Br. J. Pharmac. (1982), **77**, 549–557

THE ACTIONS OF SOME ESTERS OF 4-HYDROXYQUINUCLIDINE ON GUINEA-PIG ILEUM, ATRIA AND RAT FUNDUS STRIP

R.B. BARLOW & R. KITCHEN

Department of Pharmacology, Medical School, Bristol BS8 1TD

1 The acetyl, phenylacetyl, and diphenylacetyl esters of 4-hydroxyquinuclidine and their methiodides have been prepared.

2 4-Diphenylacetoxyquinuclidine methiodide has higher affinity for muscarinic receptors than 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP methiodide) but it is less selective. At 30°C its affinity for receptors in ileum is about 5 times that for receptors in atria, a difference similar to that found with diphenylacetoxytrophine methiodide. With 4-DAMP methiodide affinity for receptors in the ileum is over 10 times that for receptors in atria.

3 4-Diphenylacetoxyquinuclidine methiodide has higher affinity for muscarinic receptors than 3-diphenylacetoxyquinuclidine hydrochloride or its methiodide.

4 4-Acetoxyquinuclidine hydrochloride has less than one-hundredth of the activity of 3-acetoxyquinuclidine hydrochloride (acecyclidine) on guinea-pig ileum, atria, and rat fundus: however, 4-acetoxyquinuclidine methiodide is consistently more active than its 3-isomer, though it is only about 1/25 times as active as acecyclidine.

5 4-Acetoxyquinuclidine hydrochloride is only a poor substrate for electric eel acetylcholinesterase: its affinity is similar to that of acecyclidine but it is greatly reduced by methylation.

6 The relations between the structure and activity of the agonists are very different from the relations between the structure and affinity of the antagonists, which supports the view that agonists and antagonists bind to different conformations of the muscarinic receptor.

Introduction

Esters of 4-hydroxypiperidine appear to have higher affinity than their 3-isomers for muscarine-sensitive acetylcholine receptors in the guinea-pig ileum. In the phenylacetyl, diphenylacetyl, and benziloyl esters studied by Abramson, Barlow, Franks & Pearson (1974) the 4-substituted compounds had from 6 to 100 times the affinity of their 3-isomers, the biggest effect being seen with 4-diphenylacetoxy-*N*-methyl piperidine methiodide (4-DAMP methiodide; Abramson, 1964).

Affinity can also be increased by bridging the ring. 3-Phenylacetoxy-quinuclidine has nearly 100 times the affinity of 3-phenylacetoxy-*N*-methylpiperidine; 3-diphenylacetoxy-quinuclidine has 340 times the affinity of its 3-hydroxypiperidine analogue. With the benzilic esters the difference is greater than 20 fold, though the very high affinity of 3-benziloyloxyquinuclidine (QNB) makes it difficult to measure accurately. However, with the analoguous quaternary metho-salts, the affinities of the quinuclidines were only 2.5 to 6 times those of the analogous piperidines.

Although the estimates of the affinities of the 3-substituted piperidines and quinuclidines obtained by Abramson *et al.* (1974) were for the racemates, the compounds are antagonists at muscarinic receptors and the more active enantiomer cannot have more than twice the affinity of the racemate (as it would if the weaker enantiomer had no affinity at all). It is unlikely, then, that the effects of moving the ester group round the piperidine ring or of replacing 3-hydroxypiperidine by 3-hydroxyquinuclidine are very different when the comparisons involve the more active enantiomers rather than the racemates.

It is therefore of considereable interest to know the affinity of the analogous esters of 4-hydroxyquinuclidine. Can the increase in affinity obtained by moving the ester group round the ring be combined with the increase obtained by bridging the ring?

The 4-substituted compounds are also remarkable because they show some degree of selectivity for muscarinic receptors. On guinea-pig atrial pacemaker cells the 4-diphenylacetyl ester of N-methylpiperidine methiodide (4-DAMP methiodide) had between one-tenth and one-twentieth of the affinity for receptors in the guinea-pig ileum (Barlow, Berry, Glenton, Nikolaou & Soh, 1976). There were also important differences in the

0007-1188/82/110549-09 $01.00

effects of the corresponding 3- and 4-acetoxy compounds which were agonists on atria (Barlow, Burston & Vis, 1980). It is therefore also of considerable interest to know to what extent the selectivity of agonists and antagonists is affected by bridging the piperidine ring.

