REVIEW ARTICLE

PARASYMPATHOMIMETICS AND ANTICHOLINESTERASES

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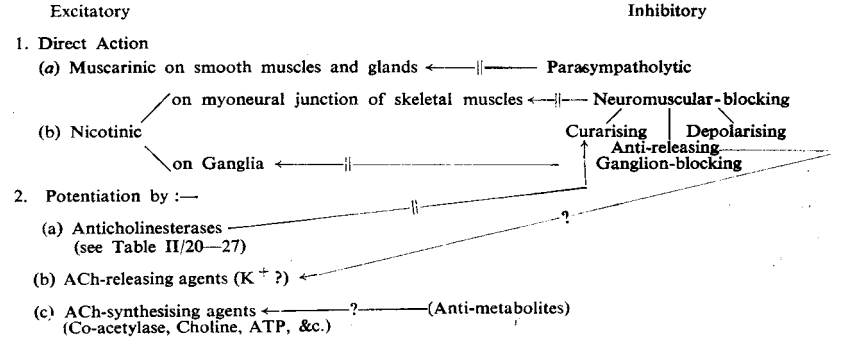
INTRODUCTION

Parasympathetic action can be produced either by stimulation of cholinergic nerve fibres, with a release of acetylcholine (ACh) as transmitter, or by the effect of certain drugs, called *parasympathomimetics*. These substances act peripherally on the same effector organs as the neurohormone itself. One distinguishes between their "muscarinic" action on smooth muscles and glands and their "nicotinic" action on skeletal muscles and autonomic ganglia. Each of these effects is known to be antagonised by groups of specific inhibitors, such as parasympatholytics and neuromuscular- and ganglion-blocking agents, respectively[1].

Parasympathetic excitation can, under certain conditions, be produced by potentiation of endogenous ACh and of some exogenous parasympathomimetics. The former can be achieved by the inhibitory action of the so-called *anticholinesterases*, blocking the hydrolysing enzymes, "true" and "pseudo" cholinesterases, which *in vivo* and *in vitro* speedily break down the neurohormone and related esters. Acetylcholine has been found to exist in the body as an inactive conjugate[2], like histamine, but while agents are known, such as di-amidines, which will release histamine, so far only potassium ions seem to assume this role in the case of acetycholine. The third possibility of potentiating parasympathetic action, namely by stimulation of acetycholine synthesis, which occurs in the living organism from choline in presence of co-acetylase[3] and is coupled with energy transfer from carbohydrate and phosphate meta-

TABLE I

ACTION ON PARASYMPATHETIC EFFECTOR CELLS



bolism, has up to the present only been demonstrated *in vitro* with homogenates or extracts of skeletal muscle or brain[4,5,6]. The relationship between direct and indirect excitatory and inhibitory effects in the field of parasympathomimetics can be gleaned from Table I.

Receptors and Esterases of Parasympathomimetics

The modern view on the mechanism of drug action is based on the assumption that certain parts of certain cells in the effector organs react most specifically with a given drug. These parts appear to be usually of a proteinous nature and have been called "active spots" or "receptors" (Pasteur, Cushny, Clark and Ing). If the drug reaches the receptors, it combines with them in a manner similar to that of a substrate-enzyme complex, and the strength of this combination depends largely on the physico-chemical character of the drug and receptor molecules. It may solely derive from the attraction of polar groups, a bonding of low energy, but, if the drug possesses ionisable groups, such as substituted

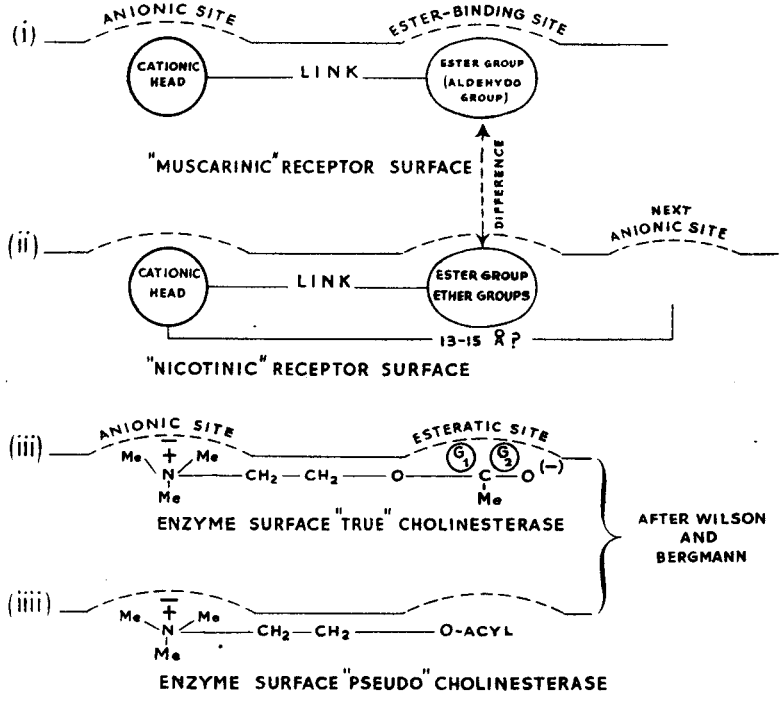


Fig. 1

amino-groups, capable of forming cations, one can assume that they will form an electrovalent bond with an anionic (COO^-, $R.S^-$, etc.) site in the receptor. Other parts of the drug molecule may be capable of hydrogen bonding ($R_1=O \leftarrow\!-\!-\!- HO.R_2$) and thus form another point of contact or attachment to the cell constituents. Again, some molecules,

