Effects of chain-length and unsaturation on affinity and selectivity at muscarinic receptors

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1 Lengthening the chain in diphenylacetylcholine decreases affinity for muscarinic cholinceptors in guinea-pig ileum. Diphenylacetoxypropyldimethylamine and its quaternary trimethylammonium salt are roughly equiactive: the dimethylamine and the piperidine have some selectivity for ileum compared with atria, but are not as active nor as selective as 4-diphenylacetoxy-*N*-methylpiperidine (4-DAMP) methobromide (MeBr). With the weaker diphenylacetoxypentyl compounds the base is more active than the quaternary salt.

2 The diphenylacetoxypentyl-, *cis*-butenyl and *trans*-butenyl compounds have similar affinities. The quaternary salts are less active than the tertiary bases, but they are less selective than the butynyl analogues studied in earlier work.

3 1,1-Diphenyl-1-hydroxy-2,4-hexadiynyl dimethylamine and its trimethylammonium salt are inactive in concentrations below 100 μ M, as are the (+)-camphor-sulphonyl ester of 4-hydroxy-*N*-methyl piperidine and its methiodide. The ($\pm$)-phenylcyclopentylacetyl ester of 4-hydroxy-*N*-methylpiperidine methobromide is more active than its cyclohexyl analogue and than 4-DAMP MeBr but it is less selective than 4-DAMP MeBr.

4 The high selectivity of *p*-fluoro-hexahydrosila-diphenidol is confirmed but this compound has relatively low affinity (for ileum log $K = 7.8$).

5 The results indicate steric constraints to binding at muscarinic receptors which could be used to check molecular modelling of the receptor based on its known amino acid sequence. The group binding the charged nitrogen is probably at the mouth of a cavity which can accommodate two large rings (as in 4-DAMP MeBr) but with a depth less than about 7 Å so that the rod-like hexadiynes cannot fit. Differences between types of receptor may only involve small changes in geometry secondary to differences in amino acids not directly involved in binding and the production of selectivity depends upon finding substituents which interfere with binding more at one type of receptor than at another.

Introduction

The binding of 4-diphenylacetoxy-*N*-methylpiperidine (4-DAMP) methobromide (MeBr) and of other antagonists at muscarinic receptors appears to involve contributions from at least three parts of the molecule, the charged (onium) nitrogen atom and the two aromatic rings. The part of the molecule linking these groups may also contribute to binding but it may be more important in determining that the rings and the onium nitrogen are held at suitable distances and suitably arranged in space. The distances will depend on the number of atoms present and the spatial arrangement will depend on whether they form part of a ring and whether the bonds are saturated or unsaturated. Results obtained with some acetylenic analogues of 4-DAMP MeBr (Barlow *et al.*, 1988) suggested that whereas 4-DAMP MeBr is more active than 4-DAMP base, the tertiary base was more active than the quaternary salt with longer compounds. Diphenyl-acetoxy-butynyl dimethylamine was more active than its trimethylammonium analogue and although it was very much less active than 4-DAMP MeBr it had similar selectivity, being about 10 times as active on ileum as on atria. In these but-2-yne the four carbon atoms lie in a straight line and the structure is rod-like. It is possible that this makes the compound less well able to fit the receptor and that this unfavourable effect is greater for receptors in atria than for those in ileum.

This paper describes results obtained with (i) saturated compounds with different chain length, diphenylacetoxypentyl and pentyl compounds (results for diphenylacetylcholine are already published), (ii) the *cis* and *trans* butenyl compounds and (iii) the diacetylenes, 1,1-diphenyl-1-hydroxy-2,4-hexadiynyl-dimethylamine and its trimethylammonium salt.

Results were also obtained with the phenylcyclopentyl acetoxy and (+)-camphorsulphonyl esters of 4-hydroxy-*N*-

methyl piperidine and with ($\pm$)-*p*-fluoro hexahydro-sila-diphenidol, which is known to be much more active at muscarinic receptors in ileum than at those in atria (Eglen *et al.*, 1989).

