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COMPARISON OF THE EFFECTS OF ESERINE AND NEOSTIGMINE ON THE LEECH MUSCLE PREPARATION

BY

B. K. BHATTACHARYA AND W. FELDBERG

From the National Institute for Medical Research, Mill Hill, London

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The relative potencies of eserine and neostigmine were determined on three preparations of cholinesterase. Eserine was found to be twice as potent as neostigmine on the pseudocholinesterase of horse plasma, half as potent on the true cholinesterase of cat central nervous system, but twelve times as potent on the true cholinesterase prepared from the leech body wall. On the leech preparation, about ten times smaller concentrations of eserine than of neostigmine were required to potentiate the acetylcholine response, but after removal of the anticholinesterase from the bath fluid, the potentiation persisted much longer after neostigmine than after eserine. The greater sensitivity of the leech muscle to eserine is fully accounted for by the fact that its cholinesterase is more sensitive to eserine than to neostigmine. The longer lasting potentiation after neostigmine on the other hand suggests that this anticholinesterase becomes more firmly attached to the cholinesterase receptors in this muscle than does eserine.

In experiments on perfused cerebral ventricles of the cat reported elsewhere (Bhattacharya and Feldberg, 1958), it became necessary to assay small amounts of eserine and neostigmine in samples of perfusate. When an attempt was made to assay these samples by their ability to sensitize the leech muscle preparation to acetylcholine, it became evident that the two anticholinesterases showed certain differences. These are reported in the present paper, which also includes a comparison of the potency of eserine and neostigmine in inhibiting preparations of pseudo- and true cholinesterase.

METHODS

Assay of Cholinesterase Inhibiting Activity.—As a source of pseudocholinesterase, samples of freeze-dried oxalated horse plasma were used. The samples had been stored in the cold for several years but had retained potent anticholinesterase activity. On the day of testing, a dried sample corresponding to 10 ml. of plasma was dissolved with frog Ringer solution so as to obtain a dilution of 10% plasma. Two preparations were used as source of true cholinesterase: either the caudate nucleus and thalamus of the cat or the dorsal body wall of the leech (*Hirudo medicinalis*). The caudate nucleus and the thalamus were removed from a freshly killed cat, weighed and homogenized with 5 ml. of distilled water/g. tissue in a small glass homogenizer The homogenate was kept in the deep freeze and was used for several days. On the day of testing, a sample of homogenate was diluted 1:50 with frog Ringer solution. The dorsal body wall of the leech was dissected free, cut into small pieces, freeze-dried and powdered. A solution of 3 mg./ml. in frog Ringer solution was prepared on the day of testing.

The method of assay was essentially that described by Burn (1952) in which a given dose of acetylcholine is incubated for a specified time with a given amount of cholinesterase and anticholinesterase and then assayed on the frog rectus muscle for acetylcholine. If the amounts of acetylcholine and cholinesterase are properly chosen the amount of acetylcholine remaining undestroyed in the sample after incubation depends on the amount of anticholinesterase added. In the present experiment, the incubation volume was 4 ml. and consisted of 1 ml. of either diluted plasma or diluted homogenate, 1 ml. acetylcholine 1:1,000,000 and 2 ml. of frog Ringer solution, alone or with varying amounts of anticholinesterase. The anticholinesterase was kept in contact with the cholinesterase for 15 min. before the acetylcholine solution was added. The mixture was incubated for 10 min. at room temperature. The enzyme activity was then stopped by the addition of 1 ml. eserine 1:40,000 so as to make the concentration of eserine in the now 5 ml. sample 1:200,000. The assay on the frog rectus muscle was made in a 5 ml. bath containing frog Ringer solution with eserine 1:200,000. Acetylcholine or the incubated samples were tested at 5 min. intervals and kept in the bath for 90 sec. With this procedure it was possible to assay a few ng. of eserine or neostigmine.

The Leech Muscle Preparation.—Two parallel strips from the dorsal body wall of the same leech

were suspended at room temperature, each in a separate 5 ml. bath. First the threshold concentration of acetylcholine was determined, which was about 1:500,000. This concentration was tested for 90 sec. at 20 min. intervals. When constant small contractions had been obtained, and 10 min. before the next dose of acetylcholine was given, eserine was added to the bath of the one and neostigmine to that of the other strip, the anticholinesterase being washed out immediately before.

