Br. J. Pharmac. (1970), **39**, 40–48.

Pharmacology of some acetylcholine homologues

B. C. BARRASS, R. W. BRIMBLECOMBE, P. RICH AND JOAN V. TAYLOR

Ministry of Defence, Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire

Summary

1. The acetates of several long chain (3 to 12 methylene groups) analogues of choline have been prepared and their pharmacological properties studied.

2. None of the compounds had a high level of activity at the post-ganglionic parasympathetic acetylcholine receptors. The lower members of the series showed weak agonist activity and the homologues with 8 to 10 methylene groups had very weak anticholinergic activity.

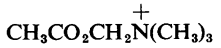
3. All the compounds had a depolarizing action at the acetylcholine receptors of the neuromuscular junction and of sympathetic ganglia. At the neuromuscular junction there were two peaks of stimulant activity, one with the hexamethylene and one with the dodecamethylene homologue, whereas at the ganglion there was only one peak, with the hexamethylene homologue.

4. The ganglion-stimulant activity of the higher members of the series was blocked by pretreatment with the anticholinesterase drug dyflos, whereas the activity of lower members was either unaffected by such treatment or slightly potentiated.

5. The results are discussed in terms of possible spatial arrangements of acetylcholine receptor units in the neuromuscular junction and the ganglion.

Introduction

Since the identification of acetylcholine as the natural cholinergic transmitter, a considerable amount of work has been done on the pharmacology, and enzymology, of this and related compounds, and also on other compounds having pharmacological actions similar to those of acetylcholine. These studies have been admirably summarized by Barlow (1964) and Triggle (1965). The major variations of the acetylcholine molecule which have been studied have involved modifications of the acyl and quaternary groups; few studies have been reported on the effects on pharmacological activity of altering the length of the choline chain. Hunt & Renshaw (1925) investigated the pharmacological activity of the acetoxymethyl trimethylammonium (I) and 3-acetoxypropyl trimethylammonium (II) ions, and showed that these compounds were less potent, as muscarinic agents, than acetylcholine.



I

II

In contrast to the paucity of information on acetylcholine homologues, it is well known that in a series of polymethylene bisodium salts (III) the pharmacological

activity is critically dependent, both qualitatively and quantitatively, on the number, *n*, of methylene groups.

$$(CH_3)_3\overset{+}{N}(CH_2)_n\overset{+}{N}(CH_3)_3 . 2X^-$$

III

Thus Paton & Zaimis (1949) showed that the compound III, $n=6$ (hexamethonium) was a potent antagonist of acetylcholine at autonomic ganglia whilst the compound III, $n=10$ (decamethonium) was a potent neuromuscular blocking agent. This series has since been extended (Barlow & Zoller, 1964) and neuromuscular blocking activity was found with some of the higher members of the series.

It is surprising that the effects of the same molecule, acetylcholine, should be blocked by different drugs at each of its major sites of action—namely, by atropine at the post-ganglionic parasympathetic (muscarinic) receptor, by hexamethonium at autonomic ganglia and by decamethonium and tubocurarine at the skeletal neuromuscular junction. It therefore seemed of interest to prepare some long chain analogues of acetylcholine and to study their interactions with muscarinic, ganglionic and neuromuscular junction receptors. This paper reports the preparation of these compounds (IV) and the results of the pharmacological tests which were carried out on them.

$$CH_3CO_2(CH_2)_n\overset{+}{N}(CH_3)_3 . X^-$$

IV, $n=3$–12

Methods

Chemical syntheses

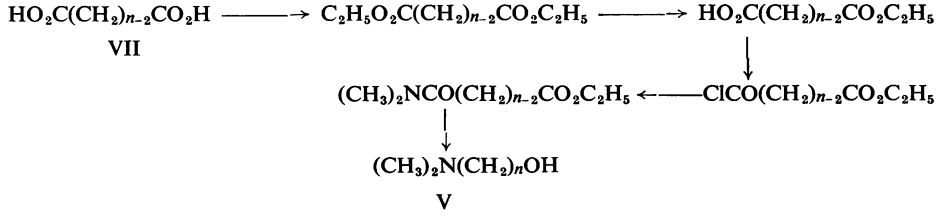
The dimethylaminoalkanols (V) were common intermediates in all of the syntheses of compounds of type IV. These syntheses therefore differed only in the way in which these intermediates were synthesized; these preparations are detailed below. 3-Dimethylaminopropanol was obtained from a commercial source.

