J. Pharm. Pharmacol. 1985, 37: 589–592
Communicated January 21, 1985

Muscarinic receptor agonist–antagonist interaction in the rat rectum: are there ways of activating the same receptors?

P. A. AKAH, M. A. ORIOWO*, *Department of Pharmacology & Therapeutics, University of Ibadan, Ibadan, Nigeria*

The interaction between the muscarinic receptor agonists, carbachol, acetylcholine (ACh) and methacholine, and antagonists, atropine, gallamine, 4-DAMP and pirenzepine, was studied on the rat isolated rectum preparation. ACh ($1{\cdot}93 \times 10^{-8}$–$1{\cdot}95 \times 10^{-6}$ M), methacholine ($8{\cdot}7 \times 10^{-8}$–$1{\cdot}1 \times 10^{-6}$ M) and carbachol ($1{\cdot}1 \times 10^{-7}$–$3{\cdot}5 \times 10^{-6}$ M) induced contractions that were reversibly antagonized by atropine ($1{\cdot}9 \times 10^{-9}$–$4{\cdot}8 \times 10^{-8}$ M), 4-DAMP ($1{\cdot}5 \times 10^{-8}$–$2{\cdot}86 \times 10^{-7}$ M) gallamine ($1{\cdot}12 \times 10^{-6}$–$1{\cdot}12 \times 10^{-4}$ M) and pirenzepine ($2{\cdot}8 \times 10^{-7}$–$7{\cdot}0 \times 10^{-6}$ M). The pA_2 values were atropine: $8{\cdot}99 \pm 0{\cdot}28$, $9{\cdot}29 \pm 0{\cdot}14$ and $8{\cdot}86 \pm 0{\cdot}05$; 4-DAMP: $8{\cdot}39 \pm 0{\cdot}10$, $8{\cdot}66 \pm 0{\cdot}15$ and $8{\cdot}26 \pm 0{\cdot}30$, gallamine: $5{\cdot}85 \pm 0{\cdot}23$, $5{\cdot}73 \pm 0{\cdot}25$ and $5{\cdot}96 \pm 0{\cdot}10$ and pirenzepine: $6{\cdot}85 \pm 0{\cdot}44$, $7{\cdot}17 \pm 0{\cdot}13$ and $7{\cdot}21 \pm 0{\cdot}03$ against ACh, methacholine and carbachol, respectively. The experimental dose-ratio (atropine + gallamine) was greater than the expected dose-ratio (as predicted by the Paton & Rang rule) for ACh and methacholine while the experimental dose-ratio closely approximates the expected dose-ratio for carbachol. It is suggested that atropine, 4-DAMP pirenzepine and gallamine act on the same receptors but gallamine allosterically altered the binding of the agonists and antagonists to varying extents.

The rat rectum contracts in response to exogenously administered acetylcholine. Even though earlier studies (e.g. Del Tacca et al 1968) have shown that acetylcholine-induced contractions were antagonized by atropine, indicating an action on muscarinic receptors, the degree of antagonism was not quantified and, in some cases, large doses of atropine (of the order of 10^{-4} g ml^{-1}) were used. More recently, at least three subtypes of muscarinic receptors have been identified based on the differential effect of certain muscarinic receptor antagonists on muscarinic receptor agonist-evoked responses in different preparations. For example, the neuromuscular blocking drugs gallamine (Clark & Mitchelson 1976; Li & Mitchelson 1980), pancuronium (Riker & Wescoe 1951) and stercuronium (Brown et al 1980; Li & Mitchelson 1980) are selective antagonists of cardiac muscarinic receptors while having no effect on ileal muscarinic receptors. On the other hand, 4-diphenylacetoxy-*N*-methyl piperidine methiodide (4-DAMP) is about 20 times more active on muscarinic receptors in the ileum than on cardiac muscarinic receptors (Barlow et al 1976; Brown et al 1980). Pirenzepine is several times more potent on muscarinic receptors in the rat ganglia than on the ileal and the cardiac muscarinic receptors (Brown et al 1980) even though it does not differentiate between functional muscarinic receptors in the ileum and in the atria (Barlow et al 1981; Szelenyi 198). Atropine and related muscarinic receptor antagonists do not differentiate between the subclasses of the muscarinic receptor. In this investigation, we have studied the interaction between muscarinic agonists and antagonists with a view to see whether or not there is a homogenous population of muscarinic receptors in the rat rectum.

