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PHARMACOLOGICAL EXPERIMENTS ON MAMMALIAN VOLUNTARY MUSCLE, IN RELATION TO THE THEORY OF CHEMICAL TRANSMISSION

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(*Received* 25 *September* 1936)

It was recently shown by Brown *et al.* [1936] that a very small dose of acetylcholine, injected directly into the empty blood vessels of a normal mammalian muscle, causes a quick contraction of the muscle, resembling a rather slow, single twitch. This contraction has now been shown by electrical records [Brown, 1936] to be a short, asynchronous tetanus, and not a contracture. It was also shown that, under suitable conditions, a small dose of eserine greatly potentiates the response of a mammalian muscle to a single maximal volley of impulses in its motor nerve, and that this potentiated "twitch" is also due to a repetitive response of the muscle, causing a brief, waning tetanus. It was suggested that these observations support the conception of a chemical transmission of excitation from motor nerve to voluntary muscle, by the release of acetylcholine at the nerve endings. The object of our experiments has been to extend these observations, and to test further their bearing on this theory.

The longer known effect of acetylcholine on denervated mammalian muscle, producing a response which is largely, at least, a contracture, was shown by Dale & Gasser [1926] to belong to that group of its actions which resemble those of nicotine [Dale, 1914]. It was of interest to know whether acetylcholine produced the rapid response of normal mammalian muscle in virtue of the same nicotine-like activity. We have therefore tested for this effect a number of choline esters and other substances having a "nicotine" action, and one choline ester of which the action is almost entirely of the "muscarine" type. Brown *et al.* attributed the potentiating action of eserine to inhibition of cholinesterase at the motor nerve endings. We have accordingly examined a series of synthetic

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substances, for which we are indebted to Dr Stedman, known to possess the inhibitory action on cholinesterase in different degrees, some being stronger and others weaker than eserine itself. We have also further examined the conditions which are required for the production of this potentiating action by eserine and its synthetic analogues, and have compared them with those under which veratrine produces a superficially similar effect.

Methods

The methods employed throughout this investigation have been essentially the same as those used by Brown *et al.* Cats and rabbits were used, under various anæsthetics, or spinal or decerebrate. The gastrocnemius muscle or the tibialis anticus was used for recording, the muscle being arranged to pull vertically on an isometric lever, with the femur or tibia and fibula immobilized by drills attached to the heavy iron bedplate of the apparatus. Muscles with natural circulation were used throughout, and injections were made, by the method earlier described in detail [Brown *et al.* 1936], into the gastrocnemius by means of a cannula in the tibial artery, the popliteal artery being occluded at the moment of injection. We have found this method to be particularly useful, not only for the rapid injections of choline esters in small amounts, but also for the direct introduction into muscle of substances which, if injected into the general circulation in sufficient amount to affect the muscle, would be lethal or very toxic to the animal under experiment. This technique, moreover, allows of more precise determination of threshold doses and, by the more rapid removal of the substance from the muscle, permits a number of observations to be made on the same preparation.

In most experiments the motor nerve to the muscle was stimulated continuously by maximal break induction shocks at a frequency of 1 in 10 sec., timed by a Lewis rotating contact breaker. When injections of a stimulating substance were made, one stimulus was omitted from the series, and the injection was made at the time when it would have occurred.

The substances used were injected in a volume of 0·5 c.c. of saline acidified to *p*H 4 with HCl—this solution by itself being without action on the muscle in this volume.

In all experiments in which injections of large doses of choline esters or of inhibitors of cholinesterase were made, the circulatory effects were avoided by previous intravenous administration of 1 mg. of atropine to the animal.

Results

(1) *Unstable choline esters*

The property of the acetyl-ester of choline in producing quick contractions of normal skeletal muscle is shared by the other unstable esters. In Fig. 1 is shown the response of the gastrocnemius of the cat to close arterial injections of butyryl-, valeryl- and propionylcholine, compared with that of 5γ of acetylcholine. It is seen that these substances produce a transient muscle twitch a little weaker in tension than that produced by acetylcholine. Like the latter, the injection is without effect upon the subsequent contractions of the muscle in response to maximal nerve stimulation. This transience of action is distinctive of the unstable esters which, in the absence of eserine, are rapidly hydrolysed by the muscle esterase.

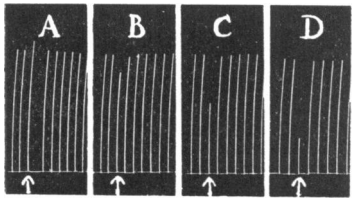

Fig. 1.

