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The affinity of some acetylenic analogues of 4-DAMP methobromide for muscarinic receptors in guinea-pig ileum and atria

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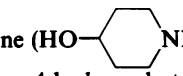
1 The replacement of 4-hydroxy-N-methyl piperidine (HO—  NMe) in 4-diphenylacetoxy-N-methyl piperidine (4-DAMP) metho-bromide by 4-hydroxy-but-2-ynylamines ($HOCH_2C{\equiv}CCH_2NR_2$) reduces the affinity for muscarine-sensitive acetylcholine receptors in guinea-pig ileum and atria. It does not abolish selectivity. The tertiary amines are more active and more selective than the corresponding quaternary salts.

2 Analogous derivatives of 4-hydroxy-but-2-ynylamines which lack the ester group (i.e. substituted 4-hydroxymethyl-propynyl amines) are less active and less selective. The quaternary compounds are more active than the tertiary bases.

3 The diphenylcarbamyl ester of 4-hydroxy-N-methylpiperidine methobromide has less than one-thousandth of the activity of the diphenylacetyl ester (4-DAMP methobromide) and is not selective.

4 Although 4-diphenylacetoxy-butynyl dimethylamine is only about one-hundredth as active as 4-DAMP methobromide it appears to have comparable selectivity. It is an interesting compound because it is a tertiary amine and should cross membranes.

Introduction

An acetylenic group is present in several compounds which are active at muscarinic receptors. These include the homologue of acetylcholine, acetoxy-but-2-ynyltrimethyl ammonium (Jacob *et al.*, 1952), oxotremorine (Cho *et al.*, 1961), McN-A-343 (Roszkowski, 1961) and arecaidine propargyl ester (Mutschler & Hultzsch, 1973). An extensive survey of acetylenic amines related to oxotremorine was made by Bebbington *et al.* (1966) and some shorter compounds, 4-dialkylamino butynols which lack an ester group, were studied by Dahlbom *et al.* (1964). Many of the compounds have been tested for their effects on intestinal smooth muscle but the main interest has been in their ability to inhibit tremors produced by oxotremorine. Possible differences between their actions on gut and on heart do not appear to have been tested.

The X-ray crystal structure of the phenylcyclohexyl glycollic ester of dimethylaminobutynol was reported by Datta *et al.* (1979). The group is rigid and this might make it more selective. If compounds such as 4-diphenylacetoxy-N-methyl piperidine (4-DAMP) methobromide have greater affinity for muscarinic receptors in ileum than for those in atria because the receptors are different, the presence of a rigid group might make it more difficult for a molecule to adapt its conformation and fit both types.

This paper describes the affinities for muscarinic receptors in guinea-pig ileum and atria of some acetylenic analogues of derivatives of 4-hydroxy-N-methyl piperidine (Barlow & Shepherd, 1985; 1986). The two amino-alcohols are superimposed in Figure 1, which is derived from the X-ray crystal structure for 4-DAMP methiodide (Barlow *et al.*, 1987) and the results of Datta *et al.* (1979). The butynol, $HOCH_2C{\equiv}CCH_2NMe_2$, is longer than 4-hydroxy-N-methyl piperidine, so as well as testing butynyl esters similar to those studied by Bebbington *et al.* (1966), we tested shorter acetylenic compounds, similar to those studied by Dahlbom *et al.* (1964). These lack the ester group and have substitutents attached directly to the 4-carbon atom so are derivatives of hydroxymethyl-propynylamine, $HOCH_2C{\equiv}CCH_2NH_2$, e.g. $Ph_2C(OH)C{\equiv}CCH_2NMe_2$. We have also tested the urethane analogue of 4-DAMP methobromide, in which the diphenylacetyl group, $Ph_2CHCOO—$, is replaced by diphenylcarbamyl, $Ph_2NCOO—$.

