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THE INTERACTION OF MUSCARINIC DRUGS WITH THE POSTGANGLIONIC ACETYLCHOLINE RECEPTOR

BY

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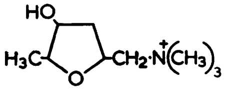
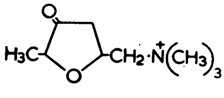
From a consideration of the chemical and pharmacological properties of muscarine and muscarone, Beckett, Harper, Clitherow & Lesser (1961) and Beckett, Harper & Clitherow (1963) have proposed a tentative picture of the muscarinic receptor based on a three-point attachment of the molecules to the receptor surface.



Muscarine

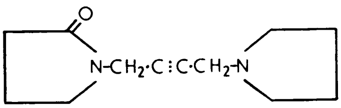


Muscarone

They suggested that the three groups involved in the interaction of muscarine with the receptor are the quaternary ammonium group, the ether oxygen atom and the hydroxyl group; in muscarone, the quaternary group, the ether oxygen atom and the ketonic carbonyl group are involved. Because of the rigidity of these molecules it was possible to draw conclusions about the nature of the receptor and it was suggested that there are three sites separated by distances which correspond approximately to the distances between the polar groups in muscarine and muscarone.

Waser (1961), however, has claimed that this explanation by Beckett *et al.* (1961, 1963) does not account for the low and inverse stereospecificity of muscarone compared with muscarine. He preferred to consider that muscarinic activity in muscarine-like compounds depends mainly on the quaternary group and a nucleophilic group which in muscarine is the ether oxygen atom and in muscarone is the carbonyl group. According to this view muscarone must have a "folded" conformation when interacting with the muscarinic receptor. Belleau & Puranen (1963) have pointed out that such a folded conformation would be unfavourable, and moreover that the molecules would depart from the maximum coplanarity which generally appears to be necessary for the effective binding of drugs to receptors. They consider that a more likely possibility is that the carbonyl group in muscarone interacts with a third site on the receptor.

The isolation and synthesis by Cho, Haslett & Jenden (1961) of oxoTremorine, and the description of its muscarinic properties (Cho, Haslett & Jenden, 1962) has led to the investigation of an interesting new class of muscarinic agent, the acetylenic amines. The activities of a number of such compounds have been interpreted by Bebbington, Brimblecombe & Shakeshaft (1966) as being due to interaction at three sites on the receptor, distributed in the manner proposed by Beckett *et al.* (1963). It was suggested that, in oxoTremorine itself, the pyrrolidine nitrogen atom, the acetylenic linkage and the carbonyl oxygen atom are involved in these interactions.



oxoTremorine

Cho *et al.* (1962), however, consider that the acetylenic linkage may not be concerned in actual interaction with the receptor. They suggest that only the pyrrolidine nitrogen atom and the carbonyl oxygen atom interact. Rotation about the C–C bonds would cause these two atoms to be separated by a distance (4 Å) which corresponds to the separation of the two sites proposed by Waser (1961).

In the present work, the muscarinic activities of a number of compounds related to both the choline ethers and acetylenic amines related to oxoTremorine have been examined in an attempt to obtain more information concerning the type of structure necessary for muscarinic activity and hence to provide additional evidence concerning the nature of the muscarinic receptor.

METHODS

Chemical syntheses

The physical properties of the compounds used in this investigation are summarized in Tables 1, 2 and 3.

Quaternary ammonium salts were in all instances prepared from the appropriate tertiary base in either acetone or ethyl methyl ketone solution.

Preparation of acetylene derivatives (compounds No. 1, 2, 3, 13 *and* 22)

Dimethylprop-2-ynylamine was prepared by the following sequence of reactions:

$$CH_2 : C(Br).CH_2.Br + NH(CH_3)_2 \longrightarrow CH_2 : C(Br).CH_2.N(CH_3)_2 \xrightarrow{KOH} CH \vdots C.CH_2.N(CH_3)_2$$

The preparation of compound No. 3 is described by Bebbington *et al.* (1965).

Compound No. 13 was obtained by the action of ethyl iodide on the sodium derivative of dimethylprop-2-ynylamine in liquid ammonia solution. Compound No. 22 was prepared similarly from 1-prop-2′-ynylpyrrolid-2-one (Bebbington & Shakeshaft, 1965).