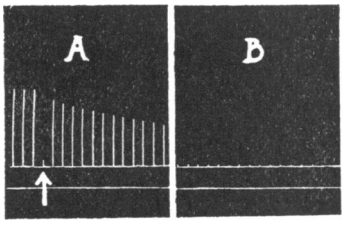

Fig. 2.

Fig. 1. Cat, chloralose 2·4 kg. Contractions of gastrocnemius in response to maximal shocks to nerve at 10 sec. intervals. A, 5γ acetylcholine by arterial injection, during intermission of one shock to nerve; B, 5γ butyrylcholine; C, 5γ valerylcholine; D, 3·5γ propionylcholine.

Fig. 2. Same experiment as Fig. 1. A, arterial injection of 50γ carbaminoylcholine; B, 7½ min. after injection.

(2) *Stable choline esters*

Brown *et al.* have shown that any process which prevents the rapid removal of acetylcholine from the muscle, when its immediate action is over, gives rise to a more or less prolonged depression of the response of the muscle to nerve stimulation. This persistence of acetylcholine was brought about by either eserine, or by the slowing of the circulatory exchange of the muscle when œdema had developed after prolonged perfusion. Should the effect, in fact, be due to the persistence of the choline ester after its exciting action is over, then the injection of a stable ester might be expected to produce such an effect in the muscle with natural circulation, and in the absence of eserine. Such conditions are fulfilled by carbaminoylcholine. In Fig. 2 is shown the effect of the

injection of 50γ of carbaminoylcholine. Its stimulant action is very weak, but it is followed by a long-lasting progressive depression of the response of the muscle. This "curarization" in the particular experiment illustrated was complete in 12 minutes and was apparently irreversible.

Acetyl-β-methylcholine is completely without action upon normal muscle. In doses from 50γ to 1 mg. it caused neither contraction of the muscle at the moment of injection, nor any subsequent depression. This is in accordance with Simonart's finding [1932] of the absence of a nicotine effect of this substance on the ganglion cell.

(3) *Other substances*

Choline itself shows, but to a very weak degree, the activity on muscle of its esters. An injection of 1 mg. produced a just visible contraction, and 5 mg. produced a rather slow contraction approximately

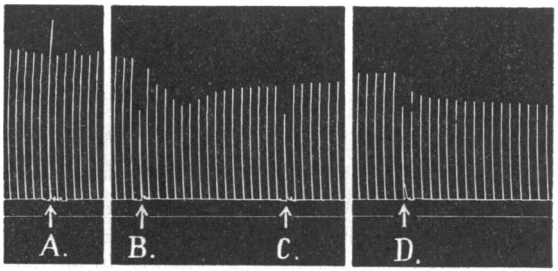

Fig. 3. Cat, chloralose. Contractions of gastrocnemius in response to maximal shocks to nerve at 10 sec. intervals. A, arterial injection of 10γ acetylcholine during intermission of one shock to nerve; B, 5 mg. choline; C, 10γ acetylcholine; D, 0·5 mg. nicotine tartrate.

equal in tension to that following injection of 5γ acetylcholine (Fig. 3 A and B). Its activity is therefore about 1/0000th of that of acetylcholine, a relation very close to that obtained with other tissues responsive to choline and acetylcholine. The contraction produced by choline is followed by some depression of the muscular response to nerve stimulation. This depression reaches its maximum in 40–50 sec. and is followed by slow recovery, complete in some 5 min.

A substance closely allied to choline chemically, and possessing the property of exciting the contracture of denervated muscle, is tetramethylammonium iodide [Dale & Gaddum, 1930]. This substance acts on normal muscle in a way almost identical with that of choline. Arterial injection of 0·5 mg. produces a twitch similar to the response to about

7γ acetylcholine, followed by a gradually developing depression and subsequent recovery of the motor nerve twitch, exactly like that following injection of choline.

Nicotine itself has a surprisingly weak effect. In amounts up to 100γ it is without any apparent effect. A quick injection of a dose of 0·5 mg., however, is followed by a rapid contraction equal in tension to that produced by 5γ acetylcholine. The contraction is followed by a steady depression (Fig. 3). This depression is remarkable, in that it is removed completely by the stimulation of the nerve for a short time with a tetanizing current, and equally effectively by the arterial injection of sufficient acetylcholine to produce a "twitch" of tension approximately equal to that produced by the tetanic stimulation.