Methods

Guinea-pig isolated ileum

The guinea-pig ileum was dissected out and set up as described by Edinburgh Staff (1974). The responses were recorded isotonicly with a load of about 0.5 g. The tissue was suspended in Krebs solution (Edinburgh Staff, 1974) aerated with a mixture of 95% O₂ and 5% CO₂ containing norphenylephrine, 5 μ M, and experiments were done at 29.8 $\pm$ 0.3°C and 37.0 $\pm$ 0.1°C.

The agonist, carbachol, was allowed to act for 30 s and added once every 90 s by relays controlled from a PET micro-computer. Alternate small and large control responses were obtained, usually to 0.1 and 0.2 μ M carbachol. When these were regular the tissue was exposed to a solution of the antagonist and the concentration of the agonist was increased to try to obtain responses which roughly matched the controls. When these were regular the approximate dose-ratio was given by the ratio of the concentrations of the agonist used in the presence and in the absence of the antagonist and an exact dose-ratio was calculated from the size of the responses by a calculation similar to a 4-point assay (Edinburgh Staff, 1974; Barlow, 1983). Usually a second concentration of antagonist was tested on each preparation and where possible a fresh set of control responses was obtained.

Guinea-pig isolated atria

The atria were dissected out as described by Edinburgh Staff (1974) and set up in Krebs solution, aerated with a mixture of

Table 1 Log affinity constant (mean $\pm$ s.e.mean and number of estimates) for the compounds and the concentrations tested