This paper describes the synthesis of the acetyl, phenylacetyl and diphenylacetyl esters of 4-hydroxyquinuclidine and their methiodides and their effects on isolated preparations from guinea-pig ileum and atria and rat fundus. The affinity of the acetyl compounds for acetylcholinesterase has also been studied.

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 0.5 g. Carbachol was used as agonist, added by machine once every 90 s and allowed to act for 30 s, as in previous work (Abramson *et al.*, 1974; Barlow & Burston, 1979). Hexamethonium (0.28 mM) was present in all experiments. The tissue was suspended in aerated Tyrode solution (Edinburgh Staff, 1974) and experiments were done at 30° and 37°C. In experiments with antagonists, 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 carbachol was increased to try to obtain responses which roughly matched the controls. When these were regular 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).

Comparisons with the agonists were made relative to carbachol but in addition the 3- and 4-substituted compounds were compared directly with each other. The equipotent molar ratio, that is the ratio of the concentrations producing the same response, was calculated as in a 4-point assay though the order of giving the drugs was not arranged according to a latin square but altered so that the responses from the tissue were alternately small and large.

Guinea-pig isolated atria

The atria were set up as described previously (Barlow *et al.*, 1976) in Ringer-Locke solution (Edinburgh Staff 1974, but with 2.16 mM calcium chloride, twice the concentration listed). This also contained 0.28 mM hexamethonium and was aerated with pure oxygen. The temperature was 30°C. The atrial contractions were recorded isometrically with a load of about 0.2 g. Action potentials were also recorded by means of external electrodes of the type used by Dr E.M. Vaughan Williams (personal communication) for stimulating the preparation. Carbachol was the agonist given once every 16 min and allowed to act for 4 min and its effect was expressed, as previously, as the percentage increase in the time required for 50 beats, calculated from the value at the end of the application of the agonist and the value just before its application. In experiments with antagonists there was a control period in which responses were obtained with carbachol, usually 0.2 and 0.4 μM; the preparation was then exposed to the antagonist and the concentration of carbachol increased, as in the experiments on ileum. Because of the much longer time cycle, however, dose-ratios were often based only on two pairs of control responses and two pairs after the action of the antagonist. The calculations of the equipotent molar ratios of the agonists were similarly based only on small numbers of responses.

Rat fundus strip (Vane, 1975)

The fundus strip was set up as described by Edinburgh Staff (1974) with the responses recorded isotonically, but with an increased load (about 2 g). Carbachol was agonist, added by machine (operated by a PET computer) once every 5 min and allowed to act for 20 min. In these conditions it was not necessary to increase the load to stretch the preparation after a contraction. The tissue was suspended in Krebs solution (Edinburgh Staff, 1974), aerated with a mixture of 95% O_2 and 5% CO_2 and the temperature was 37°C. Because the log dose-response curve with this preparation was flatter than with the ileum, four concentrations of agonist were tested, usually 0.12, 0.18, 0.28, and 0.42 μM, with the order such that the responses were alternately small and large. When these were regular the preparation was exposed to the antagonist and the concentrations of carbachol were increased, as in the experiments with the ileum. The exact dose-ratio was calculated by fitting the dependence of responses, Y, on the agonist concentration, A, by least-squares to the logistic expression,

$$Y = M \frac{A^p}{A^p + K^p}$$

assuming that the slope of the dose-response curve is unaltered by the antagonist, i.e. p and M are common to both sets and the dose-ratio is given by the ratio of the values of K (Waud & Parker, 1971; Barlow 1975)