particularly those with so-called " energy-rich bonds " or in presence of specific enzymes may undergo true chemical reaction with the formation of covalent (electron-sharing) bonds between the drug and the protein.

Summarising the results of work during recent years, to which, in Britain, Ing and his colleagues have made admirable contributions, the molecule of the protagonist of parasympathomimetic action, acetylcholine (Table II/1), consisting of a cationic head $Me_3\overset{+}{N}-$, a two-carbon link and an ester group, is capable of a two-point contact with four proteinous surfaces (see Fig. 1). Two of these belong to the receptors in the effector cells of " muscarinic " and " nicotinic " structures and two to the controlling enzymes, " true " and " pseudo " cholinesterases. It will be seen that similarities and differences exist between these four surfaces with regard to their specificity towards size, shape, bonding properties and, in the case of the cholinesterases, also towards stability, or drugs related to the neurohormone and producing parasympathomimetic excitatory effects or a block of such pharmacodynamic actions. In other words, there is evidence to show that the reacting part of " muscarinic " receptors in smooth muscles and glands and of " nicotinic " receptors in autonomic ganglia and at the myoneural junctions of skeletal muscles and of the hydrolysing enzymes will combine with acetylcholine, and its homologues and analogues, according to the physico-chemical properties of these drugs, and that such combinations, if formed, will promote a change of physiological function, the magnitude and direction of the change being dependent upon the structural characteristics of the substances employed and upon the proteinous surfaces involved. One has to keep an open mind to the possibility that some of the differences of action and of degree of potency may be due to differences in ease of access to the specific effector organs, and to the stability of the drugs in presence of cholinesterases. It will be best to consider the overall problem by first discussing the changes of the cationic head, then of the link, then of the ester group and finally to review compounds blocking the enzymes.

Cationic Head

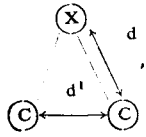
One of the points of contact of parasympathomimetic drugs is identical with the ammonium group, the " cationic head." This part of the molecule, in the case of acetylcholine $Me_3\overset{+}{N}-$, is supposed to react with an anionic site present in the receptors and the cholinesterase molecules, forming a salt by electrovalent bonding (see Fig. 1). It has been recognised that a quaternary ammonium group confers on the appropriate drug molecule not only the highest possible parasympathomimetic activity but also, dependent on the remainder of the molecule, maximum sensitivity in respect of the cholinesterases.

What happens if the size of this cationic head is altered by replacing the methyl groups with other substituents? Successive replacement by hydrogen[7,8] produces a steady fall in " muscarinic " activity (Table II/A). This may be due to two factors, decrease in size and thus bad fit, and diminution in stability of the ion which in its tertiary, secondary and

primary forms will consist, in an increasing way, of the corresponding base. Moreover, a change in one part of the molecule will reflect on the overall physico-chemical properties of the whole compound. If one increases the size of the cationic head by the introduction of ethyl groups, the series, according to Holton and Ing[8], shows revealing changes in both " muscarinic " and " nicotinic " activity. They are rapidly reduced. On the frog's heart the triethyl homologue produces an antiacetylcholine effect (see Table II/B). Similarly, the rise of blood pressure in the atropinised cat, a " nicotinic " effect, does not occur with the di- and tri-ethyl homologues. If the methyl group is replaced by acetoxyethyl groups, one can observe a similar trend[8]. Even in the series of phenyl ethers, to be mentioned later, Hey[9] has found that the triethyl homologue lacks activity. It appears that maximum effects are only achieved if at least two methyl groups are present in the cationic head, and that further alterations abolish excitatory effects.