Compound	Atria		Ileum	
	Rate	Force	30°	37°
(1) Diphenylacetoxypropyl dimethylamine HBr				
1 μ M	6.79 $\pm$ 0.01 (4)	6.67 $\pm$ 0.07 (4)	7.53 $\pm$ 0.05 (4)	7.46 $\pm$ 0.02 (4)
5 μ M	6.81 $\pm$ 0.03 (4)	6.76 $\pm$ 0.08 (4)	7.52 $\pm$ 0.05 (4)	7.44 $\pm$ 0.05 (4)
(2) Diphenylacetoxypropyl trimethylammonium bromide				
1 μ M	7.03 $\pm$ 0.04 (5)	6.97 $\pm$ 0.03 (5)	7.40 $\pm$ 0.01 (4)	7.31 $\pm$ 0.02 (4)
5 μ M	7.04 $\pm$ 0.06 (4)	6.99 $\pm$ 0.06 (4)	7.39 $\pm$ 0.02 (4)	7.29 $\pm$ 0.02 (4)
20 μ M	7.06 $\pm$ 0.06 (2)	7.05 $\pm$ 0.04 (2)	7.35 $\pm$ 0.01 (2)	7.24 $\pm$ 0.02 (2)
(3) Diphenylacetoxypropyl pyrrolidine HBr				
1 μ M	6.56 $\pm$ 0.08 (3)	6.42 $\pm$ 0.07 (5)	7.21 $\pm$ 0.03 (7)	7.22 $\pm$ 0.04 (6)
5 μ M	6.71 $\pm$ 0.07 (4)	6.34 $\pm$ 0.07 (5)	7.03 $\pm$ 0.05 (6)	7.09 $\pm$ 0.06 (5)
(4) Diphenylacetoxypropyl <i>N</i> -methylpyrrolidinium bromide				
1 μ M	6.72 $\pm$ 0.09 (5)	6.57 $\pm$ 0.07 (5)	7.16 $\pm$ 0.09 (6)	7.14 $\pm$ 0.06 (6)
5 μ M	6.79 $\pm$ 0.11 (4)	6.54 $\pm$ 0.07 (5)	7.04 $\pm$ 0.11 (6)	7.04 $\pm$ 0.06 (6)
(5) Diphenylacetoxypropyl piperidine HBr				
0.5 μ M			6.74 $\pm$ 0.17 (4)	6.79 $\pm$ 0.22 (3)
2.5 μ M	6.00 $\pm$ 0.07 (4)	5.61 $\pm$ 0.07 (5)	6.66 $\pm$ 0.14 (3)	6.64 $\pm$ 0.15 (4)
(6) Diphenylacetoxypropyl <i>N</i> -methylpiperidinium bromide				
0.5 μ M	6.31 $\pm$ 0.16 (4)	6.43 $\pm$ 0.09 (5)	7.03 $\pm$ 0.13 (5)	7.21 $\pm$ 0.14 (6)
2.5 μ M	6.52 $\pm$ 0.14 (3)	6.42 $\pm$ 0.11 (4)	6.85 $\pm$ 0.06 (4)	6.90 $\pm$ 0.04 (6)
(7) Diphenylacetoxybutyl dimethylamine HBr				
1 μ M	6.48 $\pm$ 0.10 (4)	6.47 $\pm$ 0.05 (4)	6.92 $\pm$ 0.06 (6)	6.75 $\pm$ 0.04 (5)
5 μ M	6.44 $\pm$ 0.07 (5)	6.43 $\pm$ 0.07 (5)	7.01 $\pm$ 0.05 (6)	6.77 $\pm$ 0.08 (5)
(8) Diphenylacetoxybutyl trimethylammonium bromide				
10 μ M	6.08 $\pm$ 0.03 (3)	6.03 $\pm$ 0.07 (3)	6.44 $\pm$ 0.04 (3)	6.44 $\pm$ 0.02 (3)
30 μ M	6.09 $\pm$ 0.12 (3)	6.07 $\pm$ 0.09 (3)	6.55 $\pm$ 0.09 (3)	6.49 $\pm$ 0.02 (3)
(9) <i>cis</i> Diphenylacetoxy-but-2-enyl dimethylamine HBr				
1 μ M	6.78 $\pm$ 0.03 (3)	6.70 $\pm$ 0.05 (7)	7.37 $\pm$ 0.05 (7)	7.32 $\pm$ 0.06 (7)
5 μ M	6.72 $\pm$ 0.15 (2)	6.75 $\pm$ 0.05 (5)	7.34 $\pm$ 0.08 (4)	7.30 $\pm$ 0.05 (6)
(10) <i>cis</i> Diphenylacetoxy-but-2-enyl trimethylammonium bromide				
5 μ M		5.79 $\pm$ 0.08 (5)	6.26 $\pm$ 0.02 (7)	6.15 $\pm$ 0.04 (7)
25 μ M		5.72 $\pm$ 0.05 (4)	6.27 $\pm$ 0.05 (6)	6.20 $\pm$ 0.03 (6)
(11) <i>trans</i> Diphenylacetoxy-but-2-enyl dimethylamine HBr				
1 μ M	6.61 $\pm$ 0.05 (4)	6.59 $\pm$ 0.05 (5)	7.31 $\pm$ 0.02 (4)	7.33 $\pm$ 0.02 (5)
5 μ M	6.64 $\pm$ 0.08 (4)	6.64 $\pm$ 0.05 (5)	7.39 $\pm$ 0.04 (4)	7.41 $\pm$ 0.01 (5)
(12) <i>trans</i> Diphenylacetoxy-but-2-enyl trimethylammonium bromide				
5 μ M	5.94 $\pm$ 0.04 (5)	5.86 $\pm$ 0.08 (6)	6.07 $\pm$ 0.07 (9)	6.04 $\pm$ 0.07 (9)
25 μ M	5.78 $\pm$ 0.05 (5)	5.70 $\pm$ 0.04 (5)	6.20 $\pm$ 0.08 (3)	6.21 $\pm$ 0.02 (5)
(13) 4-Phenyl-4-cyclopentylacetoxy- <i>N</i> -methyl-piperidine MeBr				
0.1 μ M	9.06 $\pm$ 0.07 (5)	8.94 $\pm$ 0.07 (5)	9.69 $\pm$ 0.10 (5)	9.56 $\pm$ 0.08 (5)
(14) 4-(+)-Camphorsulphonyl- <i>N</i> -methylpiperidine HCl				
inactive below 100 μ M				
(15) 4-(+)-Camphorsulphonyl- <i>N</i> -methylpiperidine MeBr				
inactive below 100 μ M				
(16) 1,1-Diphenyl-1-hydroxy-2,4-hexadiynyl-dimethylamine HBr				
inactive below 50 μ M				
(17) 1,1-Diphenyl-1-hydroxy-2,4-hexadiynyl-trimethylammonium bromide				
inactive below 50 μ M				
(18) ($\pm$)- <i>p</i> -Fluoro-hexahydro-sila-diphenidol				
0.1 μ M			7.77 $\pm$ 0.05 (5)	7.77 $\pm$ 0.06 (5)
1.0 μ M	5.91 $\pm$ 0.10 (6)	5.56 $\pm$ 0.19 (8)	7.53 $\pm$ 0.05 (8)	7.53 $\pm$ 0.05 (8)