Experiments with electric eel acetylcholinesterase

These were carried out as described by Barlow, Bremner & Soh (1978). The compounds were mostly

poor substrates when studied by electrometric titration (pH 7.4). Their affinity for the enzyme was measured in mixed substrate experiments with acetylthiocholine, studied spectrophotometrically (Ellman, Courtney, Andres & Featherstone, 1961), rather than by electrometric titration. The reaction was performed in 0.1 M phosphate buffer, pH 8.35 and control measurements were made, at least in duplicate, with 0.05, 0.1, 0.15, 0.2, 0.3, and 0.4 mM acetylthiocholine. Measurements were then made in the presence of the second compound and the concentrations of acetylthiocholine increased if necessary. The rate of hydrolysis of acetylthiocholine, V, was calculated from the change in optical density (at 412 nm) with time and its dependence on the concentration of acetylthiocholine, S, was fitted by least-squares directly to the equation

$$V = \frac{V_{max} S}{S + K_m}$$

where V_{max} is the maximum velocity and K_m is the Michaelis constant. In the presence of the second substrate K_m should increase and if the effect is competitive the 'apparent' K_m in the presence of a concentration I of inhibitor with a dissociation constant K' is given by $K_m(1 + I/K')$: V_{max} should be unaltered. The ratio of the estimates of K_m in the presence and in the absence of the second compound is, in fact, the dose-ratio.

The direct fit of V as a function of S avoids possible bias associated with linear transformations (such as the Lineweaver-Burk plot) and estimates of the standard errors of K_m and V_{max} can be obtained from the Gauss multipliers (Snedecor & Cochran, 1968). These estimates appear to be in the same range as those obtained from the between experiment variance. For instance standard errors of about 10% were obtained by Barlow *et al.* (1978) from four sets of electrometric titrations in which four concentrations of substrate were tested in duplicate. In similar work with mixed substrate experiments followed spectrophotometrically, Barlow (1978) observed standard errors in log affinity constant ($-\log K'$) of 0.01 to 0.05 log units (again usually based on 4 estimates).

Calculations

The least-squares fit to the logistic expression and to the hyperbola were made with a PET 2001 or 3032 computer by methods already described (Barlow, 1975; 1980).

Compounds

The synthesis of 4-hydroxyquinuclidine starting from N-benzylpiperidin-4-one is described by Grob & Brenneisen (1958). The ketone is converted into the ethyl ester of 4-hydroxy-*N*-benzylpiperidine-4-acetic acid by a Reformatzky reaction with zinc and ethyl bromoacetate. The ester is reduced with lithium aluminium hydride to 4-hydroxy-4-hydroxyethyl-*N*-benzyl piperidine which is then treated with tosyl

Table 1 Melting-points and analyses

4-Acetoxyquinuclidine HCl, m.p. 286° (dec)				
Found	C, 52.49, 52.73;	H, 7.69, 8.15	N, 6.45, 6.34;	Cl, 17.91
Theory	C, 52.56	H, 7.84	N, 6.81	Cl, 17.23%
4-Acetoxyquinuclidine methiodide, m.p. 255–256° (dec)				
Found	C, 38.70, 38.53;	H, 6.16, 6.07;	N, 4.20, 4.42;	I^-, 41.09
Theory	C, 38.60	H, 5.83	N, 4.50	I^-, 40.78%
4-Phenylacetoxyquinuclidine methiodide, m.p. 170.5–171.5°				
Found	C, 49.52, 49.88;	H, 5.95, 5.82;	N, 3.68, 3.45;	I^-, 32.92
Theory	C, 49.62	H, 5.73	N, 3.62	I^-, 32.77%
4-Diphenylacetoxyquinuclidine HCl, m.p. 259.1–259.7° (dec)				
Found	C, 70.69, 70.67;	H, 7.42, 7.35;	N, 3.70, 3.51;	Cl, 10.42, 10.33
Theory	C, 70.48	H, 6.76	N, 3.96	Cl, 9.91%
4-Diphenylacetoxyquinuclidine methiodine methiodide, m.p. 194.6–195.8°				
Found	C, 57.49, 57.51;	H, 5.67, 5.23;	N, 2.88, 2.90;	I^-, 27.47
Theory	C, 57.03	H, 5.66	N, 3.02	I^-, 27.39%

Melting-points were determined with a Mettler FP5 instrument, coupled to a potentiometric recorder: when given to 0.1°C they were obtained with a rate of heating of 0.2°C/min: with the others the rate was 1°C/min. Microanalysis for C, H, N and Cl are by Mr M. West, School of Chemistry, University of Bristol. Iodide analyses are gravimetric with samples of 50–150 mg.

