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A search for selective antagonists at M_2 muscarinic receptors

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1 Isolated preparations of guinea-pig ileum and atria have been used to estimate the dose-ratios produced by antagonists at muscarinic receptors. Experiments with 4-diphenyl-acetoxy-*N*-methyl-piperidine (4DAMP) metho-salts and with its isomer, 3DAMP methiodide, indicate that these are only slightly affected by the choice of physiological salt solution, the choice of agonist and the presence or absence of hexamethonium.

2 Methyl or chloro groups in the *p*-position of the two benzene rings in 4DAMP metho-salts markedly reduce affinity and selectivity.

3 When the two benzene rings are linked together, as in the fluorene-9-carboxylic ester, the affinity for the receptors in the atria is comparable with that of 4DAMP methobromide but that for the ileum is about half, so the selectivity is reduced. When the rings are linked as in the xanthene-9-carboxylic ester, the affinity for receptors in both tissues is greater than that of 4DAMP methobromide but there is less selectivity.

4 When two molecules of 4DAMP are linked together by a polymethylene chain of from 4 to 12 carbon atoms the effects on affinity for muscarinic receptors in the guinea-pig ileum are different from those on affinity for muscarinic receptors in guinea-pig atria. The pentamethylene compound is the most selective: compared with 4DAMP methobromide it has slightly less affinity for receptors in the ileum but much less affinity for receptors in the atria.

5 The effects of the compounds in antagonizing the actions of carbachol on atrial rate are not markedly different from their effects in antagonizing its actions on the force of the atrial contractions.

Introduction

In the last few years it has become apparent that there are subtypes of muscarinic acetylcholine receptors and relatively selective blocking agents have begun to emerge. The compound pirenzepine has much greater affinity for some muscarinic receptors, classified as M_1 and present, for instance, in hippocampus and superior cervical ganglion, than for others, classified as M_2 and present, for instance, in ileum and atria. The compound 4-diphenylacetoxy-*N*-methyl-piperidine (4DAMP) methiodide or methobromide, however, has greater affinity for muscarinic receptors in ileum than for those in atrial pacemaker cells so it appears that M_2-receptors must be further subdivided. There are suggestions that there may be differences within atrial tissue, between receptors concerned with effects on atrial rate and those concerned with effects on the force of contraction: there is also evidence for an allosteric site at muscarinic receptors in the heart, which can be blocked by some neuromuscular blocking agents, e.g. gallamine. The subject has been reviewed by Caulfield & Straughan (1983), Birdsall & Hulme (1983), and Hammer & Giachetti (1984).

This paper describes attempts to improve the selectivity of 4DAMP methiodide for subclasses of M_2-receptors. Because appreciable selectivity was also found in hyoscine methiodide it was thought that the 4-hydroxypiperidine ring might be important but although the corresponding ester of 4-hydroxyquinuclidine (4DAQ methiodide: Figure 1) has greater affinity for muscarinic receptors it is less selective (Barlow & Kitchen, 1982). A summary of the affinities and selectivities of compounds related to 4DAMP is shown in Figure 2.

This does not suggest any further changes which should be made to the piperidine ring to improve selectivity so the other end of the molecule has been altered. Chloro- and methyl groups have been introduced into the *p*-position of the benzene ring and in

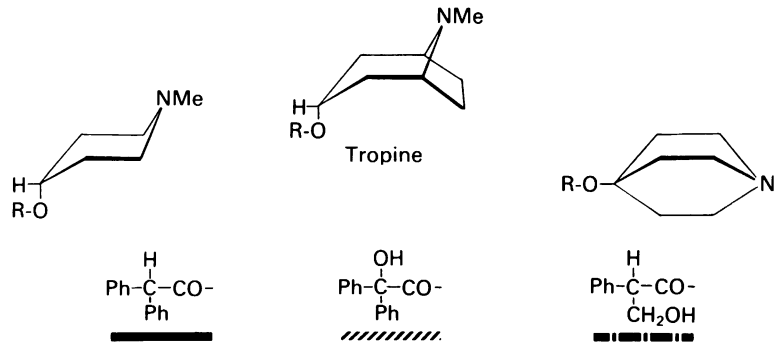

Figure 1 Structures of compounds: 4DAMP methiodide is the methiodide of the ester of 4-hydroxy-*N*-methylpiperidine (top left) with diphenylacetic acid (Bottom left): 4DAQ methiodide is the corresponding compound formed from the ester of diphenylacetic acid with 4-hydroxyquinuclidine (top right). Atropine is formed from tropine and racemic tropic acid (bottom right). Hyoscine (scopolamine) is formed from tropic acid and scopine (oscine), which is tropine with an epoxide bridge across the 5-membered ring.

