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SOME SELECTIVE INHIBITORS OF TRUE CHOLINESTERASE*

BY

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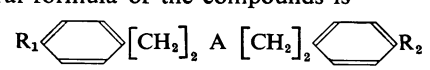
While studying certain diphenyl pentanones as possible neuromuscular blocking agents, it was found that 1:5-bis(4-trimethyl-ammoniumphenyl)-*n*-pentan-3-one diiodide (62C47, Wellcome) had a marked selective inhibitory action on "true cholinesterase." The distribution of this type of activity was therefore investigated in further members of this and analogous series. The general formula of the compounds is



wherein R_1 and R_2 are usually substituted quaternary nitrogens of varying degrees of complexity, and A is usually either CO or CHOH (Table I). Copp (1953) has described their chemical properties. The present paper deals with some of their pharmacological actions.

METHODS

Cholinesterase Inhibition.—Anticholinesterase activity was determined manometrically at 37° C. in the Warburg apparatus (Ammon, 1933) with 0.025 M-$NaHCO_3$ as medium equilibrated with 5% CO_2 and 95% N_2. Rat brain, homogenized in 15 parts by weight of 0.025 M-$NaHCO_3$, was the source of the true enzyme and horse serum that of pseudo-cholinesterase. The enzyme preparation—1 ml. rat brain homogenate or 0.3 ml. horse serum—was introduced into the main compartment of the bottle together with a fresh solution of the inhibitor dissolved in 0.025 M-$NaHCO_3$. The substrate, also dissolved in 0.025 M-$NaHCO_3$, was placed in the side arm. The total fluid volume, adjusted by the addition of 0.025 M-$NaHCO_3$, was always 3.0 ml. The final substrate concentrations were 0.003 M or 0.012 M-acetylcholine (bromide) when testing true cholinesterase activity and 0.008 M-benzoylcholine (iodide) when testing pseudo-cholinesterase inhibition. Enzyme and inhibitor were in contact for 25 min. while gaseous and thermal equilibria were being established, before the substrate was added. All the results were corrected for non-enzymic hydrolysis of the substrate, and the inhibitor values were calculated from the results obtained after equilibrium had been established between enzyme, substrate and inhibitor.

Action on Nerve-Muscle Preparations.—The rat diaphragm–phrenic nerve preparation (Bülbring, 1946) was used to investigate (*a*) the ability to increase the response to indirect stimulation by square pulses of 0.34 msec. duration, (*b*) antagonism to the paralysing action of (+)-tubocurarine chloride by the method of Mogey and Young (1949), and (*c*) paralysing activity. This last was compared directly against, and expressed as a percentage of, the activity of (+)-tubocurarine in the way described by Mogey, Trevan, and Young (1949) except that, instead of allowing drugs to act for a standard period, time was given for equilibrium to be reached. The compounds were added at intervals until activity was shown or a concentration of $10^{-3.5}$ had been obtained. The stimuli used were square wave impulses of maximal intensity, and of 0.34 msec. duration, applied once every 12 seconds.

In vivo paralysing activity was determined for some of the compounds in the cat anaesthetized with pentobarbitone sodium. The response of the gastrocnemius to sciatic nerve stimulation was recorded by a spring lever and the drugs were injected into the femoral vein of the other leg.

Toxicity.—The drugs were administered intravenously in saline to mice, each mouse receiving 0.5 ml. solution per 20 g. body weight.

Other methods, not requiring detailed description, are referred to in the most appropriate places in the text.

RESULTS

Cholinesterase Inhibition.—Table I gives estimates of the activity against the cholinesterase of rat brain (true) and that of horse serum (pseudo), expressed as *p*I 50 values (*p*I 50 = the negative logarithm of the molar concentration causing 50% inhibition). Whereas all the results reported for the activity against true cholinesterase were from experiments where 0.012 M-acetylcholine was used, some experiments were done with 0.003 M-acetylcholine. The degree of inhibition was usually greater with the lower substrate concentration. This indicates that at the higher concentrations the acetylcholine did not itself cause inhibition and

* The gist of this paper formed a communication (by Gertrude E. Glock and G. A. M.) to the British Pharmacological Society in Edinburgh, July, 1948: a few members of the series were included in a demonstration (by J. W. Trevan and G. A. M.) to the Society at Beckenham, January, 1948.