(4) *Action of eserine*

A number of experiments were made to elucidate certain points in connexion with the potentiation, by eserine, of the response of muscle to single maximal motor nerve volleys.

Source of acetylcholine. Although there is every reason to believe that eserine owes its effects to protection of the acetylcholine liberated at the motor nerve endings, it appeared possible that part, at least, of the action might be accounted for, if cholinergic sympathetic fibres were present in the nerve, and if acetylcholine liberated by the stimulation of these could, when protected by eserine, produce an effect upon the muscle. This possibility was tested by treating with eserine a muscle deprived of its sympathetic innervation. The lumbar sympathetic chain of one side was removed aseptically from a cat under ether, together with the first three sacral ganglia on both sides. After 10 days had elapsed the gastrocnemius was prepared and eserine was given. The effect of small or large doses was identical with that occurring in the completely innervated muscle (Fig. 4).

Frequency of stimulation. A few preliminary experiments have been made on the relation between frequency of stimulation and the reaction of the muscle to eserine. As shown by Briscoe [1936], a muscle poisoned with eserine is unable to maintain a contraction at frequencies of stimulation between 75 and 150 per sec. It behaves in many ways like a muscle under the influence of a small dose of curare. This effect appears to be due to the accumulation, in such a muscle, of the acetylcholine liberated by the nerve impulses, and to be analogous to that following injection of acetylcholine after eserine. In fact, in the presence of eserine or of any other inhibitor of choline esterase, such as nicotine, a short period of tetanic stimulation, interpolated in a series of maximal single

stimuli at intervals of 10 sec., reproduces with great fidelity the effect of an injection of acetylcholine similarly interpolated (Fig. 5). It is obvious from the long duration of the depression following a short

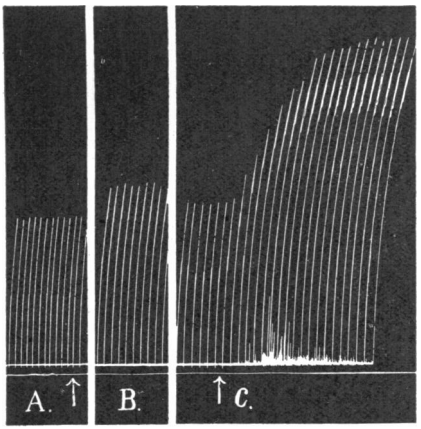

Fig. 4.

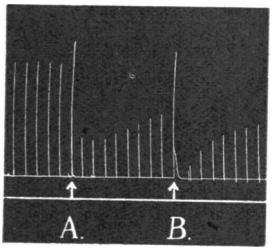

Fig. 5.

Fig. 4. Spinal cat, 9 days after lumbosacral sympathectomy. Contractions of gastrocnemius in response to maximal shocks to nerve at 10 sec. intervals. A, arterial injection of 5γ eserine; B, 10 min. later; C, arterial injection of 20γ eserine.

Fig. 5. Spinal cat. Contractions of gastrocnemius in response to maximal shocks to nerve at 10 sec. intervals, after arterial injection of 100γ miotine. A, arterial injection of 10γ acetylcholine; B, short tetanic stimulation of nerve.

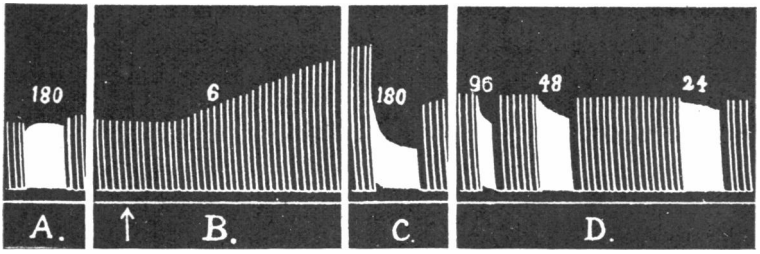

Fig. 6. Cat, chloralose 2·9 kg. Contractions of tibialis anticus in response to maximal shocks to nerve. Various frequencies of stimulation. A, 6 per min. changed to 180 per min.; B, 6 per min. At arrow, intravenous eserine 0·5 mg. per kg.; C, frequency changed from 6 per min. to 180 per min.; D, frequency changed from 6 per min. to 96, 48 and 24 per min.

tetanus, that, if a frequency of stimulation is chosen which is slightly too rapid, the effect of eserine may appear, not as a potentiation of the muscle twitch, but as a depression. The effects of frequency of stimulation are shown in Fig. 6. Stimulation was started at a frequency of 6 per min., a

large dose of eserine was given and the usual potentiation appeared. Increase of the frequency up to 180 per min. now produced a fall of the tension below the original level, and from this depression the muscle did not completely recover. At a frequency of 48 per min. the depression is less pronounced; but even at the relatively slow rate of 24 per min. the tension of each twitch is still less than when stimulation is at 6 per min.