chloride. The free tosyl ester undergoes ring closure to give 4-hydroxy-*N*-benzyl quinuclidinium tosylate from which the tosylate salt of 4-hydroxyquinuclidine is obtained on reduction with hydrogen and palladium charcoal. The yields obtained up to this stage were between half and three-quarters of those reported by Grob & Brenneisen (1958) but great difficulty was experienced with the isolation of the free 4-hydroxyquinuclidine which is extremely hydrophilic as well as being a strong base. The esters were, in fact, usually prepared by heating the tosylate salt in dimethylformamide with the acid chloride. It was then possible to isolate the tertiary bases, which were much less hydrophilic than 4-hydroxyquinuclidine, by adding alkali and extracting with ether. 4-Acetoxyquinuclidine had b.p. 108°/10 mm but went solid; 4-phenylacetoxyquinuclidine had b.p. 120–130°/0.4 mm, N_D^{20} 1.5393. No attempt was made to distil the diphenylacetyl ester. Melting points and analyses of the hydrochlorides and methiodides are shown in Table 1. The compounds were crystallized from mixtures of ethanol, acetone or butanone, and ether.

Carbachol chloride, hexamethonium bromide and electric eel acetylcholinesterase were obtained from Sigma. Acetylthiocholine iodide and Ellman's reagent were obtained from BDH Ltd.

Table 2 Effects of antagonists on guinea-pig ileum and atria and on rat fundus

	Atria	*Ileum*		*Rat Fundus*
Conc.	*30*	*30°*	*37°*	*37°*
4-Phenylacetoxyquinuclidine methiodide				
5 μM	DR 4.13 ±0.48 (8)	9.20 ±0.88 (7)	11.2 ±1.51 (8)	16.5 ±0.61 (20)
	log *K* 5.767 ±0.058	6.197 ±0.052	6.286 ±0.051	6.484 ±0.018
10 μM	DR 4.79 ±0.46 (4)	19.7 ±1.60 (4)	17.4 ±1.78 (4)	34.5 ±1.07 (7)
	log *K* 5.569 ±0.052	6.267 ±0.040	6.205 ±0.050	6.524 ±0.014
50 μM	DR 20.8 ±3.26 (4)	48.9 ±7.05 (2)	66.9 ±3.31 (3)	120 ±4.40 (4)
	log *K* 5.583 ±0.066	5.977 ±0.064	6.119 ±0.022	6.376 ±0.016
Overall mean log *K*	5.671 ±0.042 (16)	6.185 ±0.040 (13)	6.231 ±0.034 (15)	6.479 ±0.014 (31)
4-Diphenylacetoxyquinuclidine HCl				
0.5 μM	DR	5.02 ±0.14 (2)	3.19 ±0.44 (2)	6.83 ±0.51 (6)
	log *K*	6.905 ±0.015	6.634 ±0.087	7.060 ±0.034
2 μM	DR 11.2	22.4	17.5	26.8
	log *K* 6.691 ±0.059	7.020 ±0.043	6.912 ±0.034	7.100 ±0.040
Overall mean log *K*		6.987 ±0.037 (7)	6.833 ±0.060 (7)	7.081 ±0.026 (13)
4-Diphenylacetoxyquinuclidine methiodide				
0.025 μM	DR			175 ±24.5 (12)
	log *K*			9.891 ±0.045
0.05 μM	DR 35.2 ±4.40 (5)	174 ±24.2 (7)	214 ±23.6 (8)	249 ±18.9 (10)
	log *K* 8.823 ±0.049	9.506 ±0.073	9.604 ±0.063	9.685 ±0.030
Overall mean log *K*				9.798 ±0.035 (22)

The concentrations tested are shown with the mean dose-ratio (DR) and mean value of log affinity constant (log *K*). Values are given ± s.e. with the number of estimates in parentheses.

Results

The effects of the antagonists on the guinea-pig atria and ileum and on rat fundus are shown in Table 2. Where more than one concentration was tested, the values of log affinity constant appear to be independent of the concentration, i.e., the results are not inconsistent with competitive antagonism. There is some evidence that the affinity for receptors in the ileum is greater than for those in atria. The difference is greatest (0.7 log units) with 4-diphenylacetoxyquinuclidine methiodide (4-DAQ methiodide) but this is not as big as with the analogous piperidine (4-DAMP methiodide; 1.3 log units) and is similar to that found with diphenylacetyltropine methiodide (0.7 log units: Barlow *et al.*, 1976).