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so increase the apparent effectiveness of the drugs: it also suggests that the inhibition is of a competitive nature. Many workers condemn the use of substrate concentrations greater than the optimal when dealing with true cholinesterase. At low substrate concentrations, however, the results are less reliable than at the high, because the total CO_2 evolved is much less and it is difficult to maintain CO_2 production for a time sufficient to demonstrate the establishment of equilibrium before the substrate is completely hydrolysed.

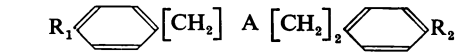
In addition, to exclude the possible effects of variation of the enzyme source, compounds 62C47, 25C48, eserine, and neostigmine were tested by using a single rat brain as the source of the true cholinesterase. The same results were obtained as when different rat brains were used. As with other cholinesterase inhibitors, atropine sulphate did not inhibit the activity of 25C48 *in vitro*: this was the only compound tested, but it was one of the most potent anticholinesterases.

Since these compounds are cholinesterase inhibitors, it was expected that their injection into mice would increase the toxicity of acetylcholine. The degree of increase would depend on the dose of the inhibitor given, and the time it was in the body before the acetylcholine was injected. Only the most potent inhibitors were examined, and Table II shows some of the results. Detailed analysis of the experiments, including observations at earlier times than those given in the table, showed that most of the compounds came into action about the same time as eserine and more rapidly than neostigmine; that 25C48, 297C50, and 298C50 were about equipotent at the time of maximal effect, and that the duration of the effect of equipotent doses was always nearly the same.

The activity of some of these compounds in increasing the response of the rat diaphragm to indirect stimulation, by square pulses, serves as further evidence of their resemblance to eserine and neostigmine. This increased response, probably due to the setting up of a repetitive discharge at the motor end plate, was abolished by concentrations of (+)-tubocurarine too low to affect the natural twitch response to short stimuli. Atropine antagonized this effect when used in doses large enough to prevent the naturally occurring repetitive responses to long stimuli. Compounds 62C47,

TABLE I

THE COMPOUNDS AND THEIR *p*I 50 VALUES ON TRUE AND PSEUDO-CHOLINESTERASE



Wellcome Code No.	R_1	R_2	A	*p*I 50	
				Rat Brain: Acetylcholine 0·012M	Horse Serum: Benzoylcholine 0·008M
71C48	$^{+}NMe_3I$	$^{+}NMe_3I$	CH_2	6·1	3·6
62C47	$^{+}NMe_3I$	$^{+}NMe_3I$	CO	6·7	3·2
25C48	$^{+}NMe_2EtI$	$^{+}NMe_2EtI$	CO	7·7	<3·0
297C50	$^{+}NMe_2AllI$	$^{+}NMe_2AllI$	CO	7·8	<3·0
298C50	$^{+}NMe_2nPrI$	$^{+}NMe_2nPrI$	CO	7·8	<3·0
312C50	$^{+}NMe_2nBuBr$	$^{+}NMe_2nBuBr$	CO	5·9	3·5
19C51	$^{+}NMe_2CH_2PhCl$	$^{+}NMe_2CH_2PhCl$	CO	5·3	4·8
455C50	$^{+}NMeEt_2I$	$^{+}NMeEt_2I$	CO	7·5	<3·0
153C47	$^{+}NMe_3I$	H	CO	4·0	3·0
26C48	$^{+}NMe_3I$	OMe	CO	4·6	3·2
143C48	$^{+}NMe_3I$	$^{+}NMe_3I$	CHOH	5·7	<3·0
101C48	$^{+}NMe_3I$	OMe	CHOH	3·9	<3·0
316C50	$^{+}NMe_2EtI$	$^{+}NMe_2EtI$	CHOH	5·9	<3·0
495C50	$^{+}NMeEt_2I$	$^{+}NMeEt_2I$	CHOH	6·3	<3·0
95C48	$^{+}NMe_3I$	$^{+}NMe_3I$	C_2H_5COH	5·0	<3·0
72C48	$^{+}NMe_3I$	$^{+}NMe_3I$	Ph.COH	4·5	3·3
142C48	$^{+}NMe_3I$	$^{+}NMe_3I$	HCOCOPh	5·6	5·0
Eserine				6·5	8·0
Neostigmine				6·6	7·6

Me=methyl, Et=ethyl, All=allyl, *n*Pr=*n*-propyl, *n*Bu=*n*-butyl, Ph=phenyl. The dibromide corresponding to 297C50 is 284C51.

