^{-6}M), the slopes of the concentration-response curves to acetylcholine were increased so a pA_2 value for atropine was only determined in the presence of a low concentration (1×10^{-9}M), 9.55±0.29 (5) (mean pA_2 value± s.e.mean, $n = 5$). When experiments were carried out in the presence of hexamethonium (1×10^{-4}M), or of phentolamine (1×10^{-6}M), the inhibitory effects of atropine (1×10^{-9}–1×10^{-6}M) on responses to acetylcholine were similar to those observed in Krebs solution alone.

Atropine (1×10^{-8}M) alone had no effect on the magnitude of the maximal responses or the slopes of the concentration-response curves to methacholine or carbachol. Responses to methacholine and carbachol were inhibited ×29.5±5.9 (10) (mean dose-ratio ± s.e.mean, $n = 10$) and 29.3±3.4 (10), respectively, by atropine, 1×10^{-8}M. Thus the pA_2 values were similar for atropine when either methacholine or carbachol was used: 9.38±0.08 (10) (mean pA_2 value ± s.e.mean, $n = 10$) and 9.42±0.05 (10), respectively.

Homatropine Homatropine (1×10^{-6}M) alone had no effect on the contractile responses to (−)-

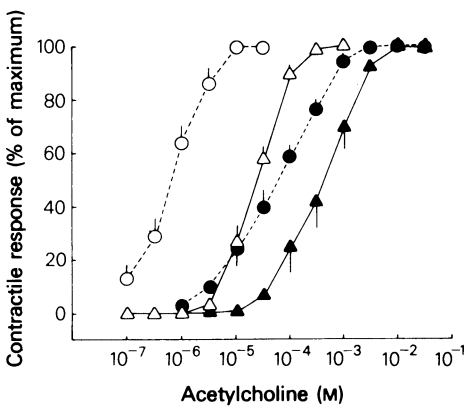

Figure 2 Concentration-response curves to acetylcholine of the rat anococcygeus: 5 tissues alone in Krebs solution (●) and paired tissues in 1×10^{-6}M homatropine (▲) and from other animals 6 tissues in 1×10^{-6}M neostigmine (○) and paired tissues in neostigmine and 1×10^{-6}M homatropine (△). See legend to Figure 1 for further details. The inhibitory effect of homatropine alone on responses to acetylcholine included an increase in the slope of the concentration-response curve whereas the inhibitory effect in the presence of 1×10^{-6}M neostigmine was greater and did not include an alteration in slope.

Table 1 The effect of atropine and homatropine on the responses of rat anococcygeus muscle to cholinomimetics

		Slope	*pD_2*
Acetylcholine			
(a)	Control	35.23 ± 4.45 (4)	4.40 ± 0.29 (4)
	Atropine, 5×10^{-10}M	36.28 ± 3.22 (4)	4.09 ± 0.25 (4)
(b)	Control	30.65 ± 1.68 (5)	3.99 ± 0.29 (5)
	Atropine, 1×10^{-9}M	39.95 ± 6.18 (5)	3.26 ± 0.33 (5)**
(c)	Control	34.43 ± 2.93 (7)	4.24 ± 0.13 (7)
	Atropine, 1×10^{-8}M	54.34 ± 6.64 (7)*	3.25 ± 0.08 (7)***
(d)	Control	34.88 ± 4.05 (6)	4.35 ± 0.18 (6)
	Atropine, 1×10^{-7}M	62.20 ± 3.79 (6)***	2.39 ± 0.16 (6)***
(e)	Control	33.22 ± 3.62 (6)	4.44 ± 0.04 (6)
	Atropine, 1×10^{-6}M	80.02 ± 11.77 (6)**	2.29 ± 0.08 (6)***
(f)	Control	36.98 ± 3.32 (5)	4.26 ± 0.14 (5)
	Homatropine, 1×10^{-6}M	56.64 ± 4.70 (5)**	3.43 ± 0.16 (5)***
Methacholine			
(a)	Control	67.86 ± 3.44 (10)	4.96 ± 0.12 (10)
	Atropine, 1×10^{-8}M	60.21 ± 8.25 (10)	3.55 ± 0.12 (10)***
(b)	Control	51.50 ± 5.15 (5)	5.25 ± 0.21 (5)
	Homatropine, 1×10^{-6}M	56.84 ± 4.66 (5)	3.83 ± 0.16 (5)**
Carbachol			
	Control	90.65 ± 7.07 (10)	5.07 ± 0.08 (10)
	Atropine, 1×10^{-8}M	95.23 ± 7.91 (10)	3.62 ± 0.08 (10)***

Each value is the mean ± s.e.mean (n), where n = number of observations.
*$P < 0.025$; **$P < 0.005$; ***$P < 0.0005$; paired t test.

