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THE ACTION OF CARBAMIC ESTERS AND TETRAETHYLPYROPHOSPHATE ON NORMAL AND CURARIZED FROG RECTUS MUSCLE

BY

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It has been suggested by many authors (see review by Augustinsson, 1948) that anticholinesterase drugs may act on skeletal muscle in two ways. Firstly, they may act by inhibiting cholinesterase, and hence preserving acetylcholine (Ach); secondly, they may exert a direct stimulating action on the endplate or muscle fibre. In the treatment of myasthenia gravis by anticholinesterase drugs some (e.g., neostigmine) have been found more effective clinically than others of equivalent anticholinesterase activity *in vitro* (e.g., eserine). This difference has been attributed partly to differences in intensity of the direct action on the muscle. Until recently it has not been possible to distinguish between the direct action and the anticholinesterase action, but with the introduction of anticholinesterases such as di*iso*propylfluorophosphonate (DFP) and tetraethylpyrophosphate (TEPP), whose actions are very little, if at all, reversible (Brauer, 1948; Jansen, Nutting, and Balls, 1948; Burgen, 1949), such analyses have been made. Riker and Wescoe (1946) and Miquel (1946) showed in the cat and frog respectively that neostigmine in suitable concentrations could produce a contracture of muscle after all the cholinesterase had apparently been inactivated by DFP. Miquel also demonstrated that under these conditions neostigmine and eserine in relatively low concentrations could still potentiate the action of Ach on the frog rectus preparation; this he attributed to direct action of these anticholinesterases on the muscle. Bacq (1947) on the other hand found that in the frog rectus, after preliminary treatment with DFP, eserine did not alter the response to Ach, while Quilliam and Strong (1949) noted in the same circumstances a reduced Ach response.

In this paper the following points have been studied on the frog rectus abdominis muscle:

(*a*) The direct stimulant action of carbamic esters such as neostigmine, eserine, Nu 683, and Nu 5130, and their influence on the sensitivity of the muscle to Ach.

(*b*) The responses to muscle stimulants other than Ach—e.g., nicotine and potassium.

(*c*) The anticurare actions of carbamic esters and TEPP.

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METHODS AND MATERIALS

The experiments were carried out in the period January to May with *Rana temporaria* frogs. The isolated rectus abdominis muscle was prepared as described by Chang and Gaddum (1933). It was set up in a bath of 2 ml. frog Ringer solution (0.6 g. NaCl, 0.0075 g. KCl, 0.01 g. $CaCl_2$, and 0.025 g. $NaHCO_3$ per 100 ml.; final *p*H 7.2). Each test was usually carried out during a period of 90 seconds, and the interval between the tests varied from 5–30 minutes depending on the rate of relaxation of the muscle, but in any case it was kept constant during any given experiment. Further details are given in the legends to the Figures and in the text relating to different experiments.

In order to produce complete inhibition of cholinesterase TEPP was added to the bath in a concentration of 3–5 μg./ml. for 30 minutes. In order to maintain this inhibition of the enzyme it was necessary to add TEPP in a concentration of 0.02 μg./ml. to the Ringer solution used during the tests of the responses to the various muscle-stimulating substances.

For estimation of cholinesterase activity the muscle was ground with sand in a mortar, suspended in Ringer's solution containing Ach in a final concentration of 1 μg./ml. The mixture was then incubated at 19° C. and the residual Ach assayed on another rectus muscle after different intervals. This method is more sensitive than the manometric technique described by Ammon (1933). It was found with this procedure that treatment with TEPP, as described above, reduced the rate of hydrolysis of Ach to less than 2 per cent of that in the absence of TEPP and hence provided the required background for the testing of the direct effects of other anticholinesterases.

In some of the experiments the isolated rat phrenic nerve diaphragm preparation (Bülbring, 1946) was used for estimation of anticurare activity of neostigmine and Nu 5130, and the manometric method (Ammon, 1933) for comparison of their anticholinesterase potencies.

TEPP was made up as a 5 per cent (v/v) stock solution in dry propyleneglycol and from this an aqueous solution was made immediately before use.

Nu 683 is the dimethylcarbamic ester of 5-phenyl-2-hydroxy-benzyltrimethylammonium bromide and Nu 5130 is the dimethylcarbamic ester of 3-hydroxymethylpyridine hydrobromide. Neostigmine methylsulphate and eserine salicylate were also used; they will be referred to as neostigmine and eserine, but all results relate to their salts.

