Brit. J. Pharmacol. (1955), **10**, 168.

A SERIES OF POLYMETHYLENE BIS-ACETOXYETHYL-DIMETHYLAMMONIUM SALTS

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(RECEIVED NOVEMBER 15, 1954)

Polymethylene bis-acetoxyethyldimethylammonium salts may be regarded as consisting of two molecules of acetylcholine (ACh) linked together by a polymethylene chain through a methyl group on each nitrogen atom—

$$CH_3COOCH_2CH_2\overset{+}{N}Me_2\text{-}(CH_2)_n\text{-}Me_2\overset{+}{N}CH_2CH_2OOCCH_3, 2X^-.$$

They were prepared because it was thought that a compound in which the polymethylene chain was of suitable length might be able to combine with two sets of receptors (on structures affected by ACh) at once. Replacement of one methyl group, attached to the quaternary nitrogen atom in ACh, by an ethyl group lowers activity to between one-half and one-fifth (Holton and Ing, 1949); it was felt, therefore, that replacement by a polymethylene chain should not completely destroy affinity for the receptors.

Such a compound, which might be called "pharmacologically bivalent," should be much more than twice as strongly adsorbed at the biological surface as ACh for two reasons (cf. Barlow and Ing, 1948). Firstly, when one end of a "pharmacologically bivalent" molecule dissociates, the drug is still held to the biological surface at the other end, and it is therefore very likely that the dissociated end will become re-adsorbed. Secondly, if the sets of receptors are some distance apart, there may be considerable Van der Waals attraction between the polymethylene chain and the biological surface, if these can fit closely together.

Ethylene bis-acetoxyethyldimethylammonium chloride was prepared and tested by Ing, Kordik, and Tudor-Williams (1952) and found to be inactive. This did not deter me from making other members of this series; I considered it unlikely that a compound with so short a chain could combine with two sets of receptors at once.

The compounds examined were tetra, penta, hexa, hepta, octa, nona, deca, undeca, and dodecamethylene bis-acetoxyethyldimethylammonium salts, and penta, octa, nona, deca, undeca, and dodecamethylene bis-hydroxyethyldimethylammonium salts. Members of the first series are called the *acetoxy* compounds, members of the second the *hydroxy* compounds.

METHODS

The compounds were tested by standard methods on the frog rectus, frog heart, spinal cat, and rat diaphragm preparations, and on the cholinesterases of dog caudate nucleus and horse serum. Solutions of the *acetoxy* compounds were freshly made up for each pharmacological experiment and made acid to *p*H 4. This eliminated complications arising from hydrolysis, which occurred if the solutions were stored for an hour or two.

In the cholinesterase investigations rates of hydrolysis were determined by measuring the volume of carbon dioxide liberated from the buffer solution (Krebs bicarbonate Ringer) during the first 6 min. Blank experiments were made to determine the extent of spontaneous hydrolysis, which was found to be small in the conditions employed (*p*H 7.2; 37° C.).

RESULTS

Experiments on Frog Rectus, Frog Heart, and Spinal Cat.—Only the nona, deca, undeca, and dodecamethylene members of the *acetoxy* series had any activity. The number of molecules of any of the other compounds required to produce an effect comparable with that of one molecule of ACh was greater than 5,000. The results are summarized in Table I. There is a sharp maximum in activity on all three preparations at the decamethylene compound.

Experiments on the Rat Diaphragm Preparation.—Members of the *acetoxy* series antagonized the actions of both decamethonium and (+)-tubocurarine chloride on this preparation. The com-

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TABLE I

ACTIVITY OF *ACETOXY* COMPOUNDS ON FROG RECTUS, FROG HEART, AND CAT BLOOD PRESSURE

$CH_3COOCH_2CH_2\overset{+}{N}Me_2\text{-}(CH_2)_n\text{-}Me_2\overset{+}{N}CH_2CH_2OOCCH_3$

n	Equipotent Molar Ratios		
	Frog Rectus	Frog Heart	Cat Blood Pressure
9	4,317±438	1,760	704
10	13·3±0·5	26·3±4·1	34·5
11	156±21	198±27	472
12	142±26	158±31	463

The table shows the number of molecules of the compound (±S.E.) required to produce effects comparable with those of one molecule of ACh. A high number indicates a low activity. Each value for the frog rectus and frog heart is the mean of 3 expts.; those for the cat blood pressure are the results of 1 expt. only.

pounds are, however, only "anticurare" agents; decamethonium has a paralysing action on the rat diaphragm preparation. When given in a dose of 0.5 mg., members of the *acetoxy* series produced between 12 and 59% reduction of the effects of 1 mg. decamethonium on the preparation. There did not appear to be any great variation in activity with the length of the polymethylene chain.

