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The relative potencies of some agonists at M_2 muscarinic receptors in guinea-pig ileum, atria and bronchi

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1 The effects of some agonists on isolated preparations of guinea-pig ileum, atria and bronchial muscle have been compared with those of carbachol. The concentrations producing comparable responses were used to estimate the equipotent molar ratio relative to carbachol.

2 Arecaidine propargyl ester was 4 to 5 times as active as carbachol on the ileum but more than 10 times as active as carbachol on atrial rate or atrial force, so the results confirm that this compound has a 2 to 3 fold selectivity for receptors in atria.

3 Ethoxyethyltrimethylammonium iodide was one-quarter to one-third as active as carbachol on ileum but only one-tenth as active as carbachol on atrial rate or atrial force and so shows a 3 to 4 fold selectivity for receptors in ileum.

4 The other compounds tested, which included acetylcholine, methacholine, *n*-pentyltrimethylammonium iodide and bethanechol showed less selectivity.

5 There were no obvious differences between effects on atrial rate and effects on atrial force, though with esters it was often difficult to obtain effects on atrial rate in the absence of an inhibitor of cholinesterase.

6 Activity on bronchial muscle was generally similar to activity on ileum.

Introduction

Though the original classification of the actions of acetylcholine by Dale (1914) was made with the agonists muscarine and nicotine, the subsequent classification of receptors has usually involved the actions of antagonists, such as atropine. The subclassification of muscarinic receptors has involved antagonists such as pirenzepine, 4-diphenyl-acetoxy-*N*-methylpiperidine (4DAMP) metho-salts or gallamine triethiodide (Birdsall & Hulme, 1983).

Less attention has been given to agonists. The muscarine-like activity of *m*-chloro-phenylcarbamyl ester of 4-hydroxy-but-2-ynyl-trimethylammonium (McN-A 343: Roszkowski, 1961) at sympathetic ganglia has been known for some time and this appears to be selective for M_1 (pirenzepine-sensitive)-receptors (Hammer & Giachetti, 1982: Mutschler & Lambrecht, 1984). There do not as yet appear to be any agonists with similar marked selectivity at subtypes of M_2-receptors. Barlow *et al.* (1980) found that compared with carbachol, 4-acetoxy-*N*-methylpiperidine methiodide (4AMP methiodide) was appreciably weaker on atria than on ileum in the guinea-pig but the difference was only three fold. The 3-substitued isomer (3AMP methiodide) was likewise stronger on ileum than on atria but produced effects on atrial size in concentrations which did not affect the atrial rate, suggesting that there might be separate muscarinic receptors involved in effects on atrial rate and on atrial force. Mutschler & Lambrecht (1984) studied the resolved forms of 3AMP methiodide and the corresponding thiane (with the piperidine nitrogen replaced by sulphur), and found that not only did the enantiomers differ in potency, indicating stereospecificity, but there were differences between tissues, indicating some selectivity. The S-isomer of 3AMP methiodide had only one tenth of the activity on guinea-pig atrial force that it had on the guinea-pig ileum. However, arecaidine propargyl ester (APE: Mutschler & Hultzsch, 1973), a tertiary base which is much more potent than carbachol, was more active on the atria than on ileum.

Furchgott & Cherry (1984) measured the affinities of agonists, as well as antagonists, for muscarinic receptors in rabbit aorta and stomach but there did not

appear to be much selectivity with compounds such as methacholine, carbachol and *n*-butyltrimethylammonium compared with acetylcholine, though pilocarpine was an antagonist on the aorta preparation, whereas it was an agonist on the stomach.

Results with agonists have nevertheless been rather fragmentary, many have been tested for effects on atrial force but not on atrial rate, for instance. This paper describes the simultaneous comparison of several muscarinic agonists which might show some degree of selectivity, on guinea-pig ileum, bronchial muscle and on atrial force and atrial rate.

Methods

The guinea-pig isolated ileum and isolated atria were set up as described in the previous paper (Barlow & Shepherd, 1985) in Krebs solution and without hexamethonium. The guinea-pig isolated bronchial strip preparation was set up in Krebs solution at 37°C, and aerated with a mixture of 95% O_2 and 5% CO_2, as described by Barlow *et al.* (1972). A single spiral strip was obtained from each animal and agonists were allowed to act for 5 min; the preparation was then washed and washed again 5 min later, with the next dose of agonist added after a further 6 min, making a cycle time of 16 min, slightly longer than in experiments with the atria (see below).