95% O₂ and 5% CO₂ containing *norphenylephrine*, 5 μ M (the same solution as was used for the ileum). The temperature was 29.8 $\pm$ 0.3°C and the spontaneous contractions were recorded isometrically with a load of about 0.2 g: action potentials were also recorded and the time required for 50 beats was continuously printed out (Barlow & Shepherd, 1986).

The agonist, carbachol, was added by relays operated from a Commodore 128 microcomputer and allowed to act for 5 min: doses were given once every 15 min, with a second wash 10 min from the start of the cycle. The effects of the agonist were expressed as the percentage inhibition of the force of contraction and the percentage increase in the time for 50 beats. As with the ileum the control responses were

usually obtained with 0.1 and 0.2 μ M carbachol. The preparation was then exposed to the antagonist, and the experiment continued as with the ileum.

These methods are the same as those used by Barlow & Shepherd (1986) and Barlow *et al.* (1988).

Compounds

The synthesis of the diphenylacetoxy propyl compounds (1, 2, 3, 4, 5 and 6) started from diphenylacetyl chloride and 3-chloro-propan-1-ol. The diphenyl-acetoxypropyl chloride formed was converted to the iodide which was treated with dimethylamine, pyrrolidine or piperidine.

The butyl compounds (7 and 8) were prepared similarly, starting from diphenylacetyl chloride and tetrahydrofuran, which gave diphenylacetoxy butyl chloride.

The *cis* butenyl compounds (9 and 10) were prepared from *cis* but-2-ene-1,4-diol (Aldrich). This was converted into the mono-chloro compound and esterified with diphenylacetylchloride. The 4-diphenylacetoxy-*cis*-but-2-enyl chloride formed was treated directly with the amine (without first converting it to the iodide).

The *trans* butenyl compounds (11 and 12) were prepared from fumaric acid which was reduced to give *trans* but-2-ene-1,4-diol and the synthesis was then similar to that for the *cis* compounds.

The phenylcyclopentyl compound (13) was obtained from phenylcyclopentylacetic acid, which was converted into the acid chloride and esterified with 4-hydroxy-*N*-methylpiperidine. The (+)-camphor-sulphonyl esters (14 and 15) were also obtained from the acid chloride (Aldrich) and 4-hydroxy-*N*-methylpiperidine.

The hexadiynes (16 and 17) were obtained starting from but-2-yne-1,4-diol. This was converted into the 1,4-dichloro-compound and when treated with sodamide in liquid ammonia this formed the sodio derivative of buta-1,3-diyne which was reacted with ethanethiol, forming 1-thioethyl-but-1-ene-3-yne. This was treated with dimethylaminomethanol in the presence of cupric acetate to form the 3-dimethylaminomethyl compound, which was converted back to a diacetylene by removal of the thioethyl group with sodamide in liquid ammonia. The 4-dimethylaminomethyl-but-1,3-diyne was isolated and treated with butyl-lithium in ether and the lithio-derivative was reacted with benzophenone, as in previous work (Barlow *et al.*, 1988) to form 1,1-diphenyl-1-hydroxy-2,4-hexadiynyl dimethylamine.

All the final products were recrystallized from combinations of ethanol, butanone and ether. Their structures were checked by nuclear magnetic resonance (n.m.r.) and they gave satisfactory analyses. A table of melting-points and analyses is available from the authors.

(±)-*p*-Fluoro hexahydro-sila-diphenidol was a gift from Dr R.M. Eglen. Carbachol chloride was bought from Sigma.