Preparation of choline ethers (compounds No. 4, 5, 6, 14, 15, 16 *and* 23)

(2-Methoxyethyl)dimethylamine was obtained from sodium methoxide and 2-dimethylaminoethyl chloride in methanol solution.

Compounds No. 14, 15 and 23 were prepared from 1-chloromethylpyrrolid-2-one and the sodium derivative of the appropriate amino-alcohol in benzene solution.

Compound No. 16 could not be obtained by the same method, as 1-(2-chloroethyl)pyrrolid-2-one is readily converted to 1-vinylpyrrolid-2-one. It was obtained from 2-dimethylaminoethyl chloride and the sodium salt of 1-(2-hydroxyethyl)pyrrolid-2-one.

TABLE 1
QUATERNARY AMMONIUM SALTS
(*d*) = With decomposition

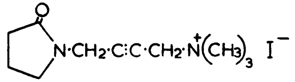
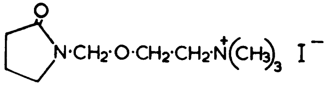
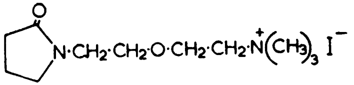
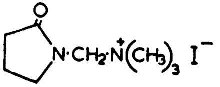
No.	Compound	Formula	Melting point (°C)	Analysis: Found	Analysis: Calculated	Yield (%)
	Acetylene derivatives					
1	Trimethylprop-2-ynylammonium iodide $CH\vdots C.CH_2.N^+(CH_3)_3I^-$	$C_6H_{12}IN$	182–184			
2	Trimethylpent-2-ynylammonium iodide $CH_3.CH_2.C\vdots C.CH_2.N^+(CH_3)_3I^-$	$C_8H_{16}IN$	175–177			
3	Trimethyl[4-(2-oxopyrrolidin-1-yl)but-2-ynyl]-ammonium iodide 	$C_{11}H_{19}IN_2O$				
	Choline ethers					
4	(2-Methoxyethyl)trimethylammonium iodide (choline methyl ether iodide) $CH_3O.CH_2.CH_2.N^+(CH_3)_3I^-$	$C_6H_{16}INO$	104–106			
5	Trimethyl{2-(2-oxopyrrolidin-1-ylmethoxy)ethyl}-ammonium iodide 	$C_{10}H_{21}IN_2O_2$	122–123	C, 36·5; H, 6·3	C, 36·6; H, 6·5	44
6	Trimethyl{2-[2-(2-oxopyrrolidin-1-yl)ethoxy]ethyl}-ammonium iodide 	$C_{11}H_{23}IN_2O_2$		C, 38·0; H, 6·9; N, 7·9	C, 38·6; H, 6·8; N, 8·2	43
	Polymethylene derivatives					
7	Trimethyl(2-oxopyrrolidin-1-ylmethyl)ammonium iodide 	$C_8H_{17}IN_2O$	160–165 (*d*)	C, 34·0; H, 6·2; N, 10·1	C, 33·8; H, 6·0; N, 9·9	80

TABLE 1—*Continued*

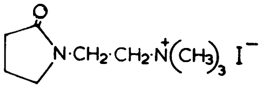
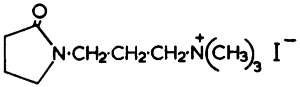
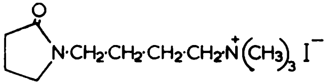
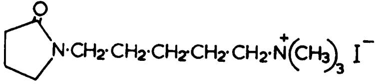
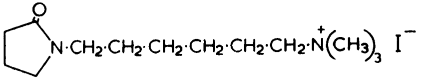
No.	Compound	Formula	Melting point (°C)	Analysis		Yield (%)
				Found	Calculated	
8	Trimethyl[2-(2-oxopyrrolidin-1-yl)ethyl]-ammonium iodide 	$C_9H_{19}IN_2O$	237–239	C, 36·5; H, 6·4; N, 9·2	C, 36·3; H, 6·4; N, 9·4	71
9	Trimethyl[3-(2-oxopyrrolidin-1-yl)propyl]-ammonium iodide 	$C_{10}H_{21}IN_2O$	120–122	C, 38·8; H, 7·2; N, 9·0	C, 38·5; H, 6·8; N, 9·0	70
10	Trimethyl[4-(2-oxopyrrolidin-1-yl)butyl]-ammonium iodide 	$C_{11}H_{23}IN_2O$	141–142	C, 40·3; H, 7·2	C, 40·5; H, 7·1	75
11	Trimethyl[5-(2-oxopyrrolidin-1-yl)pentyl]-ammonium iodide 	$C_{12}H_{25}IN_2O$				71
12	Trimethyl[6-(2-oxopyrrolidin-1-yl)hexyl]-ammonium iodide 	$C_{13}H_{27}IN_2O$				70