TABLE II

TOXICITY OF 10 MG./KG. ACETYLCHOLINE BROMIDE INTRAVENOUSLY IN MICE AFTER INTRAPERITONEAL INJECTION OF CHOLINESTERASE INHIBITORS

(This dose of acetylcholine given alone kills 2% of mice)

Inhibitor	Dose (mg./kg.)	Mortality at		
		30 min.	60 min.	120 min.
62C47 ..	1·3	9/10	1/10	0/10
25C48 ..	0·3	8/10	8/10	0/10
	0·15	8/10	3/10	0/10
	0·075	14/20	0/10	
	0·038	9/20		
	0·019	0/10		
297C50 ..	0·075	5/10	0/10	
	0·038	3/10	0/10	2/10
298C50 ..	0·075	5/10	0/10	
	0·038	5/10	1/10	0/10
Neostigmine ..	0·075	19/20	0/10	
	0·038	3/10	0/10	

25C48, 297C50, and 298C50 were almost as active as neostigmine in this respect, whereas 153C47, 26C48, and 95C48 were about one-tenth as active. Compound 72C48 was inactive as far as we could determine. Some of the others showed doubtful activity.

Reversal of Paralysis.—On the rat diaphragm, those compounds most effective in increasing the response to a short stimulus were also very active in opposing the effect of (+)-tubocurarine. The four most potent compounds gave the following figures for the AD 66/33—that is, the concentration in μg./ml. of antagonist which reduces by half the action of a dose of (+)-tubocurarine which by itself would have caused a 66% paralysis (Mogey and Young, 1949) : 62C47, 4.0; 25C48, 1.5; 297C50 and 298C50, 0.1. The less active compounds gave figures of the following order : 153C47, α; 26C48, 105; and 95C48, 78 : compound 72C48, which was relatively inactive in this respect, gave an AD 66/33 of 70. With the lower degrees of activity the AD 66/33 does not appear to give any sensitive differentiation between the members of the various series. As with neostigmine, the antagonism between these compounds and (+)-tubocurarine was not apparent when an excessive dose of the latter was used. The paralysis following (+)-tubocurarine was not reversed by any of the series in the presence of high concentrations of atropine. Because of their structural similarity to decamethonium, 71C48 and 62C47 were examined on a rat diaphragm paralysed by decamethonium. Small quantities of either drug showed no effect, whereas large amounts increased the paralysis.

Paralysing Activity.—The effect of these various compounds on the response of the rat diaphragm-phrenic nerve preparation was studied in considerable detail. As the concentration of the drug was increased, the stimulating effect, already described, gave way to a decrease in the size of the response to a short square impulse (0.34 msec.). We found no evidence of a direct action on the muscle fibre. Phrenic nerve conduction was unaffected by concentrations of 62C47 as high as 10^{-3}. Neostigmine had no effect except in high concentrations, such as $10^{-5.6}$ to $10^{-5.3}$, when it increased the paralysis. Recovery from paralysis was complete after washing and contracture was never induced. 142C48 showed approximately 9% of the paralysing activity of (+)-tubocurarine. The other members of the series showed 6% or less of the activity of (+)-tubocurarine, and most of them 2 or 1%. The degree of specificity of their action on true cholinesterase did not necessarily appear to be a factor of importance in this respect.

With compounds 62C47 and 25C48, the paralysing action was increased by small amounts of (+)-tubocurarine when either long (5.7 msec.) or short (0.34 msec.) stimuli were used. The paralysing effect of 25C48 on the responses to short stimuli was weakly antagonized by catechol ($10^{-5.3}$). Neither decamethonium nor tetramethylammonium iodide antagonized the paralysis after 62C47 or 25C48. Atropine did not reduce the paralysis following any of them. Indeed, the paralysing effect of atropine was easily elicited in the presence of these compounds. When the stimuli to the phrenic nerve were long enough to cause a repetitive discharge, i.e., over 1.25 msec. (Mogey and Trevan, 1948), the paralysing action of atropine appeared at about $10^{-4.3}$. With short stimuli, capable of producing only twitch responses, four or five times as much atropine was required, but, in the presence of paralysing concentrations of 62C47 or 25C48, much smaller concentrations of atropine produced an increase in paralysis.