* Correspondence and present address: Department of Pharmacology, College of Medicine, University of Vermont, Burlington, Vermont 05405, USA.

Materials and methods

Adult albino rats of either sex, (200–350 g), were killed by a blow to the head and then exsanguinated. An incision was made in the lower abdomen and the pelvis split to expose the rectum. Segments of the rectum about 1·0 cm were isolated and suspended in a 10·0 ml organ bath containing Tyrode solution of the following composition (mmol litre^{-1}): NaCl 136·0; KCl 2·7; $CaCl_2$ 1·8; $MgCl_2$ 1·0; NaH_2PO_4 0·3; $NaHCO_3$ 12 and glucose 5·5. The Tyrode solution was maintained at 36 °C and gassed with air. The preparation was allowed to equilibrate under a resting tension of approximately 0·5 g for at least 60 min during which the Tyrode solution was changed at 15 min intervals. Isotonic contractions (magnification ×8) were recorded through a front writing level on smoked paper. Non-cumulative concentration-response curves to acetylcholine, methacholine and carbachol were obtained in the absence and also in the presence of the antagonists (atropine, 4-DAMP, gallamine and pirenzepine). One agonist and antagonist pair was tested on any particular preparation. In all cases, responses to acetylcholine and methacholine were obtained in the presence of physostigmine ($2{\cdot}5 \times 10^{-7}$ M) to inhibit acetylcholinesterase while in some series of experiments, carbachol-induced responses were obtained in the presence of hexamethonium ($2{\cdot}5 \times 10^{-5}$ M) to block nicotinic receptors. Antagonists were allowed to equilibrate with the tissue for 30 min and for each antagonist, three ascending concentrations of the antagonists were tested on any particular tissue. The degree of antagonism was expressed in terms of pA_2 values (Arunlakshana & Schild 1959). Antagonism was regarded as competitive if the slope of the Schild regression line is not significantly different from unity.

Statistical analysis. Values are presented as mean ± s.e. of 'n' observations and where necessary mean pA_2 values were compared using the Student's *t*-test and

differences between mean pA_2 values were taken to be significant when $P < 0.05$.

Drug solutions. The following drugs were used: acetylcholine chloride, acetyl-β-methylcholine (methacholine), carbachol chloride, atropine sulphate (BDH); 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP, generously donated by Dr R. B. Barlow, Department of Pharmacology, University of Bristol); gallamine triethiodide (Flaxedil, May & Baker); hexamethonium bromide (Koch-Light), physostigmine salicylate (Burroughs Wellcome) and pirenzepine (Gastrozepin, Boehringer Ingelheim). All drugs were dissolved in distilled water.

Results

Dose-response curves to acetylcholine, methacholine and carbachol. Most of the preparations developed spontaneous rhythmic contractions within 10 min after they were set up. These spontaneous rhythmic contractions steadily declined during regular dosing of the preparations with agonists. Acetylcholine (1.93×10^{-9}–1.95×10^{-6} M) concentration-dependently contracted the rat rectum. The −log EC50 values were 7.38 ± 1.83 (n = 20) 6.23 ± 0.07 (n = 20) and 5.82 ± 0.07 (n = 20) for acetylcholine, methacholine and carbachol, respectively.

Effect of muscarinic receptor antagonists

Atropine (1.9×10^{-9}–4.8×10^{-8} M) competitively antagonized acetylcholine, methacholine and carbachol-induced contractions with no change in the slope of the concentration-response curves (Fig. 1A). The pA_2 values were 8.99 ± 0.11 (slope = 1.10) 9.29 ± 0.14 (slope = 0.94) and 8.28 ± 0.11 (slope = 0.98) (n = 5 in all cases), for acetylcholine, methacholine and carbachol, respectively. The pA_2 value for atropine against carbachol was significantly ($P < 0.05$) less than the corresponding values against acetylcholine and methacholine. Since carbachol stimulates both muscarinic and nicotinic receptors, an attempt was made to test whether the lower potency for atropine against carbachol (compared with acetylcholine and methacholine) could be due to a simultaneous action of carbachol on nicotinic receptors. This was done by studying the interaction between atropine and carbachol in the presence of hexamethonium (2.5 ± 10^{-5} M). The result shows that hexamethonium did not change the slope of the concentration-response curve to carbachol. Neither the threshold nor maximum concentration was affected. However, in the presence of hexamethonium, atropine produced a mean pA_2 value of 8.86 ± 0.10 with a slope of 1.06 against carbachol. This pA_2 value was significantly ($P < 0.05$) different from that obtained in the absence of hexamethonium but was not significantly ($P > 0.05$) different from the corresponding pA_2 values against acetylcholine and methacholine. Therefore in subsequent experiments involving carbachol, hexamethonium (2.5×10^{-5} M) was always included in the Tyrode solution. 4-DAMP (1.15×10^{-8}–2.86×10^{-7} M) concentration-dependently antagonized acetylcholine, methacholine and carbachol-induced contractions. In all cases, there was no change in the slope of the curves nor was the maximum response suppressed (Fig. 1B). The pA_2 values were 8.38 ± 0.10, 8.66 ± 0.15 and 8.26 ± 0.30 (n = 5 in all cases) respectively against acetylcholine, methacholine and carbachol, respectively. The slopes of the A–S plots were not significantly greater than 1 except for 4-DAMP against acetylcholine which was slightly but significantly ($P < 0.05$) greater than 1.