RESULTS

Inhibition of Cholinesterase.—Table I shows the amounts of eserine and neostigmine in μg. which gave the same degree of inhibition of cholinesterase by our method of testing. Neostigmine was half as potent as eserine when tested on pseudocholinesterase. The results obtained with true cholinesterase differed with the two preparations used. In the experiments with homogenate of cat central nervous system, neostigmine was found to be twice as potent as eserine, but in the experiments with powder prepared from the leech body wall, which were suggested to us by Sir Henry Dale, eserine was found to be twelve times as potent as neostigmine. This is illustrated in Fig. 1, which is part of an assay on a frog rectus muscle. The unlabelled contractions were caused by 1 μg. acetylcholine alone, the small contraction at C by 1 μg. acetylcholine after incubation with 3 mg./ml. of powder prepared from the leech body wall. This contraction thus shows the destruction of acetylcholine on incubation with this concentration of cholinesterase. The other contractions were obtained after incubation of 1 μg. acetylcholine with the same amount of cholinesterase, but after the addition of different amounts of eserine or neostigmine. It will be seen that with 0.1 μg. of neostigmine the inhibition of the cholinesterase was less, with 0.15 μg. more than with 0.01 μg. eserine, but that with 0.12 μg. neostigmine it was about equal.

Table I also includes a comparison with dyflos (DFP), a selective inhibitor of pseudocholinesterase. Compared with either eserine or neostigmine, it inhibited the pseudocholinesterase in smaller doses, and the true cholinesterase of the cat central nervous system in larger doses.

In Table II, these results are compared with those obtained by other authors using different methods. Dr. V. C. Abrahams kindly measured for us the cholinesterase-inhibiting properties of eserine and neostigmine with the Warburg technique. His results have been included in this Table, which gives the ratio of potency: eserine/neostigmine. In calculating this ratio, the molecular weights of the various salts used by different authors have been taken into account. The molecular weight of eserine is 275, of its hydrochloride 312, of its salicylate 414, and of its sulphate 649. But since the sulphate contains two moieties of eserine half the molecular weight, 324, was used. The molecular weight of neostigmine

TABLE I

THE WEIGHTS IN μG. OF ANTICHOLINESTERASE GIVING THE SAME DEGREE OF INHIBITION OF CHOLINESTERASE

The numerals in brackets are molecular weights except in the case of eserine sulphate, which contains two eserine moieties, half the molecular weight.

	True Cholinesterase		Pseudo-cholinesterase
	Cat C.N.S.	Leech	
Neostigmine methyl-sulphate (334) ..	0·04	0·12	0·04
Eserine sulphate (324)	0·08	0·01	0·02
Dyflos (185) ..	3·0		0·005

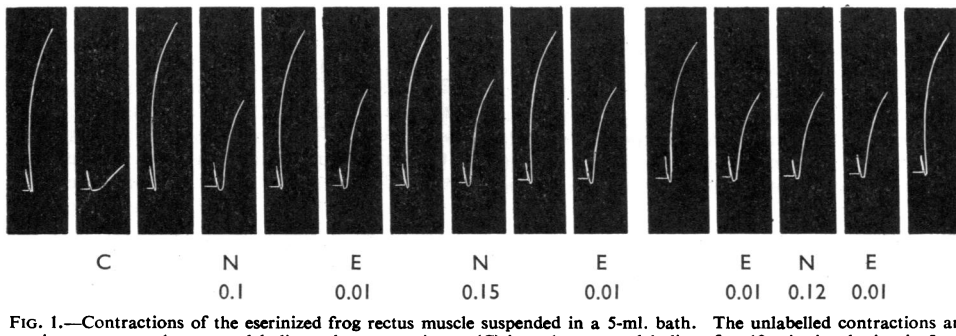

FIG. 1.—Contractions of the eserinized frog rectus muscle suspended in a 5-ml. bath. The unlabelled contractions are in response to 1 μg. acetylcholine; the contraction at (C) is to 1 μg. acetylcholine after 10 min. incubation in 5 ml. containing 15 mg. powder of leech body wall; those at (N) and (E) are to 1 μg. acetylcholine similarly incubated, but after the cholinesterase had been in contact for 15 min. with either neostigmine (N) or eserine (E), the numerals indicating in μg. the amounts of anticholinesterase used. In the assay, the contractions to the incubated samples were always followed by two contractions to 1 μg. acetylcholine, but only one of these is shown in the first part and none in the second part of the record.

is 223, of its bromide 303, and of its methylsulphate 334. Thus eserine sulphate and neostigmine methylsulphate, the salts used in the present experiments, can be compared without great error on a weight-for-weight basis.

Whereas Roepke (1937) found neostigmine 3 times, and Blaschko, Bülbring and Chou (1949) 1.5 times, as active as eserine in inhibiting pseudocholinesterase, Eadie (1942), Abrahams (personal communication) and ourselves found the reverse, that eserine was 1.6 to 2 times as active as neostigmine. On the other hand, on the true cholinesterase of the cat or dog central nervous system, we found eserine half as active as neostigmine; Hawkins and Mendel (1946) and Abrahams (personal communication) found both equally active, and Blaschko *et al.* (1949) found eserine 4 times as potent as neostigmine. On the true cholinesterase of the electric eel, Cohen and Unna (1951) found eserine 10 times as active as neostigmine. This corresponds to our results obtained on the true cholinesterase of the leech muscle; on this preparation eserine was 12 times as active as neostigmine.