The ω-dimethylaminoalkan-1-ols (V, $n=4$, 6 and 10) were prepared from the corresponding commercially available chloroalcohols (VI) by heating them in sealed tubes for 4 h at 100° C with anhydrous dimethylamine.

$$Cl(CH_2)_nOH + (CH_3)_2NH \longrightarrow (CH_3)_2N(CH_2)_nOH$$

VI V

The ω-dimethylaminoalkan-1-ols (V, $n=5$, 7, 8, 9 and 12) were prepared from the appropriate commercially available dibasic acids (VII) by the series of reactions outlined below.



The various reaction steps were accomplished by standard procedures; the final reduction of the amides to the dimethylaminoalkan-1-ols was carried out using lithium aluminium hydride in ether under reflux. 11-Dimethylaminoundecan-1-ol

(V, $n=11$) was prepared from the commercially available 11-aminoundecanoic acid as shown below.

$$H_2N(CH_2)_{10}CO_2H \longrightarrow \underset{\text{VIII}}{H_2N(CH_2)_{10}CO_2C_2H_5} \longrightarrow \underset{\text{IX}}{(CH_3)_2N(CH_2)_{10}CO_2C_2H_5}$$

$$\downarrow$$

$$(CH_3)_2N(CH_2)_{11}OH$$

The methylation of VIII to IX was carried out with formaldehyde/formic acid using the Eschweiler–Clarke procedure ; the crude product, probably a mixture of IX and the corresponding acid, was esterified by the Fischer–Speier technique without further purification to give IX in good overall yield. The final reduction was accomplished with lithium aluminium hydride.

The ω-dimethylaminoalkan-1-ols so prepared are listed in Table 1. Difficulty was experienced in obtaining satisfactory microanalyses on these compounds even though the infrared spectra were consistent with the expected structures. They were therefore characterized as their methiodides which are also listed in Table 1.

The above ω-dimethylaminoalkan-1-ols were converted into the corresponding acetates by reaction with acetylchloride in the usual manner. These acetates are listed in Table 2, together with their methiodides (IV) which form the subject of this paper.

Biological methods

Isolated tissues

All the compounds were tested for their effects on the isolated guinea-pig ileum and on isolated chick semi-spinalis muscle.

A 2 cm segment of ileum was suspended in a 5 ml organ bath containing Ringer-Tyrode solution at 37° C, through which a mixture of 95% oxygen and 5% carbon dioxide was bubbled. Contractions were recorded on a kymograph using an isotonic lever. The activities of agonist drugs were expressed as potencies relative to acetylcholine. 0·1 ml doses of acetylcholine solutions were added to the bath to give final standard concentrations of 10^{-7}M and 2×10^{-7}M acetylcholine ; the concentrations of compounds being assayed were adjusted to give contractions approximately equivalent to those produced by these concentrations of acetylcholine. Relative potencies were then calculated on the basis of four-point assays. Tests were always carried out to check that the contractions were blocked by atropine sulphate (4×10^{-8}M left in the bath for 1 min before adding the substance under test).

If preliminary tests revealed that a compound was an antagonist to the effects of acetylcholine, then a more precise estimate of its potency was obtained by determining a pA_2 value (Schild, 1957).

The semi-spinalis muscle was removed from 3–10-day-old chicks anaesthetized with ether. The preparation was used as described by Child & Zaimis (1960). Recordings of the contractions of the muscle were made in the manner described for the guinea-pig ileum. Nicotine (2 and 3×10^{-6}M) was used as the standard drug and again four-point assays were carried out. A check was always made that the contractions were blocked by hexamethonium (5×10^{-4}M left in the bath for 1 min before adding the substance under test).

