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TUBOCURARINE ANTAGONISM AND INHIBITION OF CHOLINESTERASES

BY

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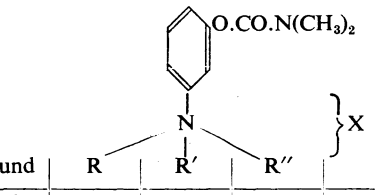
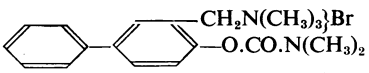
In a recent paper Bülbring and Chou (1947) compared the activities of various substances with that of prostigmine as antagonists to tubocurarine and as inhibitors of cholinesterase. The results obtained with ethyl homologues of prostigmine suggested that a parallelism might exist between the two activities, and for this reason a larger series of chemical compounds has now been investigated.

All the substances studied are given in Table I. Compounds 3392 and 3393, the mono- and di-ethyl homologues of prostigmine, are the two substances previously examined ; compound S208

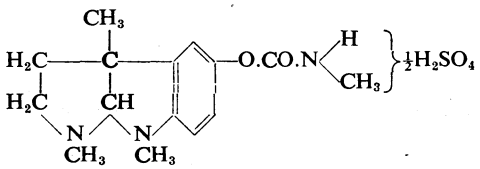
TABLE I



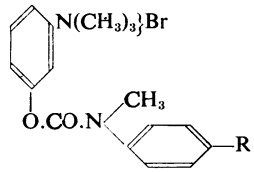
Compound	R	R′	R″	X
Prostigmine	CH_3	CH_3	CH_3	SO_4CH_3
3392 ..	CH_3	CH_3	C_2H_5	I
3393 ..	CH_3	C_2H_5	C_2H_5	I
S208 ..	C_2H_5	C_2H_5	C_2H_5	I
5220/5 ..	CH_3	CH_3	OH	Cl



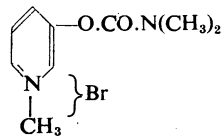
Nu 683



Eserine sulphate



Nu 1250, R = Cl
Nu 1197, R = CH_3



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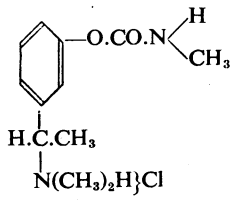
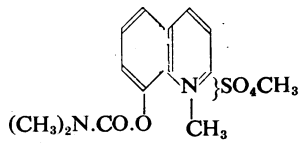


Miotine hydrochloride



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is the N-triethyl homologue of the same series. Compound 5220/5 is an amine oxide closely related to prostigmine ; this substance is of interest as a prostigmine derivative in which the quaternary ammonium group has lost its strongly basic character. Compounds Nu 1250 and Nu 1197 are known to be strong inhibitors of true cholinesterase (Aeschlimann and Stempel, 1946), and the compound Nu 683 was included because it is

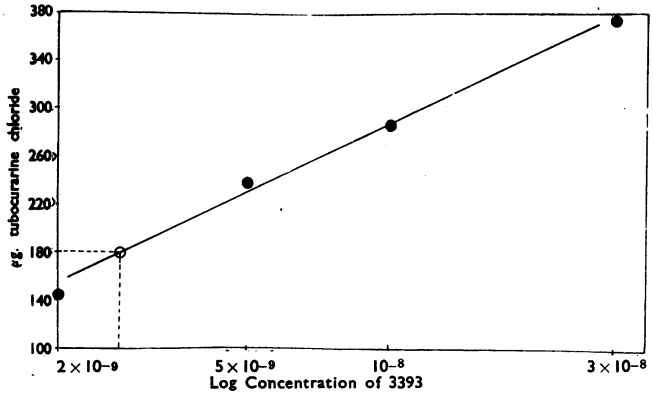

FIG. 1.—Evaluation of antitubocurarine activity on the rat's phrenic nerve-diaphragm. Ordinate = dose of *d*-tubocurarine chloride in μg. Abscissa = log concentration of 3393. The circle indicates the point from which the pD 20 value is found (see text).

known to be a specific inhibitor of pseudocholinesterase (Hawkins and Gunter, 1946).