TABLE 2

TERTIARY AMINES

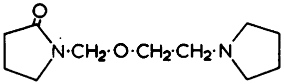
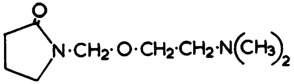
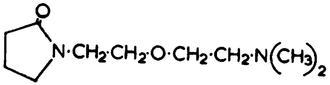
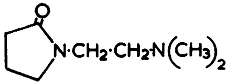
No.	Compound	Formula	Boiling point (°C)	Analysis: Found	Analysis: Calculated	Yield (%)
	Acetylene derivatives					
13	Dimethylpent-2-ynylamine $CH_3.CH_2.C\vdots C.CH_2.N(CH_3)_2$	$C_7H_{13}N$	130–135			
	Choline ethers					
14	1-(2-Pyrrolidin-1′-ylethoxymethyl)pyrrolid-2-one 	$C_{11}H_{20}N_2O_2$	121–123 (0·02 mm Hg)	C, 62·4; H, 10·0	C, 62·2; H, 9·5	60
15	1-(2-Dimethylaminoethoxymethyl)pyrrolid-2-one 	$C_9H_{18}N_2O_2$	101–103 (0·05 mm Hg)	C, 57·9; H, 10·1	C, 58·0; H, 9·7	63
16	1-[2-(2-Dimethylaminoethoxy)ethyl]pyrrolid-2-one 	$C_{10}H_{20}N_2O_2$	110–112 (0·05 mm Hg)			52
	Polymethylene derivatives					
17	1-(2-Dimethylaminoethyl)pyrrolid-2-one 	$C_8H_{16}N_2O$	126–127 (15 mm Hg)	C, 61·9; H, 10·8	C, 61·5; H, 10·3	80

TABLE 2—*Continued*

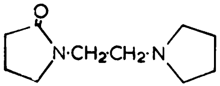
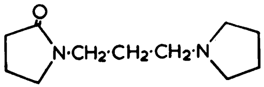
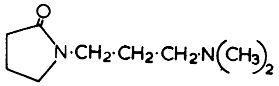
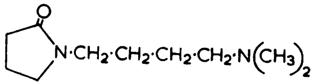
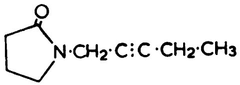
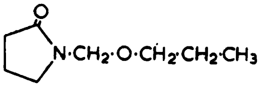
No.	Compound	Formula	Boiling point (°C)	Analysis		Yield (%)
				Found	Calculated	
18	1-(2-Pyrrolidin-1′-ylethyl)pyrrolid-2-one 	$C_{10}H_{18}N_2O$	92–89 (0·1 mm Hg)	C, 66·3; H, 10·4	C, 65·9; H, 10·0	63
19	1-(3-Pyrrolidin-1′-ylpropyl)pyrrolid-2-one 	$C_{11}H_{20}N_2O$	110–113 (0·1 mm Hg)	C, 66·8; H, 10·8	C, 67·3; H, 10·3	83
20	1-(3-Dimethylaminopropyl)pyrrolid-2-one 	$C_9H_{18}N_2O$	143–144 (20 mm Hg)			74
21	1-(4-Dimethylaminobutyl)pyrrolid-2-one 	$C_{10}H_{20}N_2O$	84–86 (0·1 mm Hg)	C, 65·3; H, 11·3; N, 15·2	C, 65·2; H, 10·9; N, 15·2	65

TABLE 3

MISCELLANEOUS COMPOUNDS

No.	Compound	Formula	Boiling point (°C)	Analysis Found	Analysis Calculated	Yield (%)
22	1-Pent-2′-ynylpyrrolid-2-one 	$C_9H_{13}NO$	84–86 (0·1 mm Hg)			43
23	1-Propoxymethylpyrrolid-2-one 	$C_8H_{15}NO_2$	116–118 (15 mm Hg)	C, 61·5; H, 10·0	C, 61·6; H, 9·6	68

Preparation of polymethylene derivatives **(*compounds No.* 7, 8, 9, 10, 11, 12, 17, 18, 19, 20 *and* 21)**

When amino-alcohols of the type $NH_2.[CH_2]_n.OH$ were readily available, the following route was adopted. The amino-alcohols were converted to 1-(ω-hydroxyalkyl)pyrrolid-2-ones by reaction with γ-butyrolactone at 200° C for 4 hr. The products were converted by thionyl chloride into the corresponding chlorides, which were treated with an excess of the required amine.