The symbols below the acids are used to identify the compounds in Figure 2.

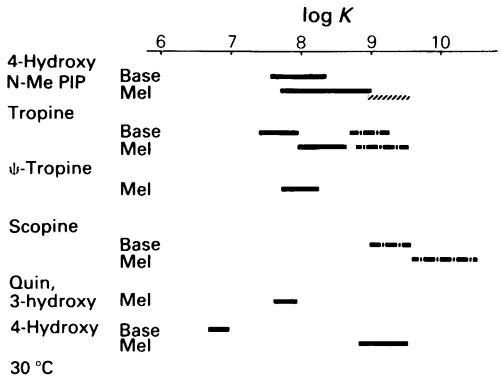

Figure 2 Estimates of the log affinity constants of some compounds related to atropine and 4-diphenyl-acetoxy-*N*-methylpiperidine (4DAMP) methiodide (Barlow *et al.*, 1976; Barlow & Kitchen, 1982). The bases are indicated on the left with 4-hydroxy-*N*-methylpiperidine at the top and 4-hydroxyquinuclidine at the bottom. The acids are identified by the same symbols in Figure 1. The right-hand end of the bar indicates log *K* for receptors in guinea-pig ileum and the left-hand end indicates log *K* for receptors in guinea-pig atrial pacemaker cells, which is lower for all the compounds in this group. The width of the bar indicates the selectivity. This is greatest in the methiodide of the ester of 4-hydroxy-*N*-methylpiperidine with diphenylacetic acid (4DAMP methiodide; second from the top) but is less in the corresponding ester of 4-hydroxyquinuclidine (4DAQ methiodide; bottom), even though this has higher affinity (lies further to the right). Note the appreciable selectivity of hyoscine methiodide (*N*-methyl scopolamine: extreme right).

The estimated errors in log *K* are 0.1 log units for the ileum and 0.15 log units for the atria (but see results).

other compounds, esters of fluorene- and xanthene-9-carboxylic acids, the rings have been locked together.

A series of polymethylene *bis*-4DAMP compounds has also been tested. Some *bis*-atropinium compounds, prepared as potential neuromuscular blocking agents and studied by Kimura *et al.* (1949), Kimura & Unna (1950) and Eckfield (1959) were found to retain atropine-like activity, though their selectivity cannot be estimated from the published results. A quaternary methyl group in 4DAMP methosalts can be replaced by higher homologues without the total loss of selectivity (Barlow & Shacklady, unpublished) so it seemed possible that the *bis*-onium salts might retain affinity for muscarinic receptors and it was hoped that the effects of chain length on affinity might be different for different subclasses.

The paper begins with an assessment of the extent to which experimental conditions affect estimates of dose-ratios and selectivity.

Methods

Guinea-pig isolated ileum

The guinea-pig ileum was set up as described by Edinburgh Staff (1974) with the responses recorded isotonically and a load of about 0.5g. The agonist was usually carbachol (see below), allowed to act for 30 s and added once every 90 s by relays controlled from a PET microcomputer. The tissue was suspended in Krebs solution (Edinburgh Staff, 1974), aerated with a mixture of 95% O_2 and 5% CO_2 and experiments were done at 29.5 ± 0.3° and 37.0 ± 0.1°C. Alternate small

and large control responses were obtained, usually with 0.1 and 0.2 μM carbachol, and when these were regular the tissue was exposed to a solution of the antagonist and the concentration of agonist was increased to try to obtain responses which roughly matched the controls. When these were regular, this could take 30 min or longer, the size of the responses could be used to obtain an estimate of the exact dose-ratio by a calculation similar to a 4-point assay (Edinburgh Staff, 1974).