Increasing the rate of stimulation intensified the block. Tetanizing stimuli (150/sec.) produced either a poorly sustained yet obvious tetanus or a twitch, depending upon the initial degree of paralysis. The twitch was followed by Wedensky inhibition for the duration of the tetanizing stimulus, and when normal rates of stimulation (5/min.) were resumed, there was a temporary reversal of the paralysis. Soon, however, the paralysis was greater than before the tetanus.

The action of adrenaline on the paralysant effect of 25C48 was examined. Small quantities ($10^{-7.5}$ to $10^{-6.5}$) increased the paralysis from 25C48, with either long or short stimuli. After washing 25C48 from the diaphragm, adrenaline produced a marked increase in the size of the response to long or short stimuli.

Some of the compounds were tested on the isolated rectus abdominis of the frog at the temperature of melting ice. 62C47 showed a nicotine stimulating type of action at concentrations from 10^{-6} to $10^{-3.5}$. The other substances showed no such action (26, 71, 101, 142, and 143C48 were not tested). The action of acetylcholine was potentiated with low concentrations of some of the drugs, but was blocked with high concentrations ($10^{-3.5}$) of all of them. Most of the compounds were readily washed from the preparation.

In the cat, compounds 62C47, 153C47, 71C48, 101C48, 297C50, 455C50, 495C50, and 19C51 all caused some paralysis of the gastrocnemius at 0.5 mg./kg. except 71C48, which required 2 mg./

kg., and 153C47, which was still ineffective at 2 mg./kg. Fine muscular fasciculation preceded the paralysis. 0.1 to 0.15 mg./kg. (+)-tubocurarine chloride under these conditions would have caused about 50% paralysis.

Ocular Actions.—With the exception of 101C48, all the drugs were examined on the rabbit eye by direct instillation, and on the mouse eye after intraperitoneal injection of, usually, one-fifth of the intravenous LD50. They all showed varying degrees of miotic effect on parenteral injection, but were inactive on direct application. Compounds 62C47, 25C48, and 71C48 were powerful miotics. Atropine antagonized the miotic effect of 25C48, the only member of the series examined for this action. The effect on intraocular tension was not studied. Compound 312C50, even when instilled in a concentration of 1%, did not produce miosis in the human subject. None of the concentrations up to 1% (in saline) caused any irritation.

Effect on Blood Pressure.—The effect on the blood pressure of the rabbit under pentobarbitone sodium varied from compound to compound. Within the intravenous dose-range 0.17 to 3.2 mg./kg., the commonest effect was a small rise followed by a fall. Similar results were found for most of the series in the anaesthetized cat (pentobarbitone sodium or chloralose). In the atropinized cat, however, 298C50 or 312C50 up to 5 mg./kg. produced no fall.

Toxicity.—The mouse toxicity figures are given in Table III. All the compounds caused symptoms of marked parasympathetic activity—salivation, urination, and defaecation being prominent. Muscular twitchings occurred and sometimes developed into generalized convulsions; after this a flaccid paralysis occasionally appeared. Death seemed to be from respiratory failure: the heart usually continued to beat for a short time after the cessation of respiration. The exhibition of atropine sulphate—20 to 80 mg./kg. intraperitoneally half to two hours in advance, or 2.0 mg./kg. intravenously two minutes before—did not have a significant effect on the toxicity of 25C48, one of the very specific inhibitors. The combined intravenous injection of 0.17 mg./kg. (+)-tubocurarine chloride (approximately the LD50) and 1.3 mg./kg. 25C48 (LD50) was more toxic than either alone: 0.075 mg./kg. (+)-tubocurarine (a non-lethal dose), given simultaneously, had no protective effect against, nor did it increase the toxicity of, 25C48. Indeed, the toxicity was not affected by the previous administration of dibenamine, pentamethonium, nicotine tartrate or a combination of nicotine and atropine.