METHODS

For the estimation of the anticurare activity the isolated phrenic nerve diaphragm preparation of the rat (Bülbring, 1946) was used. The quantitative evaluation was based on the fact that the dose of tubocurarine chloride required to cause a certain reduction of muscle contractions in the presence of an antagonist is proportional to the concentration of the antagonist. Bülbring and Chou (1947) expressed the potency of any antagonist in terms of that of prostigmine, which they used as a standard. For a comparison of anticurare activity with anticholinesterase activity it was, however, necessary to express the potency by an absolute rather than by a relative definition. This was done as illustrated in Fig. 1, in which the mean results of five experiments are shown. Each point represents the relation between a given concentration of 3393 (abscissae) and the dose of *d*-tubocurarine chloride (ordinates) which caused 20 per cent depression of the muscle contractions. A dose of *d*-tubocurarine chloride had to be chosen which was antagonized by most of the substances investigated within the range of concentrations used; this was 180 μg. The corresponding concentration of the antagonist was found by interpolation as shown in Fig. 1. For four compounds (38, Nu 683, 5150, and 5220/5) it had to be found by extrapolation. The potency of any tubocurarine antagonist was thus expressed as the negative logarithm of the concentration in the presence of which 180 μg. *d*-tubocurarine chloride caused 20 per cent depression of the muscle contractions. This is the pD 20 value; the figures are given in Table III.

The method of estimating anticholinesterase activity was the same as that described by Bülbring and Chou (1947). The inhibitory action on both true and pseudo-cholinesterase was studied. Each substance was therefore incubated with dog's brain (caudate nucleus) and acetylcholine (for its action on true cholinesterase) and also with horse serum and benzoylcholine (for its action on pseudocholinesterase). Table II gives the quantities of enzyme preparations and substrates used in our experiments.

RESULTS

For the estimation of anticholinesterase activity several different concentrations of the inhibitor were tested. An absolute value of inhibitory activity was obtained by finding an index which was called pI 50. Fig. 2 shows all the results obtained with different concentrations of prostigmine on the two enzymes. Each point corresponds to one observation, and the curves are drawn through the means for each prostigmine concentration. The abscissa of the point where the curve crosses the 50 per cent line is the negative logarithm of the molar concentration which causes 50 per cent inhibition: this is the pI 50 value. It can be seen in Fig. 2*a* that the pI 50 value obtained with dog's brain was 7.4. The actual figures in eight individual experiments were 7.2, 7.6, 7.5, 7.1, 7.5, 7.3, 7.3. With horse serum the individual figures in four experiments (Fig. 2*b*) were 7.2, 7.1, 7.35, 7.15, and the mean was thus 7.2. Koelle and Gilman (1946) used a similar index which they called pK. Whenever possible the pI 50 value for each enzyme preparation was determined with the inhibitor to be tested and with prostigmine on the same day.

TABLE II

Type of enzyme	Source and amount of enzyme preparation added to each flask (Total vol. = 3 c.c.)	Substrate
True cholinesterase	4 mg. dog's caudate nucleus	$6 \times 10^{-3}M$ acetylcholine
Pseudo-cholinesterase	2 c.c. horse serum	$6 \times 10^{-3}M$ benzoylcholine