Effect on Leech Muscle.—Neither eserine nor neostigmine caused contraction of the leech muscle when given at 10 times the concentration used for comparing their potentiating effect. It was found that 0.05 μg./ml. eserine sulphate caused either approximately the same degree of potentiation as 0.5 μg./ml. neostigmine methylsulphate, or an even more pronounced potentiation as shown in the experiment illustrated in Fig. 1. Eserine is thus at least 10 times as potent as neostigmine.

Bülbring (1946), Bülbring and Chou (1947) and Cohen and Unna (1951) have compared the effect of eserine and neostigmine on other striped muscle preparations. Their results, together with our own, are given in Table II. In potentiating the frog rectus muscle to acetylcholine, eserine was found to be only 1.8 times as potent as neostigmine and the anticurare action of eserine on the rat diaphragm was only 1.2 times greater than that of neostigmine. On the other hand, in potentiating the muscle twitch of the rat diaphragm, eserine was found to be active at about 1/10 of the concentration of neostigmine. This difference corresponds to that found for the potentiation of acetylcholine on the leech muscle. In our experiments an additional difference in the action of eserine and neostigmine on the leech muscle was observed. It concerned the duration of potentiation after washing out the anticholinesterase. After eserine, it took the muscle a shorter time to revert to its original sensitivity to acetylcholine than after neostigmine (Fig. 2).

In a few experiments of this kind the effect of dyflos caused about the same degree of potentiation as 0.5 μg./ml. of neostigmine or 0.05 μg./ml. of eserine, but after dyflos it took an even longer time for the muscle to revert to its original sensitivity to acetylcholine than after neostigmine.

TABLE II

COMPARISON OF POTENCY OF ESERINE AND NEOSTIGMINE ON CHOLINESTERASE AND STRIPED MUSCLE PREPARATIONS

Authors	Ratio of Activity Eserine/ Neostigmine	Pseudo-cholinesterase	True Cholinesterase	Method	Effect on Striped Muscle Preparations
Roepke (1937)	0·33	Horse serum		Titration	
Eadie (1942)	1·6	Dog ,,		Electrometric titration	
Blaschko, Bülbring, and Chou (1949)	0·67	Horse ,,		Warburg 50% inhibition	
Abrahams (personal communication)	1·6	Horse plasma (dried)		Warburg inhibition at equal concentration	
Bhattacharya and Feldberg (1958)	2	Horse plasma (dried)		Frog rectus assay	
Hawkins and Mendel (1946)	1		Dog caudate nucleus	Warburg 50% inhibition	
Blaschko, Bülbring, and Chou (1949)	4		Dog caudate nucleus	,, ,, ,,	
Cohen and Unna (1951) ..	10		Electric eel	,, ,, ,,	
Abrahams (personal communication)	1		Cat caudate nucleus	Warburg inhibition at equal concentration	
Bhattacharya and Feldberg	0·5		Cat caudate nucleus	Frog rectus assay	
,,	12		Leech muscle	,, ,, ,,	
Bülbring (1946)" .. " ..	10				Augmentation of muscle twitch (rat diaphragm)
Bülbring and Chou (1947) ..	1·2				Anticurare activity (rat diaphragm)
Cohen and Unna (1951) ..	1·8				Acetylcholine potentiation of frog rectus muscle
Bhattacharya and Feldberg	10				Acetylcholine potentiation of leech muscle