RESULTS

Direct stimulant action of carbamic esters on the frog rectus muscle

Neostigmine had no effect on the rectus muscle until with a concentration of 25 μg./ml. a small contracture occurred. With higher concentrations this contracture became larger. The dose-response curve, however, rapidly flattened out so that with concentrations exceeding 500 μg./ml. virtually no further increase in the contracture occurred, and even with 1 mg./ml. the contracture was less than that produced by 0.25 μg./ml. of Ach. When the muscle was exposed to neostigmine for periods exceeding 90 seconds the dose-response curve was steeper but still flattened out at a level below the height of the maximal Ach-contracture obtained on the same muscle. In Fig. 1 the dose-response curve of neostigmine is compared with that of Ach before and after sensitization of the muscle with TEPP. It will be seen that the rectus was very much less sensitive to neostigmine

than to Ach, and, as was to be expected, the sensitivity to neostigmine was quite unaffected by sensitization of the muscle with TEPP. The minimum stimulating concentration of eserine was about 1 mg./ml. It was noticed that the latent period with eserine was longer than with Ach or neostigmine. The effect of eserine was also unchanged by TEPP.

The anticholinesterases Nu 683 and Nu 5130 had no stimulating effect whatever, even in concentrations of 2 mg./ml.

Concentrations of carbamic esters required to sensitize the rectus to Ach

The action of the carbamic esters on the sensitivity of the rectus muscle to Ach was examined in the following way: an Ach-dose-response curve was first established on an untreated muscle; then one of the carbamic esters was added in a low concentration to the bath for 30 minutes and an Ach-dose-response curve again recorded. This procedure was repeated with increasing concentrations of the carbamic ester until maximal sensitization to Ach was produced. At this point the muscle was repeatedly washed and 5 μg. TEPP/ml. added to the bath for 30 minutes. The sensitization which could be achieved by TEPP was taken as 100 per cent and the effects of the different concentrations of the carbamic ester were expressed as a percentage of this. A 30-minute period of exposure to the drugs was chosen after preliminary experiments had shown that this duration was necessary to allow the sensitization to reach a steady level.

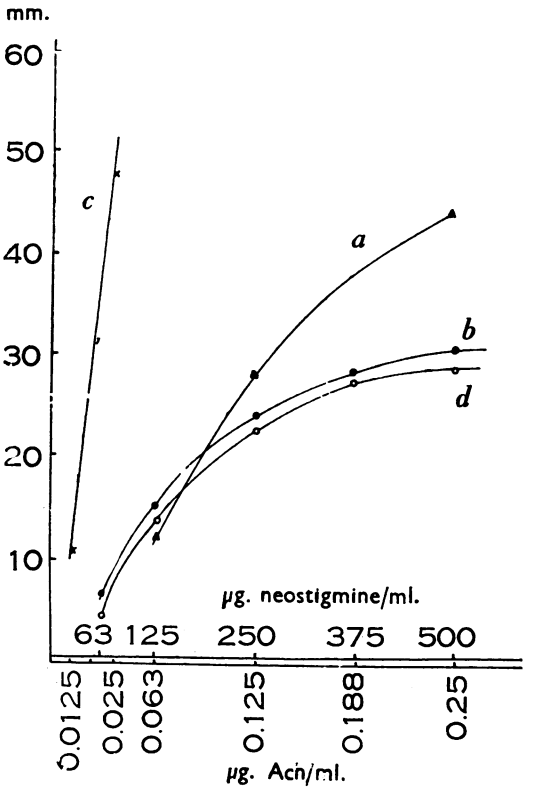

FIG. 1.—Dose-response curve of acetylcholine and neostigmine on the frog rectus abdominis muscle before and after tetraethylpyrophosphate (5 μg./ml. for 30 min.). Duration of tests 90 sec. Ordinates: height of contractures in mm. Abscissae: concentration of Ach (-chloride) and neostigmine (-methylsulphate) in μg./ml. (final concentration). (*a*) Ach before TEPP, (*b*) neostigmine before TEPP, (*c*) Ach after TEPP, (*d*) neostigmine after TEPP.

In Fig. 2 the effects of different concentrations of the four carbamic esters on the sensitivity of the rectus muscle to Ach are summarized. It will be seen that it was only possible to obtain full sensitization with neostigmine (4 μg./ml.); Nu 5130 (21 μg./ml.) produced 90 per cent, eserine (10 μg./ml.) about 60 per cent, and Nu 683 (12 μg./ml.) 20 per cent of the sensitization given by TEPP. These concentrations of the carbamic esters were optimal, and a further increase in their concentration had only a depressant effect on the sensitivity of the rectus