Dosage. We have made some observations on the effects of varying doses of eserine, using the method of local arterial injection for the administration of the drug only to the muscle under experiment. The threshold dose of eserine for the potentiation appears to be about 5γ in the cat's gastrocnemius. With this dose a small, obvious potentiation was regularly produced (Fig. 4). With increasing doses the potentiation increases *pari passu* until an amount of 25γ is reached. With doses in excess of this, e.g. 100γ, the potentiation begins as usual, but the rise is cut short after some 30 sec. and the muscle response falls to a level little above its previous value. In such a muscle the paralysing effect of a short tetanus, or of an injection of acetylcholine, is particularly obvious. The failure of the potentiation after large doses of eserine is probably to be attributed to the accumulation of acetylcholine, but it is difficult completely to exclude a paralysing action of eserine itself; against such an action is the fact that large doses of eserine have no depressant action *per se*, provided that the stimulation is sufficiently infrequent and that no acetylcholine is injected.

Effect of anæsthetics. Brown *et al.* showed that the eserine potentiation does not appear in the cat anæsthetized with ether. This we have been able to confirm and we have found, moreover, that even the injection directly into the arteries of a muscle of doses up to 20γ is without effect under such conditions (Fig. 8A). Similarly, we have failed to observe any potentiation by intravenous eserine in a rabbit anæsthetized with avertin, 0·3 g. per kg. Brown *et al.* suggested that the failure of Rosenblueth *et al.* [1936] to observe the potentiating action of eserine might be due to their use of dial as an anæsthetic. In our experience the effect of eserine can be obtained regularly in animals anæsthetized with dial (0·8 c.c. per kg. intraperitoneally), but the onset is slow, the maximum being attained only 10 min. after the injection, and the total effect is weaker than in the spinal animal. Nevertheless, the potentiation is typical and electrical records show the characteristic repetitive nature of the response of the muscle to single nerve volleys. Amytal similarly depresses the potentiation, and in one experiment we observed that

the administration of amytal (50 mg. per kg.), during a potentiation in a spinal cat, reduced the tension developed by each contraction.

The effects under urethane anæsthesia are peculiar. In rabbits and cats under urethane (1·5 g. per kg. subcutaneously), intravenous injection of eserine neither potentiates the mechanical response nor produces the repetitive character of the electromyogram. If, however, the eserine is given by direct arterial injection in the usual amounts, a potentiation is produced under urethane which is only slightly less than that seen in the spinal cat.

Under chloralose anæsthesia (80 mg. per kg.) all the effects of eserine are obtainable with as great facility as in the spinal or decerebrate animal (Fig. 7). For this reason, many of our later experiments have been made with this anæsthetic. Anæsthesia with ethyl alcohol in the rabbit does not prevent the appearance of the eserine potentiation.

Effects of other alkaloids. In the course of the experiments in which nicotine and tetramethylammonium iodide were used, we observed that, after these substances had been injected, the usual effect of a subsequent dose of eserine was absent; neither its potentiating nor its depressant effects could be observed. The action of acetylcholine was also apparently unaffected by eserine under such conditions, and its injection did not produce the depressant effect which is usually seen in the muscle after eserine.

(5) *Action of other inhibitors of choline esterase*

The following substances, having a graded inhibitory action upon cholinesterase, have been tested for their action on skeletal muscle:

(1) Methylurethane of *m*-hydroxyphenyltrimethyl ammonium iodide ("meta-compound").

(2) Methylurethane α-*m*-hydroxyphenylethyldimethylamine hydrochloride (miotine).

(3) Methylurethane of ρ-hydroxyophenyltrimethyl ammonium iodide ("para-compound").

(4) Methylurethane of hordenine hydrochloride ("M.U.H.").

(5) Ergotoxine.

If the activity per unit weight of eserine as an inhibitor of cholinesterase is taken as 1, then the activity of the five above compounds will be approximately as follows: meta-compound, "the most active inhibitor available," miotine 2, para-compound 1/5, M.U.H. 1/10 [Stedman, 1936], and ergotoxine 1/1000 [Matthes, 1930].