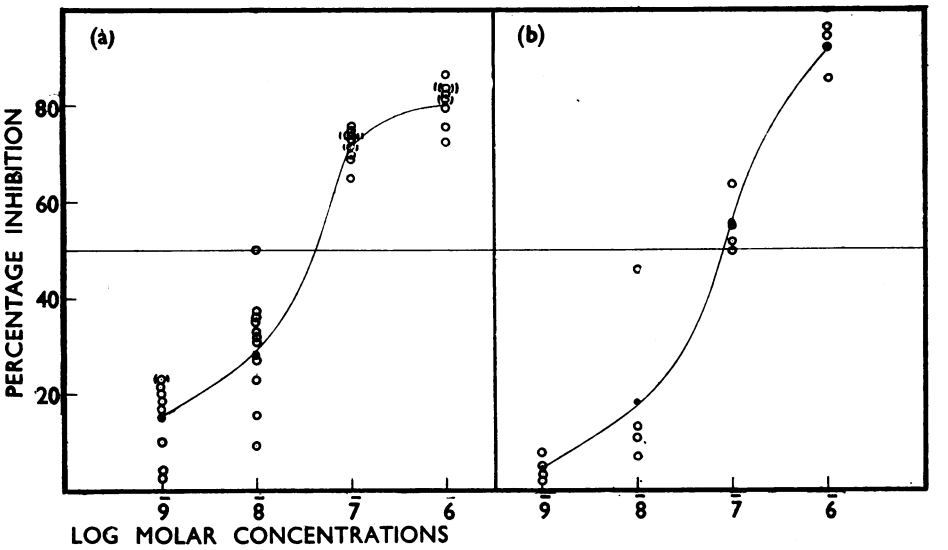

FIG. 2.—Evaluation of anticholinesterase activity of prostigmine. (*a*) Dog's caudate nucleus, (*b*) horse serum. Ordinates = percentages inhibition. Abscissae = log molar concentration of prostigmine. The pI 50 value is found from the point where the 50 per cent line crosses the curves.

The ethyl homologues of prostigmine.—Since the earlier publication of Bülbring and Chou (1947) the triethyl homologue of prostigmine (S208) has become available. It had been found previously that the substitution of one methyl group on the quaternary nitrogen by ethyl increased the activity both as inhibitor of the two cholinesterases and as antagonist to tubocurarine. The introduction of the second ethyl led to a still further increase in activity. We have now examined S208 and have found that the introduction of the third ethyl group decreases the activity in both respects below that of prostigmine itself. The figures will be found in Table III, in which all the results are summarized.

The amine-oxide homologue of prostigmine (5220/5).—This compound was without any anticurare activity in the concentration tested. The pD 20 value was determined by extrapolation and was the lowest of all. Its inhibitory action on both enzymes was also weak. It is known that the amine oxides of the general structure

TABLE III

Compounds	Mol. weight	Anticurarine activity pD 20 value	Anticholinesterase activity pI 50 value	
			On true cholinesterase	On pseudo-cholinesterase
Prostigmine ($RNMe_3$)*	334	7.60	7.4	7.2
3392 ($RNMe\ Et$)	336	8.19	8.0	7.3
3393 ($RNMeEt_2$)	350	8.57	8.2	8.0
S208 ($RNEt_3$)	392	6.59	7.2	7.4
Miotine HCl	254	7.85	7.2	6.4
Eserine sulphate	648	7.21	7.1	7.7
No. 38	354	6.23	7.1	7.6
Nu 1250	339.7	7.26	7.4	7.9
Nu 1197	379.3	7.31	6.9	7.1
Nu 683	388	5.44	6.2	8.5
5130	251	5.26	6.4	5.8
5220/5 ($RNMe_2OH$)	240.5	4.77	4.5	4.4

*R = m-$(CH_3)_3N.CO_2C_6H_4$-

$R_3NOH\}OH$ (where R is an alkyl or aromatic radical) are bases with very small dissociation constants and in aqueous solution they exist almost entirely as undissociated molecules. For instance, the salts of trimethylamine oxide, $Me_3NOH\}I$, show an acid reaction in solution. Our results therefore reveal that the strongly basic character of the phenolic nitrogen radical is indispensable for a substance with strong anticholinesterase and anticurare activity.