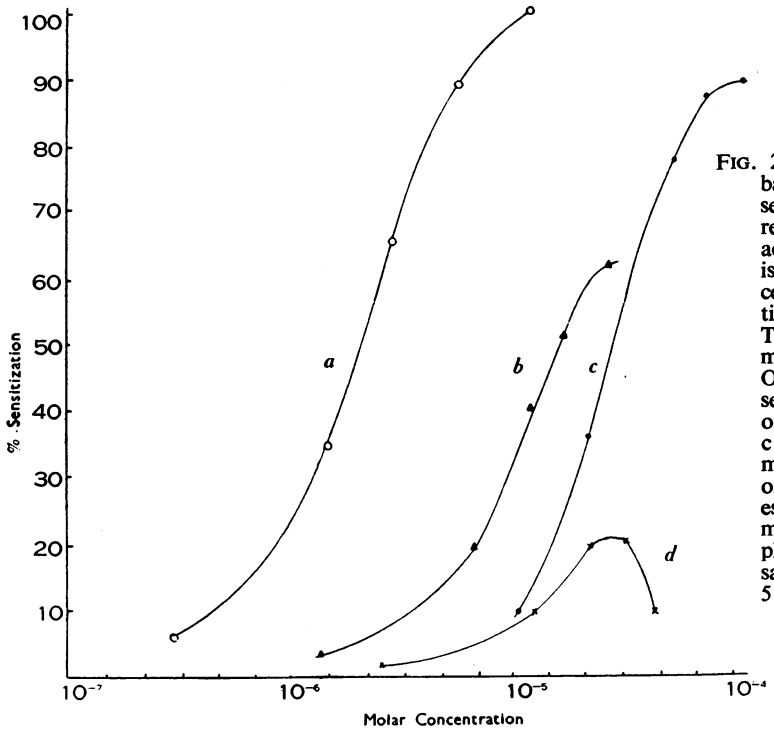

FIG. 2.—Effect of carbamic esters on the sensitivity of the frog rectus to Ach. The action of each drug is expressed in per cent of the sensitization produced by TEPP on the same muscle (see text). Ordinates: Per cent sensitization (effect of TEPP = 100 per cent). Abscissae: molar concentration of the carbamic esters. (*a*) Neostigmine methylsulphate, (*b*) eserine salicylate, (*c*) Nu 5130, (*d*) Nu 683.

to Ach. It can be further seen from Fig. 2 that the concentrations of neostigmine and eserine which are required to give an optimal sensitization are considerably lower than those which produce a direct stimulating effect on the muscle. The ratio: $\frac{\text{Direct stimulating dose}}{\text{Maximal sensitizing dose}}$ was for neostigmine about 6–8, for eserine about 100, and for Nu 683 and Nu 5130 it must be more than 100.

That the difference in the degree of sensitization by the four carbamic esters is not due to inadequate inhibition of the muscle cholinesterase was indicated by experiments in which ground rectus muscles were incubated at room temperature (19–21° C.) for 30 minutes with each of these drugs. Ach in a final concentration of 1 μg./ml. was then added and the Ach breakdown tested on a sensitized rectus after different intervals. The results obtained are shown in Table I and represent for each group the means of three experiments.

These experiments show that all four carbamic esters, in the concentrations used, were about equally potent as inhibitors of the muscle cholinesterase.

Modification of the response of the fully sensitized rectus to Ach by the carbamic esters

The concentrations of neostigmine, eserine, and Nu 5130, shown previously to produce maximal sensitization of untreated muscle to Ach, did not influence the response to Ach of a muscle fully sensitized by TEPP.

TABLE I

HYDROLYSIS OF ACETYLCHOLINE BY GROUND RECTUS MUSCLE IN PRESENCE OF CARBAMIC ESTERS

60 mg. ground rectus muscle used in each experiment. Final volume 10 ml. See text

Carbamic ester	Final concn. μg./ml.	% Ach hydrolysis at			Maximal sensitizing concn. μg./ml.
		25 min.	50 min.	70 min.	
Neostigmine methylsulphate	5	8	20	32	4
Eserine salicylate	10	5	15	28	10
Nu 683	15	10	25	35	12
Nu 5130	25	9	20	34	21

If, however, *neostigmine* was added to the bath 5 seconds before Ach in concentrations which by themselves produced a contracture, the response of the muscle depended on the amount of Ach applied. With low concentrations of Ach the contracture was now larger than that given by Ach alone. With higher concentrations of Ach the contracture to Ach plus neostigmine was smaller than that given by Ach alone, as may be seen in Fig. 3 (curve *C*). As the concentration of neostigmine was increased this transformation was accentuated so that the