Results for the agonists are shown in Table 3. Values for racemic 3-acetoxyquinuclidine agree reasonably with those obtained by Lambrecht & Mutschler (1974), who found that it was about 1/10 as active as acetylcholine on guinea-pig ileum. Weinstein, Maayani, Srebrenik, Cohen & Sokolovsky (1975) obtained the same value. Barlow & Casy (1975) obtained results with the separate enantiomers which indicated that it was about one-eleventh as active as carbachol on this preparation. The low activity of the corresponding methiodide is known from the work of Robinson, Belleau & Cox (1969), in which it appears to be about 1/1000 as active as acetylcholine on the guinea-pig ileum. Weinstein *et al.* (1975) found it to be about 1/2000 as active as acetylcholine. The results of Barlow & Casy with the enantiomers indicate that it is about 1/250 as active as carbachol.

The results of the experiments with acetylcholinesterase are shown in Table 4. Values for racemic 3-acetoxyquinuclidine are again similar to those obtained previously: Pyttel & Robinson (1973) estimated K_m for ox acetylcholinesterase to be 1.1 mM with V_{max} about 0.2 times that for acetylcholine. Weinstein *et al.* (1975) found $K_m = 0.5$ mM for acetylcholinesterase from human red calls. For the corresponding methiodide Robinson *et al.* (1969) obtained $K_m = 0.8$ mM for this enzyme. The results obtained in this work, however, suggest that it is not competing with acetylthiocholine and this was not apparent from previous studies. The effects of some of the other compounds on V_{max} and the dependence of estimates of K' on concentration suggest that they, too, are not acting strictly competitively.

Discussion

The results obtained with acetylcholinesterase indicate that hydrolysis of the agonists is not likely to affect their pharmacological properties to any great extent. It is the 3-substituted compounds that are susceptible to hydrolysis and affinity is slightly increased by methylation. Racemic 3-acetoxy-*N*-methyl piperidine methiodide, however, appears to have much the same affinity as racemic 3-

Table 3 Effects of agonists on guinea-pig ileum and atria and on rat fundus

	Atria	*Ileum*		*Fundus*
	30°	30°	37°	37°
Acetoxyquinuclidine HCl				
3–	8.0	6.9	6.0	12.4
	±0.90 (4)	±1.3 (4)	±0.60 (4)	± 1.6 (6)
4–	752	1385	981	2126
	±46 (3)	±174 (5)	±129 (5)	±414 (6)
4:3	110	193	145	183
	±22 (3)	±34 (4)	±10 (4)	±22 (6)
Acetoxyquinuclidine methiodide				
3–	1385	909	577	1605
	±696 (2)	(1)	±58 (4)	±69 (3)
4–	182	269	146	95
	±16 (4)	±17 (6)	±16 (6)	±8.3 (4)
4:3	0.250	0.461	0.606	0.173
	±0.044 (3)	±0.037 (2)	±0.061 (2)	±0.022 (3)

Numbers are the mean equipotent molar ratio (±s.e., number of estimates) relative to carbachol, or for the 4-compound relative to its 3-isomer in experiments in which they were compared directly (denoted 4:3). Note that the direct comparisons of the isomers give similar values to what would be expected from the comparisons of each separately with carbachol. Large equipotent molar ratios indicate low activity: for instance with the hydrochlorides the 4-acetoxycompound has between 0.5 and 1% of the 3-acetoxy compound, whereas with the methiodides the 4-acetoxy compound is between 1.7 and 5.8 times as active as the 3-acetoxy compound, depending on the tissue and temperature.

Table 4 Effects of acetoxy compounds on electric eel acetylcholinesterase

A Followed by electrometric titration (pH 7.4). The relative maximum velocity was calculated with the rate for the hydrolysis of acetylcholine at 37°C taken as 1; *n* indicates the number of points on the graph of velocity against substrate concentration. Estimates of the standard error of K_m and V_{max} were calculated from the Gauss multipliers (Snedecor & Cochran, 1968) and show, for instance, that the effects of temperature are real.