Members of the *hydroxy* series were more active. Approximately the same effect—between 14 and 50% reduction of the effects of 1 mg. decamethonium—was produced by doses as small as 10 μg. of the penta, nona, deca, undeca, and dodecamethylene compounds (those tested). Dr. E. J. Zaimis has kindly examined decamethylene bis-hydroxyethyldimethylammonium in some detail and finds that its "anticurare" properties are suggestive of moderate anticholinesterase activity (see below). The compound did not antagonize the effects of decamethonium on the cat tibialis preparation.

Experiments on Cholinesterases.—Members of the *acetoxy* series were tested as substrates of the cholinesterases of dog caudate nucleus and horse serum. The rates of hydrolysis of the compounds (10^{-3}M) were compared with that of ACh (10^{-3}M); it must be borne in mind that the compounds contain two ester links whereas ACh contains only one. Both the links were found to be hydrolysed by the enzyme preparations and the total amount of carbon dioxide was about twice that liberated when ACh was the substrate. Comparison of the rates of hydrolysis, however, is complicated by the fact that the hydrolysis of the *acetoxy* compounds must proceed via the monoester.

With the preparation from dog caudate nucleus, there was a sharp maximum in the rates of hydrolysis at the octamethylene compound (Table IIA); this was hydrolysed at about 60% of the rate of ACh.

There was no similar sharp maximum in the rate of hydrolysis by horse serum (Table IIB); there was a rise from the tetramethylene to the pentamethylene compound, but the rate of hydrolysis of the higher members was much the same as that of the pentamethylene compound.

Experiments were also performed in which an *acetoxy* compound (10^{-3}M) was incubated together with ACh (10^{-3}M). With the cholinesterase of dog caudate nucleus the rate of liberation of CO_2 from the buffer solution was less than that during an action on ACh alone. When horse serum was used, the rate of liberation of CO_2 by mixtures of ACh and the lower members (tetramethylene to octamethylene) was slightly greater than that during an action on ACh alone; from mixtures containing the higher members the rate was less. If the *acetoxy* compound did not affect the hydrolysis of ACh (or vice versa), the amount of CO_2 liberated during the first 6 min. should be the sum of that produced by each compound separately during that time. In Table II, column (ii), the volumes of CO_2 produced by hydrolysis of the mixtures during the first 6 min. are expressed as

TABLE II

HYDROLYSIS OF *ACETOXY* COMPOUNDS BY CHOLINESTERASES

$CH_3COOCH_2CH_2\overset{+}{N}Me_2\text{-}(CH_2)_n\text{-}Me_2\overset{+}{N}CH_2CH_2OOCCH_3$

n	Rate of Hydrolysis		
	Of Compound Alone (i)	Of Mixtures: As % of ACh Rate (ii)	Of Mixtures: As % of Sum of ACh Rate and that of Compound (iii)
A. By cholinesterase of dog caudate nucleus:			
4	0 (2)	92±4	92±4
5	4±2 (3)	92±3	91±1
6	8±2 (3)	80±4	72±4
7	30±4 (3)	70±3	51±4
8	61±2 (2)	58±14	29±16
9	36±2 (3)	39±8	28±5
10	29±5 (3)	31±1	23±2
11	12±5 (3)	19±5	18±6
12	9±1 (2)	12±4	9±3
B By cholinesterase of horse serum:			
4	25±5 (4)	106±2	90±6
5	72±2 (3)	127±10	75±5
6	67±5 (3)	100±0	61±1
7	88±2 (4)	106±2	57±1
8	77±1 (2)	102±0	57±1
9	84±9 (3)	91±14	50±4
10	71±7 (3)	82±13	49±4
11	76±5 (3)	81±7	47±2
12	72±9 (3)	87±11	52±3