TABLE III

INTRAVENOUS TOXICITY OF ANTICHOLINESTERASES IN ALBINO MICE

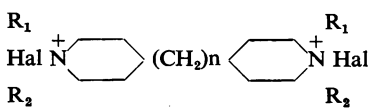
Code No.	LD50 (mg./kg.)	Limits (P=0·95)
71C48	4·2	4·0 –4·4
62C47	2·6	2·5 –2·7
25C48	1·35	1·28–1·41
297C50	2·1	1·96–2·28
298C50	2·0	1·83–2·12
312C50	1·3	1·18–1·41
19C51	0·48	0·42–0·54
455C50	1·0	0·88–1·13
153C47	11·5	10·3 –13·0
26C48	5·8	5·25–6·47
143C48	4·2	3·8 –4·8
101C48	8·25	7·94–8·55
316C50	2·6	2·38–2·86
495C50	1·25	1·07–1·46
95C48	4·4	4·13–4·75
72C48	3·4	3·04–3·75
142C48	1·4	1·3 –1·49
Eserine SO_4 ..	0·39	0·35–0·43
Neostigmine $MeSO_4$	0·27	0·25–0·30

DISCUSSION

This series of compounds presents certain analogies and physical similarities to the polymethylene bistrimethyl ammonium compounds of the general formula

$$\bar{I}Me_3\overset{+}{N}(CH_2)n\overset{+}{N}Me_3\bar{I}$$

examined by Willstätter and Heubner (1907), Barlow and Ing (1948), and Paton and Zaimis (1949). These last authors showed that activity as paralysing agents was greatest when n=9 to 12, and that all compounds where n>7 showed some activity as anticholinesterases, the dodecyl member being the most potent against the true enzyme. In our group of specific cholinesterase inhibitors the distance between the quaternary nitrogens is about equivalent to that in the 1:12 dodecyl bistrimethylammonium ion. Despite the chemical analogies between the two series, the anticholinesterase activity of our compounds is much more prominent than the paralysing effect. Another series, of the general form



and thus intermediate to decamethonium and those compounds described here, was tested by Randall (1952). Paralysing activity was again uppermost. However, the members of another closely related series, examined by Funke and Depierre (1950), and mentioned later, were predominately anticurare agents.

Neuromuscular Block.—The neuromuscular blocking action of anticholinesterases has been described frequently (Briscoe, 1936; Rosenblueth, Lindsley, and Morison, 1936; Lehmann, 1946; Krop, 1947) and has usually been ascribed to an accumulation of undestroyed acetylcholine. At first this was thought to be a likely mode of action of our series, but cholinesterase inhibition and paralysing activities are not simply related. It seems that the double quaternary nature of the molecule is not essential, for compounds with only one nitrogen (e.g., 153C47, 26C48, 101C48) are as potent paralysing agents as those with two, but are weaker inhibitors of " true " cholinesterase. The paralysis was due to an action at the myoneural junction, since the muscle response to direct stimulation was unaffected, and conduction along the phrenic nerve was not blocked by concentrations of 62C47 which reduced the diaphragmatic twitch.

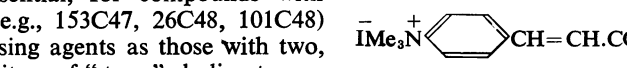
The block from our compounds resembles that of (+)-tubocurarine in some respects, as, for example, in failure of the diaphragm to maintain a tetanus and in the action being reversed after tetanic stimulation; but it is unlike (+)-tubocurarine in not being antagonized by neostigmine. However, these paralysing compounds are themselves anticholinesterases.

Barnes and Duff (1953) have recently shown that inhibition of cholinesterase *per se* does not block neuromuscular transmission. Our evidence, too, suggests, that the paralysing action of these cholinesterase inhibitors is independent of the inhibition of the enzyme—whereas, in a series of compounds with a polymethylene chain uniting two *iso*quinoline groups, curariform activity paralleled inhibition of the true enzyme (Smith, Pelikan, Maramba, and Unna, 1953).

Curare Antagonism.—The anticurare action resembles that of neostigmine more than the direct stimulating effect of 3-hydroxyphenyltrimethylammonium iodide, and of its mono-ethyl derivative (Randall, 1950), or of catechol (Mogey and Young, 1949).

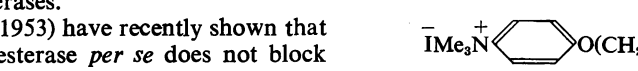
Most of our compounds with only one quaternary nitrogen were very weak antagonists. It may be that two such nitrogens are required for maximal anticurare activity.