noradrenaline or on the magnitude of the maximal responses to acetylcholine (Figure 2) or methacholine. Homatropine (1×10^{-6}M) increased the slopes of the concentration-responses to acetylcholine but not to methacholine (Table 1). At the level of the 50% maximum response (Table 1) homatropine (1×10^{-6}M) inhibited responses to acetylcholine to a lesser extent ($\times 7.0 \pm 1.7$ mean dose-ratio ± s.e.mean, $n = 5$) than to methacholine ($\times 34.5 \pm 12.4$, $n = 4$). The pA_2 value for homatropine against methacholine was 7.39 ± 0.18 (5) (mean ± s.e.mean, $n = 5$).

The effects of neostigmine

Neostigmine (1×10^{-6}M) did not alter the contractile responses to (−)-noradrenaline or the magnitude of the maximal responses to acetylcholine, methacholine, or carbachol. The slopes of the concentration-response curves to acetylcholine (Figure 3a) and methacholine were increased and those to carbachol were unaltered by neostigmine (1×10^{-6}M) (Table 2). At the level of the 50% maximum response neostigmine (1×10^{-6}M) potentiated responses to acetylcholine and methacholine $\times 53.0 \pm 10.2$ (8) (mean dose-ratio ± s.e.mean, $n = 8$) and $\times 2.3 \pm 0.3$ (8), respectively, and inhibited responses to carbachol, $\times 1.6 \pm 1.1$ (8) (see Table 2 for mean pD_2 values).

The effects of antimuscarinic agents in the presence of neostigmine

Neither atropine (1×10^{-6}M) nor homatropine (1×10^{-6}M), in the presence of neostigmine (1×10^{-6}M), altered responses to (−)-noradrenaline. The effects of atropine (1×10^{-6}M) and homatropine (1×10^{-6}M), in the presence of neostigmine (1×10^{-6}M), on responses to acetylcholine are illustrated in Figures 3b and 2, respectively.

In the presence of neostigmine, atropine (1×10^{-9}–1×10^{-6}M) did not affect the magnitude of the maximal responses or the slopes of the concentration-response curves to acetylcholine but reduced the pD_2 values to acetylcholine (Table 2). These responses were inhibited $\times 13.9 \pm 11.0$ (4) (mean dose-ratio ± s.e.mean, $n = 4$), $\times 31.1 \pm 5.0$ (6), $\times 738.8 \pm 340.3$ (6), and $\times 7{,}034 \pm 2.422$ (6) by 1×10^{-9}, 1×10^{-8}, 1×10^{-7} and 1×10^{-6}M atropine, respectively. Under these conditions it was possible to determine pA_2 values for atropine with each individual concentration. The pA_2 values for atropine were independent of the concentration and were approx. 9.60 (Table 2). In the presence of neostigmine (1×10^{-6}M), atropine (1×10^{-8}M) had no effect on the magnitude of the maximal responses or on the slopes of the concentration-response curves to methacholine and carbachol (Table 2) but the pD_2 values were reduced. The pA_2 values for atropine (Table 2) were similar, irrespective of whether

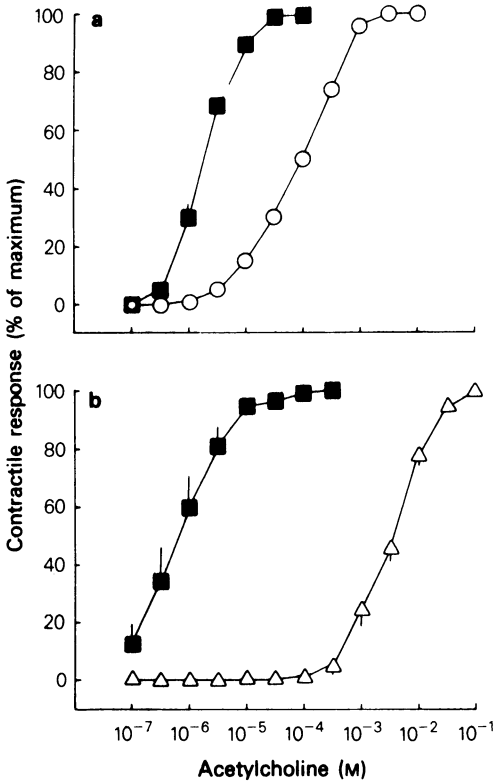

Figure 3 Concentration-response curves to acetylcholine of the rat anococcygeus: (a) 8 tissues alone in Krebs solution (○) and paired tissues in 1×10^{-6}M neostigmine (■); (b) 6 tissues in 1×10^{-6}M neostigmine (■) and paired tissues in neostigmine and 1×10^{-6}M atropine (△). See legend to Figure 1 for further details. The potentiating effect of neostigmine, 1×10^{-6}M, on the response to acetylcholine included an increase in the slope of concentration-response curve. In the presence of neostigmine, atropine, 1×10^{-6}M, inhibited responses to acetylcholine without altering the slope of the concentration-response curve.

methacholine, acetylcholine or carbachol were used as agonists, approx. 9.80, and greater than in the absence of neostigmine.