TABLE 1. *Dimethylaminoalkanols* $(CH_3)_2N(CH_2)_nOH$ *and methiodides* $(CH_3)_3\overset{+}{N}(CH_2)_nOH\,.\,I^-$

Dimethylaminoalkanols			Methiodides								
			m.p.°	Recrystallization solvent	Molecular formula	Analysis					
						Calculated			Found		
n	b.p.°/mmHg	n_D^{25}				C	H	N	C	H	N
4	90/17	1·4390	127–128	Ethanol	$C_7H_{18}INO$	35·9	6·7	4·7	35·7	6·8	5·0
5	101/13	1·4432	126–128	Acetone/Ethanol	$C_8H_{20}INO$	35·2	7·4	5·1	35·3	7·4	5·3
6	115/14	1·4462	118–120	Acetone/Ethanol	$C_9H_{22}INO$	37·6	7·7	4·9	37·4	7·6	4·8
7	84/0·2	1·4496	140–141	Ethanol	$C_{10}H_{24}INO$	40·0	8·0	4·7	40·2	8·1	4·9
8	89/0·2	1·4505	142–143	Acetone/Ethanol	$C_{11}H_{26}INO$	41·9	8·3	4·4	41·9	8·2	4·3
9	97/0·15	1·4519	154–155	Acetone/Ethanol	$C_{12}H_{28}INO$	43·8	8·6	4·3	43·8	8·6	4·4
10	100/0·1	1·4542	158–160	Acetone/Ethanol	$C_{13}H_{30}INO$	45·5	8·8	4·1	45·1	8·7	4·4
11*	110/0·3	1·4543									
12†	130/0·2	Solid									

* Analysis. Found: C, 72·6; H, 13·8; N, 6·1%. $C_{13}H_{29}NO$ requires C, 72·5; H, 13·6; N, 6·5%.
† Analysis. Found: C, 73·4; H, 13·8; N, 6·2%. $C_{14}H_{31}NO$ requires C, 73·3; H, 13·6; N, 6·1%.

TABLE 2. *Dimethylaminoalkyl acetates* $(CH_3)_2N(CH_2)_nOCOCH_3$ *and methiodides* $(CH_3)_3\overset{+}{N}(CH_2)_nOCOCH_3\,.\,I^-$

Dimethylaminoalkyl acetates			Methiodides								
			m.p.°	Recrystallization solvent	Molecular formula	Analysis					
						Calculated			Found		
n	b.p.°/mmHg	n_D^{25}				C	H	N	C	H	N
3	78/14	1·4208	85–87	Acetone	$C_6H_{16}INO_2$	33·5	6·3	4·9	33·3	6·3	5·0
4	85/6	1·4223	138–139	Acetone	$C_9H_{20}INO_2$	35·9	6·7	4·6	35·7	6·8	5·0
5	94/11	1·4289	95–97	Acetone	$C_{10}H_{22}INO_2$	38·1	7·0	4·4	37·8	6·9	4·2
6	54/0·1	1·4352	53–56	Acetone/Benzene	$C_{11}H_{24}INO_2$	40·1	7·4	4·3	40·5	7·6	4·4
7	68/0·05	1·4339	71–72	Acetone/Ether	$C_{12}H_{26}INO_2$	42·0	7·6	4·1	42·2	7·6	4·2
8	80/0·1	1·4369	76–78	Acetone/Ether	$C_{13}H_{28}INO_2$	43·7	7·9	3·9	44·0	8·1	4·0
9	86/0·2	1·4375	83–85	Acetone/Ether	$C_{14}H_{30}INO_2$	45·3	8·1	3·8	45·4	8·1	3·9
10	100/0·1	1·4418	83–85	Acetone/Ether	$C_{15}H_{32}INO_2$	46·8	8·4	3·6	46·5	8·6	4·0
11	113/0·2	1·4420	102–104	Acetone	$C_{16}H_{34}INO_2$	48·1	8·6	3·5	48·3	8·7	3·7
12	113/0·2	1·4442	104–105	Acetone/Ether	$C_{17}H_{36}INO_2$	49·4	8·8	3·4	49·0	8·6	3·7