When the amino-alcohols could not readily be obtained, the products (compounds No. 10, 11, 12 and 21) were obtained from an αω-dibromoalkane and the potassium salt of pyrrolid-2-one in toluene suspension. The 1-(ω-bromoalkyl)pyrrolid-2-ones produced were converted to the 1-(ω-dialkylaminoalkyl)pyrrolid-2-ones by the addition of an excess of a secondary amine in benzene solution.

Pharmacological tests

Toxicity. The LD50s of the compounds were determined using male albino mice (20 to 30 g). Four groups of five mice were used in each test and the compounds were injected into the tail vein. LD50s were calculated using Thompson's (1947) method of moving averages employing the tables calculated by Weil (1952). The maximum dose used in the toxicity tests was 50 mg/kg.

Muscarinic activity. This was measured using the isolated guinea-pig ileum and an anaesthetized cat preparation.

About 2 cm of ileum was taken from a freshly killed guinea-pig at a point about 5 cm from the ileo-caecal junction. It was suspended in a 5-ml. organ-bath of Ringer-Tyrode solution at 37° C and a mixture of 95% oxygen and 5% carbon dioxide was passed through the solution. Contractions were recorded on a kymograph using an isotonic lever. Potencies relative to acetylcholine were measured using standard doses of 0.1 and 0.2 μM-acetylcholine and four-point assays were carried out. Tests were always made to confirm that the contractions produced were blocked by atropine sulphate (0.04 μM, left in the bath for 1 min before adding the test substance).

Cats were anaesthetized with a mixture of chloralose and urethane (2.5 ml./kg intraperitoneally of a solution containing 25 mg/ml. of chloralose and 250 mg/ml. of urethane). Arterial blood pressure was recorded from a carotid artery using a mercury manometer. Compounds were injected into a femoral vein in a volume of 0.1 ml./kg and washed in with 2 ml. of 0.9% saline. The hypotensive effects produced by the compounds were compared with those from standard doses of acetylcholine (usually 0.5 and 2.5 mμM/kg) and relative potency was calculated on the basis of a four-point assay. The cats were then given atropine sulphate (3.6 μM/kg) and the compounds were retested; block of their hypotensive actions confirmed that the effects were muscarinic.

TABLE 4

TOXICITIES AND MUSCARINIC ACTIVITIES OF QUATERNARY AMMONIUM SALTS

See Table 1 for formulae of compounds. Inactive means <0·001 relative to acetylcholine. LD50s are intravenous in mice

		Muscarinic activity (acetylcholine=1)	
Compound No.	LD50 (mg/kg)	Guinea-pig ileum	Cat blood pressure
1	35	Inactive	Inactive
2	4·3	0·22	0·13
3	0·27	1·74	1·49
4	10·0	0·01	0·01
5	0·55	0·03	0·02
6	0·64	0·005	0·005
7	>50	Inactive	Inactive
8	>50	Inactive	Inactive
9	3·05	Inactive	Inactive
10	2·90	0·002	0·002
11	3·60	Inactive	Inactive
12	7·41	Inactive	Inactive

RESULTS

The results obtained with the quaternary ammonium salts (compounds No. 1 to 12) are given in Table 4. All the tertiary amines listed in Table 2 (compounds No. 13 to 21) and the two miscellaneous compounds in Table 3 (compounds No. 22 and 23) are devoid of muscarinic activity. Among these tertiary amines and miscellaneous compounds, only two compounds have LD50s to mice of 50 mg/kg or less. They are compound No. 14 (LD50=50 mg/kg) and compound No. 19 (LD50=35 mg/kg).

DISCUSSION

The lack of activity of the compounds in Table 3 compared with the relatively high activities of compounds No. 3 and 5 indicates that an essential requirement for muscarinic activity is the presence of a positively charged group or a group which can be protonated at physiological *p*H.

Although a number of tertiary amines having high muscarinic activity are now known (Bebbington *et al.*, 1966), in the series of compounds examined in this work quaternization of the basic nitrogen atom was found to be essential for high activity (compounds No. 13 and 15 are inactive, whereas the corresponding quaternary ammonium derivatives, compounds No. 2 and 5, are active). In the following structure-activity discussion only the quaternary ammonium salts are therefore considered.

