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THE INHIBITION OF CHOLINESTERASES BY ANTAGONISTS OF ACETYLCHOLINE AND HISTAMINE

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A well-recognized group of drugs has the property of inhibiting cholinesterases. The present paper is concerned mainly with the inhibition of cholinesterases by drugs which do not primarily fall into this group but which appear to influence the action of acetylcholine.

Some 50 compounds were studied in respect of their ability to inhibit the true and pseudo cholinesterases, as typified by homogenates of rat brain and rat intestinal mucosa respectively (Ord and Thompson, 1950).

A summary of this work was communicated to the symposium on anticholinesterases, organized jointly by the British Pharmacological Society and the Society of Chemical Industry (Fine Chemicals Division), and held at the Wellcome Research Institution on March 27 and 28, 1953.

METHODS

The cholinesterase activity was estimated by a modification of Ammon's (1933) method. The details are as follows:

Technique.—The substrate and the inhibitor were mixed in the well of the Warburg flask and the enzyme was placed in the side-arm. This served to minimize the delay in the attainment of maximum enzymic activity noticed by Goldstein (1944). After tipping, at least five minutes were allowed for temperature re-equilibration before the first reading was taken. The intervals between readings were three minutes for the brain enzyme and six minutes for the intestinal mucosa enzyme. The results were calculated by the method of Aldridge, Berry, and Davies (1949).

Enzyme Preparations.—Male albino rats of 250–350 g. were used throughout. The animals were killed by fracture of the neck. The brain was dissected free, washed in saline, and homogenized immediately in a high-speed homogenizer in Krebs-Henseleit saline (18.5 ml. saline per g. wet weight). The small intestine was removed, washed through with saline, and split longitudinally; the mucosa was scraped off with a spatula and suspended in 20 ml. Krebs-Henseleit saline. This was homogenized and then diluted as required, 1 in 4–6, according to the activity of the preparation.

Using 0.5 ml. per flask, the output from these preparations was 200–300 μl. CO_2 per hour.

Substrates.—Acetylcholine chloride (Roche) was used in all experiments. Solutions were made up in distilled water and brought to *p*H 4 to minimize hydrolysis before use. A final concentration of 0.0025 M was employed with the brain preparation, and of 0.0625 M with the mucosa; each of these concentrations is close to the optimum value. When comparing the activities of true and pseudo cholinesterases against the physiological substrate most workers have used the same substrate concentration —usually 0.015 M—with both enzymes. Augustinsson (1949) has, however, criticized the use of a substrate concentration above the optimum with the true cholinesterase; our experience fully supports his criticism (Todrick, Fellowes, and Rutland, 1951).

Drugs.—These were dissolved in Krebs-Henseleit saline in concentrations 50% higher than those finally required. The addition of 2.0 ml. to the remaining reagents gave the correct concentration. Some drugs were insufficiently soluble to produce the desired 50% inhibition. Where extrapolation could reasonably be carried out, an approximate result has been quoted and indicated as such.

Controls.—In the earlier experiments, the enzyme blank was determined both in the absence of inhibitor and in the presence of the highest inhibitor concentration used; the value for the substrate blank was taken from a previous paper (Todrick *et al.*, 1951). In later experiments the first of the two controls, for which the value lay between 0 and 16 mm.^{3}/hr., was omitted, since it formed part of the second control. The flasks thus freed were used for a direct estimation of the substrate blank. This procedure is safer for the measurement of enzyme activity, but makes it rather less easy to recognize the effect of the homogenate on the drug when the effect is small.

Calculation of Results.—The inhibitory potency of each drug was determined at four or five concentrations, the total number of estimations on the inhibited enzyme being not less than twelve. From these results, the percentage inhibition-log concentration curve was drawn by hand, and the concentration causing

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50% inhibition was read off. This was converted to its negative logarithm.

The ratio of the 50% inhibition concentrations for the two enzymes has been calculated in the form [I]50 brain/[I]50 mucosa, subsequently abbreviated to " B/M ratio." This index is a measure of the specificity of the drug for one enzyme or the other. Since a high [I]50 denotes a low specificity, a B/M ratio >1 indicates preferential inhibition of the pseudo cholinesterase.