	Concentration range	K_m	V_{max}	*Relative* V_{max}	n
Acetylcholine					
25°C	0.05–1.9 mM	0.255 ±0.033	31.7 ± 1.4	0.70	18
37°C	0.05–0.8 mM	0.369 ±0.068	45.3 ± 4.5	1	23
Acetylthiocholine					
25°C	0.05–1.6 mM	0.150 ±0.021	30.8 ± 1.3	0.68	18
37°C	0.05–0.8 mM	0.182 ±0.048	42.4 ± 3.4	0.96	6
(±)-3-Acetoxy-N-methylpiperidine methiodide					
25°C	0.05–1.6 mM	0.299 ±0.055	9.6 ± 0.6	0.21	18
37°C	0.05–1.6 mM	0.428 ±0.061	18.3 ± 1.1	0.40	21

The rates of hydrolysis of 3-acetoxyquinuclidine HCl and 4-acetoxy-*N*-methylpiperidine methiodide were too slow to be fitted to the hyperbolic expression.

acetoxyquinuclidine methiodide, suggesting that the extra methylene groups do not contribute to the total binding, though they lead to a change in conformation at the receptor which makes hydrolysis more difficult.

Although 4-acetoxyquinuclidine appears to have much the same affinity for the enzyme as racemic 3-acetoxyquinuclidine, this is reduced by methylation which suggests that the nitrogen atom is not interacting with the same part of the active site of the enzyme in the two compounds. In fact the compounds have remarkably low affinity for the enzyme. From studies of the affinities of antagonists for muscarinic acetylcholine receptors it is found that one methylene group can increase log *K* by up to about 1 log unit (Abramson *et al.*, 1974) so either only a small part of the molecule is interacting with the enzyme, or, perhaps more likely, the main bulk of the molecule is reducing binding sterically.

From the results with the agonists shown in Table 3 it is possible to speculate that a similar situation may occur at muscarinic acetylcholine receptors and to suppose that 3-acetoxyquinuclidine, or rather its (+)-**S** enantiomer, just fits the receptor, whereas the methiodide, being bigger, has to bind in a quite different way. This is consistent with the inversion of stereospecifically on methylation: the **R**-methiodide is more active than the **S**-methiodide. It may not be correct to equate activity with affinity, however, and the results of the experiments with the antagonists do not support the view that the 3-substituted compounds bind better than the 4-substituted compounds. The affinity constants of antagonists are summarized in Table 5, which includes published results for tropines and N-methyl-hydroxypiperidines. The affinity of 4-diphenylacetoxyquinuclidine hydrochloride is remarkably low compared with that of its methiodide but large differences are also found between related compounds, such as the benziloyltropines (log *K* 9.5 for benziloyltropine, 10.4 for its methiodide) and pseudotropines (log *K* 8.8 for benziloylpseudotropine, 9.8 for its methiodide: Abramson *et al.*, 1974). With quaternary compounds the derivatives of 4-hydroxyquinuclidine have higher affinity than their 3-isomers and the diphenylacetyl compound has higher affinity than the corresponding tropine or piperidine. It is unfortunate that the benzilic ester of 4-hydroxyquinuclidine has not yet been made because it and/or its methiodide would be expected to have very high affinity for muscarinic receptors, though there is some uncertainty because is it not clear whether the reduction in affinity obtained by methylating QNB is because it is a 3-substituted compound or because there is perhaps some overall limit to affinity. The difference between the structure-affinity relations of these antagonists

Table 4

B Followed by mixed-substrate experiments with acetylthiocholine in which the hydrolysis was studied spectrophotometrically (pH 8.4; 25°C). The relative maximum velocity was calculated from the appropriate control value for acetylthiocholine; there are two groups of experiments, separated by over a year. K^1 indicates the inhibitor constant for the second substrate, calculated from its effect on the Michaelis constant. Estimates of the standard error of K_m and V_{max} were calculated from the Gauss multipliers as above. Some compounds have definite effects on V_{max} and are not behaving strictly competitively.