Substances with predominantly inhibitor action on pseudo-cholinesterase.—Hawkins and Gunter (1946) found that the substance Nu 683 was a strong inhibitor of pseudo-cholinesterase, whereas it affected true cholinesterase only in very high concentrations. In the living animal it produced symptoms of acetylcholine poisoning only in doses which significantly reduced the action of the true enzyme. The authors concluded that these results indicated that pseudo-cholinesterase played no essential part in the hydrolysis of acetylcholine *in vivo*. We found that both the anticurare action of Nu 683 and its inhibitory action on true cholinesterase were low, whereas its action on pseudo-cholinesterase was high.

The parallelism between anticurarine activity and inhibitory action on true cholinesterase.—Inspection of Table III will show that the different substances do not always have the same inhibitory action on both esterases; some inhibit pseudo-cholinesterase more strongly than true cholinesterase and vice versa. On the whole, however, there is good agreement between the degree of inhibitory activity on true cholinesterase and that of antitubocurarine activity, whereas no such correlation exists between the action on pseudo-cholinesterase and tubocurarine antagonism. We are very grateful to Dr. W. Perry for the statistical analysis of the results in Table III, by which it was established that a significant correlation exists between the values for anticurare activity and those for anti-true-cholinesterase activity ($P<0.001$), whereas there is no indication of a similar correlation between anticurare and anti-pseudo-cholinesterase activities ($P>0.1$).

Discussion

Our results show that there is a significant correlation between antitubocurarine activity and inhibitory action on true cholinesterase. This supports the view of Hawkins and Mendel (1947) that it is the true cholinesterase which is responsible for the destruction of acetylcholine at the site of its physiological action.

Aeschlimann and Stempel (1946) have estimated the inhibition of a purified specific cholinesterase preparation from the electric eel by Nu 1250 and Nu 1197. They found that the anticholinesterase activity of Nu 1250 was five times, and that of Nu 1197 more than equal to, that of prostigmine. We find that the first substance has the same activity as prostigmine, and the second substance has less activity. A possible reason for this discrepancy is the different source of the enzyme; we have used a suspension of the dog's caudate nucleus and consequently our tests both for anticurare activity and for anticholinesterase activity were carried out with mammalian tissues.

It is known that structural changes of the prostigmine molecule sometimes increase the affinity for one enzyme and decrease it for the other. One example is Nu 683, which is of special interest here as the decline in inhibitory action on true cholinesterase finds its parallel in weaker anticurare activity.

An important result of our experiments is the finding that a strongly basic nitrogen radical in the phenolic moiety of the molecule is indispensable not only for a strong anticurare activity but also for a strong anticholinesterase activity.

The ethyl homologues of prostigmine provide an interesting group of compounds: substitution of the methyl groups on the quaternary nitrogen of prostigmine by ethyl at first increases both the antitubocurarine and the anticholinesterase activity, but the peak is reached at the diethyl compound and the activity of the triethyl compound is again much less.

Summary

1. The antitubocurarine activity of a series of compounds related to prostigmine was compared with their inhibitory action on the true cholinesterase of dog's caudate nucleus and on the pseudo-cholinesterase of horse serum.

2. Significant correlation was found between the inhibition of true cholinesterase and the antagonism to *d*-tubocurarine.

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References

Aeschlimann, J. A., and Stempel, A. (1946). *Roche Jubilee Vol. for Emil Barell*, Basle.

Bülbring, E. (1946). *Brit. J. Pharmacol.*, **1**, 38.

Bülbring, E., and Chou, T. C. (1947). *Brit. J. Pharmacol.*, **2**, 8.

Hawkins, R. D., and Gunter, J. M. (1946). *Biochem. J.*, **40**, 192.

Hawkins, R. D., and Mendel, B. (1947). *Brit. J. Pharmacol.*, **2**, 173.

Koelle, G. B., and Gilman, A. (1946). *J. Pharmacol.*, **87**, 421.