In a further series of experiments the activities of the drugs in the presence of the anticholinesterase drug edrophonium (4×10^{-6}M in the Ringer solution) were measured. It was not feasible to use nicotine or acetylcholine as a standard in these studies because the dose-response lines were not parallel; the potencies of the compounds were therefore expressed in terms of the activity of the C_6 homologue (IV, $n=6$).

Anaesthetized cat experiments

Cats were anaesthetized with chloralose-urethane (2·5 ml/kg intraperitoneally of a solution containing chloralose 25 mg/ml and urethane 250 mg/ml). Blood pressure was recorded from a femoral artery using a Statham Physiological Transducer Model P23AA. Changes in tension of the nictitating membrane were recorded using an E and M Myograph Type C (maximum sensitivity 5 g) with a resting tension on the membrane of 1 g. Signals were suitably amplified and recorded on a pen recorder (Physiograph 'Six', E and M Instrument Co. Inc.). The cat was also tracheotomized with a stainless steel tube and the larynx and oesophagus were reflected forwards to leave a cervical well which was filled with liquid paraffin. The cervical sympathetic trunk was dissected free of the vagus, cut at a point about 1·5 cm caudal to the superior cervical ganglion and placed on platinum stimulating electrodes. Supramaximal electrical stimulation was applied using an Attree stimulator delivering rectangular pulses of 4 ms duration at a frequency of 10 Hz.

Drugs were administered either intravenously via a cannula in the femoral vein or, where it was desired to study their effects specifically on the superior cervical ganglion, intra-arterially via a cannula in the external carotid artery. For the latter type of experiment the common carotid artery was dissected away from the vagus and some small branches tied off. The lingual artery was tied off and a polyethylene cannula inserted into the central end of the external carotid artery. When injections were made the common carotid artery was occluded for the period of the injection but opened immediately after. The injected material was then directed towards the superior cervical ganglion passing mainly along the internal carotid artery.

It was difficult to obtain precise values for the ganglion-stimulating activity of the compounds because with repeated dosing there was a gradual decrease in sensitivity. The procedure was to give two doses of acetylcholine (usually 100 and 25 nmol) which produced large, but submaximal, and small contractions of the nictitating membrane respectively, and then to find a single dose of the test compound which gave a contraction intermediate in magnitude. An approximate potency relative to acetylcholine could then be determined. The effects of pretreatment of the ganglion with either atropine (29 nmol intra-arterially) or hexamethonium (2·8 μmol intra-arterially) on the response to the stimulant drugs were also investigated. The blocking drugs were given 1 min before the stimulant drugs.

In some experiments a dose of 3 μmol of the anticholinesterase drug dyflos was injected intra-arterially into the ganglion 15 min before applying the test compound by the same route of administration.

Some of the compounds being investigated produced a fall in blood pressure, blocked by atropine sulphate (7·2 μmol/kg intravenously 10 min before). These were assayed for their activity in this respect using acetylcholine as a standard.

Effects on chickens

Chickens aged between 2 and 4 weeks were used to distinguish between non-depolarizing and depolarizing neuromuscular blocking agents. The method used was essentially that described by Buttle & Zaimis (1949) in which the test compound was injected into a wing vein. Non-depolarizing blocking drugs gave a flaccid paralysis with head-drop while depolarizing blocking drugs gave a spastic paralysis with a typical retraction of the head. An initial dose of 2 mg (about 10 μmol) was used and if this had no effect a further dose of 4 mg (about 20 μmol) was given 5 min later.