Concentration range	K_m	V_{max}	*Relative* V_{max}	n
(i) Acetylthiocholine				
0.05–0.4 mM	0.098 ±0.009	62.2 ± 2.0	1	69
+ 4-acetoxy-*N*-methylpiperidine methiodide				
1 mM (K^1 = 1.3 mM)	0.173 ±0.027	58.5 ± 4.1	0.94	16
3 mM (K^1 = 2.8 mM)	0.203 ±0.016	46.7 ± 1.9	0.75	16
+ 3-acetoxyquinuclidine HCl				
1 mM (K^1 = 3.6 mM)	0.125 ±0.011	54.9 ± 1.9	0.88	23
3 mM (K^1 = 5.5 mM)	0.151 ±0.014	48.9 ± 2.0	0.79	24
(ii) Acetylthiocholine				
0.05–0.4 mM	0.093 ±0.012	38.9 ± 1.8	1	33
+ 3-acetoxyquinuclidine methiodide				
2 mM (K^1 = 0.35 mM)	0.623 ±0.142	17.0 ± 1.8	0.44	12
+ 4-acetoxyquinuclidine HCl				
1 mM (K^1 = 2.9 mM)	0.125 ±0.025	34.5 ± 2.8	0.89	8
3 mM (K^1 = 2.1 mM)	0.223 ±0.040	36.0 ± 3.3	0.92	22
+ 4-acetoxyquinuclidine methiodide				
3 mM (K^1 = 23 mM)	0.105 ±0.017	25.0 ± 1.5	0.64	20

and the structure-activity relations of the agonists gives some support to the view expressed by Burgen, Birdsall & Hulme (1979) that muscarinic agonists and antagonists are binding to different conformations of the receptor.

The selectivity of 4-DAMP methiodide has not been increased by the incorporation of the two extra methylene groups in 4-DAQ methiodide. Possibly this is because the piperidine rings will be locked in the boat conformation. However, the results with the agonists are interesting because 4-acetoxyquinuclidine and its methiodide are more active on ileum than atria, whereas with 3-acetoxyquinuclidine and its methiodide the difference, if any, is the other way round. The results also indicate differences between guinea-pig ileum and rat fundus and support the view that there are differences between muscarine-sensitive acetylcholine receptors in different tissues, even though none of these particular compounds is very selective.

References

ABRAMSON, F.B. (1964). The synthesis and pharmacological properties of some alicyclic compounds related to acetylcholine. *Ph.D. Thesis, University of Edinburgh.*

ABRAMSON, F.B., BARLOW, R.B., FRANKS, FIONA M. & PEARSON, J.D.M. (1974). Relationships between chemical structure and affinity for postganglionic acetyl-

Table 5 Affinity for muscarinic receptors: summary

	Atria	*Ileum*		
	30°C	30°C	37 C	Δ 3–4
Phenylacetoxy-				
3-quinuclidine MeI			5.50	
4-quinuclidine MeI	5.67*	6.18*	6.23*	0.73
3-*N*-methylpiperidine MeI			5.11	
4-*N*-methylpiperidine MeI			6.19	1.08
Tropine MeI			6.96	
Diphenylacetoxy-				
3-quinuclidine HCL			9.29	
4-quinuclidine HCl	6.69*	6.99*	6.83*	−2.46
3-*N*-methylpiperidine HCl			6.76	
4-*N*-methylpiperidine HCl			8.36	1.60
Tropine HCl	7.41	7.96	8.11	
3-quinuclidine MeI	7.62	7.95	7.86	
4-quinuclidine MeI	8.82*	9.51*	9.60*	1.74
3-*N*-methylpiperidine MeI			7.09	
4-*N*-methylpiperidine MeI	7.70	9.00	9.06	1.97
Tropine MeI	7.95	8.67	8.67	

Estimates of log *K* for receptors in the ileum and atria are from Abramson *et al.* (1974), Barlow *et al.* (1976) and from the present work (marked with an asterisk). Differences greater than 0.1 log units are likely to be significant (see Table 2). The effect on log *K* (ileum, 37°C) of changing the ester from the 3- position to the 4- position is indicated by Δ 3–4: selectivity can be assessed by comparing values for atria with those for ileum at 30°C.

choline receptors of the guinea-pig ileum. *Br. J. Pharmac.*, **51**, 81–93.