Experiments were done on the ileum at 30°C, for comparison with experiments on the atria, and at 37°C, for comparison with the bronchial strip. The agonists were allowed to act for 30 s and the cycle time was 2 min. Alternate large and small responses were obtained with carbachol, usually 0.1 and 0.2 μM, and when these were regular similar responses were obtained with the test agonist. The agonist was then changed back to carbachol and the process repeated with another test agonist. Up to 6 agonists were tested on any one preparation with 6 pairs of responses to

Table 1 The numbers are the mean of the equipotent molar-ratio relative to carbachol (± s.e. and number of estimates)

	Atria 29.5°C		*Ileum* 29.5°C	37°C	*Bronchi* 37°C	
	Rate	*Force* (F)	(I)			F/I
Arecaidine propargyl ester						
(i)	0.079 ±0.020(5)	0.077 ±0.005(5)	0.156 ±0.010(5)	0.185 ±0.012(5)	0.404 ±0.056(5)	0.49
(ii)	0.077 ±0.007(8)	0.097 ±0.012(8)	0.263 ±0.011(9)	0.250 ±0.001(9)		0.37
Pooled	0.078 ±0.008(13)	0.089 ±0.008(13)	0.225 ±0.016(14)	0.227 ±0.011(14)		0.40
Acetylcholine	0.233 ±0.016(3)	0.193 ±0.031(3)	0.624 ±0.032(5)	0.543 ±0.042	0.371 ±0.202(2)	0.31
Methacholine	0.276 ±0.025(2)	0.274 ±0.077(2)	0.568 ±0.034(5)	0.690 ±0.032(5)	0.262 ±0.138(2)	0.48
Ethoxyethyltrimethylammonium bromide	10.2 ±1.62(4)	9.13 ±0.88(5)	2.56 ±0.17(5)	3.07 ±0.11(5)	3.42 ±0.33(5)	3.6
n-Pentyltrimethylammonium iodide	15.1 ±0.98(4)	13.2 ±0.34(4)	8.38 ±0.34(5)	9.65 ±0.30(5)	7.67 ±0.35(5)	1.6
Bethanechol chloride	20.1 ±1.01(5)	20.7 ±1.55(4)	10.4 ±0.63(5)	13.0 ±0.48(6)	16.7 ±1.08(5)	2.0
4-Acetoxy-*N*-methylpiperidine methiodide (4AMP)		27.0 ±3.45(4)	10.7 ±0.35(5)	11.6 ±0.60(5)		2.5

All tissues were suspended in Krebs solution without hexamethonium: selectivity is indicated by F/I

low and high doses of agonist compared with 6 pairs to carbachol (3 pairs taken from just before, and 3 pairs from just after, the test agonist). The concentrations of agonists were chosen so that the responses roughly matched those produced by carbachol and a calculation similar to that used in a 4-point assay (Edinburgh Staff, 1974) was used to obtain the concentrations which should produce exactly the same response. The result was expressed as an equipotent molar ratio relative to carbachol: this will be less than 1 if the compound is more active than carbachol but greater than 1 if it is weaker.

In the experiments on atria the time cycle was 14 min, with the agonist allowed to act for 5 min, with a second wash after 4.5 min. The concentrations of carbachol tested were usually 0.75, 1.5 and 3.0 μM, producing slight, moderate and marked effects but only two concentrations of test agonist were tested. Because of the interval between doses only two agonists could be compared with carbachol on any one preparation and only 2 pairs of responses to the test compound and to carbachol were used for the calculation of the equipotent molar ratio.

In the experiments on bronchial muscle at least three pairs of responses were used and usually up to three agonists could be compared with carbachol on any one preparation.

With all the preparations the order in which the agonists were tested was arranged so that they were as evenly distributed as possible. Experiments with acetylcholine and methacholine on the atria and bronchial strip were made in the presence of 0.2 μM neostigmine bromide. The compounds all appeared to be full agonists and the comparison of 4-acetoxy-*N*-methylpiperidine methiodide with carbachol did not appear to be affected by hexamethonium (0.28 mM).

Acetylcholine iodide, methacholine chloride, carbachol chloride and bethanechol chloride were obtained from Sigma. Arecaidine propargyl ester hydrochloride was a gift from Dr G. Lambrecht.