TABLE II

$X.CH_2.CH_2.O.\overset{+}{\underset{\|}{C}}.Me$ (MOLAR RATIOS)

X	Fall of Cat's B.P. (M)	Contraction gut (M)	Slowing frog's heart (M)	Frog's rec. abd. (N)	Rates hydrol. horse serum	enzym. in μl. CO_2 caud. nucl.
A. After Stehle *et al.*						
Me_3N	1	1	1	1	61	49
Me_2HN...	50	>40	>50	—	—	—
MeH_2N	>500	>1,000	>500	—	—	—
H_3N	>2,000	20,000	40,000	—	—	—
B. After Holton and Ing						
Me_2EtN	3	2·5	2	3	44	53
$MeEt_2N$	400	700	1,500	400	30	47
Et_3N	>2,000	1,700	reversal	>2,000	16	46
$Me_2AcOCH_2CH_2N$	150	100	75	150	51*	52*
$(AcOCH_2CH_2)_2$ MeN ...	>20,000	>20,000	12,500	>20,000	5	11
C. After Welch and Roepke						
Me_3P	13	12	12	6	—	—
Me_3As	66	90	83	37	—	—
	X	d,Å	d',Å	—	—	—
	N	1·47	2·4	—	—	—
	P	1·87	3·05	—	—	—
	As	1·98	3·23	—	—	—

* Two ester groups.

It is not without interest to consider the impact of such changes on the velocity of hydrolysis by the two groups of cholinesterases : while the rate achieved by the " true " enzyme from the caudate nucleus of the dog remains roughly the same whether there are three methyl groups or an increasing number of ethyl radicals, the " pseudo " esterase from horse serum is more sensitive to such structural alterations. On the other hand, the presence of acetoxyethyl groups diminishes the efficiency of both enzyme systems; particularly is the hydrolysis of the triacetoxyethyl homologue extremely slow (see Table II/B).

A change in size of the cationic head can also be achieved by replace-

ment of the nitrogen atom by phosphorus or arsenic, both these atoms being larger. Such compounds show less " muscarinic " and " nicotinic " activity[10]. This is, according to Holton and Ing[8], due to an overall increase of the size of the cationic head in that the larger central atoms cause a greater spread of the methyl groups (see Table II/C).

A most interesting contribution to the problem of the influence of size and shape, even in esters lacking nitrogen and having in its place a carbon atom, has been made by Whittaker *et al.*[11] who showed that the rate of hydrolysis by " true " and " pseudo " cholinesterases was, apart from the character of the acyl group, dependent upon the kind of substituent of the terminal carbon atom of the alcohol moiety. Thus 3 : 3′-dimethylbutyl acetate, which is spatially reminiscent of the acetylcholine molecule, showed hydrolysis rates in presence of cholinesterases which were very near that of the neurohormone itself (Table III). Even in

TABLE III

AFTER WHITTAKER *et al.*

Aliphatic esters	Rate of hydrolysis as per cent. of acetylcholine	
	Pseudo	True
$Me_3.C.C.C.O.C.Me$ (O)	35	60
$Me_2.C.C.C.O.C.Me$ (O)	27	24
$Me_2(Et).C.C.C.O.C.Me$ (O)	23	—
$Me.C.C.C.O.C.Me$ (O)	11	16

the case of choline homologues, choline itself being very much less active than its acetate (1/200th to 1/100,000th of its activity), Dallemagne and Philippot[12] have reported quantitative and qualitative alterations of the

TABLE IV

AFTER DALLEMAGNE AND PHILIPPOT. $R.Me_2.\overset{+}{N}.CH_2.CH_2.OH$

R	Muscarinic	Nicotinic	Ganglion block			Neuromusc. block	
				mg/kg.	per cent.	mg/kg	
Me ...	+ +	+ +	0	—	—	—	0
Et ...	+	+ +	0	6	0	3	0
C_4H_9 ...	+ +	+	+	3	72	3	+
C_6H_{13} ...	+	+ + +	+ +	3	80	9	0
C_8H_{17} ...	0	0	+ + +	3	100	6	+
$C_{10}H_{21}$ ...	0	0	+ + +	3	100	3	+ +
$C_{12}H_{25}$ ...	0	0	+ +	3	96	3	+ + +
$C_{14}H_{29}$ ...	0	0	+	10	60	9	0
$C_{16}H_{33}$ ...	0	0	+	10	50	12	0
$C_{18}H_{37}$ ...	0	0	+	10	50	3	0

pharmacological effects if one of the three methyl groups was replaced by a homologous series of alkyl radicals (see Table IV). If that radical went beyond C_6, " muscarinic " and " nicotinic " activity were lost while, with further lengthening, ganglion- and neuromuscular-blocking effects

were obtained. In the case of quaternary ammonium salts, changes in the size of the -onium group are followed by alterations in pharmacological activity as older work by Clark, Raventos and Ing has demonstrated, but it is impossible to do full justice in the space of this article to these earlier contributions.

Looking again at Figure 1 which, as mentioned before, represents schematically the surfaces of the receptors and enzymes (drawn after Wilson and Bergmann[13]), one can see, perhaps in an over-simplified manner, that the cationic head has to fit into an anionic site. From the evidence above one can conclude that there exists an optimal size for this head and, with it, an optimum of coulomb forces to bind the two structures sufficiently together at this point.