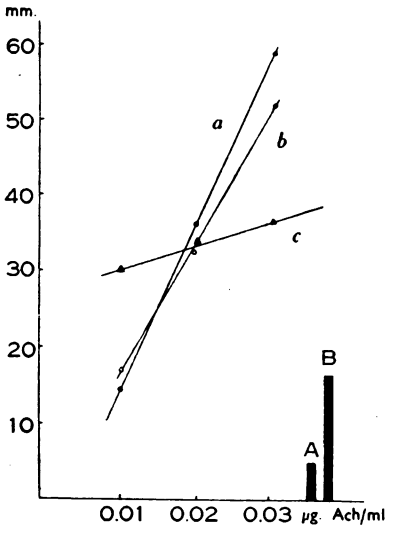

FIG. 3

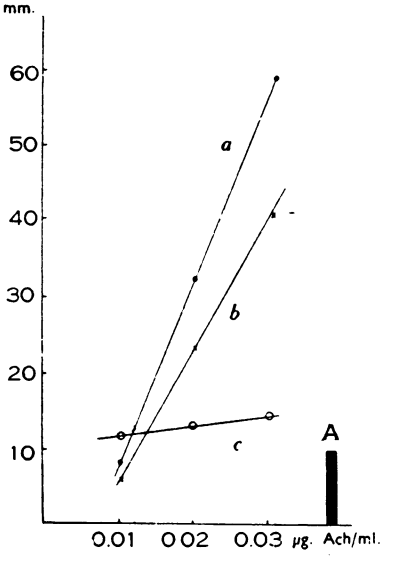

FIG. 4

FIG. 3.—Effect of neostigmine on the sensitivity to Ach of a rectus previously treated with TEPP. Neostigmine was added 5 sec. before Ach. Duration of tests 90 sec. Ordinates: height of contractures in mm. Abscissae: concentrations of Ach-chloride in μg./ml. (*a*) Control Ach dose-response curve after TEPP. (*b*) Ach + neostigmine (65 μg./ml.), (*c*) Ach + neostigmine (250 μg./ml.). The height of contracture by neostigmine alone is given in A (65 μg./ml.) and B (250 μg./ml.).

FIG. 4.—Effect of eserine on the sensitivity to Ach of a rectus previously treated by TEPP. Eserine was added 5 sec. before Ach. Duration of tests 90 sec. Abscissae: concentrations of Ach-chloride in μg./ml. (*a*) Ach dose-response curve after TEPP, (*b*) Ach + eserine (0.1 mg./ml.), (*c*) Ach + eserine (2 mg./ml.). (A) is the height of contracture by eserine alone (2 mg./ml.).

slope of the Ach-dose-response curve became more and more flattened. After *eserine* this change in the response of the muscle to Ach was somewhat different. Here with concentrations below those producing a direct stimulating effect the response to Ach was diminished over the whole range of Ach-dosage, as may be seen in Fig. 4. When doses, which by themselves produced a contracture, were used the response of the muscle to Ach was similar to that described for neostigmine, but the change in slope was even more marked.

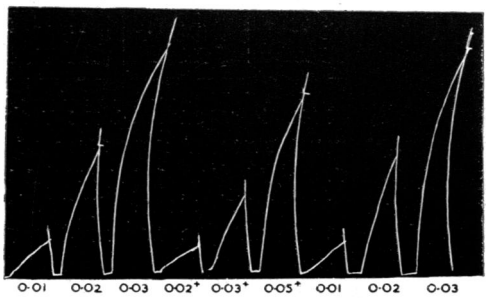

FIG. 5

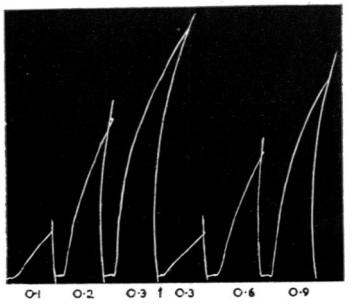

FIG. 6

FIG. 5.—Record of the effect of Nu 683 on the sensitivity to Ach of the frog rectus sensitized by TEPP. Abscissae: concentration of Ach-chloride in μg./ml. Duration of tests 90 sec. In the tests marked by + 15 μg. Nu 683/ml. was added to the bath 5 sec. before Ach.

FIG. 6.—Record of the effect of *d*-tubocurarine on the sensitivity of the frog rectus to Ach. Abscissae: concentrations of Ach-chloride in μg./ml. At ↑ *d*-tubocurarine (0.5 μg./ml.) was added to the bath for 30 min. and Ach tested again in its presence. Duration of tests 90 sec.

With Nu 683 and Nu 5130, as noted previously, no direct stimulating action could be demonstrated, and both these substances exerted only a depressant effect on the response of fully sensitized muscle to Ach. Nu 5130 was as potent as eserine, but with Nu 683 the depressant action occurred with much lower concentrations than with the other two substances (Fig. 5). The blocking effect of the carbamic esters on the sensitivity of the rectus to Ach closely resembled that produced by *d*-tubocurarine on non-sensitized muscle (Fig. 6).