Results

The results are summarized in Table 1. The (+)-camphor sulphonic esters (14 and 15) were inactive in concentrations up to 100 µM so their log affinity constants must be less than 4 and the diacetylenes (16 and 17) must have log *K* less than 4.3. Most of the compounds were only tested at two concentrations but the mean estimates of log *K* are similar, which is consistent with the idea that the antagonism is competitive. The propyl compound (2) was tested at three concentrations and the slopes (± s.e.mean and number of degrees of freedom) of the Schild plots were 1.02 (± 0.06; 8) for atrial rate, 1.06 (± 0.05; 8) for atrial force, 0.97 (± 0.02; 9) for ileum at 30°C and 0.95 (± 0.02; 9) for ileum at 37°C.

The results with *p*-fluoro-hexahydro-sila-diphenidol (18) on ileum suggested that a check should be made that it acts competitively, as reported by Eglen *et al.* (1989). It was not possible to test a wide range of concentrations, so 1.0 µM solutions were tested together with 0.01 µM 4-DAMP methobromide. On ileum these concentrations separately produce dose-ratios of about 36 (DR₁) and 10 (DR₂); thus, the expected combined

dose-ratio should be 45 (DR₁ + DR₂ - 1) if both were acting competitively at the same site (Paton & Rang, 1965; Abramson *et al.*, 1969). The results are shown in Table 2 and confirm that this is so.

The selectivity has been calculated from the difference between the log *K* for effects on ileum (the overall mean for all results at 30°C) and log *K* for effects on atria (the overall mean for all results, both effects on atrial rate and effects on atrial force). The effects of chain length are shown in Figure 1a in which the butyl compounds are compared with the corresponding propyl compounds and with diphenylacetylcholine (Barlow *et al.*, 1976). Lengthening the chain decreases the affinity but whereas the butyl base is stronger than its metho salt this is not true for the corresponding propyl compounds and the propyl base has some selectivity. Increasing the size of the onium group from dimethylamino to pyrrolidino, however, reduces affinity. This is similar to what is seen with the corresponding butynes (Barlow *et al.*, 1988) but unlike what is observed with choline derivatives (Abramson *et al.*, 1969). These compounds all have much less affinity than 4-DAMP methobromide. The dimethylamine had some selectivity but less than that of 4-DAMP methobromide. It is interesting that the piperidine also had some selectivity because this ring occurs in diphenidol and its derivatives.

The effects of saturation can be seen in Figure 1b, which compares the butyl, butenyl and butynyl compounds. With all these the base is consistently more potent than the quaternary salt but there is remarkably little difference between the compounds, except in selectivity. It is surprising that the *cis* and *trans* compounds are so similar but there is no reason to doubt their chemical identity. The *trans* compound was prepared from fumaric acid; the *cis* compound was obtained from the *cis* diol by methods which should not lead to isomerization and the base would not crystallize as the hydrobromide. The n.m.r. spectra are quite distinct.

The effects of replacing one phenyl ring in 4-DAMP methobromide by cyclopentyl are shown in Figure 1c. There is an increase in affinity (more than obtained by replacing phenyl by cyclohexyl; Barlow & Shepherd, 1986), but there is a loss of selectivity. The very high selectivity of *p*-fluoro-hexahydro-sila-diphenidol can also be seen but the compound has relatively low affinity, less than that of silabenzhexol or silaprocyclidine (Barlow & Shepherd, 1986). The value for receptors in ileum (log *K* = 7.6) is comparable with that of diphenylhydroxymethyl-prop-3-ynyl-trimethyl-ammonium (log *K* = 7.3; Barlow *et al.*, 1988), which has a C—C≡C—C chain instead of Si—C—C—C, but this compound has no selectivity (for atria log *K* = 7.4).