Gallamine (1.12×10^{-6}–1.12×10^{-4} M) produced parallel rightward shifts of the concentration response curves to acetylcholine, methacholine and carbachol (Fig. 1C). The pA_2 values were 5.83 ± 0.23, 5.73 ± 0.25 and 5.96 ± 0.10 (n = 5 in all cases) for acetylcholine, methacholine and carbachol, respectively. In all cases, the antagonism, though surmountable, was probably non-competitive as judged by the slopes of the Schild regression lines—0.47, 0.42 and 0.41 for acetylcholine, methacholine and carbachol, respectively. Pirenzepine (2.8×10^{-7}–7.0×10^{-6} M) produced parallel rightward shifts of the concentration response curves to acetylcholine, methylcholine and carbachol (Fig. 1D). The pA_2 values were 6.85 ± 0.04 (slope = 1.06 ± 0.04); 7.17 ± 0.13 (slope = 1.08 ± 0.20) and 7.21 ± 0.03 (slope = 0.92 ± 0.04) (n = 5 in all cases) against acetylcholine, methacholine and carbachol, respectively.

Combination of atropine and gallamine

These series of experiments were performed to test whether competitive antagonists and gallamine have a common site of action. Atropine was used as a standard competitive muscarinic receptor antagonist (see Discussion). Concentration response curves were obtained for acetylcholine, methacholine and carbachol. Three concentrations of atropine in the presence of a fixed concentration of gallamine were then tested against agonist-induced contractions. Only one concentration of atropine was tested on any particular preparation. Table 1 shows the dose-ratios obtained for atropine against acetylcholine, methacholine and carbachol induced contractions in the presence of 1.12×10^{-4} M gallamine. The experimental dose-ratio (combination antagonist) was greater than the expected dose-ratio at all concentrations of atropine.

Discussion

These results show that acetylcholine, methacholine and carbachol are potent muscarinic receptor agonists in the rat rectum with the potency order; acetylcholine > methacholine > carbachol. This difference in agonist potency might suggest that there are subtypes of muscarinic receptors in the rat rectum, however, this was not supported by results obtained with the antagonists. For example, gallamine, which is supposed to be a specific allosteric antagonist at cardiac muscarinic