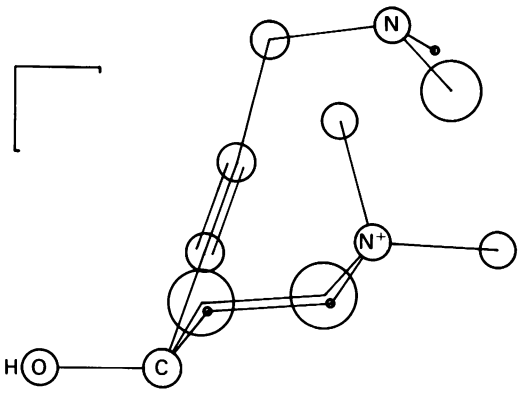


Figure 1 4-Hydroxy-N-methyl-piperidine methiodide with 4-hydroxy-but-2-ynyl-dimethylamine superimposed. The structures were taken from crystallographic data for 4-DAMP methiodide (Barlow *et al.*, 1987) and for dimethylamino-butynyl-cyclohexyl-hydroxy-phenyl-acetate (Datta *et al.*, 1979). They are arranged with the hydroxyl group lying along the X-axis, the carbon to which it is attached as the origin and with the nitrogen atoms lying in the vertical plane, i.e. in the plane of the paper. The O-C distance is the same in both compounds. The piperidine ring is viewed from one side with the nearer atoms indicated by the larger circles. The atoms forming the butynyl group lie roughly along a straight line. The C to N distances are 2.90 (piperidonol) and 4.92 Å (butynylamine): the O to N distances are 4.20° and 5.84 Å. The scale shows 1 Å.

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. The agonist, carbachol, was 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, containing *nor*phenylephrine, 5 μM (Barlow & Shepherd, 1986), and experiments were done at 29.8 $\pm$ 0.3° and 37 $\pm$ 0.1°C.

Alternate small and large control responses were obtained, usually to 0.1 and 0.2 μM carbachol. 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 the approximate dose-ratio is given by the ratio of the concentrations of 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 set up in Krebs solution (Edinburgh Staff, 1974) aerated with a mixture of 95% O_2 and 5% CO_2, containing *nor*phenylephrine, 5 μM (the same solution as was used for the ileum). The temperature was 29.8 $\pm$ 0.3° 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 the contraction and the percentage increase in the time for 50 beats. As in the experiments on ileum, the control responses were usually obtained with 0.1 and 0.2 μM carbachol. The tissue was then exposed to the antagonist and the experiment continued as with the ileum.

These methods are the same as used by Barlow & Shepherd (1986).

Compounds

The esters of 4-hydroxy-but-2-ynyl amines were prepared from 1-chloro-4-hydroxy-but-2-yne. This was esterified with the appropriate acid chloride and the chloro-butynyl ester then reacted with the appropriate base (dimethylamine or pyrrolidine).

The shorter compounds, lacking the ester group, were obtained by treating dimethyl propargylamine with butyl lithium and reacting the lithio-derivative with the appropriate ketone (such as benzophenone). The products are all butynes but in Table 1 compounds 11 to 15 are described as hydroxy-methyl propynes and, with compounds 16 to 21, the fourth carbon atom in the butyne chain forms part of a cyclic structure.

The urethane analogue of 4-DAMP methobromide (compound 22) was obtained from 4-hydroxy-N-methyl-piperidine and diphenylcarbamyl chloride, just as in the preparation of the diphenylacetyl ester (4-DAMP).