In these experiments the rates of hydrolysis of ACh (10^{-3}M) and of *acetoxy* compound (10^{-3}M), alone and together, were measured. Column (i) shows the amount of carbon dioxide liberated during the first 6 min. expressed as a percentage of the amount liberated in the same conditions by ACh. Each value is the mean of a number of experiments (indicated by the figures in parentheses) ±S.E. Columns (ii) and (iii) show the amount of CO_2 liberated during the first 6 min. by action of the enzyme on a solution containing both the compound and ACh expressed as a percentage of: column (ii), the amount liberated during that time by ACh alone; column (iii), the sum of the amounts liberated during that time by ACh and the compound separately. Each result is the mean of two experiments ±S.E.

percentages of the volume produced by hydrolysis of ACh alone in the same time; in column (iii) they are expressed as percentages of the sums of the volumes produced by hydrolysis of ACh and *acetoxy* compounds separately. This last column can be taken as an approximate index of the affinity of the *acetoxy* compounds for the enzyme. A low percentage may indicate that the less rapidly hydrolysed *acetoxy* compound is strongly adsorbed by the enzyme.

Affinity for the enzyme of dog caudate nucleus appears to increase steadily up the series, though the change in increase appears to be greatest between the penta and hexa, hexa and hepta, and hepta and octamethylene compounds. Affinity for the enzyme of horse serum appears to increase only to the hexamethylene compound and to remain steady thereafter.

The *hydroxy* compounds, which are products of the hydrolysis of the *acetoxy* compounds, inhibited the hydrolysis of ACh by the two enzyme preparations (Table III). It appears that the affinity for both enzymes rises with increasing length of the

TABLE III

INHIBITION OF CHOLINESTERASES BY *HYDROXY* COMPOUNDS

$HOCH_2CH_2\overset{+}{N}Me_2\text{-}(CH_2)_n\text{-}Me_2\overset{+}{N}CH_2CH_2OH$

n	5	8	9	10	11	12
% inhibition:						
Of dog caudate nucleus esterase	0	78±5	80±5	89±5	100	100
Of horse serum esterase ..	33±1	62±2	82±6	93±1	94±2	98±2

The percentage inhibition is calculated from the amount of CO_2 liberated during the first 6 min.; both ACh and the *hydroxy* compounds were present in concentrations of 10^{-5}M. Each value is the mean of 2 expts. ±S.E.

polymethylene chain. For the cholinesterase of dog caudate nucleus the *p*I 50 of the decamethylene compound was 4.9; for the dodecamethylene compound it was 6.0.

DISCUSSION

One of the chief difficulties in the assessment of these results is to predict whether increased adsorbability at the biological surface should lead to increased activity. Clark and Raventos (1937) concluded, from experiments with ACh and tetramethylammonium on the rat intestine, frog auricle, and frog rectus preparations, that equiactive doses of the two drugs formed the same number of drug-receptor complexes—i.e., that tetramethylammonium has only one-thousandth of the activity of ACh because one thousand times as many molecules of tetramethylammonium are needed to form the same number of drug-receptor complexes. If the formation of a drug-receptor complex is simply, or strictly analogous to, a physicochemical adsorption, then increased adsorbability should lead to increased activity. The maximum in the pharmacological activity of the members of the *acetoxy* series at the decamethylene compound might, then, be taken to indicate a sharp rise in adsorbability at this chain length. Likewise, the maximum in the rate of hydrolysis by the cholinesterase of dog caudate nucleus at the octamethylene compound might be taken to imply increased adsorbability at the enzyme surface when this chain separates the two quaternary groups. The increased adsorbability might well be caused by the simultaneous attachment of both ends of these molecules.

There are two difficulties in the way of accepting this idea.

Firstly, adsorbability cannot be the only factor governing activity. Compounds closely related to ACh, but differing from it in some important respect, may antagonize it by competing with ACh for the receptors. The more strongly adsorbed the compound, the more powerful an antagonist it should be. It is difficult to know, therefore, whether high adsorbability should be indicated by high agonistic or high antagonistic activity.