The Link Between Cationic Head and Ester Group or Equivalent

In the course of work and speculations in this field, two rules have been established by Pfeiffer[14] and Ing[15] respectively. While the second point of contact of the drugs with the proteinous surfaces is the ester group or its equivalents, to be discussed later, Pfeiffer has claimed that for maximal activity an optimal distance exists between the cationic head and the esterified oxygen, amounting to about 5 Å. On the other hand, and its implications will become clearer further on, Ing has proposed that the number of atoms between the cationic head and the end of the drug molecule should, again for maximal effect, reach the number 5.

Considering more particularly the link between the cationic head and the ester group, it has in fact been found that the two-carbon chain of acetylcholine represents the optimal length. This is, to a certain extent, demonstrated by the fact that the lower and higher homologues of acetylcholine, namely formocholine acetate and homocholine acetate (Table V/4a and b), particularly the former, are very much weaker than acetylcholine itself. Unfortunately, comparative data for derivatives of formocholine and homocholine, complying with Ing's five-atom rule for total length, are not available; thus, it would be interesting to see whether formocholine propionate and homocholine formate would still show a lack of activity as compared with the neuro-hormone. On the other hand, the inactivity of a phenyl ether derivative of homocholine, as confirmed by Hey[9] (Table VI, B_1,n=3), supports the thesis of an optimal distance between the trimethylammonium group and the alcoholic oxygen.

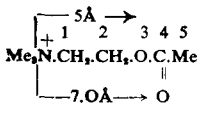
Remarkable alterations in activity are noted if the linking chain carries a branched methyl group; while such a group next to the alcoholic oxygen, as in mecholyl (Table V/5), seems to disturb the bonding with the " nicotinic " site, leaving that with the " muscarinic " site unimpaired, α-methylcholine ester (Table V/6) is weak as a " muscarinic " agent but fairly strong in its " nicotinic " blood pressure effects in the atropinised cat[16].

Attention is again drawn to the fact that some of these esters show different sensitivity to the cholinesterases and it is only possible for the worker in this field, or the reviewer, to compare the results if experiments

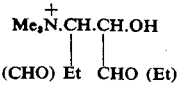
TABLE V

PARASYMPATHOMIMETICS AND ANTICHOLINESTERASES

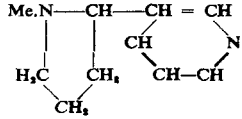
(M = muscarinic, N = nicotinic, CES = cholinesterase sensitive, CEI = cholinesterase inhibiting)



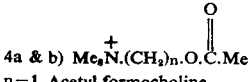
1) Acetylcholine
(M+N, CES+++)



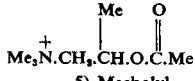
2) Muscarine (M)



3) Nicotine (N)

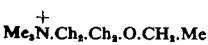


4a & b) $Me_3\overset{+}{N}.(CH_2)_n.O.C.Me$
n=1 Acetyl formocholine
n=3 Acetyl homocholine



5) Mecholyl
(M+n; CES+)

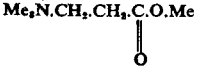
6) α-Methyl ACh
(m+N)



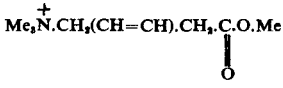
7) Choline ether
(m+N; CES −)

8) R = phenyl, substituted phenyl (N)

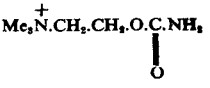
9) Keto derivatives (N)



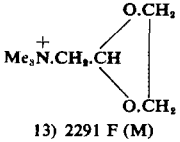
10) β-Homobetaine ester
(M+N; CES −)



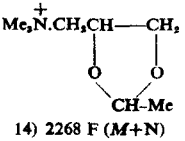
11) Crotonbetaine ester
(M+N; CES −)



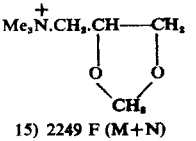
12) Carbachol
(m+N, CES −)



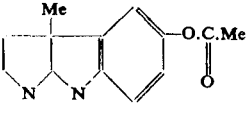
13) 2291 F (M)



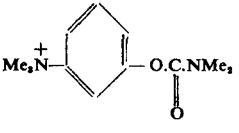
14) 2268 F (M+N)



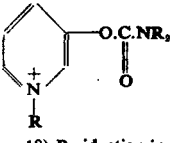
15) 2249 F (M+N)



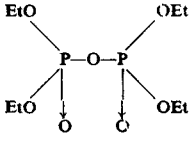
Me Me
16) Eserine, Physostigmine
(CEI rev.)



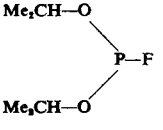
17) Prostigmin, neostigmine
(CEI rev.)



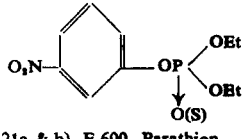
18) Pyridostigmine
e.g. Ro 1-5130
(CEI rev.)



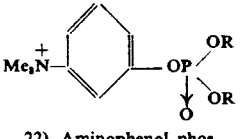
19) TEPP (CEI irrev.)



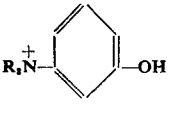
20) DFP (CEI irrev.)



