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Muscarinic receptor sub-type in the rat anococcygeus muscle

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Muscarinic agonists contract the anococcygeus muscle of the rat (Gillespie 1972; Gillespie & McGrath 1974), dog (Dehpour et al 1980) and mouse (Gibson & Wedmore 1980) but relax that of the rabbit (Creed et al 1977) and cat (Gillespie & McGrath 1974; Li & Mitchelson 1980). Both types of responses (contraction and relaxation) are blocked by atropine and are therefore assumed to involve similar receptors. Recently at least three subclasses of muscarinic receptors have been identified using more selective muscarinic receptor antagonists (Barlow et al 1976, 1980; Brown et al 1980; Hammer et al 1980; Li & Mitchelson 1980). These have been classified as M_1, M_2 and M_3 muscarinic receptors (Barlow et al 1980). M_1 receptors are present in smooth muscles such as the guinea-pig ileum, urinary bladder etc. and are blocked more preferentially by 4-diphenylacetoxy-*N*-methyl piperidine methiodide (Barlow et al 1976; Brown et al 1980). M_2 and M_3 receptors are present in the atria associated with effects on rate and force of contraction, respectively. (Barlow et al 1980). The neuromuscular blocking drugs gallamine (Clark & Mitchelson 1976; Li & Mitchelson 1980) stercuronium (Li & Mitchelson 1980) and pancuronium (Riker & Wescoe 1951) are more specific on these receptors. Atropine and related compounds are non-specific and are equipotent on all types of muscarinic receptors.

The present study compares the effects of gallamine, 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP) and atropine on acetylcholine induced contractions of the rat anococcygeus muscle. The antagonists were also studied on the guinea-pig ileum for comparison.

Method

Paired anoccygii were removed from adult male rats (200–300g) according to Gillespie (1972) and set up in Tyrode solution (NaCl 137; KCl 2·7; NaH_2PO4 0·3; $MgCl_2$ 0·9; $CaCl_2$ 1·8; $NaHCO_3$ 11·9 and glucose 5·6 mmol litre^{-1}). The tension on the tissue was approximately 1·0 g. Ileal segments were removed from killed guinea-pigs and set up (tension—0·5 g) in Tyrode solution (at 37 °C) contained in a 20·0 ml organ bath. Isotonic contractions in response to added acetylcholine were recorded through a frontal writing level on smoked paper.

The drug was added and allowed to act for 90 s (on the anococcygeus muscle) and 30 s (on the guinea-pig ileum) before wash out. In both the anococcygeus muscle and the ileum preparations there was a rest period of at least 5 min between contractions. Dose-response curves to acetylcholine were obtained in the absence and also in the presence of different concentrations of the antagonists. The tissues were allowed to equilibrate with the antagonists for 30 min before repeating the dose-response curves. Only one concentration of each antagonist was tested on each preparation. From the dose response curves, EC50 values were obtained for acetylcholine in the absence and also in the presence of the antagonists. Dose-ratios (DR) were calculated and pA_2 values were obtained from a plot of log (DR-1) against negative molar concentration of the antagonists (Arunlakshana & Schild 1959).

The drugs used were: acetylcholine chloride (BDH), physostigmine salicylate (Wellcome-Borough), gallamine triethiodide (May & Baker), atropine sulphate (BDH), and 4-DAMP methiodide (4-diphenylacetoxy-*N*-methylpiperidine methiodide generously donated by Dr R. B. Barlow—Pharmacology Department, Bristol University).

Results

Rat anococcygeus muscle. Contractions to acetylcholine were obtained in the presence of physostigmine (2·5 ×

Table 1. Effect of muscarinic receptor antagonists on ACh-induced contractions of the rat anococcygeus muscle.[a]

Antagonist[b]	n	Dose–Ratio	pA_2[c]	Slope[c]
Atropine				
10^{-9} M	4	2·97 ± 0·3 31·2 ± 5·9	9·10	1·10
10^{-8} M	4	510·0 ± 45·0		
10^{-7} M	4			
4-DAMP MeI				
10^{-8} M	8	6·5 ± 1·0		
10^{-7} M	8	49·1 ± 10·3	8·75	0·91
10^{-6} M	8	333·3 ± 50·0		
Gallamine				
10^{-6} M	4	Potentiation		
10^{-5} M	4	Potentiation		
10^{-4} M	4	1·2		

[a] Experiments were carried out in the presence of Physostigmine (2·5 × 10^{-7} M) to inhibit cholinesterase.
[b] Only one concentration of each antagonist was tested on any particular tissue. A 30 min (antagonist-tissue) equilibration period was allowed.
[c] Obtained from a plot of log (DR-1)vs negative log (molar) concentration of the antagonist.