Secondly, the ability of the *acetoxy* and *hydroxy* compounds to inhibit the hydrolysis of ACh by the cholinesterase of dog caudate nucleus appears to increase with chain length. However, in the *acetoxy* series there is a fairly sharp rise in this activity up the series to the octamethylene compound—Table II, column (iii)—and it is conceivable that there is some other factor, besides ability to combine with two receptors, which is responsible for further increases in the activity of higher members. The variation with chain length of the ability of polymethylene bis-trimethylammonium salts to inhibit the hydrolysis of acetyl-β-methylcholine by the " true " cholinesterase of laked rabbit red blood cells (Paton and Zaimis, 1949) is remarkably similar; the dodecamethylene compound appears to be the most active. In this series, however, there is no sharp rise to the octamethylene compound, and it is possible that this sharp rise may only occur in a series of compounds which, like the *acetoxy* compounds, contain an ester group and so may be attached to the extremely important " esteratic " site on the enzyme (Wilson and Bergmann, 1950) as well as at the " anionic " site.

These two objections, then, do not rule out the possibility that the receptors in the pharmacologi-

cal preparations studied are so placed that the decamethylene member of the *acetoxy* series can combine with two sets at once, and the receptors in the enzyme of dog caudate nucleus so placed that the octamethylene compound can combine with two sets at once.

If the idea is correct, it might be possible to obtain powerful antagonists of ACh or powerful anticholinesterases by replacing the acetyl groups by suitable " blocking " groups, such as benzilyl, dibutylcarbamyl, or carbamyl. Kimura and Unna (1950) have, in fact, studied such a compound—decamethylene bis-atropinium—but they report that it is only about twice as powerful an antagonist of the muscarine-like effects of ACh as atropine itself (or as methylatropinium, which is about as active as atropine). This might be accounted for by supposing that, perhaps because of the large size of the atropine molecule, the chain length in the pharmacologically bivalent compound must be rather more than ten methylene groups. It is also possible that the presence of the chain on the quaternary nitrogen atom destroys much of the affinity for the receptors—this is certainly true for the quaternary nitrogen atom in ACh—and that the two fragments should be linked at some less important point.

Three things therefore seem desirable. First, the nona, undeca, and dodecamethylene members of the bis-atropinium series should be examined in order to locate the peak in atropine-like activity. Second, a compound such as decamethylene bis-atropinium, which is pharmacologically bivalent, should be compared with the corresponding pharmacologically " univalent " compound, which, in this instance, would be *N*-*n*-amylatropinium and not *N*-methylatropinium. Third, linkage through the quaternary nitrogen atom should be avoided, not only because of the need to preserve the integrity of the quaternary group, but also because, if the sets of receptors are all orientated in the same way, substances such as the *acetoxy* compounds could only combine with two sets transversely and not with two sets in line.

Chemical Section

Analyses are by Mr. J. M. L. Cameron and Miss M. W. Christie; m.p.s are uncorrected.

The compounds were prepared by one of three methods:

1. Polymethylene dibromide was condensed with an excess of acetoxyethyldimethylamine dissolved in ethanol.

2. Polymethylene dibromide was condensed with an excess of methylaminoethanol dissolved in ethanol. The polymethylene bis-hydroxyethylmethylamine was liberated with alkali, extracted with ether, distilled, and treated with methyl iodide. The polymethylene bis-hydroxyethyldimethylammonium iodide was esterified with acetic anhydride and a few drops of hydriodic acid.

3. Polymethylene di-iodide was condensed with an excess of dimethylaminoethanol dissolved in ethanol. The polymethylene bis-hydroxyethyldimethylammonium iodide was esterified with acetic anhydride and a few drops of hydriodic acid.

The analyses and preparative details are shown in Table IV.