21a & b) E 600, Parathion
(CEI irrev.)



22) Aminophenol phosphates e.g. Ro 3-0412 (CEI)



23) e.g. Ro 2-3198
2-2561

have been carried out on the same organ or in the same animal species—and also before and after treatment with substances such as eserine which hold the hydrolysing enzymes in check.

The Ester Group or Substitutes

The difference between " muscarinic " and " nicotinic " sites becomes more pronounced if one looks at the results obtained with products in which certain alterations of the $-OCOMe$ group have been effected. If the alkyl group Y (see Table VI/A) is elongated[17,18], the pure " muscarinic " action falls rapidly while the effects on leech muscle and frog's rectus abdominis, both " nicotinic," show different maxima. A similar observation was made by Hey[9] (see Table VI/B_2) who demonstrated that the " nicotinic " blood pressure effect was rather independent of the character of the acyl group; propionyl-, *iso*butyryl-, trimethylacetyl-, benzoyl- and phenylacetyl- esters of choline all showed pronounced " nicotinic " activity as measured by the rise of blood pressure in the atropinised cat. Likewise, the behaviour of the two cholinesterases is different in their specific activity on various esters of choline; while the " true " cholinesterase acts most efficiently on the acetate, the butyrate is hydrolysed at the rate of 1/66th of that of the former. On the other hand, this butyrate is twice as rapidly hydrolysed in the presence of " pseudo " esterase. It is therefore not impossible that the ester-binding or esteratic site (see Fig. 1) in the " muscarinic " receptors and in the " true " cholinesterase requires a more strict compliance with the 5-atom rule of Ing than does the corresponding site in the " nicotinic " receptors and in the " pseudo " esterase. However, in addition to this effect of size, the influence of the radical Y on the bonding properties of the $-O-COY$ grouping, and on the overall physical properties of the total molecule, may play a not inconsiderable part in causing these differences.

Although not possessing an ester group and thus no second point of contact, trimethylalkylammonium salts, as observed by Welsh and Taub[19], whose further work will be mentioned below, and who continued on the lines of Raventos[20] and Alles and Knoefel[21], show a definite maximum of " nicotinic " effect. In this homologous series where the alkyl group, so to speak, replaces the acetoxyethyl group of acetylcholine, the greatest activity was found with *N*-amyltrimethylammonium salts, the amyl radical corresponding in number of atoms to $-CH_2.CH_2.O.CO.Me$ and thus obeying Ing's rule.

Pfeiffer[14] stipulated on theoretical grounds that for optimal parasympathomimetic action two oxygen atoms have to be present at a maximal distance of 5 and 7 Å respectively from the ionic group. Ing[15] answered this statement by pointing out that the fit of the drug and its molecular groups is at least as important and that there are drugs in existence which do not possess two oxygen atoms, although they still exert noticeable activity. The issue is somewhat complicated by the fact that complete data, particularly on " nicotinic " actions, are not available and that investigations by various authors have been carried out on different organs and under different conditions. It is correct that

the aliphatic choline ethers (see Table V/7), with regard to their "muscarinic" action are weaker than acetylcholine, yet the ethyl homologue is not only the most potent in this respect, thus complying with the 5-atom rule, but is a relatively powerful "nicotinic" drug[22]. This

TABLE VI

A. After Chang and Gaddum and after Ing. $Me_3.\overset{+}{N}.CH_2.CH_2.O.CO.Y$

Y	Rabbit gut M	Rabbit's blood pressure M	Leech M + N	Frog's Rectus Abdominis N
Me	100	100	100	100
Et	3	4	45	550
Prop.	0·24	0	90	90
But....	0·2	0	0·9	25
ø	0	0	—	+
øCH₂ – ...	0	0	—	+
ø ø>C— OH	100	100	—	—

B₁. After Hey (i) $R_3\overset{+}{N}.(CH_2)_n.O.R_1$ and (ii) $Me_3\overset{+}{N}.CH_2.CH_2.\overset{+}{O}=\overset{-}{R_2}$

R	(i) R_1	n	(ii) R_2	Atropinised Cat's B.P. rise Rel. activity	60 mm. rise moles
Et	phenyl	2	—	0	—
Me	phenyl	3	—	0	—
Me	phenyl	(.CH₃CHMe)	—	0	—
B_2.	Rel. Act.	Moles	phenyl	1	$1·44x10^{-7}$
ACh	0·013	$1·1x10^{-5}$	*m*-cresyl	0·13	$1·1\ x10^{-6}$
Prop. Ch	0·021	$6·7x10^{-6}$	3 : 5-xylenyl-	0·052	$2·3\ x10^{-6}$
*iso*But. Ch	0·039	$3·7x10^{-6}$	*p*-cresyl-	0·004	$3·4\ x10^{-6}$
TriMe-ACh	0·210	$6·7x10^{-7}$	*m*-chloro-phenyl	2·1	$7·0\ x10^{-9}$
Benz. Ch	0·024	$5·9x10^{-6}$	*m*-bromo-phenyl	3·2	$4·7\ x10^{-6}$
Phenyl-ACh	0·072	$2·0x10^{-6}$	3 : 5-dibromo-phenyl	3·0	$4·7\ x10^{-9}$
Carbachol	—	$2·0x10^{-6}$	*p*-chloro-phenyl	0·1	$1·4\ x10^{-6}$