Results

The results of the isolated tissue studies are given in Table 3. On the isolated guinea-pig ileum the compounds with 3 to 7 methylene groups were weak agonists, their effects being blocked by atropine. The remaining compounds, octamethylene and upwards, were weak antagonists of acetylcholine.

On the isolated chick semi-spinalis muscle all the compounds were stimulants, but peaks of activity were observed with the hexamethylene and dodecamethylene analogues. There was little evidence for the compounds being substrates for cholinesterase because the same general pattern of relative activities was seen in the experiments where the anticholinesterase drug edrophonium was present in the Ringer's solution. *In vitro* studies confirmed that the compounds were not substrates for either acetylcholinesterase or cholinesterase.

The results from the anaesthetized cat experiments are summarized in Table 4. The effects on blood pressure are similar to those obtained in the isolated guinea-pig ileum experiments in that the homologues with short methylene chains displayed weak muscarinic activity. No effects were seen with the remaining compounds. The compounds with 3–10 methylene groups were all ganglion stimulants with a

TABLE 3. *Effects of acetylcholine homologues (general formula* $CH_3CO_2(CH_2)_nN^+(CH_3)_3$*) on isolated tissue preparations*

	Isolated guinea-pig ileum	Isolated chick semispinalis	
n	Potency relative to acetylcholine (ACh) with 95% limits or pA_2 value	Potency relative to nicotine with 95% limits	Potency relative to $(CH_2)_6$ compound in presence of edrophonium (4×10^{-6}M)
3	0·02 (0·01—0·03)	0·009 (0·008—0·01)	0·05 (0·04—0·06)
4	0·004 (0·003—0·006)	0·067 (0·062—0·072)	0·17 (0·13—1·4)
5	0·01 (0·008—0·013)	0·069 (0·057–0·077)	0·25 (0·20–0·30)
6	0·001 (0·008–0·11)	0·46 (0·43–0·49)	1
7	<0·001	0·23 (0·22–0·24)	0·33 (0·30–0·38)
8	pA_2 4·9	0·078 (0·066–0·087)	0·20 (0·19–0·21)
9	pA_2 5·0	0·12 (0·11–0·13)	0·34 (0·26–0·51)
10	pA_2 5·3	0·22 (0·20–0·25)	0·61 (0·55–0·66)
11	pA_2 <4·5	0·53 (0·48–0·57)	1·3 (1·2–1·4)
12	pA_2 <4·5	0·84 (0·74–0·95)	2·0 (1·95–2·04)

peak activity at the hexamethylene analogue. The compounds with 11 and 12 methylene groups produced ganglion blockade at doses very close to those which stimulated the ganglia. Ganglion blockade was also produced by the lower analogues but only at doses above those which produced stimulation. It appeared that the ratio of blocking dose to stimulant dose decreased with increasing chain length though it was difficult to quantitate this with any degree of precision.

The sensitivity of the dyflos-pretreated ganglion to acetylcholine was regularly increased about one-hundred-fold. No such potentiation was seen with any of the acetylcholine homologues. The responses to the compounds with 3–6 methylene groups were little affected by dyflos-pretreatment and the sensitivity to the hepta-methylene analogue was decreased by about 5 times. The effects of compounds with 8 to 10 methylene groups were virtually completely blocked by dyflos-pre-treatment. The undecamethylene and dodecamethylene analogues produced a ganglion blockade themselves ; this was unaffected by dyflos pretreatment.

In the experiments on chickens all the compounds showed activity typical of depolarizing neuromuscular blockers, producing a head-retraction. In all cases a total dose of 4 mg (about 20 μmol) was required to obtain a clear effect.

Discussion

None of these homologues of acetylcholine showed a high level of activity at the post-ganglionic parasympathetic acetylcholine receptors (muscarinic sites). Beckett, Harper & Clitherow (1963) and Bebbington & Brimblecombe (1965) proposed a model for the muscarinic receptor in which three sites, termed 1, 2 and 3, were considered to interact with the quaternary nitrogen atom, the ester oxygen atom and the carbonyl oxygen atom of acetylcholine respectively. Although in these homologues it is theoretically possible for the carbon chain to fold so that the nitrogen atom and the ester oxygen atom are separated by the appropriate distance, this would not represent the preferred conformation of these compounds.