This curare antagonism may not be simple. It may be due (*a*) to anticholinesterase effects, or (*b*) to a direct stimulant action at the myoneural junction, or (*c*) to a combination of these, or (*d*) to some other effect. (*a*) Significant correlation between anticurare and anticholinesterase actions was found by Blaschko, Bülbring, and Chou (1949) in some neostigmine analogues, and also by Hobbiger (1950) for tetraethylpyrophosphate, physostigmine, neostigmine, and two homologues. On the other hand, Aeschlimann and Stempel (1946) and Randall and Lehmann (1950) found no such correlation in closely related series. Correlation between anticurare and anticholinesterase actions, although positive, was not statistically significant in our series. (*b*) Compound 62C47 and an unsaturated derivative not so far mentioned (729C47,



had a nicotine stimulating type of action on the frog rectus, whereas 25C48, 297C50, and 298C50 had not. These last three, although devoid of stimulating action on frog rectus, were among the most potent as anticurare agents and cholinesterase inhibitors. Randall and Lehmann (1950) found that the direct stimulating action on denervated muscle of phenyl-trimethylammonium bromide and analogues did not correlate with anticurare activity, but that these two actions were closely related in muscle stimulated through its nerve.

A series of compounds examined by Funke and Depierre (1950), very similar in structure to our series, had the general formula



These were found to be anticurare agents, and when n=3, anticholinesterase activity and curare antagonism were very marked. They did not say whether their compounds showed any selectivity for true cholinesterase, although they did report that they inhibited the red cell esterase which, in most species, is predominantly true cholinesterase. As with our compounds, but unlike " Tensilon " (3-hydroxyl-phenyl dimethyl-ethyl ammonium iodide), none of their series antagonized a massive dose of (+)-tubocurarine.

The finding that atropine did not reduce the inhibition of cholinesterase *in vitro* by these compounds is in agreement with Frey's results (1948) for physostigmine, although high concentrations of atropine prevented the anticurare action, as with neostigmine. This is of interest in view of the synergism reported by McDowall (1949) between atropine and (+)-tubocurarine. Atropine blocked some of the actions of these anticholinesterases (e.g., salivation and miosis in the mouse and rabbit), although it did not reduce the toxicity. Kimura and Unna (1950) showed that 2.0 mg./kg. atropine sulphate intravenously more than doubled the LD50 of physostigmine.

Cholinesterase Inhibition.—The outstanding characteristic of the series is cholinesterase inhibition, several members having the same order of activity as eserine and neostigmine on true cholinesterase when acetylcholine is used as substrate. Some—e.g., 25C48, 297C50, and 298C50—show little or no inhibition of pseudo-cholinesterase, although they are potent inhibitors of true cholinesterase. In fact, the characteristic most common to this group of anticholinesterases is their low activity against pseudo-cholinesterase. The activity against true cholinesterase varies within very much wider limits, thus causing the degree of specificity to fluctuate from ten-fold or so to about one hundred thousand-fold.

The most specifically effective are compounds 62C47, 25C48, 297C50, 298C50, and 455C50. These are all symmetrical molecules, and the distance between the two quaternary nitrogens is of the order of that obtaining in 1 : 12 dodecyl-bis-trimethylammonium. The less active compound 142C48 is also symmetrical and has a similar inter-nitrogen length ; but the centre around which it is symmetrical is not CO but HCOCOPh. Even when the central group is CO, attachment of a radical larger than propyl to the quaternary nitrogens lowers the specificity. Though some specificity persists in those compounds with nearly the same total length of central chain, but with a single quaternary nitrogen, it is much less than in the five compounds mentioned above, and the activity against both enzymes is of a lower order.

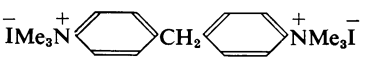
The selective reversible inhibition of the di-allyl compound, 297C50, was described under the number 284C51 by Austin and Berry (1953), and that of 62C47 by Burgen (1949b) and Todrick (1954).

The selectivity shown for true cholinesterase is much greater than that described by Adams and Thompson (1948), who found $\beta\beta'$-dichloro-diethyl-N-methylamine (DDM)—the first example, apart from caffeine (Zeller and Bissegger, 1943), of a selective inhibitor of true cholinesterase—to be 10 to 30 times as active on pigeon brain esterase as on human plasma enzyme. The selectivity is greater than that found by Paton and Zaimis for the polymethylene bis-trimethylammonium series, and by Hawkins and Mendel (1949) for the neostigmine analogue, Nu 1250.