RESULTS

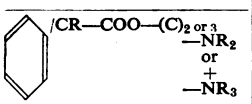
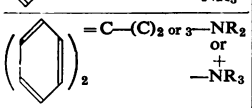
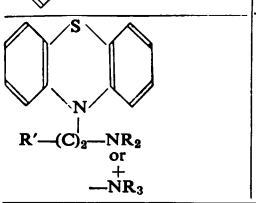
Forty-six substances have been examined for inhibitory action on the two cholinesterases. The drugs can be classified as:

1. Neuromuscular blocking agents.
2. Antimuscarinic agents.
3. Drugs effective in Parkinsonism.
4. Antihistaminic agents.
5. Anticholinesterases.
6. Miscellaneous substances.

The chemical formulae of the drugs in the first three classes are derived from one of the four basic structures shown in Table I.

TABLE I

BASIC CHEMICAL STRUCTURES FOUND AMONG NEUROMUSCULAR BLOCKING AND ANTI-MUSCARINIC DRUGS AND DRUGS EFFECTIVE IN PARKINSONISM

Basic Chemical Structure	Notes	Structure Code Used in Tables II to IV
$R_3\overset{+}{N}-(C)_{9-11}-\overset{+}{N}R_3$	Some of the C atoms may be part of ring systems; one or two can be replaced by ether linkages	A
	R is often another phenyl group; the phenyl groups can be replaced by other cyclic structures	B
	The phenyl groups can be replaced by other cyclic structures	C
	R′ is either H or a methyl group	D

The individual compounds examined in each class are listed in alphabetical order in Tables II–VII respectively, together with the code letters indicating the basic structure (Tables II–IV only), and the experimental results.

Each drug has been classified in accordance with its best recognized action. The distinction between antimuscarinic substances and drugs effective in Parkinsonism rests not so much on the lack of antimuscarinic properties in the latter as upon their greater effectiveness in this syndrome. It is possible that this effect is merely a manifestation of antimuscarinic action at a central rather than a peripheral site. Alternatively, drugs effective in Parkinsonism may possess additional actions, probably related to their antagonism to the central effects of nicotine (Bovet and Longo, 1951).

Many of the drugs have relatively powerful secondary actions and one, promethazine hydrochloride (" Phenergan "), seems to possess three major actions—antihistaminic, antimuscarinic, and effectiveness in Parkinsonism. Account must be taken of this in considering the experimental results.

The Effect of Cholinesterases on Drugs.—Bovet-Nitti (1949) showed that succinylcholine and other synthetic paralysing drugs were hydrolysed by cholinesterases; it was therefore considered desirable to determine whether, and to what extent, this occurred with the drugs and enzyme preparations used in this work. Hydrolysis would introduce two errors: it would appear as an increase in the enzyme activity and it would cause a fall in the inhibitor concentration.

It was found that three compounds were acted upon by the enzyme preparations, with the formation of acids or CO_2, and, as they were alkamine esters (atropine, lachesine, and IS337), it seemed probable that the cholinesterase was responsible. The hydrolysis only occurred at a measurable rate at very high concentrations (*ca.* 10^{-2}M), and it could be shown, for example with lachesine, that the percentage destruction of the inhibitor did not amount to more than 2% in 30 min. at 38° C.; thus the effect on the inhibitor concentration during the experiment was negligible.

It should be made clear, however, that the hydrolysis of drugs by the enzymes has not been measured at concentrations higher than are necessary to produce nearly complete inhibition. Nevertheless, it would not be expected that a further increase in drug concentration beyond this point would materially increase its hydrolysis rate.

The Inhibition of Cholinesterases by Drugs.—Tables II, III, and IV give the *p*I 50 values and B/M ratios for the neuromuscular blocking and antimuscarinic drugs, and for those effective in Parkinsonism. The compounds are arranged in alphabetical order.