Choline methyl ether (compound No. 4) can be considered to interact at two receptor sites involving the quaternary ammonium group and the ether oxygen atom. The high activity of trimethylpent-2-ynylammonium iodide (compound No. 2) suggests that interaction may again take place at two sites. It is suggested that, in this case, the triple bond, which can be regarded as a region of high electron density, is capable of interaction with a receptor site in a similar manner to the ether oxygen atom of choline methyl ether (compound No. 4). Waser (1961), in his review of muscarine-like compounds, concludes that for maximal muscarinic activity the quaternary ammonium group must be separated from a nucleophilic group by a distance of approximately 4 Å. Examination of molecular models reveals that in trimethylpent-2-ynylammonium iodide (compound No. 2) the acetylenic linkage is separated from the quaternary ammonium group by a distance not exceeding 3 Å. This distance corresponds well with the separation of the two sites proposed by Beckett *et al.* (1963) for interaction with the quaternary groups and ether oxygen atoms in muscarine and muscarone. It is interesting to note that trimethylprop-2-ynylammonium iodide (compound No. 1) is much less active than its homologue (compound No. 2). This behaviour parallels that previously observed in the choline ethers in which choline itself was found to be much less active than choline ethyl ether (Chang & Gaddum, 1933).

The activity of trimethyl[4-(2-oxopyrrolidin-1-yl)but-2-ynyl]ammonium iodide (compound No. 3) has been interpreted by Bebbington *et al.* (1966) on the basis of interaction of the quaternary ammonium group, the acetylenic linkage, and the amide carbonyl group at three receptor sites. The activity of the corresponding ether (compound No. 5) can be interpreted similarly, the ether oxygen atom interacting at the same site as the acetylenic linkage. The higher activity of compound No. 3 compared with trimethylpent-2-ynylammonium iodide (compound No. 2), and the higher activity of compound No. 5 compared with choline methyl ether (compound No. 4), are readily explained on the basis of an additional interaction of the amide carbonyl group at a third receptor site.

The introduction of an extra methylene group in the choline ether as in compound No. 6 results in a decrease in activity. The amide carbonyl group in this compound is less favourably disposed for interaction at a third site than the amide carbonyl group in the other compounds which have been considered.

In the series of *N*-(ω-dialkylaminoalkyl)lactams, the only compound which shows any muscarinic activity has a four-carbon chain (compound No. 10), in which the distance between the amide carbonyl group and the quaternary ammonium group in the extended form of the molecule (5 to 6 Å) compares well with the distance between receptor sites 1 and 3 according to Beckett *et al.* (1963). The activity of compound No. 10 is, however, very much less than that of the corresponding compounds containing an ether oxygen atom (compound No. 5) or an acetylenic linkage (compound No. 3). It can be concluded that, on the basis of the receptor model proposed by Beckett *et al.* (1963), interaction at site 2 is necessary for high activity.

The evidence obtained from the activities of these compounds suggests that three-point interaction at the cholinergic receptor gives maximal muscarinic activity. Barlow, Scott & Stephenson (1963) have suggested that the action of acetylcholine at the postganglionic parasympathetic receptors in the guinea-pig ileum depends on the presence of the carbonyl group (and presumably the onium group) for affinity and on the ether oxygen atom for efficacy. In the compounds examined here, the acetylenic derivatives have higher activities than the corresponding choline ethers and so, on the basis of the hypothesis of Barlow *et al.* (1963), it can be suggested that the efficacy associated with a triple bond is greater than that associated with an ether oxygen atom.

SUMMARY

1. The muscarinic activities of some compounds related to oxoTremorine and the choline ethers have been examined using as responses contraction of the guinea-pig isolated ileum and hypotension in anaesthetized cats.

2. It is suggested that the muscarinic activities of these compounds can be satisfactorily explained on the basis of a three-point interaction with the acetylcholine receptor.

3. Muscarinic activity is present in compounds having groups capable of interacting at two sites only, separated by approximately 3 Å, but an increase in activity always occurs when the molecule contains, in addition, an amide carbonyl group suitably placed to interact at a third site.

4. A comparison of the muscarinic activities of acetylenic compounds and choline ethers suggests that the efficacy associated with a triple bond is greater than that associated with an ether oxygen atom.

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