Table 1 The numbers are the mean dose-ratios (± s.e. and number of estimates) produced by 0.1 μM 4-diphenylacetoxy-*N*-methyl-piperidine (4DAMP) metho-salts

	Atria		*Ileum*	
A				
	29.5°C		29.5°C	37°C
	Rate	*Size*		
	APE		APE	APE
	10.1 ± 1.5 (6)	9.8 ± 1.0 (7)	103 ± 11.2 (6)	62.6 ± 3.5 (6)
	CCh		AMP	AMP
	13.3 ± 2.2 (4)	10.3 ± 1.4 (4)	103 ± 12.3 (6)	88.9 ± 8.9 (6)
B				
	29°C CCh		29°C CCh	37°C CCh
	7.3 ± 1.1 (4)		132 ± 5.4 (6)	63.3 ± 7.2 (7)
	5.0 ± 0.1 (3)		72.8 ± 2.1 (4)	49.9 ± 3.4 (4)
	30°C CCh		30°C CCh	
	7.1 ± 0.4 (4)	7.5 ± 0.6 (3)	92 ± 12.0 (2)	
C	3-DAMP methiodide			
	29.5°C CCh		29.5°C CCh	37°C CCh
1 μM				
	12.8 ± 3.7 (4)	14.8 ± 2.8 (4)	14.4 ± 2.3 (4)	14.1 ± 1.8 (4)
5 μM				
	70.4 ± 17.4 (4)	53.2 ± 5.5 (4)	54.2 ± 11.7 (4)	64.1 ± 10.6 (4)
Mean log *K* (8 estimates)				
	7.055 ± 0.083	7.065 ± 0.017	7.054 ± 0.055	7.093 ± 0.016

Values in section A were obtained with 4DAMP methobromide and with the agonists indicated (CCh = carbachol; APE = arecaidine propargyl ester; AMP = 4-acetoxy-*N*-methylpiperidine methiodide). All the tissues were suspended in Krebs solution without hexamethonium.

Values in section B are taken from Barlow *et al.* (1976: two sets) and Barlow *et al.* (1980) with 4DAMP methiodide and with carbachol as the agonist and hexamethonium (0.28 mM) in the physiological saline, which was Ringer-Locke solution for atria and Tyrode solution for the ileum.

Values in section C were obtained with 3DAMP methiodide in concentrations of 1 and 5 μM and include estimates of the mean value of log affinity constant. The value of ileum at 37°C should be compared with 7.094 ± 0.006 (6), obtained by Abramson *et al.* (1974) with carbachol as agonist and in the presence of hexamethonium.

Guinea-pig isolated atria

The atria were set up in Krebs solution (Edinburgh Staff, 1974), aerated with 95% O_2 and 5% CO_2. The temperature was 29.5 ± 0.3°C and the spontaneous contractions were recorded isometrically with a load of about 0.2g: the action potentials were also recorded (Barlow & Kitchen, 1982). The agonist was usually carbachol, allowed to act for 4 min and given once every 16 min and its effect was expressed, as previously, as the percentage increase in the time required for 50 beats, calculated from the values at the end of the application of the agonist and the values just before its application. These were recorded by a device produced by Mr R.O. Morris, which counted the action potentials and automatically printed out the time for 50 impulses. The action potentials were also displayed on an oscilloscope and the correct functioning of the apparatus was occasionally checked by counting the potentials or the beats with a stop-watch.

As in the experiments on ileum, there was a control period in which responses were obtained with alternate low and high concentrations of carbachol, usually 0.1 and 0.2 μM: the preparation was then exposed to the antagonist and the concentrations of carbachol increased. The responses in the presence of the antagonist appeared to be regular after about 30 min, i.e. the response to the first application of agonist, 12 min after exposure to the antagonist, was ignored. However, because of the much longer time-cycle dose-ratios were usually based only on two pairs of control responses and two pairs in the presence of the antagonist.

These methods are similar to those previously used (Barlow *et al.*, 1976; 1980; Barlow & Kitchen, 1982) except that Krebs solution, aerated with 95% O_2 and 5% CO_2, was used for all tissues and no hexamethonium was present.

In some experiments the antagonists were only tested in one concentration, chosen so that the dose-ratios were in the range 10 to 250, but in others, including the polymethylene *bis*-4DAMP bromides, two concentrations were tested. Wherever possible the experiments were done simultaneously with the same solutions on all three preparations.

Compounds

Fluorene-9-carboxylic acid and xanthene-9-carboxylic acid were purchased from Aldrich. Di-(*p*-chlorophenyl)acetic acid was obtained by the hydrolysis of DDT. Di-*p*-tolylacetic acid was obtained from *p*-tolualdehyde by way of the corresponding benzoin, benzil, and benzilic acid, which was reduced to the diphenylacetic acid.