All the starting materials were obtained from Aldrich. The final products were crystallized from combinations of ethanol, acetone, ethylmethyl ketone and ether. Their structures were checked by n.m.r. and they gave satisfactory analyses. A table of

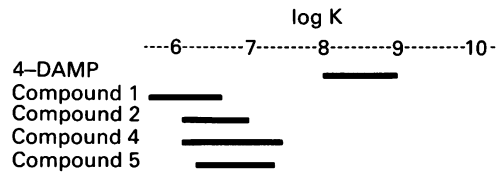


Figure 2 Selectivity, indicated by the difference between log K for atria and for ileum. The scale is shown at the top and the value for atria is obtained by pooling all results, i.e. it is assumed that there is no difference between effects on rate and effects on force. All the other compounds in Table 1 were less selective than those shown. Note that although compound 4 has much less affinity, the results indicate selectivity comparable to that of 4-DAMP methobromide.

metling-points and analyses is available from the authors.

Results

The results are summarized in Table 1, which shows the mean estimate of log affinity constant ± s.e. and number of results, based on the pooled results for the range of concentrations indicated.

Although both types of compound, the butynol esters and the shorter compounds have been studied previously, all the compounds in Table 1 are new except for number 16. This was prepared (as a hydrochloride) by Dahlbom *et al.* (1964) but its biological activity was not reported.

With the butynol esters (compounds 1–10) it appears that the tertiary compounds are more active than their quaternary analogues (from 1.5 times to almost 10 times as active: compare compound 1 with 4, 3 with 5, or 8 with 9). This agrees with the results of Inch & Brimblecombe (1971) for the R(−)-phenylcyclohexylglycollyl esters (log K 9.18 for the base compared with 8.34 for the methiodide). With the analogous compounds of the shorter type they found the reverse, that both the R(−)- and S(+)-bases (log K 7.26, 7.30) were less active than the quaternary derivatives (log K 7.98, 8.11). A similar difference is seen between compounds 14 and 15 where quaternization increases affinity about 3 fold. Although there is no compound with which direct comparisons can be made, the results in Table 1 appear to be consistent with those already published.

The dramatic effect of replacing diphenylacetyl by diphenylcarbamyl in 4-DAMP methobromide can be seen from the results for compound 22. Affinity for receptors in the ileum is reduced more than 1000 fold and selectivity is lost.

The selectivity of the compounds is illustrated in Figure 2, which shows the mean estimates of log K for ileum at 30°C and for pooled values for effects on both rate and force for the atria. Only compounds where the difference is greater than 0.7 log units (5 fold) are shown. These are all butynol esters; the shorter compounds are not only less active, they are less selective.

Discussion

The apparent high selectivity of a tertiary base (compound 4) is of interest because this should cross membranes, unlike 4-DAMP methobromide, which is a quaternary salt. The effect of the butynyl group can be assessed by comparing this compound with diphenylacetylcholine, which has log K for receptors in ileum = 7.16 (at 37°C: Abramson *et al.*, 1969; Edinburgh Staff, 1974). This value is slightly less than the value for compound 4 on ileum, though the trimethylammonium analogue (compound 1) is definitely weaker. It appears that the greater length, which makes the compound fit less well, can be offset by reducing the number of methyl groups from three to two. Diphenylacetylcholine does not appear to have been tested on atria but the corresponding ether, diphenylethoxyethyl trimethylammonium bromide, had virtually no selectivity (Barlow *et al.*, 1976). It is possible, therefore, that the stiffness and greater length of the butynol ester makes the dimethylammonium compound (4) less able to fit receptors in atria.

From the results of the compounds tested it is unlikely that more selective compounds will be obtained by making and testing more examples, particularly those of the shorter type. The effects of substituents have only been studied in a fragmentary, rather than a systematic, fashion but some comments are possible. An α-methyl group increased affinity (about 2 fold: compare compounds 1 and 2). For 4-DAMP methobromide and its α-methyl analogue log K = 9.0 and 9.7, respectively for ileum at 30°C (Barlow & Shepherd, 1986). This makes it difficult to accept the view that the high affinity of α-hydroxy compounds can be ascribed to hydrogen bonding unless such compounds interact with a different part of the receptor.

The greater affinity of the tertiary amines compared with their quaternary analogues in the butynyl esters is compatible with the idea that the greater length of these compounds makes it more difficult for them to fit the receptors than the piperidinols.