Discussion

The propyl base (1) can be regarded as 4-DAMP MeBr with an incomplete piperidine ring, which might explain why it is the most selective of the series ethyl, propyl, butyl (Figure 1a) but it is very much weaker. For ileum it has log *K* about 1.5 log units less than that of 4-DAMP MeBr, which indicates a difference of 2.1 kcal (8.7 kJ) mol⁻¹ in the free energy of adsorption. This does not seem to be too big a contribution to be expected from two methylene groups (Abramson *et al.*, 1974) but it suggests that the introduction of these groups does not greatly disturb existing binding (Abramson *et al.*,

Table 2 Test for competition: dose-ratios (mean ± s.e.mean and number of estimates) for the solution indicated

Rate	Atria		Ileum	
	Force		30°C	37°C
Dose-ratios with (±)- <i>p</i> -fluoro-hexahydrosiladiphenidol (1.0 µM)				
	1.91 ± 0.16 (6)	1.51 ± 0.10 (8)	36.8 ± 4.11 (8)	36.0 ± 3.51 (8)
Dose-ratios with (±)- <i>p</i> -fluoro-hexahydrosiladiphenidol (1.0 µM) + 4-DAMP MeBr (0.01 µM)				
	4.42 ± 0.49 (3)	3.66 ± 0.24 (4)	48.2 ± 5.03 (5)	41.7 ± 3.42 (4)

Figure 1 Selectivity, indicated by the difference between mean log affinity constant for atria (pooled results for effects on rate and effects on force at all concentrations) and mean log affinity constant for ileum (pooled results at all concentrations): temperature 30°C. With all compounds the affinity is lower for atria. The scale indicates log K and each asterisk (*) indicates a difference of 0.1 log unit.

(a) The effect of chain length. The esters of aminobutanol (BUTYL-ME₂N = compound 7, BUTYL-ME₃N = compound 8) have lower affinity than the esters of aminopropanol (1, 2, 3 and 4), which have lower affinity than diphenylacetylcholine (Barlow *et al.*, 1976), which is weaker than 4-DAMP methobromide (4DAMPMEBR). Increasing the size of the onium group in the propyl esters decreases activity (PROPYL-PYRR = compound 3, PROPYL-MEPYRR = compound 4) but the piperidine appears to have some selectivity (PROPYL-PIP = compound 5, PROPYL-MEPIP = compound 6).

(b) Effects of unsaturation. The butyl compounds (7 and 8) are compared with their *cis* analogues (9 and 10), their *trans* analogues (11 and 12) and the butynes (Barlow *et al.*, 1988). Their overall affinities are similar but the butyne base (BUTYNE-ME₂N) has appreciable selectivity.

(c) Replacing phenyl by cyclopentyl in 4-DAMP methobromide (4DAMPMEBR) (compound 13) increases affinity more than replacing phenyl by cyclohexyl (Barlow & Shepherd, 1988). High selectivity is found with *p*-fluoro-hexahydro-sila-diphenidol (compound 18) but it has relatively low affinity (lower than sila-benzhexol). The results with this compound are similar to those of Eglen *et al.* (1989).

1969), i.e. that binding of the diphenylacetyl and onium parts of these molecules is very similar but with 4-DAMP MeBr there is an additional large contribution from the ethylene bridge which forms one side of the piperidine ring. The maximum observed effect of a phenyl group on affinity in these compounds (Abramson *et al.*, 1974; Barlow, 1980) is an increase of 3.5 log units so with two phenyl groups log K might be around 7. The charge might contribute 1 log unit to binding (Barlow & Tubby, 1974; Barlow, 1980) and, with 1.5

log units from the two methylene groups (see above), log K would be 9.5 for 4-DAMP MeBr. This is only 0.5 log units more than the experimental value for ileum, suggesting that these groups are so arranged that they can exert almost their maximal effects and the structure of 4-DAMP MeBr may be a good template for the structure of part of the muscarinic receptor in ileum.

The absence of a difference between the *cis* and *trans* butenyl compounds (Figure 1b) could be explained if these are not bound in an extended form. If the N—C—C—C—C—chain has N—C—C—C *trans* and C—C—C—C *cis*, the length will be similar if the arrangement is N—C—C—C *cis* and C—C—C—C *trans*. From the low affinity of these compounds compared with 4-DAMP, it seems likely that these parts of the molecule are not much involved in binding and that they merely link the main binding groups, the onium atom and the two large (phenyl/cycloalkyl) rings. From measurements on Dreiding models the nitrogen to (end) carbon distance is around 4 Å. In the amino-butyne, which are held extended by the acetylenic bond, this distance is nearer to 5 Å.