All these substances have been found to exert an action on muscle distinguishable from that of eserine only quantitatively. The threshold

doses for a potentiating effect, determined by arterial injection into the gastrocnemius of the cat, were as follows:

Meta-compound	0·25γ	Para-compound	20γ
Miotine	2·5γ	M.U.H.	200γ
Eserine	5γ	Ergotoxine	500γ

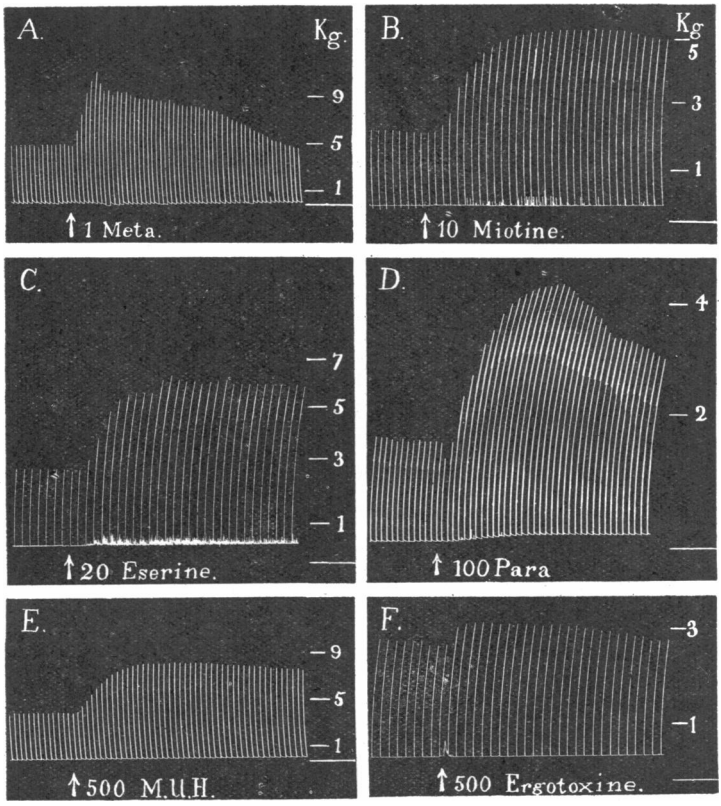

Fig. 7. Cats, chloralose. Contractions of gastrocnemius in response to maximal shocks to nerve at 10 sec. intervals. Action of inhibitors of choline esterase injected arterially at the arrows. A, 1γ meta-compound; B, 10γ miotine; C, 20γ eserine; D, 100γ para-compound; E, 500γ M.U.H.; F, 500γ ergotoxine. As different strengths of myograph springs were used, reference should be made to the calibration shown on each record.

In Fig. 7 is shown a series of effects of these compounds in doses sufficient to give approximately the maximum potentiation, except in the case of ergotoxine, in which only threshold doses were given on account of the very low activity of the substance. Here again the order of the relative

activities in potentiating the muscular contraction corresponds closely to that of the relative potencies of the substances as inhibitors of cholinesterase. It is difficult to avoid the conclusion that the potentiating action is due to the prevention of the destruction of the acetylcholine liberated at the motor nerve ending. Meta-compound and miotine, on account of their high activities, have been studied in more detail. The dose of eserine which was found to be the most effective in producing potentiation, when given intravenously, was 0·3 mg. per kg. [Brown *et al.*]. Meta-compound in doses of 0·03 mg. per kg. intravenously to the cat, and miotine in doses of 0·15 mg. per kg. in both the cat and the rabbit, produce a potentiation equivalent to that of the above-mentioned dose of eserine. Excessive doses of miotine (100γ intra-arterially) or of meta-compound (5–10γ) produce very clearly the state of excessive sensitivity to the depressant action of rapid nerve stimulation, and of injection of acetylcholine (Fig. 5). With such doses of these compounds, as with large doses of eserine, the potentiation, even with such a low frequency of stimulation as 6 per min., may be irregular.

Brown *et al.* have shown that eserine is without effect upon the denervated or curarized muscle stimulated directly. This applies also to the meta-compound (Fig. 9), doses up to 25γ by arterial injection being completely ineffective. Meta-compound, therefore, like the other inhibitors of esterase, and unlike veratrine (see later), acts only when the transmitting mechanism is intact. Meta-compound appears to differ from eserine in that the effect of successive weak doses is additive when the effect of the first dose has apparently passed off. Dr Stedman informs us that the para- and meta-compounds are themselves destroyed gradually by the esterase, and have accordingly a more transient action than the other inhibitors. This applies also to their potentiating action on the response of muscle to nerve stimulation (Fig. 7).