C. After Welsh and Taub. $Me_3.\overset{+}{N}.(Z).Me$

Z	Equiact. mol. ratios on *Venus mercenaria* heart	Z	Equiact. mol. ratios
$CH_3.CH_2O.\overset{O}{\overset{\|}{C}}$-(ACh)	1	$CH_3.CH_2.\overset{O}{\overset{\|}{C}}.CH_2$—	160
$CH_3.CH_2.CH_2.\overset{O}{\overset{\|}{C}}$-	12	$CH_3.\overset{O}{\overset{\|}{C}}.CH_2.CH_2$–	580

D. After Welsh and Taub

Preparation	After Eserine
Frog rectus abdominis (N)	ACh> 4-ketoamyl > carbachol
Leech Muscle (M + N)	ACh> carbachol > 4-ketoamyl
Frog heart (M)	ACh> carbachol > mecholyl> 4-ketoamyl
Venus heart (Ng)	ACh> 4-ketoamyl > carbachol> mecholyl

" nicotinic " effect appears to go considerably beyond that of acetylcholine in a series of aromatic ethers (Table V/8) which, when carrying a strong electronegative group, namely phenyl with electronegative substituents, have been found to be 8 to 250 times more potent than acetylcholine on the blood pressure of the atropinised cat. While the first observation on the phenyl ether of choline came from Hunt and Renshaw[23], who have made many contributions in the field of parasympathomimetics, it was Hey[9] who continued systematic studies in this field. From Table VI/B_1 it may be seen how the " nicotinic " activity is increased if halogen is introduced into the benzene ring, an exception being the *p*-chloro compound, and diminished with the introduction of methyl groups. It seems to be not unlikely that the polarity set up between ring and ether oxygen largely contributes to the fixation of this group on the ester binding-site preferentially in the " nicotinic " receptors.

Attention has been drawn before to the fact that even in this series the optimal rules, referring to the cationic head and the two-carbon link, hold good. The absence of " nicotinic" action in a compound of this kind, with a β-methyl branch, agrees with the weak " nicotinic " action of its ester analogue mecholyl (Table V/5).

While the exchange of the carbonyl, C=O for an alkyl or aryl-group produced, as was just pointed out, an extraordinarily specific nicotinic action, a similar phenomenon is observed if the alcoholic oxygen is replaced by a methylene group (Table V/9). Welsh and Taub[24] have investigated this problem with the help of the isolated heart of a mollusc, *Venus mercenaria*, which has nervous structures comparable with those of autonomic ganglia; acetylcholine depresses the beat but this action can be antagonised only by ganglion-blocking agents and not by curare. The American authors found (Table VI/C) that while the 4-ketoamyl derivative (the $-CO-$ in the same place as in acetylcholine) was the most potent, its activity was still below that of acetylcholine itself. However, a considerable fall of activity was noted when testing compounds which had a carbonyl group closer to the cationic head. The relative activity, in comparison with other drugs on various organs after eserine, can be gleaned from Table VI/D. It should be stated that the same workers investigated the alcohols corresponding to the ketones and found them 1/1500th as active as acetylcholine.

Summarily, one can say, and this is borne out by further observations, that a change in the ester group of acetylcholine reflects more on the combination and subsequent events as far as the " muscarinic " receptors are concerned than on the corresponding " nicotinic " receptors. It appears that fit, size, shape and bonding properties of the cation, and the distance from it to the second group, together with the overall length and overall physico-chemical properties, play an essential part in most of these pharmacodynamic happenings.

Miscellaneous Compounds

The study of compounds in which the cationic head is situated in the acid moiety should contribute considerably to the subject of structure-

activity-relationships in the field under discussion. In a recent paper, Bass *et al.*[25] reported on the pharmacological properties of the reversed carboxyl analogue of acetylcholine, a β-propionic acid derivative (Table V/10). While they found great similarities between the two, particularly in respect of the " muscarinic " action on the gut, they noted various differences. The maximal and minimal distances between the cationic head and the alcoholic or carbonyl oxygen are of the same but a reversed order.

Welsh and Taub[24] on their *Venus* heart preparation found its " nicotinic " action similar to that of the 4-ketoamyl derivative which gives the impression that, as far as the " nicotinic " receptors are concerned, the compound has only one oxygen. Its behaviour towards cholinesterase is interesting because, although stable to its hydrolytic effect, it inhibits the enzyme relatively slightly (50 per cent. inhibition, $1{\cdot}6 \times 10^{-2}$)[26].