Homatropine (1×10^{-6}M), in the presence of neostigmine (1×10^{-6}M), had no effect on the slopes of the concentration-response curves and inhibited the responses at the 50% maximum level to acetylcholine and methacholine equi-effectively $\times 40.7 \pm 5.1$ (6) and $\times 33.5 \pm 6.8$ (6) respectively. In the presence of neostigmine, the pA_2 values for homatropine were independent of the agonist used (Table 2). Under these conditions homatropine ($pA_2 = 7.6$) was approximately $100\times$ less potent than atropine as an antimuscarinic agent.

Discussion

The contractile responses to acetylcholine in the rat anococcygeus muscle are mediated solely by muscarinic receptors, being abolished by atropine (Gillespie, 1972) and unaffected by phentolamine, destruction of noradrenergic nerves (Doggrell & Paton, 1978) or as shown in this study by hexamethonium. Atropine (1×10^{-8}–1×10^{-6}M) and homatropine, competitive antagonists at muscarinic receptors, increased the slopes of the concentration-response curves to acetylcholine. Under these conditions, pA_2 values for atropine and homatropine could not be determined. This effect is not related to ganglionic or noradrenergic transmission, as similar effects with atropine were obtained in the presence of hexamethonium or phentolamine. Nor did this effect reflect an action of atropine or homatropine at a postsynaptic site other than a muscarinic receptor, as these agents had no effect on the responses to exogenously applied (−)-noradrenaline. An ability of antimuscarinic agents, e.g. atropine, to increase the slopes of concentration-response curves to acetylcholine has not been observed previously (e.g. Arunlakshana & Schild, 1959).

Although histochemical techniques have failed to demonstrate butyryl or acetylcholinesterase in the rat anococcygeus (Gillespie, 1972; Burnstock *et al.*, 1978), cholinesterase activity has been demonstrated colorimetrically (Gibson & Pollock, 1975; Smith & Spriggs, 1979) and the ability of neostigmine to potentiate responses to acetylcholine (but not to carbachol) confirmed (Gillespie & McGrath, 1974). The present study demonstrates that the potentiating effects of neostigmine were limited to the contractile responses to cholinomimetics and appeared to be correlated with a susceptibility to cholinesterase, i.e. a greater potentiation of the responses to acetylcholine than to methacholine was observed. The small inhibition of the responses to carbachol by neostigmine may be due to an antimuscarinic activity. Neostigmine also increased the slopes of the concentration-response curves to acetylcholine and methacholine but not to carbachol. These changes in slope could be due to the presence of a removal process for acetylcholine in this tissue, i.e. degradation by acetylcholinesterase. Thus atropine and homatropine had no effect on the slopes of concentration-response curves to acetylcholine in the presence of neostigmine.

The presence of removal processes for agonists can produce erroneous estimations of the pA_2 values for antagonists. This has been fully discussed in relation to the influence of neuronal and extraneuronal uptake of agonists on the potencies of competitive antagonists at adrenoceptors (Furchgott, 1972). This study demonstrates for the first time that the same

Table 2 (**A**) The effect of neostigmine on the response of the rat anococcygeus to cholinomimetrics; (**B**) the effect of atropine and homatropine on the response to cholinomimetics in the presence of neostigmine

A	*Slope*	*pD_2*
Acetylcholine		
Control	43.66 ± 2.40 (8)	4.06 ± 0.06 (8)
Neostigmine, 1×10^{-6}M	70.67 ± 2.82 (8)**	5.75 ± 0.04 (8)**
Methacholine		
Control	67.79 ± 3.41 (8)	5.25 ± 0.14 (8)
Neostigmine, 1×10^{-6}M	76.17 ± 4.21 (8)*	5.58 ± 0.12 (8)**
Carbachol		
Control	78.15 ± 3.41 (8)	5.29 ± 0.11 (8)
Neostigmine, 1×10^{-6}M	78.20 ± 2.97 (8)	5.08 ± 0.11 (8)**