It is to be noted that there is some discrepancy between the activity of M.U.H. as an inhibitor of esterase and the threshold dose required for potentiation. Dr Stedman informs us that the action of M.U.H. on the esterase takes a long time to attain its maximum, and this peculiarity probably explains the necessity for the use of proportionately larger doses of this compound, in relation to its final anti-esterase action, than of the other compounds with a relatively rapid action.

(6) *The action of veratrine*

Veratrine, administered to a muscle by arterial injection in doses of 15–25γ, produces a potentiation of the response to maximal single motor

nerve volleys which bears a suggestive resemblance to the action of the esterase inhibitors (Fig. 8B). On the other hand, veratrine, in concentrations of $1 : 10^{-4}$, has no detectable action on cholinesterase. Unlike

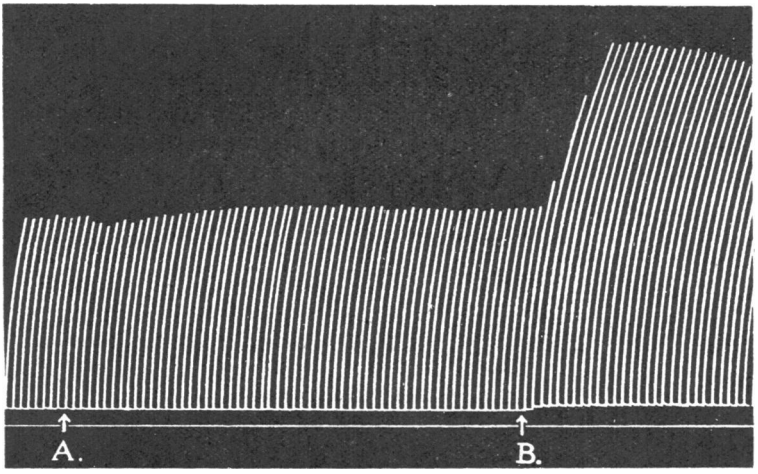

Fig. 8. Cat, ether. Contractions of gastrocnemius in response to maximal shocks to nerve at 10 sec. intervals. A, arterial injection of 20γ eserine; B, arterial injection of 25γ veratrine.

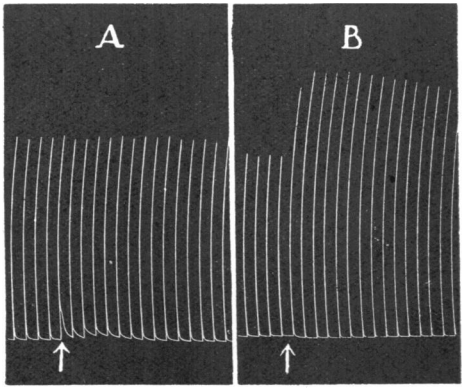

Fig. 9. Cat, chloralose. Contractions of gastrocnemius, denervated 9 days previously, in response to direct maximal shocks. Arterial injection of A, 5γ "meta"-compound; B, 25γ veratrine.

eserine, again, it does not sensitize the isolated rectus abdominis of the frog to acetylcholine. The resemblance in the potentiating effects, however, is superficial: the action of veratrine, when carefully analysed, is

found to be entirely different from that of the esterase inhibitors. It acts equally well on the muscle of an animal anæsthetized with ether, when eserine, even in large doses, is ineffective (Fig. 8). Denervation of the muscle (9 days after nerve section) and complete curarization do not alter the action of veratrine on it, the potentiation of its responses to direct stimulation being obtained with as great facility as that of the normal muscle to nerve stimulation (Fig. 9). The optical isometric myogram has a form which is distinctive, and quite unlike that after eserine (Fig. 10).