TABLE 4. *Effects of acetylcholine homologues (general formula* $CH_3CO_2(CH_2)_nN^+(CH_3)_3$*) on blood pressure and nictitating membrane responses in anaesthetized cats*

n	Muscarinic activity. Potency relative to acetylcholine in producing falls in blood pressure	Ganglionic activity. Approximate potency relative to acetylcholine in producing nictitating membrane response (Drugs given i.a.)	Effects of pretreatment with dyflos (3 μmol i.a.) on ganglion stimulant activity
3	0·1	1	Slight potentiation $<2\times$
4	0·001	2	No effect
5	0·005	1	No effect
6	$<0{\cdot}001$	4	No effect
7	No effect	2·5	Decrease in sensitivity ($5\times$)
8	No effect	1	Blockade ($>100\times$ decrease in sensitivity)
9	No effect	0·75	Blockade ($>100\times$ decrease in sensitivity)
10	No effect	0·5	Blockade ($>100\times$ decrease in sensitivity
11	No effect	$<0{\cdot}01$ (produced ganglionic blockade)	—
12	No effect	$<0{\cdot}01$ (produced ganglionic blockade)	—

The main pharmacological action of all these compounds is to produce a depolarization at the neuromuscular junction and at the ganglionic synapses. There are, however, important differences between their effects at these two pharmacological sites where acetylcholine exerts its so-called nicotinic effects

At neither site was there any appreciable potentiation of effects by an anticholinesterase drug. This finding was not unexpected because *in vitro* studies had shown that the homologues were not substrates for cholinesterase. At the ganglion there was, however, an unexpected result, in that the stimulant effects of the compounds containing 8 to 10 methylene groups were blocked by pretreatment with the anticholinesterase drug dyflos. This phenomenon is not really understood although a possible explanation is that, in addition to the muscarinic and nicotinic types of receptors known to be present in sympathetic ganglia, there could be a third type of receptor capable of interaction with the long chain homologues. Dyflos may then be assumed to interact directly with this receptor to produce a blockade.

The other main differences between the effects of these homologues at the ganglion and at the neuromuscular junction is that at the former site a peak of stimulant activity was found with the hexamethylene homologue while at the neuromuscular junction two such peaks were observed with the hexamethylene and dodecamethylene homologues respectively.

If it is assumed that acetylcholine is the natural transmitter at both the neuromuscular junction and the autonomic ganglia, then a possible explanation for the maximal activity of 6-acetoxy-*n*-hexyltrimethylammonium at the ganglia and of this compound as well as 12-acetoxy-*n*-dodecyltrimethylammonium at the neuromuscular junction, may reside in the differing spatial arrangements of cholinergic receptors at these two sites. In support of this it has been recognized for some time (Paton & Zaimis, 1949) that in a series of polymethylene *bis*-trimethylammonium salts, ganglion blocking activity is maximal at hexamethonium, whereas neuromuscular blocking activity is maximal at decamethonium. The blocking activities of these compounds are greater than those of simple monoquaternary compounds. The inference to be drawn from these observations is that in both the ganglia and the neuromuscular junction there are pairs of anionic sites capable of interacting with quaternary ammonium groups. In the ganglia the distance between these pairs of anionic sites is 8·5 to 9·0 Å (the interquaternary distance of hexamethonium), whilst at the neuromuscular junction the corresponding distance is 14 Å (the interquaternary distance of decamethonium). It is highly probable that these anionic sites are those which interact with the quaternary ammonium group of the natural transmitter, acetylcholine, and are part of the acetylcholine receptors.