Modification by carbamic esters of the response of the fully sensitized rectus to other stimulating drugs

The carbamic esters modified the response of sensitized muscle to stimulants such as nicotine, carbamylcholine, and decamethonium iodide in the same way as they altered the response of the muscle to Ach. However, stimulating concentrations of these esters enhanced the effect of KCl additively (Fig. 7), while depressant concentrations of the esters for Ach did not significantly reduce the response to KCl (Fig. 8).

Anticurare action of the carbamic esters and TEPP

The anticurare action of the anticholinesterases has been examined in the following way. A dose-response curve to acetylcholine was first established on the non-sensitized muscle (Fig. 9, curve A); the muscle was then soaked in Ringer solution containing 0.5 μg. *d*-tubocurarine chloride/ml. for 30 min., after which

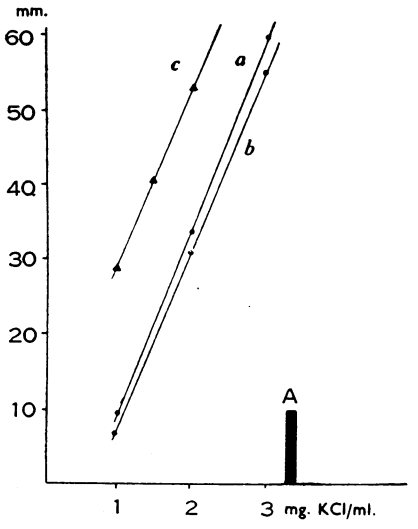

FIG. 7

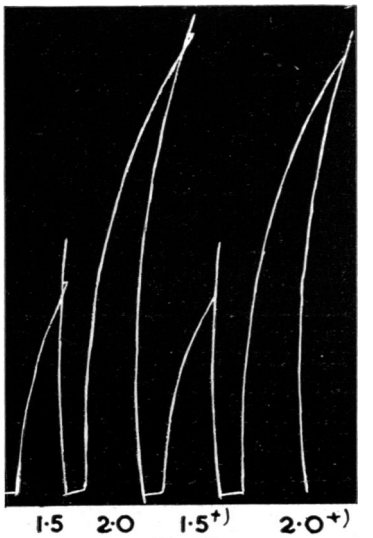

FIG. 8

FIG. 7.—Effect of neostigmine on the stimulating action of KCl on the frog rectus. Abscissae: concentrations of KCl in mg./ml. Duration of tests 90 sec. Interval between tests 20 min. (*a*) KCl dose-response curve, (*b*) KCl + neostigmine methylsulphate (150 μg./ml.), (*c*) KCl after addition of *d*-tubocurarine (0.5 μg./ml.) for 30 min. (A) Gives the height of contracture by neostigmine (150 μg./ml.) on the same muscle.

FIG. 8.—Record of the effect of Nu 683 on the stimulating action of KCl on the frog rectus. Abscissae: concentrations of KCl in mg./ml. Duration of tests 60 sec. Interval between the tests 25 min. In the tests marked by +) 20 μg. Nu 683/ml. were added 5 sec. before KCl.

time a constant degree of depression had been produced. The dose-response curve to acetylcholine was then retested in the presence of this amount of *d*-tubocurarine (Fig. 9, Curve B). It will be seen that both the absolute sensitivity and the slope of the response were diminished (see also Fig. 5). The muscle was then repeatedly washed until the sensitivity to acetylcholine had returned to the same level as before addition of *d*-tubocurarine. The muscle was now sensitized with an anticholinesterase and the sensitivity to acetylcholine tested once again (Fig. 9, curve C) and finally the muscle was soaked again in *d*-tubocurarine and the sensitivity tested in the presence of both *d*-tubocurarine and the anticholinesterase (Fig. 9, curve D). Provided that the dose of anti-

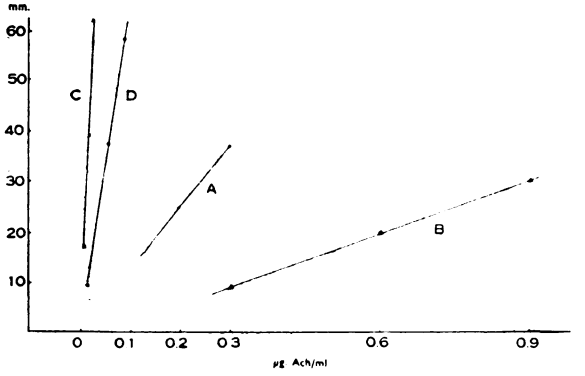

FIG. 9.—Effect of *d*-tubocurarine on the sensitivity to Ach of the sensitized and not-sensitized frog rectus. Dose-response curves for Ach: (A) in unsensitized muscle; (B) in presence of tubocurarine; (C) in muscle sensitized with TEPP; and (D) in presence of both TEPP and tubocurarine.