The acids were converted to the acid chlorides with thionyl chloride and these were reacted with a slight excess of 4-hydroxy-*N*-methylpiperidine dissolved in chloroform. The free esters were isolated and distilled (the yields were all greater than 50%) and treated with methyl bromide in acetone or butanone.

The *bis*-onium salts were obtained by refluxing the polymethylene dibromide (typically 1g) with an excess (2.5 equivalents) of 4-diphenylacetoxy-*N*-methylpiperidine (DAMP base; b.p.186°/1 mm) in butanone for one week. The product separated as an amorphous solid (for the trimethylene to octamethylene and dodecamethylene chains) or as a gummy oil (for the nonamethylene, decamethylene and undecamethylene chains). The oils were solidified by the addition of diethylether (50 ml) followed by trituration. The products were filtered, washed with a 1:1 mixture of acetone and ether, and dried under vacuum. To purify them they were dissolved in ethanol (approximately 15 ml per g of salt) and reprecipitated by dropwise addition of diethylether. These *bis*-onium compounds are extremely hygroscopic and do not melt but sinter over a wide range of temperature.

The structures of all the compounds were checked by their i.r. and n.m.r. spectra. All the analyses for carbon are lower than the theoretical but the analyses for bromide, hydrogen and nitrogen are satisfactory, except for the *bis*-compound with $n = 4$. There appear to be systematic errors in the estimation of carbon: they occur with the mono-analogues of 4-DAMP as well as with the *bis*-compounds. Low carbon might indicate incomplete formation of the *bis*-compounds but this should be accompanied by low bromide, nor was there any suggestion that this was occurring from the n.m.r. spectra.

4-(Di-*p*-tolyl)acetoxy-*N*-methylpiperidine:
b.p.205–210°/1 mm, m.p.80–82°.
Methobromide m.p.241.5–243.5°:
found C,63.3; H,7.10; N,3.18; Br,19.0:
theory C,63.8; H,6.95; N,3.24; Br,18.5%

4-(Di-*p*-chlorophenyl)acetoxy-*N*-methylpiperidine:
b.p. 208–210°/0.7 mm, m.p.85–86°.
Methobromide m.p.207–208°:
found C,52.8; H,5.19; N,2.70; Br,17.0:
theory C,53.4; H,5.08; N,2.96; Br,16.9%

4-(Fluoren-9-carboxy)-*N*-methylpiperidine:
b.p.185–190°/0.77 mm
Methobromide m.p.185–195°:
found C,60.2; H,5.92; N,3.36; Br,19.7:
theory C,62.6; H,5.97; N,3.48; Br,19.9%

4-(Xanthen-9-carboxy)-*N*-methylpiperidine:
b.p.200°/1 mm
Methobromide m.p.219–221°:
found C,59.7; H,5.77; N,2.97; Br,19.1:
theory C,60.3; H,5.75; N,3.35; Br,19.1%

Polymethylene-*bis*-(4-(diphenylacetoxy)-*N*-methylpiperidinium) bromides: melting-points were taken at a rate of heating of 10 degrees min^{-1}.

	C	H	N	Br%
n = 4: m.p. 220–225°				
Found	62.0	6.49	3.05	21.2
Theory	63.3	6.47	3.36	19.2
n = 5: m.p.175–180°				
Found	62.3	6.70	3.36	18.4
Theory	63.7	6.60	3.30	18.9
n = 6: m.p.215–220°				
Found	63.6	6.57	3.57	18.5
Theory	64.0	6.73	3.25	18.5
n = 7: m.p.185–190°				
Found	63.7	6.90	3.16	18.4
Theory	64.4	6.85	3.19	18.3
n = 8: m.p.190–210°				
Found	63.9	7.15	3.13	18.3
Theory	64.7	6.97	3.14	18.0
n = 9: n.p.180–200°				
Found	64.5	7.25	2.94	17.6
Theory	65.0	7.08	3.10	17.7
n = 10: m.p.195–200°				
Found	64.8	7.15	3.10	17.5
Theory	65.4	7.20	3.04	17.5
n = 11: m.p.180–200°				
Found	65.3	7.27	3.04	17.4
Theory	65.7	7.30	3.00	17.2
n = 12: m.p.80–90°				
Found	64.2	7.44	2.70	16.9
Theory	66.0	7.40	2.96	16.8

Results

Errors associated with estimates of selectivity

Table 1 shows the results of experiments carried out to check the effects of the choice of salt solution, of agonist, and of hexamethonium on estimates of the dose-ratio. Section A shows the mean values (± s.e.) obtained with 0.1 μM 4DAMP methobromide, when all experiments were done in Krebs solution with no hexamethonium present. On the ileum the agonist was 4-acetoxy-*N*-methylpiperidine methiodide (4AMP methiodide; Barlow *et al.*, 1980) or arecaidine propargyl ester, which has very low nicotine-like activity (Mutschler & Hultzsch, 1973), instead of carbachol. Results were obtained with both agonists (in alternate order) on each piece of ileum. It was not possible to perform similar experiments on atria because of the time required.