All these findings are not dissimilar to those with a compound having a longer link between the $Me_3\overset{+}{N}-$ and reversed ester group, namely croton betaine ester (Table V/11), which was studied by Burgen and Hobbiger[27] after being synthesised by the Roche Research Department[28]. In this case, the " muscarinic " effects were roughly 1/10th to 1/2 of those of acetylcholine but some of the " nicotinic " effects were stronger. The overall length rule, although not obeyed by the nitrogen-carbonyl distance, is somewhat maintained in that the methyl ester is the most potent of a homologous series, as found by German workers[29].

There is a group of parasympathomimetic substances which, like the alkaloid muscarine (Table V/2), the protagonist of " muscarinic " action, are either derived from aldehydes or from reversed aldehyde derivatives. The structural formula of muscarine as proposed by Koegl in 1931[30], leaves open the question of whether it is an α-aldehydo derivative of a β-ethylcholine or a β-aldehydo derivative of an α-ethylcholine. Betaine aldehyde, for a long time identified with muscarine, has feeble actions[16] but a cyclic acetal with glycol, 2291 F (Table V/13)[16] shows, like the natural alkaloid, no " nicotinic " action but has a " muscarinic " effect, especially on the heart, of the order of 1/10th to 1/100th of acetylcholine.

Fourneau and his collaborators[31] have also produced reversed aldehydo-derivatives, 2268 F (Table V/14) from acetaldehyde and 2249 F (Table V/15) from formaldehyde and a hydroxyhomocholine. While the first substance was considered to possess intense " muscarinic " action, 10 to 100 times more powerful than acetycholine in this respect, the second compound was found to exert " muscarinic " and " nicotinic " actions which were weaker than those of ACh. It was Ambache[32] who drew attention to the fact that 2268 F also shows a number of " nicotinic " actions on the superior cervical ganglion and on muscles of frog and leech, the latter antagonised by *d*-tubocurarine. It is interesting to note that the higher homologues of 2249 F and 2268 F show, in contrast to the higher acyl derivatives of choline, no " nicotinic " action while, at the same time, their " muscarinic " effects fall gradually[31]. Thus aldehydo-derivatives, in the widest sense, appear to have a greater affinity with " muscarinic " receptors.

To sum up: one can say that, as far as direct parasympathomimetic activity is concerned, apart from certain atomic distances and the constitution of the cationic head, the fixation, if any, on the second point of contact depends on a hydrogen-bonding unit such as $-O-CO-$ or any physico-chemical equivalent. It is at this point that "muscarinic" and "nicotinic" affinities seem to diverge most distinctly. Whether acetylcholine and related compounds can be considered as a coenzyme of an enzyme regulating membrane polarity and permeability is, as Welsh and Taub[24] suggested tentatively, a matter for further studies.

Anticholinesterases

A number of parasympathomimetically active substances are resistant to the effects of cholinesterases. This is in many cases due to the absence of an appropriate ester group in the drug molecule but it has been shown that even in the case of esters there exists a specificity of the enzyme surfaces for the structure of the cationic head, the carbon link and the ester and acyl groups. According to Nachmansohn, Wilson and Bergmann[13,33], the ester group of acetylcholine, apart from the fixation of the cationic head on the anionic site, shows a two-fold bonding at what they call the "esteratic site" and which was mentioned above under the name "ester-bonding site" (see Fig. 1/iii). They have found that not only does one of the two oxygens undergo a hydrogen bonding but that the carbon of the carbonyl group possesses an electrophilic character and is fixed to a corresponding sub-contact (G_1) point of the overall "esteratic" site. It has already been mentioned that while the reversed carboxyl analogue of acetylcholine, croton betaine ester, carbachol, and, according to Bergmann *et al.*[34] amino acid esters, show in addition to their resistance a relatively weak blocking action, there is known a group of substances which produces, by competition with the parasympathomimetic agent for the enzyme, such a degree of blocking that they allow the accumulation of acetylcholine at the effector cells. Thus, apart from their own pharmacological action, these anticholinesterases exert their parasympathomimetic effects mainly through their protection of acetylcholine against breakdown in the presence of the enzymes.

The first group of such drugs consists of carbamic acid esters of phenols which are somewhat related to carbachol and possess certain features which make them akin to acetylcholine itself. Although the tertiary nitrogen in eserine or physostigmine (Table V/16) and the quaternary in prostigmin or neostigmine (Table V/17) are separated from the O-carbamate group by four and three carbon atoms respectively, the resonance of the benzene ring, of which they form a part, shortens the distance sufficiently to make them fit on to the enzyme surface. As far as the *in vitro* activity is concerned, see Table VII[26,27,35,36].

If the nitrogen forms part of the ring, as in Ro 1 – 5130[37] (Table V/18), the inhibitory effect, as measured by Blaschko *et al.*[36] is somewhat diminished. In all cases, the reaction with the enzyme is reversible and competitive with the substrates. Investigation of the kinetics[39] show it to be a monomolecular reaction, and there is no doubt, particularly

after the careful physico-chemical study of Wilson and Bergmann[39], that the inhibitors react with the same spots on the enzyme as does acetylcholine.