TABLE II

ANALYSIS OF DOSE-RESPONSE CURVES IN FIG. 9

Anticholinesterase drug	Concn. μg./ml.	Mean ratio of slopes (range in parentheses)		AD/BC	No. of exps.
		A/B	C/D		
Neostigmine methylsulphate	4	3.66 (3.23–4.09)	3.20 (2.74–3.66)	1.14	11
Eserine salicylate	10	3.52 (3.32–3.72)	3.26 (3.05–3.47)	1.08	4
TEPP	5	3.67 (3.29–4.05)	3.29 (2.88–3.70)	1.11	8
Nu 5130	21	3.48 (3.23–3.73)	3.31 (3.01–3.61)	1.05	3

cholinesterase was not higher than that which produced optimal sensitization, there was no appreciable difference between the results obtained with TEPP, neostigmine, eserine, and Nu 5130. Analysis of the dose-response curves obtained in this way gave the figures recorded in Table II.

These results show that doses of the carbamic esters and TEPP which produce optimal sensitization (see Fig. 2) are equipotent in their antagonism towards *d*-tubocurarine. Nu 683 in concentrations up to 9 μg./ml. acted similarly.

These anticholinesterases did not prevent the antagonism of *d*-tubocurarine towards nicotine, carbamylcholine, and decamethonium iodide.

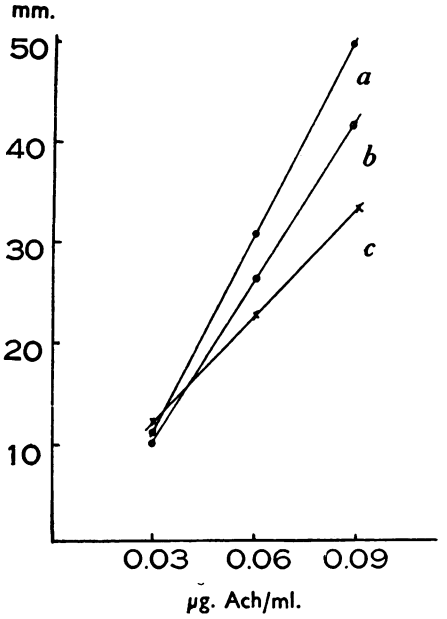

FIG. 10.—Effect of neostigmine on the sensitivity of the frog rectus to Ach after TEPP and *d*-tubocurarine. Abscissae: concentration of Ach-chloride in μg./ml. Duration of tests 90 sec. (*a*) Ach dose-response curve, (*b*) Ach + neostigmine methylsulphate (75 μg./ml.), (*c*) Ach + neostigmine methylsulphate (150 μg./ml.).

It should also be mentioned here that the direct stimulating effect of neostigmine and eserine on sensitized and non-sensitized muscle could be antagonized by *d*-tubocurarine. Pentamethonium iodide in a concentration of 50 μg./ml. behaved in the same way as 0.5 μg. *d*-tubocurarine/ml.

The effect of high concentrations of the carbamic esters on curarized, sensitized muscle

In these experiments the rectus muscle was fully sensitized with TEPP and treated with *d*-tubocurarine (0.5 μg./ml. for 30 min.). All tests were then carried out in the presence of *d*-tubocurarine. The addition of neostigmine in a concentration of 50 μg./ml. or more, 5 seconds before the addition of Ach, reduced the sensitivity of the preparation towards Ach (Fig. 10). In these circumstances the blocking action of neostigmine against Ach could still be seen, but its direct stimulating action was considerably reduced.

Similar effects were seen with eserine and Nu 5130 in concentrations above those which give a maximal sensitization of the muscle. The curarizing effect of Nu 683 occurred in concentrations above 10 μg./ml.

Discussion

The rate of hydrolysis of acetylcholine by ground frog rectus muscle can be reduced to less than 2 per cent of normal by TEPP. The sensitization to Ach by the carbamic esters neostigmine, eserine, Nu 683, and Nu 5130 has been compared with the effect of TEPP with the following result: neostigmine sensitizes the rectus to the same extent as TEPP, and Nu 5130 is only very little weaker. Eserine can only produce about 60 per cent and Nu 683 about 20 per cent of the full sensitization produced by TEPP. It has been shown (see Table I) that these differences in sensitizing capacity are not due to differences in cholinesterase inhibition. It must further be emphasized that with TEPP a rapid irreversible combination with cholinesterase occurs (Augustinsson and Nachmansohn, 1949). However, with the carbamic esters, which combine reversibly with cholinesterase, the figures for cholinesterase inhibition in our experiments with 90 seconds' exposure to Ach or other drugs are higher than those summarized in Table I, since sufficient time is not allowed for the establishment of an equilibrium between the reacting components cholinesterase, Ach and inhibitor (Goldstein, 1944; Goldstein *et al.*, 1949).