TABLE II

THE INHIBITION OF CHOLINESTERASES BY NEUROMUSCULAR BLOCKING AGENTS

Compound	Structure Type (See Table I)	*p*I 50		B/M Ratio	Pharmacological Properties (References)
		Rat Brain Cholinesterase	Rat Intestinal Mucosa Cholinesterase		
Decamethylene α:ω-bis-6: 7-dimethoxy-1: 2: 3: 4-tetrahydro*iso*quinolinium methiodide (" No. 14," A and H)	A	5·10	4·40	0·19	Taylor and Collier, 1950
Gallamine triethiodide (" Flaxedil," M and B)		2·40	3·05	4·2	Bovet, Depierre, Courvoisier, and de Lestrange, 1949
Bis-(2-diethylaminoethyl) phthalate diethiodide (" IS 302 ")	AB	2·35	3·60	18	Bovet, Bovet-Nitti, Guarino, Longo, and Marrotta, 1949
Bis-(2-diethylaminoethyl) *iso*phthalate, diethiodide (" IS 337 ")	AB	5·35	1·90	0·00035	Bovet, Bovet-Nitti, *et al.*, 1949
8: 8'-(pentamethylenedioxy) bis [1-ethyl-quinolinium iodide] (" RP 3381 ")	A	7·20	4·45	0·0019	Bovet, Courvoisier, Ducrot, and Horclois, 1949
Pentamethylene bis-oxy-*o*-phenylene bis-[trimethylammonium iodide] (" RP 3565 ")	A	5·05	2·80	0·0057	Bovet, Courvoisier, *et al.*, 1949
(+)-Tubocurarine	A	3·30	2·25	0·087	—

TABLE III

THE INHIBITION OF CHOLINESTERASES BY ANTIMUSCARINIC COMPOUNDS

Compound	Structure Type (See Table I)	*p*I 50		B/M Ratio	Pharmacological Properties (References)
		Rat Brain Cholinesterase	Rat Intestinal Mucosa Cholinesterase		
Atropine sulphate	B	1·50	3·20	51	—
Atropine methyl nitrate	B	1·70	3·45	57	—
3: 3-Diphenylpropan-3-ol diethylamine methiodide (" 186C47," Wellcome)	C	2·35	3·20	7·4	White, Green, and Hudson, 1951
Hyoscine hydrobromide	B	1·80	2·20	2·3	—
2'-Diethylaminoethyl-2: 3: 3-triphenyl propionate hydrochloride (" J 4," T. and H. Smith)	B	1–2	3·25	>18	—
N-2-(Diphenylmethylthio)-ethyl piperidine hydrochloride (" J 7," T. and H. Smith)	B	<2·65	*ca.* 3·65	>10	—
Lachesine hydrochloride	B	1·80	2·65	7·0	—
3-N-Piperidino-1: 1-di(2'-thienyl) propan-1-ol (" 5C48," Wellcome)	C	2·35	3·35	10	Adamson and Green, 1950
1-Piperidino-3: 3-diphenyl propan-3-olamine (" 369C46," Wellcome)	C	1–2	3·25	>18	White, Green, and Hudson, 1951
2-Diethylaminoethyl diphenylacetate hydrochloride (" Trasentin," Ciba)	B	2·35	3·00	4·5	Graham and Lazarus, 1940
2-Diethylaminoethyl-α-phenyl cyclohexyl acetate hydrochloride (" Trasentin 6H," Ciba)	B	<2	3·60	>40	Graham and Lazarus, 1940

TABLE IV

THE INHIBITION OF CHOLINESTERASES BY DRUGS EFFECTIVE IN PARKINSONISM

Compound	Structure Type (See Table I)	*p*I 50		B M Ratio	Pharmacological Properties (References)
		Rat Brain Cholinesterase	Rat Intestinal Mucosa Cholinesterase		
1-Cyclohexyl-1-phenyl-3 (1-piperidyl) propan-1-ol (" Artane," Lederle)	C	*ca.* 2	4·00	*ca.* 100	Doshay and Constable, 1949
1-Cyclohexyl-1-phenyl-3-pyrrolidinopropan-1-ol (" Kemadrin," B. W.)	C	2·35	3·95	40	Montuschi, Phillips, Prescott, and Green, 1952
Diethazine hydrochloride (" Diparcol," M. and B.)	D	2·55	5·45	760	Sigwald, Bovet, and Dumont, 1946
Ethopropazine hydrochloride (" Lysivane," M. and B; " RP 3356 ")	D	2·80	6·25	2,800	Durel, 1949; Sigwald, 1949
Ethopropazine methosulphate	D	2·45	5·90	2,800	—
1-Phenylcyclopentane carboxylic acid 2-diethyl aminoethyl ester (" Parpanit " Geigy)	B	2·60	5·30	490	Grünthal, 1946; Schwab and Leigh, 1949
Promethazine hydrochloride (" Phenergan," M. and B.)*	D	2·70	4·45	62	Durel, 1949; Sigwald, 1949
Promethazine methosulphate	D	2·80	4·80	100	—

* Also included in Table V.