Section B shows previous estimates obtained with 0.1 μM 4DAMP methiodide obtained with carbachol as agonist and in the presence of hexamethonium (0.28 mM), with Tyrode solution used for the ileum and Locke solution used for the atria. It was not expected that the use of 4DAMP methobromide, rather than the methiodide, would affect the results.

Section C shows results for 3DAMP methiodide, which had not previously been tested on atria and showed no selectivity.

There are differences between the values in sections A and B, but these are not large. In section C the mean estimate of kog K for ileum at 37° agrees well with the estimate obtained by Abramson *et al.* (1969) in Tyrode solution with carbachol as agonist and in the presence of hexamethonium. With this preparation the nicotinic activity of carbachol does not appear seriously to affect the dose-ratios, at least in the range up to 100. This conclusion is supported by further experiments on ileum, shown in Table 2, in which dose-ratios were obtained with both carbachol and arecaidine propargyl ester as agonist.

There is considerable variation between estimates of the variance in different batches of results and in experiments on ileum. Abramson *et al.* (1969) and Barlow *et al.* (1973) suggested that differences in log K less than 0.1 log units were probably insignificant. There is far greater variation between estimates in experiments on atria and perhaps the error to be expected in estimates of log K should be 0.2 log units, rather than 0.15 log units (Barlow *et al.*, 1976). This would give a possible error of up to 0.3 log units in estimates of selectivity.

Table 1 shows no obvious differences between dose-ratios calculated from the effects of carbachol on the force of contraction and those calculated from effects on rate. This is also seen in Table 2. All the mean estimates of the dose-ratios for 4DAMP methobromide at 37 degrees, however, are lower than the corresponding estimate at 29° or 30°C.

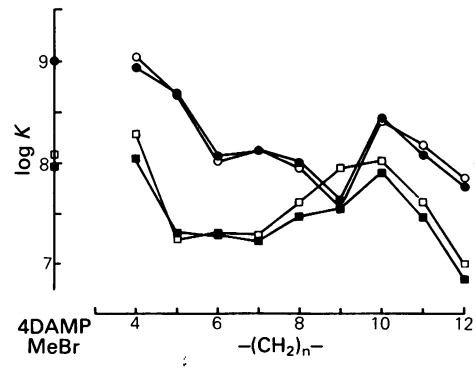

Figure 3 Estimates of the log affinity constants (at 30°C) plotted against the chain length of polymethylene *bis*-4DAMP bromides: values for 4DAMP methobromide are shown on the left. Filled circles indicate results for the ileum with 0.1 μM solutions: open circles indicate results with 0.5 μM solutions. These points should be superimposed if the compound behaves competitively. Open squares indicate estimates using the effects of carbachol on atrial rate: filled squares indicate estimates using effects on atrial force: both are the means of estimates with 0.1 and 0.5 μM solutions.

Table 2 The numbers are the mean dose-ratios (± s.e. and number of estimates) produced by the concentrations of the compounds indicated.

	Atria		*Ileum*	
	29.5°C		29.5°C	37°C
	Rate	*Size*		
4-(Di-(*p*-tolyl)acetoxy)-*N*-methylpiperidine methobromide 1.0 μM				
	CCh		CCh	CCh
	13.6 ± 2.8 (4)	11.8 ± 1.4 (4)	16.0 ± 0.8 (4)	14.4 ± 0.9 (4)
			APE	APE
			17.0 ± 1.9 (4)	15.0 ± 0.8 (4)
4-(Di-(*p*-chlorophenyl)acetoxy)-*N*-methylpiperidine methobromide 1.0 μM				
	CCh		CCh	CCh
	4.7 ± 0.4 (4)	5.4 ± 0.9 (4)	23.8 ± 0.8 (4)	27.7 ± 2.2 (4)
4-(Fluoren-9-carboxy)-*N*-methylpiperidine methobromide 0.1 μM				
	CCh		CCh	CCh
	11.0 ± 2.3 (5)	15.1 ± 3.2 (5)	42.8 ± 3.8 (5)	46.4 ± 8.0 (5)
			APE	APE
			39.4 ± 2.1 (5)	47.0 ± 5.4 (5)
4-(Xanthen-9-carboxy)-*N*-methylpiperidine methobromide 0.1 μM				
	CCh		CCh	CCh
	54.6 ± 8.3 (5)	57.5 ± 5.6 (5)	154 ± 20.9 (5)	203 ± 7.9 (5)