TABLE IV

CHEMICAL DATA

$$ROCH_2CH_2\overset{+}{N}Me_2\text{-}(CH_2)_n\text{-}Me_2\overset{+}{N}CH_2CH_2OR \quad 2X^-$$

X	R	*n*	Method (See Text)	m.p.	Crystallized From	Found			Calculated		
						C	H	N	C	H	N
Br	CH_3CO	4	(i)	204–5° (dec.)	EtOH	40·1	7·16	5·88	40·2	7·18	5·86
I	H	5	(iii)	Dec. above 258°	EtOH/Et_2O	31·3	6·21	5·68	31·1	6·44	5·58
I	CH_3CO	5	(iii)	136–7°	EtOH	35·0	6·14	4·93	34·8	6·21	4·78
I	H	6	(ii)*	162–3°	EtOH/MeOH	32·8	6·37	5·63	32·6	6·65	5·43
I	CH_3CO	6	(ii)	140°	EtOH/EtCOMe	36·1	6·25	4·85	36·0	6·40	4·67
I	H	7	(ii)*	115–6°	EtOH	34·3	6·62	5·38	34·0	6·79	5·28
I	CH_3CO	7	(ii)	120°	EtOH	37·1	6·37	4·87	37·1	6·57	4·56
I	H	8	(iii)	111°	EtOH	35·6	6·71	5·39	35·3	7·06	5·15
I	CH_3CO	8	(iii)	150°	EtOH	38·1	6·95	4·43	38·2	6·76	4·46
I	H	9	(iii)	Sinters 94°, m.p. 124°	EtOH	36·4	7·08	5·12	36·6	7·24	5·02
I	CH_3CO	9	(iii)	Sinters 91°, m.p. 105°	EtOH	39·1	6·79	4·16	39·3	6·92	4·36
I	H	10	(ii)*	104°	EtOH/Et_2O	37·6	7·67	5·01	37·8	7·42	4·90
I	CH_3CO	10	(ii)	Sinters 114°, m.p. 221°	EtOH/MeCOMe/Et_2O	40·4	7·20	4·38	40·2	7·08	4·27
I	H	11	(iii)	147–8°	EtOH/MeCOMe/Et_2O	38·8	7·47	4·99	38·9	7·59	4·78
I	CH_3CO	11	(iii)	114°	EtOH/Et_2O	41·3	7·22	4·30	41·2	7·24	4·18
I	H	12	(iii)	138–9°	EtOH/Et_2O	40·2	7·50	4·69	40·0	7·75	4·67
I	CH_3CO	12	(iii)	127–8°	EtOH/MeCOEt/Et_2O	41·9	7·24	3·93	42·1	7·39	4·10

* *Hexamethylene bis-hydroxyethylmethylamine*, b.p. 184–190°/2 mm. *Heptamethylene bis-hydroxyethylmethylamine*, b.p. 158–162°/0·6 mm. *Decamethylene bis-hydroxyethylmethylamine*, b.p. 183–7°/0·8 mm.

SUMMARY

1. A series of polymethylene bis-acetoxy and bis-hydroxy ethyldimethylammonium salts has been prepared and tested on the frog rectus, frog heart, spinal cat, and rat diaphragm preparations, and on the cholinesterases of dog caudate nucleus and horse serum.

2. There was a sharp maximum in activity on the frog rectus, frog heart, and spinal cat preparations at the decamethylene member of the acetoxy series of compounds. Members of the hydroxy series were inactive.

3. There was a maximum in the rate of hydrolysis of the acetoxy series of compounds by the cholinesterase of dog caudate nucleus at the octamethylene member.

4. Members of the hydroxy series slowed the hydrolysis of acetylcholine by the cholinesterase of dog's caudate nucleus ; members of the acetoxy series did so to a lesser extent.

5. These results are not inconsistent with the idea that decamethylene bis-acetoxyethyldimethylammonium can combine with two sets of receptors at once in the frog rectus, frog heart, and spinal cat preparations, and that the octamethylene compound can combine with two receptors at once on the enzyme. This, however, is not the only explanation, and there is evidence that it may be incorrect.

I wish to thank most sincerely Professor J. H. Burn, F.R.S., and Miss Roneen Hobbs, who performed all the pharmacological experiments, Dr. H. Blaschko and Miss Jean M. Himms, who performed all the enzyme experiments, and Mr. N. A. Dobson, who synthesized the octamethylene compounds. I am also grateful to Professor Burn and Dr. Blaschko for their valuable comments on the manuscript. The work was done during the tenure of an I.C.I. Fellowship. Some of the results were communicated to the British Pharmacological Society in July, 1954.

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