It will be seen that, while the individual *p*I 50 values have no particular significance, there appears to be a correlation between the B/M ratio of a drug and its pharmacological action. This is more clearly demonstrated by Fig. 1, which brings out the fact that the neuromuscular blocking agents are, with two exceptions, specific inhibitors of the brain enzyme, whereas the antimuscarinic drugs and those effective in Parkinsonism are specific inhibitors of the mucosa enzyme. For the antimuscarinic drugs the specificity is only moderate, but for those effective against Parkinsonism it is high.

Table V gives the results obtained with histamine and a number of antihistaminic substances. Histamine itself shows no specific inhibitory action, and the same is true of most of the antihistamines tested. Diphenhydramine hydrochloride, promethazine hydrochloride and its isomer " Lergigan," are exceptional in having B/M ratios of 6.0, 62, and 73 respectively.

Three anticholinesterases have been examined (Table VI). Eserine and neostigmine were studied because it was suggested (independently by Professor J. H. Gaddum and Dr. D. Grob) that they would be of special interest since they were specific inhibitors of the pseudo cholinesterase, yet did not possess antimuscarinic activity. However, under the conditions of test used here, eserine has a B/M ratio of 0.064 and neostigmine a ratio of 0.013: both are thus specific inhibitors of the true cholinesterase. In view of this, the inhibition of human serum cholinesterase by these drugs was examined, since this was the enzyme studied by Grob (1949). With eserine there was a large difference in the *p*I 50 for the two pseudo enzymes, the value for human serum being 6.10, and that for rat mucosa 5.25. With neostigmine, the serum enzyme was only twice as sensitive as the mucosa enzyme, and the B/M ratio (0.026) remained considerably less than unity. A major cause of the discrepancy between these and earlier results undoubtedly lies in the substrate concentrations used Grob (1949) used the same substrate concentration for both enzymes, whereas in the present study the concentration used with mucosa and serum was 25 times that used with brain.

The specific anti-true-cholinesterase activity of 62C47 (Burgen, 1948; Austin and Berry, 1953) has been confirmed.

Table VII lists the anticholinesterase activity of a number of miscellaneous drugs. Apart from the two analgesics, none possesses marked specific inhibitory