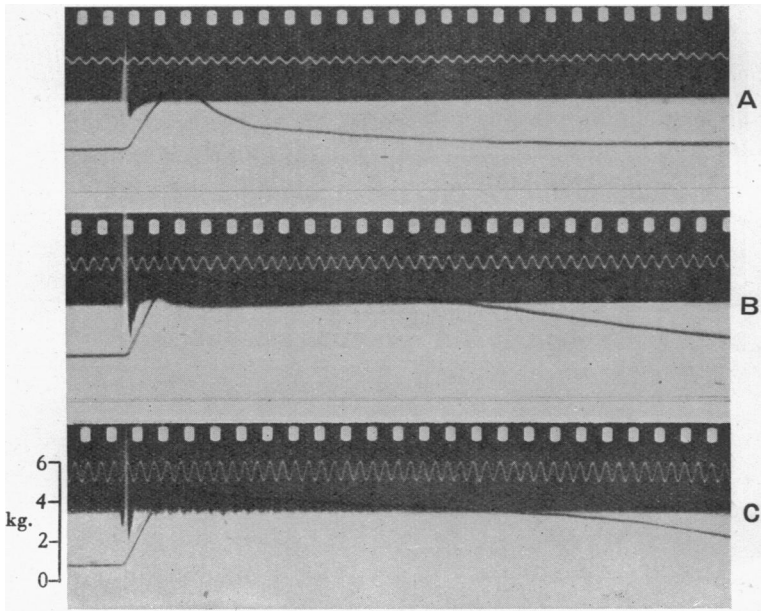

Fig. 10. Optical isometric myograms and action potentials of cat's gastrocnemius. A, normal response to single maximal shock to nerve. Electrical lead, belly to tendon; B, 1 min. after arterial injection of 15γ veratrine. Higher amplification of action potential; C, 1 hour later, 5 min. after a second arterial injection of 15γ veratrine. Concentric needle electrode. Time 10 msec.

The electromyogram shows that the veratrine potentiation is due to repetitive contraction of the muscle fibres, but the character of the discharge is unlike that under eserine. The action potential recorded in Fig. 10 B does not show the series of synchronous waves which is so characteristic of the eserine effect. With belly to tendon leads only a small irregular shift of the base-line after the main deflection is seen. Stricter localization of the electrical lead, as with concentric needle electrodes,

reveals that this shift is due to the asynchronous firing of small fibre groups (Fig. 10 C).

The contrast between these records and those of the muscle after eserine is obvious if Fig. 10 of this paper is compared with Figs. 12 and 13 of the paper by Brown *et al.* [1936], in which identical methods of recording were used.

Discussion

From these experiments it is clear that the property of exciting normal mammalian voluntary muscle is common, not only to the unstable esters of choline, but also to other substances possessing a nicotine-like action, viz. choline, nicotine and tetramethylammonium iodide. A similarity between the pharmacological affinities of denervated striated muscle and those of the cells of the sympathetic ganglia had long been recognized. This similarity now appears to apply also to normally innervated muscle, the only discrepancy being in the relative sensitivities of the two tissues to certain exciting substances. For instance, Feldberg & Vartiainen [1934] found that nicotine was approximately as effective as acetylcholine as a stimulant of ganglion cells. In the muscle, nicotine, in order to produce a contraction of equal tension to that produced by an injection of acetylcholine, must be injected in a dose about 200 times as great.

All the substances which possess the property of stimulating muscle to contraction have also a secondary, paralysing action, if they are allowed to persist in contact with the muscle fibres. This paralysing effect is absent in the case of the unstable choline esters in ordinary circumstances, on account of their rapid hydrolysis. Prevention of their destruction, by eserine or œdema, allows this action to become evident [Brown *et al.*]. The stable carbaminoyl-ester, choline itself, nicotine and tetramethylammonium iodide, all show to a greater or less degree the depression following excitation. The peculiar condition observed after nicotine, in which either a short tetanic stimulation or an injection of acetylcholine removes the depression, we are unable to explain; but the phenomenon is of interest in that it provides yet another example of the very close parallel between the effects of nerve stimulation and those of acetylcholine injection. In the course of the present experiments we have come across other examples of this close similarity, such as the depressant effects of a short tetanus and of acetylcholine on a muscle poisoned with any inhibitor of cholinesterase, and the absence of these effects after veratrine; also the absence of depression by tetanus and acetylcholine in a muscle

which has received eserine after nicotine or tetramethylammonium iodide. In only one instance is the parallelism between the effects of nerve stimulation and of injected acetylcholine incomplete. When the response of the muscle to nerve stimulation is depressed as a result of the injection of choline, or carbaminoylcholine, the excitatory effect of injected acetylcholine is even much more depressed (Fig. 3). Apparently this greater susceptibility of the effect of injected acetylcholine is common to curariform paralysis by different agents, being seen with curarine itself [Brown *et al.* 1936].