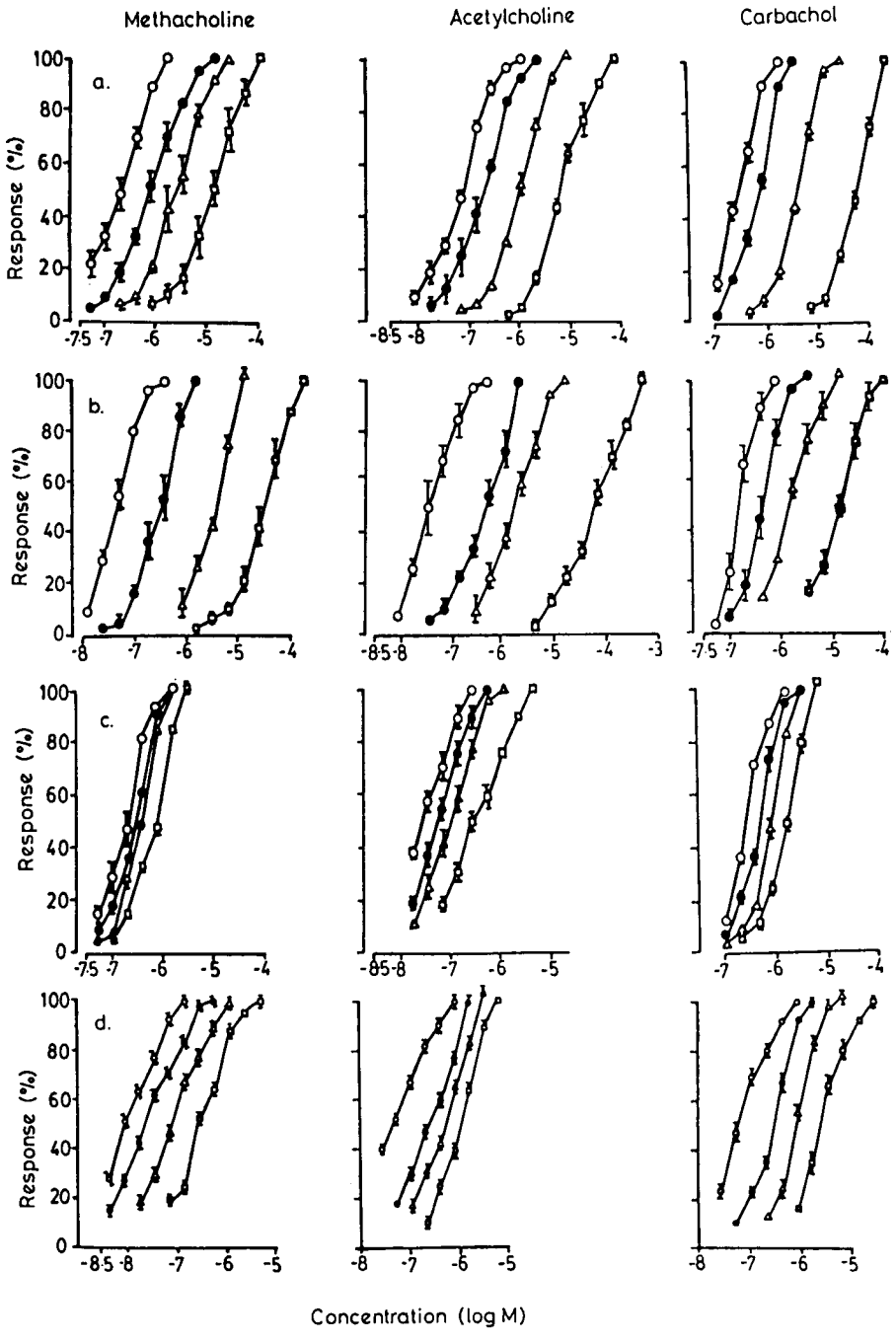

FIG. 1. Effect of (a) atropine (● $1{\cdot}9 \times 10^{-9}$, △ $9{\cdot}7 \times 10^{-9}$, □ $4{\cdot}8 \times 10^{-8}$ M), (b) 4-DAMP (● $1{\cdot}15 \times 10^{-8}$, △ $5{\cdot}75 \times 10^{-8}$, □ $2{\cdot}86 \times 10^{-7}$ M), (c) gallamine (● $1{\cdot}12 \times 10^{-6}$, △ $1{\cdot}12 \times 10^{-6}$, □ $1{\cdot}12 \times 10^{-4}$ M), (d) pirenzepine (● $2{\cdot}8 \times 10^{-7}$, △ $1{\cdot}4 \times 10^{-6}$, □ $7{\cdot}0 \times 10^{-6}$ M), on acetylcholine-, methacholine- and carbachol-(○) induced contraction of the rat rectum. Each point represents mean ± s.e. of 4 determinations. Responses to carbachol were obtained in the presence of hexamethonium ($2{\cdot}5 \times 10^{-5}$ M).

Table 1. Dose-ratios produced by atropine ($1{\cdot}93 \times 10^{-9}$–$4{\cdot}85 \times 10^{-8}$ M) and gallamine ($1{\cdot}1 \pm 10^{-4}$ M) alone and in combination with acetylcholine, methacholine and carbachol as agonists.