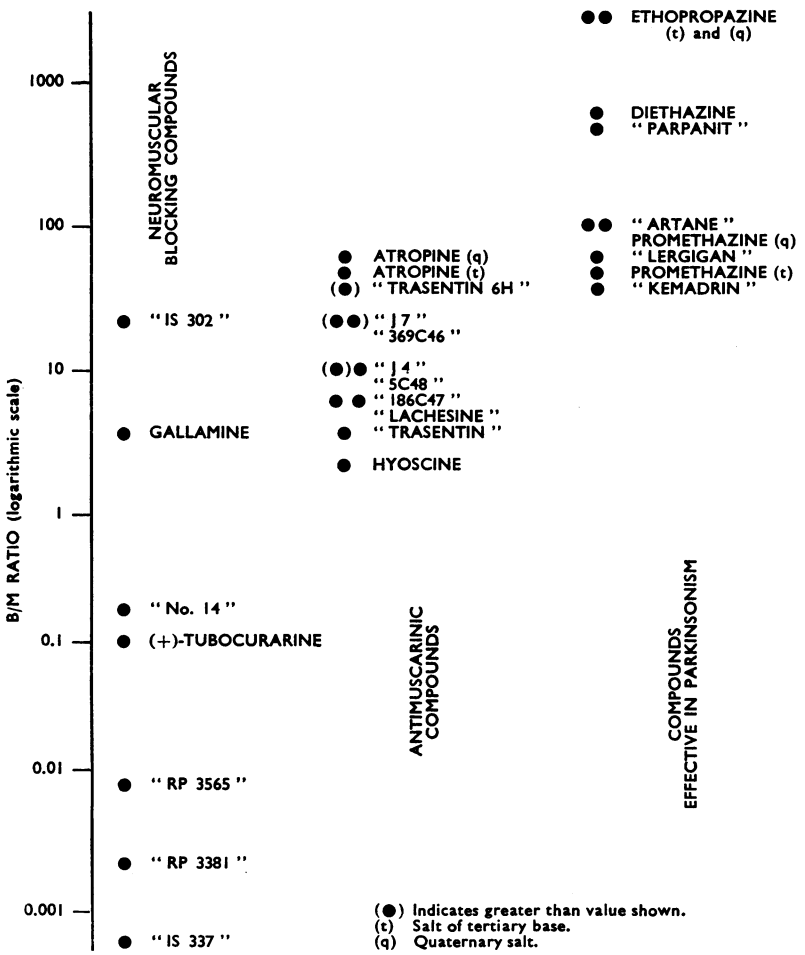


FIG. 1.—The relationship between the specific inhibitory activity of a drug against true or pseudo cholinesterase and its pharmacological action. The B/M ratio is [I]50 brain cholinesterase/[I]50 mucosa cholinesterase. A B/M ratio >1 denotes that the pseudo cholinesterase is preferentially inhibited, a ratio < 1 that true cholinesterase is preferentially inhibited.

TABLE V

THE INHIBITION OF CHOLINESTERASES BY ANTIHISTAMINE DRUGS

Compound	pI 50		B/M Ratio	pA_2	
	Rat Brain Cholinesterase	Rat Intestinal Mucosa Cholinesterase		Acetylcholine[1]	Histamine
Antazoline hydrochloride (" Antistin," Ciba)	3·90	4·10	1·5	5·47[1]	7·67[1]
trans-1-(4'Chlorophenyl)-1-(2'pyridyl)-3-pyrrolidinoprop-1-ene maleate (" 405C49," Wellcome)[3]	2·60	2·50	0·7	—	—
10-(1'-Dimethylamino 2'-propyl) phenothiazine hydrochloride (" Lergigan,"[6] AB Recip)	2·55	4·40	73	—	—
Diphenhydramine hydrochloride (" Benadryl," P.D. & Co.)	2·05	2·85	6·0	6·57[2]	8·02[2]
Phenindamine phosphate (" Thephorin," Roche)	2·80	3·20	2·5	—	—
Promethazine hydrochloride[5] (" Phenergan," M. and B.)	2·70	4·45	62	7·62[1]	9·18[1]
Mepyramine maleate (" Anthisan," M. and B.)	3·25	3·05	0·66	4·86[2] 5·03[1]	9·32[1] 9·46[2]
Tripellenamine hydrochloride (" Pyribenzamine," Ciba)	3·70	2·95	0·18	—	—
Histamine	2·15	2·30	1·5	—	—

[1] Figures taken from Reuse (1948). [2] Figures taken from Schild (1947). [3] Adamson, Barrett, Billinghurst, Green, and Jones (1951). [4] pA_2 for atropine is 8·61 (Schild, 1947). [5] Also included in Table IV. [6] Gernandt and Schmiterlöw (1953).

TABLE VI

THE INHIBITION OF CHOLINESTERASES BY CERTAIN ANTICHOLINESTERASES

Compound	pI 50		B/M Ratio	Pharmacological Properties (References)
	Rat Brain Cholinesterase	Rat Intestinal Mucosa Cholinesterase		
Eserine	6·45	5·25	0·064	—
Neostigmine methosulphate	6·70	4·80	0·013	—
1 : 5-Bis-(4'-trimethyl ammoniumphenyl)pentan-3-one diiodide (" 62C47," Wellcome)	7·20	2·45	0·00002	Mogey, 1952

TABLE VII

THE INHIBITION OF CHOLINESTERASES BY MISCELLANEOUS DRUGS

Compound	Pharmacological Action	pI 50		B/M Ratio	Pharmacological Properties (References)
		Rat Brain Cholinesterase	Rat Intestinal Mucosa Cholinesterase		
Arecoline	Parasympathetic stimulant	2·40	1·85	0·28	—
3-Diethylamino-1 : 1-di(2'-thienyl) but-1-ene (" 191C49," Wellcome)	Analgesic	3·10	4·10	9·4	Adamson and Green, 1950
3-Dimethylamino-1 : 1-di(2'-thienyl) butane (" 489C49," Wellcome)	Analgesic	2·90	3·90	10	Adamson, Duffin, and Green, 1951
Hexamethonium bromide	Antagonist to decamethonium bromide	1·75	1·75	1·2	Paton and Zaimis, 1949
Lobeline	Carotid sinus stimulant	3·15	3·30	1·5	—
Mephenesin (" Myanesin," B.D.H.)	Spinal cord depressant	—*	—*	—*	Berger and Bradley, 1946
Nicotine	Autonomic ganglion stimulant and depressant	2·75	2·35	0·40	—
Pilocarpine	Parasympathetic stimulant	2·65	1·80	0·15	—

* See text.

action, or indeed much inhibitory action at all. Mephenesin (" Myanesin ") behaves in an interesting manner. Firstly, it is incapable of causing more than 30% inhibition of either enzyme at a concentration as high as 0.03M ; secondly, it even activates the mucosa enzyme slightly over the concentration range 10^{-4}–10^{-2}M, whereas it does not activate the brain enzyme.