Results

The results are shown in Table 1. Two separate sets of experiments were done with arecaidine propargyl ester and the values of the equipotent molar-ratios relative to carbachol obtained on guinea-pig ileum are appreciably different, more than would be expected from the estimates of the variance within each group. They are also appreciably larger than the values, 0.023 for atria and 0.091 for ileum, calculated from estimates of the log EC_{50} given by Mutschler & Lambrecht (1984). It is clear, nevertheless, that the compound is considerably more potent than carbachol and is about 3 times as active on atria as it is on the ileum. In the presence of neostigmine, acetylcholine and methacholine are also more active on atria than on ileum, but if cholinesterase is not blocked these compounds are only feebly active on atria. The results with 4AMP methiodide were surprisingly different from those of Barlow *et al.* (1980): effects on atrial rate could not be measured.

The ratio F/I in Table 1 gives some idea of the relative selectivity of the compounds.

Discussion

It seems likely that hydrolysis by cholinesterase accounts for differences between estimates of equipotent molar ratios for some esters, such as acetylcholine, methacholine and 3AMP methiodide, and may explain why Barlow *et al.* (1980) could not obtain effects on atrial rate with 3AMP methiodide in concentrations that decreased the force of the atrial contractions. Effects on atrial force reach a steady state much more quickly than effects on rate. It was, however, surprising that in this work similar results were obtained with 4AMP methiodide because this compound is only hydrolysed slowly by acetylcholinesterase from electric eel (Barlow & Kitchen, 1982). Although it is possible to do experiments in the presence of an anticholinesterase it is preferable not to have to do this and to work with compounds which are not hydrolysed.

The results obtained by Ing *et al.* (1952) on guinea-pig ileum and rabbit atria suggested that *n*-pentyltrimethyl ammonium iodide should be relatively more active at muscarinic receptors in ileum than at those in atria and that ethoxyethyl-trimethylammonium iodide (EOE) should be less selective. We have confirmed that both are relatively more active on ileum than atria but ethoxyethyltrimethylammonium iodide was appreciably more selective, being 3 to 4 times as active on ileum as on atria: it is also more selective than bethanechol, which has been used as a stimulant of M_2-receptors in the oesophageal sphincter of the oppossum by Rattan & Goyal (1984).

As in the previous paper (Barlow & Shepherd, 1985), the results show no convincing differences between effects on atrial rate and effects on atrial force and if the actions of cholinesterase account for the observations of Barlow *et al.* (1980), it seems that the muscarinic receptors involved in effects on rate may, after all, be the same as those involved in effects on force. Mutschler & Lambrecht (1984), however, observed large differences in effects on rate and force with a bridged-ring derivative of arecaidine propargyl ester. Although this is an ester and a partial agonist, it is probably advisable to consider the question as unsettled and to continue to measure effects on both force and rate.

Only limited results were obtained on bronchial

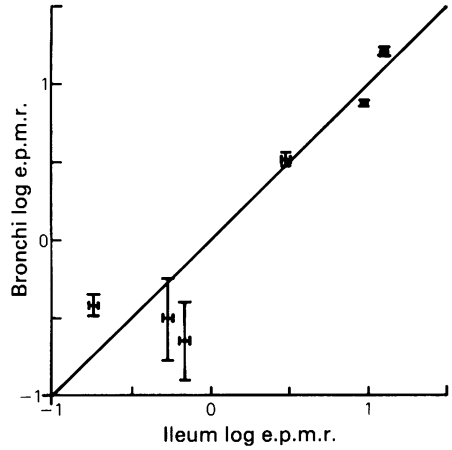

Figure 1 Estimates of the log equipotent molar ratio relative to carbachol on bronchial muscle at 37°C plotted against the log equipotent molar ratio relative to carbachol on ileum at the same temperature. The standard, carbachol, would have the values 0,0. The bars indicate the s.e. of the estimates.

muscle. From the work with antagonists by Barlow *et al.* (1974) it appeared that the receptors are not dissimilar. When values of log.equipotent molar ratios for ileum are plotted against those for bronchial muscle (Figure 1) the results lie fairly close to the line of identity, though there are large errors attached to results for esters, such as acetylcholine and methacholine, associated with the difficulties of making measurements over long periods in the presence of an anticholinesterase.

The most selective of the compounds tested are therefore APE, which is more selective for receptors in atria, and EOE, which is more selective for receptors in ileum and it would be interesting to use both these compounds as agonists in experiments with antagonists. Furthermore, because the compounds are agonists, the selectivity can arise from differences in efficacy, as well as from differences in affinity. The measurements of equipotent molar ratios described in this paper do not make it possible to assess these separately and it might be worth supplementing this work with radio-ligand binding studies.

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