Equally selective inhibitors of pseudo-cholinesterase are now known. There are, for example, *iso*OMPA—which, according to Aldridge (1952), is 10,000 times more active against pseudo- than true cholinesterase—and mipafox, which Davison (1953) has reported to be even more selective for pseudo-cholinesterase (on chicken enzymes). It should be noted, however, that these inhibitors of pseudo-cholinesterase have a practically irreversible action, whereas the effects of those inhibitors of true cholinesterase described in this paper are reversible.

It is possible that the action of these compounds on cholinesterase is due to the two quaternary ammonium groups (cf. Sanz, 1945), since compound 83C48 (not in Table I : structure

gave a *p*I 50 of 3.0 for true cholinesterase and 2.0 for pseudo-cholinesterase ; and since compounds 153C47, 26C48, and 101C48, with but one quaternary nitrogen each, are among the weakest of our series. Nevertheless, the bisquaternary compound



is not an anticholinesterase. However, increasing the distance between the benzene rings by the insertion of four more methylene groups, so giving 71C48, decreases its paralysing effect and introduces anticholinesterase activity (Glock, Mogey, and Trevan, 1948).

It has been suggested (Bergmann, Wilson, and Nachmansohn, 1950a) that the surface of true or acetylcholinesterase has two active centres, one an anionic site bearing a unit negative charge which combines with the cationic head of the substrate or inhibitor, and the other an esteratic site which has two functioning groups. One of the esteratic groups combines with the electophilic carbon of the ester carbonyl of acetylcholine, and the other with one of the two oxygens. The flexibility of the central chain of some of our compounds might allow them to become attached to some of such centres tailored to the measurements of acetylcholine. It is, however, surprising that 729C47 should also fit equally well, for its spine is somewhat more rigid : the double bonds here will, of course, introduce electron fluidity. The absence of a ketone group in the central chain is associated with a decrease in the selective affinity for true cholinesterase, although 71C48 still shows the typical effects. A greater drop of activity results from the removal of one of the quaternary nitrogens.

These observations support the later suggestion of Bergmann, Wilson, and Nachmansohn (1950b) that the true cholinesterase molecule has two anionic sites as well as the esteratic focus. The distance between these sites may be spanned by

the inhibitor. The Coulomb attraction between the onium groups of the compound and the anionic centres of the enzyme is probably the important factor in retaining the inhibitor in position. Van der Waals' forces will add to this attraction : these will increase, with the addition of radicals larger than methyl on the quaternary nitrogen, until a size is reached which hinders attachment because of its bulk. One butyl group appears sufficient to do this. But if this applies to the active site of cholinesterase it is difficult to see why it does not also apply to the point of action of acetylcholine at the neuromuscular junction.

The results show that our most selective compounds have activities of the orders of those of neostigmine and eserine on true cholinesterase. With some substrates (e.g., acetyl-β-methyl choline) they were less active. Burgen (1949b) found that 62C47 was less active than eserine *in vitro*, but he was concerned only with acetyl-β-methylcholine as substrate (private communication). We can confirm this. He also found it (1949a) to be more active than eserine in potentiating the red tear response in rats.

Tests for correlation between the various types of action show that paralysing activity bears no relationship to the others. There is a significant correlation between toxicity and anticholinesterase activity. The positive correlation that exists between anticurare and anticholinesterase activities is fairly good, although not statistically significant —due, possibly, to the small number of observations, or perhaps because this action may depend on more than one effect.

It is difficult to see new therapeutic applications for these compounds, but they will serve a useful purpose as heuristic tools—especially if used in conjunction with selective inhibitors of pseudocholinesterase.

Summary

1. A series of compounds, whose chief chemical characteristic is an arrangement of two quaternary nitrogens joined by a spine, which varies in composition but not much in length, has been examined for various pharmacodynamic effects.

2. The outstanding effect is selective reversible inhibition of true cholinesterase.

3. Some of the compounds are as active as neostigmine or eserine.

4. Some are the most selective inhibitors of true cholinesterase so far described.

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