DISCUSSION

The experimental data summarized in Fig. 1 suggest that there may be a correlation between the pharmacological action of the compounds examined and their specificity as cholinesterase inhibitors. The majority of the neuromuscular blocking agents appear to be specific inhibitors of the brain or true cholinesterase, whereas anti-

muscarinic compounds and those effective in Parkinsonism tend to be specific inhibitors of the intestinal mucosa or pseudo cholinesterase.

The results obtained with a series of antihistaminic compounds support this idea. Of the eight compounds examined, three—promethazine, its isomer lergigan, and diphenhydramine—preferentially inhibit the pseudo cholinesterase. Promethazine and diphenhydramine are known to possess antimuscarinic activity (cf. the pA_2 values against acetylcholine, determined by Reuse (1948) and Schild (1947) respectively, which are quoted in Table V, col. 5). Lergigan is also said to possess "very strong anti-acetylcholine action" (Gernandt and Schmiterlöw, 1953).

Further evidence favouring the idea of a correlation between pharmacological action and anticholinesterase specificity can be obtained from the published results of other workers.

Blaschko, Chou, and Wajda (1947) found that a series of synthetic esters with atropine-like actions markedly inhibited horse serum cholinesterase at a concentration of 6×10^{-3}M, whereas the cholinesterase of the dog caudate nucleus was affected only slightly or not all all by this concentration.

Paton and Zaimis (1949) showed that the neuromuscular blocking agent dodecamethylene bis-trimethylammonium iodide (C12) is a specific inhibitor of true cholinesterase, the ratio for the 50% inhibition concentrations (human erythrocyte/human plasma) being 0.002; 62C47 (Wellcome), which possesses neuromuscular blocking activity (Mogey, 1952), is also a highly specific inhibitor of the true cholinesterase (Burgen, 1948; Austin and Berry, 1953; see also Table VI).

Acknowledgment must be made of published work on the inhibition of cholinesterases by compounds which I also have examined—such as antazoline and mepyramine (Payot, 1946); diethazine (Gordon, 1948); (+)-tubocurarine (Harris and Harris, 1944); RP3381 and RP3565 (Bovet, Courvoisier, Ducrot, and Horclois, 1949). Some of the published data are qualitative rather than quantitative; nevertheless, when account is taken of the different sources of enzyme and the different substrates and substrate concentrations used, there seems to be no evidence of disagreement between these results and mine.

Whatever the reason for this correlation may be, it should be stressed that the absolute inhibitory activities of many of the compounds are low, and there can be no question of explaining their pharmacological action in terms of their anticholinesterase activity.

The two most obvious exceptions to the apparent correlation between pharmacological action and anticholinesterase specificity are the structurally related compounds gallamine triethiodide and IS302; the former is the pyrogallol triether and the latter the phthalic diester of the same substituted ethanolamine. Though both are stated to possess neuromuscular blocking activity (Bovet, Depierre, Courvoisier, and de Lestrange, 1949; Bovet, Bovet-Nitti, Guarino, Longo, and Marrotta, 1949), they preferentially inhibit the pseudo-cholinesterase, their B/M ratios being 4.2 and 18 respectively.

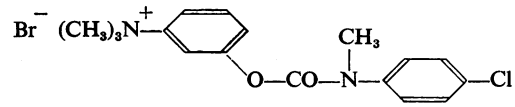
However, Marbury, Artusio, Wescoe, and Riker (1951), in a clinical study of gallamine, have described a vagolytic action which was noticeable before the onset of curare-like symptoms; this is in keeping with the previous finding of Riker and Wescoe (1951) that in cats "the vagal action exceeds the neuromuscular blocking action not only in potency but also in duration." But they also conclude that gallamine "manifests no other atropine-like action."

Hyoscine is another exception, since its B/M ratio 2.3 is the lowest found for any antimuscarinic compound. No explanation for this exception can be offered.

So far, this discussion has centred on the relationship of pharmacological and biochemical properties. It is perhaps worth while to consider shortly the question of chemical structure.