Studies of analogues of 4-DAMP methobromide suggested that there are steric constraints in the binding of the diphenylacetyl part of the molecule (Barlow & Shepherd, 1986) and these appear to be confirmed with the results for the butynyl esters. An increase in size is associated with decreased affinity.

Table 1 Mean estimates of log affinity constant for compounds tested in the concentration range indicated

	Atria		*Ileum*	
Rate	*Force*		*30°*	*37°*
	$-CO-O-CH_2C{\equiv}CCH_2-N^+\lessgtr$			
1 4-Diphenylacetoxy-but-2-ynyltrimethylammonium bromide (1–5 μM)				
5.50 ± 0.15 (6)	5.78 ± 0.03 (7)		6.59 ± 0.03 (7)	6.53 ± 0.04 (7)
2 4-Diphenyl-methyl-acetoxy-but-2-ynyltrimethylammonium bromide (0.5–5 μM)				
6.10 ± 0.06 (7)	6.09 ± 0.02 (7)		6.99 ± 0.06 (10)	6.79 ± 0.04 (10)
3 4-Diphenylacetoxy-but-2-ynyl-methylpyrrolidinium bromide (2–5 μM)				
5.96 ± 0.05 (8)	5.95 ± 0.02 (8)		6.35 ± 0.01 (8)	6.28 ± 0.03 (8)
4 4-Diphenylacetoxy-but-2-ynylidimethylamine HBr (1–5 μM)				
6.08 ± 0.08 (11)	6.17 ± 0.04 (11)		7.38 ± 0.04 (10)	7.38 ± 0.06 (10)
5 4-Diphenylacetoxy-but-2-ynylpyrrolidine HBr (1–5 μM)				
6.33 ± 0.08 (10)	6.29 ± 0.06 (10)		7.30 ± 0.02 (10)	7.19 ± 0.03 (10)
6 Anthracene-9-carboxy-but-2-ynyltrimethylammonium bromide (5–10 μM)				
5.63 ± 0.07 (4)	5.59 ± 0.03 (4)		5.87 ± 0.05 (3)	5.74 ± 0.13 (4)
7 9:10-dihydroanthracene-9-carboxy-but-2-ynyl dimethylamine HBr (1–5 μM)				
7.08 ± 0.08 (7)	6.90 ± 0.07 (7)		7.34 ± 0.03 (10)	7.27 ± 0.04 (10)
8 Xanthene-9-carboxy-but-2-ynyltrimethylammonium bromide (5–10 μM)				
6.28 ± 0.03 (8)	6.41 ± 0.06 (8)		6.47 ± 0.02 (8)	6.44 ± 0.02 (8)
9 Xanthene-9-carboxy-but-2-ynyldimethylamine HBr (1–5 μM)				
6.80 ± 0.07 (6)	6.76 ± 0.08 (8)		7.22 ± 0.02 (8)	7.23 ± 0.04 (8)
10 Thioxanthene-9-carboxy-but-2-ynyldimethylamine HBr (1–5 μM)				
6.38 ± 0.14 (6)	6.55 ± 0.04 (8)		7.05 ± 0.03 (8)	7.05 ± 0.03 (8)
	$-C(OH)C{\equiv}CCH_2-N^+\lessgtr$			
11 3-Diphenylhydroxymethyl-prop-3-ynyltrimethyammonium bromide (1–5 μM)				
7.43 ± 0.07 (7)	7.42 ± 0.04 (7)		7.33 ± 0.04 (6)	7.44 ± 0.03 (8)
12 (±)-3-(*p*-tolyl-phenyl-hydroxy)-methyl-propynyldimethylamine HBr (1–5 μM)				
6.10 ± 0.07 (9)	6.25 ± 0.07 (10)		6.67 ± 0.04 (9)	6.63 ± 0.05 (9)
13 3-Dibenzylhydroxymethyl-prop-3-ynyltrimethylammonium bromide (5 μM)				
5.03 ± 0.22 (3)	5.04 ± 0.19 (4)		5.19 ± 0.05 (5)	5.25 ± 0.05 (5)
14 3-Di(p-fluoro-phenyl)-hydroxymethyl-propynyltrimethylammonium bromide (1–5 μM)				
6.70 ± 0.13 (4)	6.57 ± 0.07 (7)		6.87 ± 0.02 (7)	6.84 ± 0.03 (7)