Neostigmine and eserine in concentrations above those necessary to produce optimal sensitization produce a contracture of the muscle by some direct action. With neostigmine this stimulating concentration is only 6–8 times greater than that which produces optimal potentiation of the response to Ach, but with eserine this ratio is about 100; with Nu 683 and Nu 5130 there was no stimulation at all in concentrations up to 2 mg./ml.

Concentrations of the carbamic esters exceeding those which produce optimal sensitization towards Ach, modify the responses to Ach in muscle fully sensitized by TEPP. It has been shown that Nu 683, Nu 5130, and substimulant doses of eserine have a depressant action on the response to Ach of muscle sensitized by TEPP. A similar blocking effect of eserine towards Ach has been described by Hobbiger and Werner (1948) for the leech muscle. With stimulant doses of eserine and neostigmine (above 25 μg./ml.) the resultant effect is the sum of their direct stimulant action and a depressed Ach-response (see Figs. 2 and 3). Like the depression of the Ach-response by *d*-tubocurarine, not only is the response to a dose of Ach diminished in the presence of these higher concentrations of carbamic esters, but the slope of the Ach-dose-response curve is also decreased. That this depressant action of the carbamic esters is essentially analogous to that of *d*-tubocurarine can be seen from the experiments in which potassium chloride was used as a muscle stimulant. On the cat muscle the action of potassium is not antagonized by *d*-tubocurarine (Brown, 1937). The experiments on the frog rectus, here described, have shown that 0.5 μg. *d*-tubocurarine/ml., which very considerably reduced the response to Ach (Fig. 6), had only a very slight effect on the action of KCl (Fig. 8). These findings are in agreement with the results of Buchthal and Lindhard (1942) on lizard muscle. If now the depressant action of the carbamic esters were analogous to that of *d*-tubocurarine, we should expect

to find that when they were administered together with potassium only addition of the stimulating actions, with practically no depression, would occur. This was indeed the case, and the results obtained agree well with those to be expected from simple addition. The stimulating effect of neostigmine or eserine was furthermore antagonized by *d*-tubocurarine ; hence we should expect that to some extent their stimulating action must be self-limited owing to the development of a depressant action. This may explain the dose-response curve for neostigmine shown in Fig. 1 which flattens out well below a maximum contracture.

These rather complex effects of carbamic esters on the actions of Ach and other parasympathomimetic drugs may also explain some of the results obtained by other workers, since the experiments described here give no evidence for a non-specific sensitizing action of eserine or neostigmine, which was assumed by them. Feldberg and Vartiainen (1934) found that on the perfused superior cervical ganglion of the cat eserine greatly potentiated the response not only to injected Ach but also to nicotine, choline, or KCl. They attributed these effects to a non-specific sensitizing action of eserine. Banister, Hebb, and Konzett (1949) on the other hand showed that eserine had no potentiating effect on the action of KCl or NH_4Cl on the denervated ganglion. It seems therefore possible that this non-specific effect of eserine on the innervated ganglion and also on other structures could be due to some accumulation of Ach in the presence of eserine.

The apparent discrepancies between the work of Miquel (1946) on the one hand, and Bacq (1947) and Quilliam and Strong (1949) on the other hand, as to whether eserine increases or decreases the sensitivity of the rectus to Ach after inactivation of the cholinesterase, can now be discussed. With 100 μg./ml. of eserine only a depressant action on the response of a TEPP-sensitized muscle to Ach was observed. With 2 mg./ml., however, an increase in response could be demonstrated on the lower part of the Ach range (Fig. 3). The manometric method of determination employed by Miquel is so insensitive that a discrimination between a complete inactivation of the cholinesterase and 80–90 per cent inhibition is extremely uncertain. This fact could readily account for Miquel's results as has been suggested also by Quilliam and Strong (1949). But it must also be remembered that the inactivation of cholinesterase by DFP at room temperature is partly reversible for several hours, as shown by Bullock, Grundfest, Nachmansohn, and Rothenberg (1947).