The relationship between pharmacological activity and chemical structure among neuromuscular blocking and atropine-like drugs has been intensively studied (cf. Barlow and Ing, 1948; Kimura, Unna, and Pfeiffer, 1949; Paton and Zaimis, 1953, for neuromuscular blocking drugs; and Gaddum, 1944; Pfeiffer, 1948; Ing, Dawes, and Wajda, 1945, for atropine-like drugs). The evidence suggests that neuromuscular blocking agents are, with the exception of the erythrina alkaloids, all quaternary ammonium salts—or onium salts in general—but that the most potent compounds contain two quaternary N-atoms separated by a relatively long chain, as in decamethonium and gallamine triethiodide, or a bulky ring structure as in tubocurarine. Atropine-like compounds are mostly alkamine esters of acids containing one or two aromatic nuclei, and the basic N-atom (which may be tertiary or quaternary) is usually separated from the ester group by a short chain of 2 or 3 C-atoms; White, Green, and Hudson (1951) have, however, found potent atropine-like compounds of the general structure $Ar_2(C)_3NR_2$ which do not contain an ester group.

If the conclusions drawn in the earlier part of the discussion are correct, true cholinesterase inhibitors should possess the structures associated with the neuromuscular blocking drugs, whereas pseudo cholinesterase inhibitors should possess those of the atropine-like compounds. This, broadly speaking, is true if the irreversibly inhibitory organic phosphates are left out of account. There is one well-recognized exception; that is the true cholinesterase inhibitor Nu 1250 (Hawkins and Mendel, 1949), which has the following structure:



In recent years Nachmansohn and Whittaker and their co-workers have reached certain conclusions regarding the chemical nature of the active centres of the two cholinesterases; the main difference between them seems to lie in the possession by the true cholinesterase of one or more anionic groups which are absent from the pseudo cholinesterase. Bergmann, Wilson, and Nachmansohn (1950) suggest, on the basis of experiments comparing the inhibition of mono- and bis-quaternary salts, that true cholinesterase probably possesses two anionic groups capable of binding quaternary nitrogen groups separated by a distance of 12–14 Å—that is, structures similar to the basic structure of the most potent neuromuscular blocking drugs (cf. also Paton and Zaimis, 1953). That the neuromuscular blocking drugs appear to be specific inhibitors of true cholinesterase is additional evidence for the view put forward by these workers.

An idea, which seems to be implicit in their conclusions that the active centre of true cholinesterase must resemble in structure the acetylcholine receptor at the neuromuscular junction, was first suggested in rather more general terms by Roepke in 1936 (cf. Wescoe and Riker, 1951).

In an analogous manner, it could be argued that the correlation of antimuscarinic activity with specificity for the pseudo cholinesterase reflects a structural resemblance between the acetylcholine receptors in the parasympathetic nervous system and the active centre of the pseudo cholinesterase. It is perhaps of interest, though the connexion is by no means clear, that Ord and Thompson (1950) have found that pseudo cholinesterase is present in excess of acetylcholinesterase in those tissues of the rat in which acetylcholine exerts a muscarinic action; further, Koelle, Koelle, and Friedenwald (1950) and Burn, Kordik, and Mole (1952) have suggested that pseudo cholinesterase is of physiological significance in the intestine of the rat.

Summary

1. The inhibition of the cholinesterases of rat brain and intestinal mucosa by a series of 46 pharmacologically active compounds has been studied.

2. The ratio of the 50% inhibition concentrations for the two enzymes tends to show some correlation with the predominant pharmacological action of the compound; thus, for most neuromuscular blocking agents the ratio [I]50 brain/[I]50 intestinal mucosa has a value less than 0.2; for antimuscarinic agents the ratio lies between 5 and 60; while for drugs effective in Parkinsonism the ratio is greater than 40.

3. Two neuromuscular blocking drugs possess ratios greater than unity. One, gallamine triethiodide, also exhibits antimuscarinic activity; the other, IS302, has a chemical structure intermediate between those of neuromuscular blocking and antimuscarinic drugs.

4. Most antihistaminic drugs show no tendency to inhibit either enzyme specifically; three, which exhibit marked antimuscarinic action, have higher ratios than the average.

5. Hyoscine does not show any correlation between pharmacological action and specificity of cholinesterase inhibition.

6. The significance of these findings is discussed.

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