15 3-Di(p-fluoro-phenyl)-hydroxymethyl-propynyldimethylamine HBr (1–5 μM)				
6.15 ± 0.25 (3)	5.84 ± 0.08 (3)		6.37 ± 0.05 (2)	6.27 ± 0.07 (2)
16 3-(9-Hydroxyfluorenyl)-propynyl-dimethylamine HBr (5 μM)				
5.31 ± 0.29 (2)	5.33 ± 0.27 (2)		5.60 ± 0.10 (4)	5.59 ± 0.07 (4)
17 3-(Hydroxy-dibenzocycloheptyl)-propynyl trimethyl-ammonium bromide (5–10 μM)				
6.36 ± 0.02 (2)	6.31 ± 0.05 (6)		6.18 ± 0.03 (9)	6.17 ± 0.03 (9)
18 3-(Hydroxy-dibenzocycloheptenyl)-propynyl-trimethyl ammonium bromide (5–20 μM)				
5.73 ± 0.07 (8)	5.71 ± 0.06 (8)		5.80 ± 0.02 (8)	5.79 ± 0.02 (8)
19 3-(Hydroxy-dibenzocycloheptenyl)-propynyl dimethylamine HBr (5 μM)				
5.52 ± 0.23 (4)	5.53 ± 0.25 (4)		5.75 ± 0.07 (4)	5.72 ± 0.09 (5)
20 3-(Hydroxy-9-xanthyl)-propynyltrimethyl ammonium bromide (10–40 μM)				
5.65 ± 0.05 (8)	5.74 ± 0.05 (8)		6.00 ± 0.02 (8)	6.04 ± 0.03 (8)
21 3-(Hydroxy-9-thioxanthyl)-propynyl trimethyl-ammonium bromide (1–5 μM)				
6.23 ± 0.04 (8)	6.36 ± 0.07 (8)		6.33 ± 0.07 (10)	6.41 ± 0.03 (9)
22 4-Diphenylcarbamyloxy-N-methylpiperidine methobromide (carbamyl 4DAMP MeBr: 1–5 μM)				
5.72 ± 0.09 (8)	5.72 ± 0.03 (7)		5.81 ± 0.05 (12)	5.83 ± 0.03 (13)

Values are given ± s.e.mean with number of results in parentheses

This seems also to be true for the shorter type of compound: the unsubstituted compound (11) has the highest affinity of those tested. This is surprising, if it is thought that with the shorter compounds there might be more space in which substituents could be accommodated.

The very low activity of the diphenylcarbamyl analogue of 4-DAMP methobromide requires further study. The replacement of ester by amide has dramatic effects on the activity of compounds related to acetylcholine (Barlow *et al.*, 1978) but in the carbamyl esters, as in carbachol, the ester group is

retained. It is not impossible that the difference in affinity can be ascribed to differences in the polarization of the carbamyl group as compared with ester. It is detectably less lipophilic when tested by paper chromatography, but the differences in R_F are small (0.65 compared with 0.73 for the solvent system butanol-ethanol-water, 5:5:2). It is possible, therefore, that the difference in affinity is at least partly caused by differences in size and shape. The two benzene rings could well be arranged differently about the ester group in diphenylcarbamyl from the arrangement in diphenylacetyl and an X-ray crystal crystallographic study of the diphenylcarbamyl compound should be made.

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