BARLOW, R.B. (1975). Use of the logistic function for the calculation of dose-ratios and potency ratios. *Br. J. Pharmac.*, **53**, 139–141.

BARLOW, R.B. (1978). The effects of a hydroxyl group on some chemical and biological properties of *n*-pentyl ammonium salts. *J. Pharm. Pharmac.*, **30**, 703–710.

BARLOW, R.B. (1980). *Quantitative Aspects of Chemical Pharmacology*. pp. 224–231. London: Croom Helm.

BARLOW, R.B., BERRY, K.J., GLENTON, P.A.M., NIKOLAOU, N.M. & SOH, K.S. (1976). A comparison of affinity constants for muscarine-sensitive acetylcholine receptors in guinea-pig atrial pacemaker cells at 29°C and in ileum at 29°C and 37°C. *Br. J. Pharmac.*, **58**, 613–620.

BARLOW, R.B., BREMNER, J.B. & SOH, K.S. (1978). The effects of replacing ester by amide on the biological properties of compounds related to acetylcholine. *Br. J. Pharmac.*, **62**, 39–50.

BARLOW, R.B. & BURSTON, K.N. (1979). Temperature coefficients of affinity and entropie of adsorption for enantiomeric pairs of compounds acting at muscarinic receptors in the guinea-pig ileum. *Br. J. Pharmac.*, **66**, 581–585.

BARLOW, R.B., BURSTON, K.N. & VIS ANNE (1980). Three types of muscarinic receptors? *Br. J. Pharmac.*, **68**, 141–142P.

BARLOW, R.B. & CASY, A.F. (1975). Inversion of stereospecificity by methylation of compounds acting at acetylcholine receptors. *Mol. Pharmac.*, **11**, 690–693.

BURGEN, A.S.V., BIRDSALL, N.J.M. & HULME, E.C. (1979). Muscarinic receptors and the nature of their reaction with agonists and antagonists. In *Medicinal Chemistry* VI. ed. Simkin, A. pp. 25–35. Oxford: Cotswold Press.

EDINBURGH STAFF (1974). *Pharmacological Experiments on Isolated Preparations*, 2nd edition. pp. 2, 25–28, 58–62, 88–89. Edinburgh: Churchill Livingstone.

ELLMAN, G.L., COURTNEY, K.D., ANDRES, V. & FEATHERSTONE, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmac.*, **7**, 88–95.

GROB, C.A. & BRENNEISEN, P. (1958). Die synthese von 4-brom und 4-hydroxy-chinuclidin. *Helv. chim. acta.*, **41**, 1184–1190.

LAMBRECHT, G. & MUTSCHLER, E. (1974). Struktur- und Konformations-Wirkungs-Beziehungen bei zyklischen Acetylcholinanaloga der Piperidin-und Chinuclidin-Reihe. *Arzneimittel-Forschung*, **24**, 1725–1729.

PYTTEL, R. & ROBINSON, J.B. (1973). Interaction of 3-quinuclidinol and its derivatives with acetylcholinesterase. *J. Pharm. Sci.*, **62**, 684–5.

ROBINSON, J.B., BELLEAU, B. & COX, B. (1969). 3-Acetoxyquinuclidine methiodide. Resolution, absolute configuration and stereospecificity of interaction with acetylcholine binding sites. *J. med. Chem.*, **12**, 848–851.

SNEDECOR, G.W. & COCHRAN, W.G. (1968). *Statistical Methods*, 6th ed. pp. 390–391. Iowa: Iowa State University Press.

VANE, J.R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12,** 344–349.

WAUD, D.R. & PARKER, R.B. (1971). Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. II Competitive antagonists. *J. Pharmac. exp. Ther.*, **177,** 13–24.

WEINSTEIN, H., MAAYANI, S., SREBRENIK, S., COHEN, S. & SOKOLOVSKY, M. (1975). A theoretical and experimental study of the semirigrid cholinergic agonist 3-acetoxyquinuclidine. *Mol. Pharmac.*, **11,** 671–689.

(*Received May 3, 1982. Revised June 11, 1982.*)