B	*pD_2*	*pA_2*
In the presence of neostigmine, 1×10^{-6}M		
Acetylcholine		
(a) Control	6.47 ± 0.19 (4)	9.56 ± 0.41 (4)
Atropine, 1×10^{-9}M	5.95 ± 0.13 (4)*	
(b) Control	6.27 ± 0.06 (6)	9.45 ± 0.06 (6)
Atropine, 1×10^{-8}M	4.79 ± 0.08 (6)**	
(c) Control	6.14 ± 0.09 (6)	9.70 ± 0.16 (6)
Atropine, 1×10^{-7}M	3.44 ± 0.11 (6)**	
(d) Control	6.19 ± 0.19 (6)	9.71 ± 0.16 (6)
Atropine, 1×10^{-6}M	2.49 ± 0.07 (6)**	
(e) Control	6.22 ± 0.11 (6)	7.58 ± 0.06 (6)
Homatropine, 1×10^{-6}M	4.63 ± 0.67 (6)*	
Methacholine		
(a) Control	5.57 ± 0.08 (4)	9.94 ± 0.13 (4)
Atropine, 1×10^{-8}M	3.68 ± 0.13 (4)**	
(b) Control	5.82 ± 0.15 (6)	7.57 ± 0.05 (6)
Homatropine, 1×10^{-6}M	4.24 ± 0.18 (6)*	
Carbachol		
Control	5.40 ± 0.11 (4)	9.65 ± 0.12 (4)
Atropine, 1×10^{-8}M	3.74 ± 0.05 (4)**	

Each value is the mean ± s.e.mean (n), where n = number of observations.
*$P < 0.0125$; **$P < 0.0005$; paired t test.

can apply with competitive antagonists at muscarinic receptors. The dose-ratio is a major determinant of the pA_2 value and variations in this ratio will produce erroneous pA_2 values. The dose-ratios in the presence of 1×10^{-8}, 1×10^{-7}, and 1×10^{-6}M atropine were two, five, and forty fold greater, respectively, in the presence than in the absence of neostigmine. Also in the situation where acetylcholinesterase was fully active, the inhibitory effect of atropine (1×10^{-8}–1×10^{-6}M) was not compatible with competitive antagonism as it included an increase in the slope of concentration-response curves to acetylcholine, whereas when acetylcholinesterase was inhibited by neostigmine the pA_2 values for atropine could be calculated and were independent of both concentration and agonist used. The use of methacholine, an agonist that is only slowly hydrolysed, did not fully overcome the influence of this inactivation process on the potency of atropine. Thus although atropine (1×10^{-8}M) did not alter the slopes of the concentration-response curves to methacholine, neostigmine increased the slopes. As a consequence the dose-ratio and pA_2 values for atropine using methacholine as an agonist were smaller in the absence than in the presence of neostigmine. Similarly, smaller dose-ratios and pA_2 values for homatropine were obtained using acetylcholine or methacholine as the agonist in the absence than in the presence of neostigmine.

In the presence of a low concentration of atropine (1×10^{-9}M) alone, the slopes of the concentration-response curves to acetylcholine were not altered. Also the pA_2 value obtained for atropine in the presence of this low concentration of atropine alone was similar to those obtained with a wide range of concentrations of atropine in the presence of neostigmine. Thus it may be possible to use a low concentration of atropine alone to determine the pA_2 value

with this agent. However, as a general method for determining pA_2 values of antimuscarinic agents, the use of a concentration of antagonist which has only a small inhibitory effect is unreliable. Although homatropine (1×10^{-6}M) alone only had a small inhibitory effect on responses to acetylcholine it also increased the slope of the concentration-response curve. Thus in the rat anococcygeus muscle the potencies of antagonists at muscarinic receptors should be determined in the presence of an acetylcholinesterase inhibitor. Using this method the present study illustrates that homatropine is 100 times less potent than atropine as an antimuscarinic agent.

It is not known whether the rapid hydrolysis of acetylcholine prevents the criteria for competitive antagonism being met by antimuscarinic agents in tissues other than the rat anococcygeus muscle. In the guinea-pig ileum a wide range of concentrations of atropine caused a parallel shift of the middle range of the concentration-response curve to acetylcholine in the absence of an acetylcholinesterase inhibitor (Arunlakshana & Schild, 1959). A smaller pA_2 value for atropine was obtained in the guinea-pig ileum and air-perfused lung in the absence of an acetylcholinesterase inhibitor, <9.0 (Arunlakshana & Schild, 1959) than the present study. This suggests either the presence of a different type of muscarinic receptor in the guinea-pig tissues and rat anococcygeus muscles or that the pA_2 values for the guinea-pig tissues were smaller due to the activity of acetylcholinesterase. In many other studies (e.g. using the cat's nictitating membrane: Langer & Trendelenburg, 1969), the potency of antimuscarinic agents has been determined routinely in the presence of an acetylcholinesterase inhibitor.

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