TABLE VII

	50 per cent. inhibition of Cholinesterases			
	Red blood cell	Human serum	Caudate nucleus	Horse serum
	Mol. conc.	Mol. conc.	pI	50
Croton betaine ester	2×10^{-3} (23 per cent.)	2×10^{-3} (18 per cent.)	—	—
γ-Carbmethoxy-ethyl trimethyl ammonium salt	$1{\cdot}6 \times 10^{-2}$	—	—	—
Eserine	*ca.* $1{\cdot}18 \times 10^{-7}$	more active	7·1	7·7
Prostigmin	$1{\cdot}14 \times 10^{-7}$	$6{\cdot}9 \times 10^{-7}$	7·4	7·2
Ro 1-5130	—	—	6·4	5·8
TEPP	$1{\cdot}2 \times 10^{-8}$	8×10^{-10}	—	—
DFP	$1{\cdot}6 \times 10^{-7}$	—	—	—
Ro 3-0412	$7{\cdot}5 \times 10^{-6}$	$6{\cdot}3 \times 10^{-9}$	—	—

A different mechanism of action has to be assumed for the second group of anticholinesterases which were originally developed as war gases and insecticides[39,40,41]. They are, chemically, organic phosphoric acid derivatives lacking completely the cationic head. The best known are TEPP (Table V/19), tetraethylpyrophosphate, DFP (Table V/20), diisopropylfluorophosphonate, and *p*-nitrophenol derivatives, E 600 and Parathion (Table V/21 a and b). They are all lipophilic, as far as their solubilities are concerned, and produce (DFP particularly) a completely irreversible reaction with the enzyme which, however, is still competitive with acetylcholine and other substrates including the reversible inhibitors of the "stigmine" type. The latter are, of course, not hydrolysed.

Kinetically, their reaction with the cholinesterases[31] is bimolecular and the difference between these phosphates and the "stigmines" must be assumed to be due to the following: The enzyme combines with the anticholinesterase but, while this combination is reversible in the case of the "stigmines," having a two-point contact, the phosphates, after having formed a one-point contact at the ester-bonding site, react more profoundly with this part of the enzyme molecule; the inhibitor splits into two moieties, one remains attached to the enzyme and the other is lost.

$$E + I \rightleftharpoons (EI) \rightarrow (EI/_{x_1}) + I/_{x_2}$$

rev. irrev.

In the case of TEPP, the stability of the phosphorylated enzyme may not be very great so that, after a certain time has elapsed, TEPP interactions seems to be of a reversible nature. It appears that the alkyl groups of the phosphate anticholinesterases have a profound effect on

the process of phosphorylation. In this connection the observation of Saunders and Stacey[42] is of importance, namely that dimethyl- and diethyl-fluorophosphonates are less active on the eye than the di*iso*propyl homologue and that the duration of action is shorter and more comparable with that of TEPP.

So far, the only direct proof that these compounds are capable of phosphorylating proteinous enzymes has been produced by Jansen *et al.*[43] who, using radioactive phosphorus in diisopropylfluorophosphonate, have shown that this prosphorus can be spotted in α-chymotrypsin together with the *iso*propyl groups, but no fluorine is found.

In connection with the influence of the alkyl group, it is worth while mentioning another series of anticholinesterases which have recently been investigated biochemically and pharmacologically by Burgen, Hobbiger and Keele[44], and which were synthesised in the Roche Research Department [45]. They represent a hybrid between the " stigmines " and the phosphates described above (see Table V/22), being formed from a quaternary aminophenol and dialkyl phosphoric acid. While the dimethyl homologue shows some reversibility of action, the diethyl derivative behaves more like TEPP and the diisopropyl compound like DFP. However, they do not penetrate as the other anticholinesterases do into the central nervous system and their penetration into the eye is slower. On the other hand, they are, relatively speaking, less toxic. It should be mentioned that the anticholinesterases, apart from their indirect and direct parasympathomimetic effects, are useful antagonists to the actions of curare and those neuro-muscular-blocking agents, which produce their effects in a curariform manner. In this respect, it is interesting to note that the quaternary aminophenols, such as the compound shown on Table V/23, possess this antagonist activity to a desirable degree[46,47].

Conclusion

The study of structure-activity relationships in the group of parasympathomimetic amines and anticholinesterases appears to be very useful but, whatever conclusions one may draw from it, such deductions can only have academic value if they are not confirmed by results *in vivo*, particularly in man. Although the main motive of the scientist is to search continually for the truth, and truth alone, from a more utilitarian point of view, the value of his endeavours would be diminished if one could not, or could only to a certain extent, relate his scientific findings to the effectiveness of the drugs in human therapy. But this is where the applied pharmacologist and clinician come in and where the pharmacological chemist, the biochemist and the pharmacologist have to leave the stage.

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