From the above inferences it is possible to account, in the present series of compounds, for the ganglion stimulant activity being maximal at 6-acetoxy-*n*-hexyltrimethylammonium and for neuromuscular depolarizing activity being maximal at 12-acetoxy-*n*-dodecyltrimethyl ammonium. It is suggested that 6-acetoxy-*n*-hexyltrimethylammonium interacts simultaneously through its quaternary group with the anionic site of one acetylcholine receptor and through its acetyl group with the appropriate group on an adjacent acetylcholine receptor. Similar interactions would account for the activity of 12-acetoxy-*n*-dodecyltrimethylammonium at the neuromuscular junction, the only difference being that anionic sites in the neuromuscular junction are separated by a greater distance than are those in the ganglia.

It is difficult, on this hypothesis, to account for the relatively high stimulant activity of 6-acetoxy-*n*-hexyltrimethylammonium at the neuromuscular junction and it seems necessary to postulate that, whereas in the ganglion the acetylcholine receptors are arranged in pairs as suggested above, in the neuromuscular junction they are arranged functionally in trios with two of the anionic sites separated by the interquaternary distance of hexamethonium and two sites separated by the interquaternary distance of decamethonium. This immediately raises the question of the lack of blocking activity of hexamethonium at the neuromuscular junction and at present no satisfactory explanation of this apparent anomaly can be presented.

Although the above hypothesis can be used to account for the findings reported here, other possible explanations cannot at present be excluded. One possibility is that at least some of the groups with which these drugs interact do not, in contrast to the above suggestions, form part of the acetylcholine receptor but are relatively non-specific " anchoring sites ". Alternatively it is tempting to speculate that the natural neurotransmitter at the two sites is not the same. Indeed, McLennan (1963) and Kewitz (1959) have both drawn attention to the possibility that compounds other than acetylcholine may be involved in chemical transmission at cholinergic synapses, but too much weight cannot be given to such suggestions until such compounds are isolated from animal tissues.

REFERENCES

BARLOW, R. B. (1964). In *Introduction to Chemical Pharmacology.* London: Methuen.

BARLOW, R. B. & ZOLLER, A. (1964). Some effects of long chain polymethylene bisonium salts on junctional transmission in the peripheral nervous system. *Br. J. Pharmac. Chemother.*, **23**, 131–150.

BEBBINGTON, A. & BRIMBLECOMBE, R. W. (1965). Muscarinic receptors in the peripheral and central nervous system. *Adv. drug Res.*, **2**, 143–172.

BECKETT, A. H., HARPER, N. J. & CLITHEROW, J. W. (1963). The importance of stereoisomerism in muscarinic activity. *J. Pharm. Pharmac.*, **15**, 362–371.

BUTTLE, G. A. H. & ZAIMIS, E. J. (1949). The action of decamethonium iodide in birds. *J. Pharm. Pharmac.*, **1**, 991–992.

CHILD, K. J. & ZAIMIS, E. J. (1960). A new biological method for the assay of depolarising substances using the isolated semispinalis muscle of the chick. *Br. J. Pharmac. Chemother.*, **15**, 412–416.

HUNT, R. & RENSHAW, R. R. (1925). On some effects of arsonium, stibonium, phosphonium and sulphonium compounds on the autonomic nervous system. *J. Pharmac. exp. Ther.*, **25**, 315–355.

KEWITZ, H. (1959). Nachweis von 4-Amino-*n*-butyrylcholin in Warmblütgehirn. *Arch. exp. Path. u. Pharmak.*, **237**, 308–318.

MCLENNAN, H. (1963). In *Synaptic Transmission.* Philadelphia: Saunders.

PATON, W. D. M. & ZAIMIS, E. J. (1949). The pharmacological actions of polymethylene bistrimethylammonium salts. *Br. J. Pharmac. Chemother.*, **4**, 381–400.

SCHILD, H. O. (1957). pA, a new scale for the measurement of drug antagonism. *Br. J. Pharmac. Chemother.*, **2**, 189–206.

TRIGGLE, D. J. (1965). In *Chemical Aspects of the Autonomic Nervous System.* London: Academic Press.

(*Received January* 8, 1970)