Anticurare action of anticholinesterases

The observations on the anticurare action of the carbamic esters and TEPP yield several points of interest. Firstly, with doses up to those which gave a maximum anticurare action there was good agreement between capacity to sensitize the muscle to Ach, and antagonism towards curare. Secondly, when the anticholinesterase effect was irrelevant, as when carbamylcholine or nicotine was used as the excitant, no anticurare action was produced by carbamic esters or TEPP. Thirdly, when the cholinesterase was first inactivated by TEPP the anticurare effect of the carbamic esters completely disappeared and in higher concentrations some additive effect with curare occurred. This last mentioned effect was not equal for all the carbamic esters but was seen especially with Nu 683 and explains why this drug is relatively weak in its sensitizing capacity to Ach

(Fig. 2). It is interesting that Blaschko, Bülbring, and Chou (1949) found that for neostigmine and eserine a reasonably good correlation exists between *in vitro* anticholinesterase activity and anticurare action on the rat phrenic-nerve-diaphragm preparation. They further noted that Nu 683 and Nu 5130 were relatively less potent in their antagonism to curare if inhibition of true cholinesterase was taken for comparison ; but this difference was not significant. The experiments described in this paper suggest that the curariform action of Nu 683 might be responsible for such a peculiarity. But Nu 5130 was as powerful as neostigmine in its antagonism to curare on the rectus, and further experiments have shown that neostigmine and Nu 5130 are equipotent also in their antagonism to curare on the rat phrenic-nerve-diaphragm preparation.

Lehmann (1946) described a curare-like action of neostigmine analogues on dog and cat muscle, but concluded that it was somewhat different from the action shown by curare itself. In our opinion it is difficult to draw such conclusions from experiments in the whole animal, since in the presence of higher concentrations of anticholinesterases not only will a curare-like action of these drugs be noticed but also the accumulation of Ach will produce some neuromuscular block. It is the advantage of the isolated rectus preparation that this second possibility is nearly completely ruled out.

Eccles and MacFarlane (1949) examined the changes in endplate potential of the curarized frog sartorius muscle when various anticholinesterases were applied ; they found that with lower concentrations there was an increase in the height and duration of the endplate potential, but with higher concentrations the height of the endplate potential declined. This decline may in part be due to the mechanism described here, but it must also be borne in mind that with high concentrations of anticholinesterases some decrease in Ach output may occur, owing to blocking of conduction in the terminal nerve fibres.

From these experiments it can be concluded that—

(1) Carbamic esters, in low concentrations, sensitize the frog rectus muscle to Ach by inhibition of cholinesterase.

(2) Neostigmine and eserine, in concentrations well above those necessary for sensitization to Ach, have a direct stimulating effect on the muscle.

(3) The anticurare effect of these drugs is due solely to inhibition of cholinesterase.

(4) After complete inhibition of cholinesterase by TEPP, higher concentrations of the carbamic esters have a curare-like blocking action towards Ach.

SUMMARY

The actions of the carbamic esters, neostigmine, eserine, Nu 683, and Nu 5130 have been studied on the isolated frog rectus abdominis muscle and were found to be as follows:

1. *Sensitization of the rectus response to Ach*:—5 μg. neostigmine/ml. gave the same degree of sensitization as 5 μg. TEPP/ml. This concentration of TEPP completely inhibited the cholinesterase in ground muscle. Nu 5130 was nearly as active as neostigmine, but eserine produced only about 60 per cent and Nu 683 about 20 per cent of the sensitization produced by TEPP.

2. *Direct stimulating action*:—Neostigmine in a concentration of 25 μg./ml. and higher, and eserine in a concentration over 1 mg./ml., produced some contracture of the muscle which was unaffected by previous treatment of the muscle with TEPP; this stimulant action was antagonized by *d*-tubocurarine. Nu 683 and Nu 5130 had no stimulating actions in concentrations up to 2 mg./ml.

3. *Blocking action against Ach*:—Nu 683, Nu 5130, and eserine in concentrations below 1 mg./ml. decreased the response of fully sensitized muscle to Ach. Higher concentrations of eserine, or neostigmine above 25 μg./ml., which stimulated the muscle by themselves, had on the fully sensitized muscle some additive effect with lower concentrations of Ach, but depressed the response to higher concentrations of Ach. Similar results were obtained if, instead of Ach, other parasympathomimetic drugs or nicotine were used. The depressant actions of the carbamic esters were analogous to that of *d*-tubocurarine in that they did not antagonize the stimulant actions of KCl.

4. *Anticurare action*:—Good agreement was shown between sensitizing effect and anticurare action of the carbamic esters and TEPP. On muscle fully sensitized by TEPP the carbamic esters no longer had an anticurare action, but their blocking effect on the response of the muscle to Ach could still be demonstrated. TEPP and the carbamic esters had no anticurare action when nicotine or other parasympathomimetic drugs were used as muscle stimulants.

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