Agonists	Atropine (M)	Atropine Dose-ratio (DR_A)	Gallamine (0·11 mM) Dose-ratio (DR_G)	Expt. comb. Dose-ratio (DR_G)	Expected combined Dose-ratio $DR_G + DR_G - 1$
Acetylcholine	$1{\cdot}93 \times 10^{-9}$	4·0 ± 1·2	12·1 ± 2·2	35·3 ± 5·0	15·1
	$9{\cdot}70 \times 10^{-9}$	24·2 ± 1·6	,,	83·1 ± 15·0	35·0
	$4{\cdot}85 \times 10^{-8}$	187·0 ± 13·7	,,	1200·0 ± 201·0	198·0
Methacholine	$1{\cdot}93 \times 10^{-9}$	4·7 ± 0·7	5·8 ± 1·2	25·0 ± 6·5	9·5
	$9{\cdot}70 \times 10^{-9}$	14·6 ± 2·3	,,	33·0 ± 6·3	19·4
	$4{\cdot}85 \times 10^{-8}$	84·3 ± 12·0	,,	140·0 ± 23·5	89·1
Carbachol	$1{\cdot}93 \times 10^{-9}$	2·8 ± 0·8	8·0 ± 0·9	14·7 ± 3·6	9·8
+ hexamethonium	$1{\cdot}93 \times 10^{-8}$	16·2 ± 3·4	,,	40·9 ± 4·9	23·2
($2{\cdot}5 \times 10^{-5}$ M)	$1{\cdot}93 \times 10^{-7}$	241·0 ± 40·0	,,	267·0 ± 7·6	247·0

receptors, was equipotent against acetylcholine, methacholine and carbachol. Also, as in atria (Clark & Mitchelson 1976), gallamine was not a competitive antagonist, judging from the slopes of the A–S plots, which for the rat rectum all ranged between 0·41 and 0·47. Similarly, 4-DAMP, a preferential antagonist of ileal muscarinic receptors (Barlow et al 1976), was equipotent against all three agonists. Pirenzepine also was equipotent against acetylcholine, methacholine and carbachol and the pA_2 values were similar to values reported on the atria and the ileum (Barlow et al 1981; Szelenyi 1982), supporting the fact that pirenzepine does not differentiate between ileal and cardiac muscarinic receptors. It therefore appears that the muscarinic receptors in the rat rectum possess characteristics similar to ileal and cardiac muscarinic receptors. The question then arises: are the antagonists acting at the same site? This was investigated by studying the interaction between atropine (used because of its potency and, at least in this preparation, the same spectrum of action as pirenzepine and 4-DAMP) and gallamine on acetylcholine-, methacholine- and carbachol-induced contractions. The rationale was that gallamine, being a metaffinoid antagonist (Clark & Mitchelson 1976), would differentially alter the binding of agonists and competitive antagonists, and as a result the experimental dose-ratio of the combination would either be less or greater than the expected dose-ratio (Paton & Rang 1965) depending on whether the binding of the antagonists was altered more or less than that of the agonists (e.g. Clark & Mitchelson 1976). Our results show that gallamine possibly altered the binding of acetylcholine and methacholine more than it altered the binding of atropine (thus the experimental dose-ratio was significantly greater than expected), while the binding of carbachol and atropine was altered almost to a similar extent (the experimental dose-ratio approximated the expected dose-ratio). These results suggest there could be a homogenous population of muscarinic receptors in the rat rectum and that the antagonists produce their characteristic effects by inducing different conformations of the receptor site.

REFERENCES

Arunlakshana, O., Schild, H. O. (1959) Br. J. Pharmacol. 14: 48–58

Barlow, R. B., Berry, K. S., Glenton, P. A. M., Nikolaou, N. M., Soh, K. S. (1976) Ibid. 58: 613–620

Barlow, R. B., Caulfield, M. P., Kitchen, R., Roberts, P. M., Stubley, J. K. (1981) Ibid. 182–184P

Brown, D. A., Forward, A., Marsh, S. (1980) Ibid. 71: 362–364

Clark, A. L., Mitchelson, F. (1976) Ibid. 58: 323–331

Del Tacca, N., Lecchini, S., Frigo, G. N., Benzi, G. (1968) Eur. J. Pharmacol. 4: 188–197

Li, C. K., Mitchelson, F. (1980) Br. J. Pharmacol. 70: 313–321

Paton, W. D. M., Rang, H. P. (1965) Proc. R. Soc. B. 163: 1–44

Riker, W. F., Wescoe, W. C. (1951) Ann. N.Y. Acad. Sci. 54: 373–392

Szelenyi, I. (1982) Br. J. Pharmacol. 77: 567–569