Table 2. Effect of muscarinic receptor antagonists on ACh-induced contractions of the guinea-pig ileum.

Antagonist[a]	n	Dose–Ratio	pA_2[b]	Slope[b]
10^{-9} M	4	2·78 ± 0·5		
10^{-8} M	4	39·1 ± 5·2	9·15	1·15
10^{-7} M	4	407·0 ± 40·5		
4-DAMP MeI				
10^{-8} M	4	2·8 ± 0·5		
10^{-7} M	4	17·3 ± 2·2	8·30	0·91
10^{-6} M	4	125·0 ± 10·0		
Gallamine				
10^{-6} M	4	Potentiation		
10^{-5} M	4	Potentiation		
10^{-4} M	4	1·1		

[a] Only one concentration of each antagonist was tested on any particular tissue (only one antagonist on one preparation). The tissue was allowed to equilibrate with the antagonist for 30 min before adding the agonist.

[b] Obtained from a plot of log (DR-1) vs negative log (molar) concentration of the antagonist.

10^{-7} M) to inhibit acetylcholinesterase. Under the conditions, acetylcholine (7×10^{-7}–$2{\cdot}2 \times 10^{-5}$ M) produced concentration-dependent contractions of the rat anococcygeus muscle. Atropine (10^{-9}–10^{-7} M) produced parallel right shifts of the dose-response curves to acetylcholine with no alteration in the slope. There was also no suppression of the maximum response to acetylcholine. The pA_2 value (obtained according to Arunlakshana & Schild 1959) was 9·10 (slope = 1·10, n = 4). 4-DAMP methiodide produced effects qualitatively similar to atropine but was less potent. The pA_2 value was 8·75 (slope = 0·91, n = 8). Gallamine (10^{-6}–10^{-4} M) had little or no effect on acetylcholine-induced contractions. At the lower concentrations, of gallamine (10^{-6}–10^{-5} M), there was a slight potentiation of acetylcholine-induced contractions (Table 1).

Guinea-pig ileum. Acetylcholine ($2{\cdot}7 \times 10^{-8}$–$4{\cdot}4 \times 10^{-7}$ M) produced concentration-dependent contractions of the guinea-pig ileum. Atropine (10^{-9}–10^{-7} M) and 4-DAMP methiodide (10^{-8}–10^{-6} M) displaced the concentration-response curves to the right with neither a suppression of the maximum response nor an alteration in the slope of the concentration-response curves. The pA_2 values were 9·15 (slope = 1·15 n = 4) and 8·30 (slope = 0·91 n = 4) for atropine and 4-DAMP methiodide respectively. The pA_2 value (8·3) obtained for 4-DAMP methiodide on this tissue was very close to the pA_2 value (8·36) obtained by Barlow et al (1976) on the same tissue and at the same temperature. Gallamine (10^{-6}–10^{-4} M) did not antagonize acetylcholine induced contractions. There was a slight potentiation of the acetylcholine-induced contractions at the lower concentrations (10^{-6}–10^{-5} M) of gallamine (Table 2).

Discussion

The above results demonstrate that atropine and 4-DAMP methiodide are potent muscarinic receptor antagonists in the rat anococcygeus muscle and the guinea-pig ileum. The pA_2 values were comparable in both tissues suggesting that the receptors are similar. Gallamine which is selective for muscarinic receptors in the atria (Clark & Mitchelson 1976) and autonomic ganglia (Brown et al 1980) was without effect (at concentrations up to 10^{-4} M) in the anococcygeus muscle as well as the guinea-pig ileum preparation. Similar results have been obtained with gallamine on the guinea-pig ileum by Li & Mitchelson (1980). It is therefore concluded that muscarinic receptors in the rat anococcygeus muscle are similar to those in the guinea-pig ileum. According to the classification of Barlow et al (1980), the muscarinic receptors are of the M_1-type. This finding is interesting in view of the report by Li & Mitchelson (1980) that gallamine produced a parallel right shift of the inhibitory response to carbachol on the rat anococcygeus muscle. The pKB (negative logarithm of the dissociation constant) value obtained on the cat anococcygeus was comparable to values obtained for gallamine against carbachol on the atria and the rabbit ear artery, thus suggesting that muscarinic receptors in the cat anococcygeus are similar to those in the atria and the autonomic ganglia. It therefore appears that different muscarinic receptors mediate the contractile response to muscarinic agonists in the rat anococcygeus and the inhibitory response in the cat anococcygeus muscle.

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