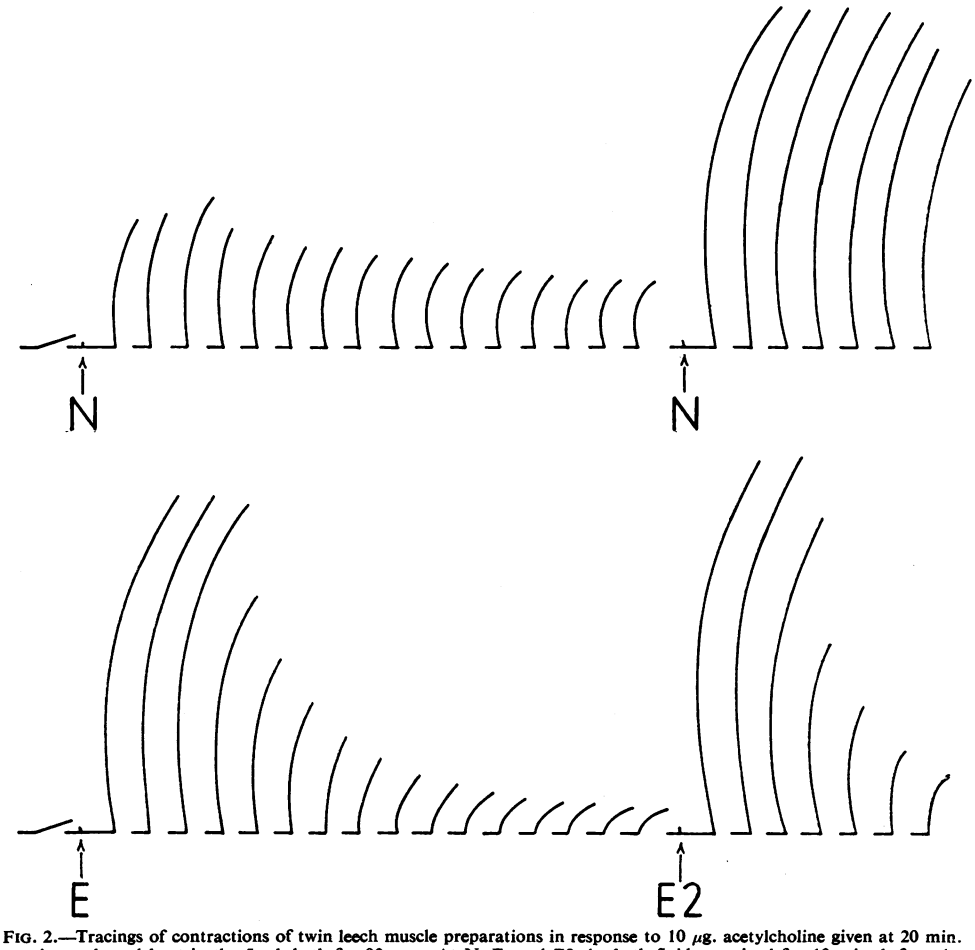

FIG. 2.—Tracings of contractions of twin leech muscle preparations in response to 10 μg. acetylcholine given at 20 min. intervals and kept in the 5-ml. bath for 90 sec. At N, E, and E2 the bath fluid contained for 10 min. before the subsequent acetylcholine contractions: 2.5 μg. neostigmine (N), 0.25 μg. eserine (E), and 1·25 μg. eserine (E2). The anticholinesterases were washed out immediately before the next acetylcholine contraction.

DISCUSSION

Eserine and neostigmine are generally looked upon as being anticholinesterases of about equal potency. However, in those experiments in which actual comparisons have been made, divergent results have been obtained. Some authors found eserine, others neostigmine, more potent in inhibiting pseudocholinesterase; in our experiments eserine was about twice as potent as neostigmine.

When the drugs were tested as inhibitors of true cholinesterase some authors found no difference between the two, others found eserine more active than neostigmine. We found a difference according to the source of the true cholinesterase. On homogenate of cat caudate nucleus and thalamus, eserine was only half as active as neostigmine, but on the true cholinesterase prepared from the leech body wall eserine was 12 times as active as neostigmine. The finding that eserine potentiates the acetylcholine contraction of the leech muscle in about 1/10 of the dose of neostigmine is thus fully accounted for by the greater sensitivity of the cholinesterase of this muscle to eserine. It would be interesting to know whether the cholinesterase of the rat diaphragm is also more sensitive to eserine than to neostigmine, because Bülbring (1946) found that eserine augmented the muscle twitch on this preparation in 1/10 of the dose of neostigmine.

The fact that the relative potencies of eserine and neostigmine vary so much on different preparations of true cholinesterase has to be taken into

account when trying to interpret in terms of anticholinesterase activity responses obtained on whole organs with these anticholinesterases. In addition, other factors, such as penetration into the tissue, a firmer attachment to the enzyme receptors may influence the response. For instance, the finding that the effect of neostigmine in potentiating the leech muscle to acetylcholine persisted longer than the potentiating effect of eserine after the anticholinesterase had been washed out may indicate that neostigmine becomes more firmly attached to the enzyme receptors and is thus a less reversible inhibitor than eserine. Firmer attachment may also occur when neostigmine is brought into contact with cholinesterase preparations *in vitro*, but it need not necessarily be revealed in measuring the inhibition by the usual methods. On the other hand, it may be a feature characteristic for the organization of the enzyme in the structure of the muscle fibre.

If the longer duration of the potentiating effect of neostigmine on the leech muscle, which is suggestive of a firmer attachment to the receptors of the muscle cholinesterase, were to apply to striated muscles and motor endplates in general, and particularly to those of human beings, this might explain why neostigmine is more effective in myasthenia gravis than eserine.

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