All measurements were made with the tissues suspended in Krebs solution without hexamethonium: in some experiments on ileum, arecaidine propargyl ester (APE) was used as agonist as well as carbachol (CCh).

Effects of changing the diphenylacetic acid group

The results in Table 2 shows that the introduction of either two methyl or two chloro groups in the *p*-position markedly reduces affinity but whereas the methyl compound has almost no selectivity, the chloro compound retains it to some extent.

When the two phenyl rings are locked together in the fluorenyl-9-carboylic acid the affinity and selectivity are less than in 4DAMP metho-salts, the compound has rather less than half the affinity of 4DAMP methobromide on the ileum but very slightly more on the atria (compare with the results in Table 1). When the benzene rings are also joined together by an oxygen atom in a xanthene ring the compound has higher affinity than 4DAMP methobromide for muscarinic receptors in the ileum but there is an even more marked increase in the effects on the atria and reduced selectivity.

Polymethylene bis-*4DAMP bromides*

All the compunds were tested at 0.1 and 0.5 μM concentrations and Table 3 shows the mean of the estimates of $\log K$ corresponding to the dose-ratio obtained. Because the variance in experiments on atria is much larger than in experiments on ileum, the mean was calculated from the pooled results for the two concentrations: separate means were calculated for the two concentrations in experiments on ileum. If the

Table 3 Affinities of polymethylene *bis*-4-diphenyl-acetoxy-*N*-methyl-piperidine (4DAMP) bromides for muscarinic receptors in guinea-pig ileum and atria

	Atria 29.5°C		*Ileum* 29.5°C		37°C	
	Rate	*Size*	*a*	*b*	*a*	*b*
$n = 4$	8.297 ± 0.111 (8)	8.052 ± 0.047 (8)	8.960 ± 0.091 (4)	9.051 ± 0.048 (4)	8.848 ± 0.016 (4)	8.975 ± 0.050 (4)
$n = 5$	7.251 ± 0.119 (10)	7.317 ± 0.084 (10)	8.702 ± 0.021 (6)	8.679 ± 0.011 (6)	8.500 ± 0.021 (6)	8.522 ± 0.026 (6)
$n = 6$	7.311 ± 0.206 (3)	7.284 ± 0.294 (3)	8.103 ± 0.018 (5)	8.035 ± 0.021 (5)	8.073 ± 0.018 (5)	8.089 ± 0.011 (5)
$n = 7$	7.302 ± 0.151 (7)	7.237 ± 0.119 (7)	8.141 ± 0.088 (5)	8.141 ± 0.067 (5)	7.893 ± 0.032 (5)	7.852 ± 0.013 (5)
$n = 8$	7.626 ± 0.073 (8)	7.486 ± 0.058 (8)	8.039 ± 0.103 (4)	7.974 ± 0.083 (4)	7.948 ± 0.030 (4)	7.956 ± 0.031 (4)
$n = 9$	7.972 ± 0.080 (8)	7.579 ± 0.290 (8)	7.663 ± 0.062 (4)	7.571 ± 0.048 (4)	7.672 ± 0.047 (4)	7.620 ± 0.009 (4)
$n = 10$	8.043 ± 0.189 (10)	7.922 ± 0.149 (12)	8.474 ± 0.046 (4)	8.436 ± 0.107 (9)	8.417 ± 0.018 (4)	8.353 ± 0.052 (10)
$n = 11$	7.639 ± 0.065 (8)	7.484 ± 0.056 (8)	8.119 ± 0.053 (4)	8.206 ± 0.047 (4)	8.199 ± 0.027 (4)	8.228 ± 0.063 (4)
$n = 12$	7.021 ± 0.113 (8)	6.859 ± 0.133 (8)	7.796 ± 0.047 (4)	7.882 ± 0.030 (4)	7.754 ± 0.022 (3)	7.800 ± 0.025 (3)