In the hexadiynes (16 and 17) the end-to-end carbon distance is almost 7 Å and the nitrogen-to-(end) carbon distance is greater than this. Their inactivity suggests a definite limit to the size of group which can be accommodated: in 4-DAMP methiodide the distance between the nitrogen and the carbon of the acetyl group is only 6.5 Å in the crystal (Barlow *et al.*, 1987). The inactivity of the camphor sulphonates also suggests that there is a limit to the bulk of the ring which is acceptable. It has long been known that the combination of phenyl and cyclohexyl rings leads to high affinity but the phenylcyclopentyl-acetoxy analogue of 4-DAMP MeBr is actually more potent than its phenylcyclohexyl analogue (Figure 1c). The carbon-carbon distances in a phenyl ring are approximately 2.8 Å lengthways and 2.3 Å across (from the crystal structure for 4-DAMP methiodide (MeI), Barlow *et al.*,

Figure 2 Possible binding of 4-DAMP methiodide at muscarinic receptors, based on the crystal structure (Barlow *et al.*, 1987). There are possible contributions from: (A) the onium nitrogen atom, (B) one large (phenyl or cyclohexyl) ring, (C) a second ring and (D) the two ethylene groups in the piperidine ring. As drawn the postulated binding groups in the receptor (indicated by shading) are towards the viewer so the drug will approach from the rear. The distance between the onium and acetyl groups in the crystal is 6.5 Å: in the diacetylenes, which are inactive, the comparable distance is about 7.0 Å. It is suggested that the onium binding site (A) is on the outer side (more easily accessible from solution) and that with longer molecules a better fit is obtained with the smaller dimethylamines than with trimethylammonium salts. There are probably size limits (possibly about 3 Å) to groups binding at sites B and C, because the camphorsulphonate esters, in which some distances in the ring are around 3.8 Å, are inactive. The relative positions of sites B and C are important. The 'claw-like' structure of many muscarinic antagonists was commented on by Hamor (1977) and the replacement of tetrahedral carbon by trigonal nitrogen in the carbamate analogue of 4-DAMP MeBr produces a compound which appears to bind only to one site, not to both (Barlow & Johnson, 1989). Reasons for believing that site D is particularly important in the binding of 4-DAMP are discussed in the text.

1987): for a cyclohexyl group the corresponding distances are approximately 2.9 Å and 2.5 Å (from the crystal structure for phenylcyclohexyl glycolloyl ethyl piperidine methiodide, Hamor, 1977); for camphor-10-sulphonic acid the distance between the 8 (or 9) methyl group and carbon atoms on the far edge (2, 3, 5 or 6) is nearer 3.8 Å (from the crystal structure, Koch *et al.*, 1975), again suggesting that there are limits to the size of the group which can be accommodated. In Figure 2 an attempt has been made to collect these ideas together, based on the crystal structure of 4-DAMP MeI.

Although this picture can only be conjecture, the size limits should help to check molecular modelling of the muscarinic receptor based on its amino acid sequence which is now known (Kubo *et al.*, 1986a; 1986b; Bonner *et al.*, 1987; Peralta *et al.*, 1987; 1988). It is possible to speculate about the actual groups involved in the binding of muscarinic antagonists and work with propylbenzyl choline mustard by Curtis *et al.* (1989) suggests that the binding of the onium group involves an aspartate residue (D, 105) in transmembrane helix

3. There are tryptophan (W) and tyrosine (Y) residues nearby which might be involved in binding phenyl or cycloalkyl rings in the antagonists, but the aminoacids present in this part of the sequence are very similar (CDLWL**DYV*) in all four receptor types studied by Bonner and colleagues. Although the relative positions of these groups is not known (or even whether they are the ones actually involved), this may explain why it is difficult to achieve selectivity. Differences in the geometry of the receptors, which allow a drug to be selective by fitting one shape much better than another, may depend on differences in the arrangements of the same groups caused indirectly by differences in other groups in the sequence.

In these compounds it seems that there are definite steric constraints and that selectivity is achieved by introducing groups which reduce binding but have a greater adverse effect at receptors in atria than at those in ileum.

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