The numbers are the means of estimates of log affinity constant (± s.e. and number of estimates): n is the length of the polymethylene chain. In experiments in ileum separate estimates are shown for the two concentrations of antagonist tested (0.1 and 0.5 μM): these are indicated by a and b. If the results fit the Gaddum-Schild equation these should be the same. All measurements are made with the tissues suspended in Krebs solution without hexamethonium and with carbachol as agonist.

results fit the Gaddum-Schild equation, the estimates of log K should be the same, whatever the concentration. The results with the ileum can therefore be used to see whether the compound behaves competitively and the pooling of results at different concentrations is justified. The differences between the entries in columns a and b are all less than 0.1 log units.

The effects of chain length on affinity for muscarinic receptors in atria and ileum are illustrated in Figure 3, which includes values for 4DAMP methobromide shown as $n = 0$. Lengthening the chain decreases affinity but this then rises before falling once more. The pattern for experiments on ileum is different from the pattern for experiments on atria and the difference between the two is greatest with the pentamethylene compound, which appears to have greater selectivity than 4DAMP methobromide.

Discussion

The results in the first part of this work do not show any appreciable difference between experiments carried out with Krebs solution for both ileum and atria, rather than Tyrode solution for ileum and Locke solution for atria. This is consistent with the observations of Butt (1972), who found little difference between estimates of the affinity of a compound for receptors in ileum in Tyrode solution and in Krebs solution, though lengthening the agonist contact time could increase estimates of log K by up to 0.2 log units. If it is accepted that estimates of the variance are underestimates of the real error, the results obtained with carbachol, acetylcholine or arecaidine propargyl ester as agonists are not very different from each other, nor does the presence or absence of hexamethonium

make much difference. This is what would be expected from the observations on ileum with different agonists made by Abramson *et al.* (1969) and from studies of the effects of hexamethonium on estimates of affinity made by Barlow *et al.* (1972). This does not imply that there may not be differences with other agonists or other tissues,but the results obtained in this work indicate that the background 'noise' associated with estimates of affinity will make them difficult to detect. For differences between ileum and atria it seems that the limit of detection is about 0.3 log units (a factor of 2) and differences in log K must exceed this to indicate any selectivity.

The results obtained with 3DAMP methiodide, which had not previously been tested on atria, are a check that the selectivity of 4DAMP metho-salts is real but attempts to improve selectivity by altering the diphenylacetic acid part of the molecule have failed. There are still changes which could be made: the effects of altering the ester group and of increasing the size will be investigated. Joining two molecules of 4DAMP together, however, shows definite differences between the muscarinic receptors in guinea-pig ileum and atria. The pentamethylene compound appears to be more selective than 4DAMP methobromide but the increase in selectivity does not occur because the compound fits the receptors in the ileum better. It fits them slightly worse than 4DAMP methobromide but it fits those in the atria much worse. Increased selectivity is therefore accompanied by decreased activity, but the compound is still quite potent, being between one-third and half as active as atropine on the ileum. It seems likely, therefore, that it could be used without producing effects at nicotinic receptors in ganglia or at the neuromuscular junction. It produces a dose-ratio of around 50 on the ileum at a concentration of 0.1 μM: log K for hexamethonium and nicotinic receptors in ileum is around 5.4 (Barlow & Franks, 1971). Some of the *bis* atropinium compounds studied by Kimura *et al.* (1949, 1950) and Eckfield (1959) had neuromuscular blocking activity comparable with (+)-tubocurarine but log K for (+)-tubocurarine chloride and nicotinic receptors in frog muscle is around 6.5 (Jenkinson, 1960), so unless pentamethylene *bis*-4DAMP bromide turns out to be an exceptionally potent neuromuscular blocking agent, it should be much more active at muscarinic receptors in ileum than at any nicotinic receptors.

The results obtained in all sections of this work do not show any unambiguous differences between dose-ratios (and affinities) obtained from the effects of agonists on atrial rate and those obtained from effects on atrial force. Differences in log K of up to 0.4 log units could probably be attributed to experimental errors, with 0.2 log units for each set, and even the results obtained with nonamethylene *bis*-4DAMP bromide are less than this. The possibility that the muscarinic receptors involved in effects on atrial rate are not identical with those involved in effects on atrial force cannot, however, be ruled out.

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