We have been able in these experiments to reproduce with regularity the potentiating effects of eserine on the response of muscle to single maximal motor nerve volleys. The failure of Rosenblueth *et al.* [1936] to observe this phenomenon is not entirely due to their use of dial as an anæsthetic, as Brown *et al.* suggested. In animals under dial anæsthesia, and using a dose of 0·3 mg. to 0·5 mg. eserine per kg., we have been able to demonstrate with ease that the mechanical response is increased and that the electrical record shows the typical repetitive discharge observed in the spinal or chloralosed animal. But, in all our experiments, the frequency of stimulation was kept at 6 per minute. The use of a higher frequency of stimulation, as employed by Rosenblueth *et al.* (two pairs of make and break shocks per second), is sufficient, as shown in Fig. 6, to produce a depression, and to obscure completely the potentiating action. A further factor which probably contributed to the absence of potentiation from Rosenblueth *et al.* results is their use of rather large doses of eserine (0·3–1·0 mg. per kg.) which, as we have shown, may facilitate the appearance of depression.

In our experiments with eserine and substances of similar action we have seen that there is a very suggestive correspondence between activity in potentiating the responses of a muscle to maximal nerve volleys and strength of anti-esterase action. It was shown by Brown *et al.* [1936], in the case of eserine, that this potentiation was due to repetitive response of the muscle to a single volley; and they interpreted this as indicating that eserine produced this action by inhibiting the cholinesterase at the nerve endings, and thus allowing the acetylcholine released by an impulse to persist in a concentration sufficient to restimulate the motor end plate of the muscle fibre through several successive refractory periods. The correspondence between potentiating and anti-esterase actions over a series of compounds strongly reinforces this interpretation. It is, of course, conceivable that the correspondence might be accidental; but in that case it would be necessary to suppose that the action of these sub-

stances was on the muscle fibre, so changing its physiological condition that it would give a repetitive response to a single stimulus, as frog's muscle does under the influence of unbalanced sodium ions [Adrian & Gelfan, 1933]. We have ourselves given an example of an action of this kind in the case of veratrine, which has no anti-esterase action. But veratrine causes a similar repetitive response of the completely curarized or denervated muscle to directly applied induction shocks, whereas the substances of the eserine series have no action at all on the response of muscle to direct stimulation under these conditions. The only feature of the action of these substances which might suggest a change of the excitability of the muscle fibres themselves is the appearance of small, irregular twitchings, which they all produce. This twitching, however, is not produced in the denervated or curarized muscles, and, like the potentiation of responses to maximal nerve volleys, it is produced by the different members of the series in the same order as their anti-esterase actions. The only exception to this correspondence is provided by the aberrant case of ergotoxine, which causes twitchings out of proportion to its very weak anti-esterase and potentiating actions. For the closer analogues of eserine there seems to be good reason for attributing the spontaneous twitching, like the repetitive response to a single nerve volley, to the anti-esterase action, causing the excitation of small groups of muscle fibres by the persistence of acetylcholine liberated by random impulses passing down the divided nerve. It may be noted, further, that in the case of veratrine, which certainly increases the excitability of the muscle fibres themselves, no twitchings have been produced by the doses which we employed.

Altogether, the evidence which we have put forward in this paper is not merely compatible with the transmission of excitation from motor nerve endings to voluntary muscle fibres by liberation of acetylcholine; it is difficult to find any other conception which gives a logical significance to all the facts.

Summary

1. The excitant effects of a number of choline esters on normal mammalian muscle have been compared with those of acetylcholine.

2. The excitatory action of acetylcholine on normal mammalian muscle is a property common to other substances possessing a nicotine-like action (choline, nicotine, tetramethylammonium iodide).

3. The excitation of the muscle is followed by depression of the effect of nerve stimulation when stable substances are used (carbaminoyl

choline, choline, nicotine and tetramethylammonium iodide). This depression is similar to that produced by the unstable esters when their hydrolysis is prevented by eserine.

4. The potentiation by eserine of the response of normal mammalian muscle to single maximal motor nerve volleys has been investigated further:

(*a*) It is present in the muscle deprived of its sympathetic innervation.

(*c*) It is only demonstrable if very low frequencies of nerve stimulation are used.

(*c*) It is completely suppressed by ether and avertin anæsthesia, slowed and depressed by barbiturates, but unaffected by chloralose anæsthesia or by alcohol.

5. The potentiating actions of five eserine substitutes on the response of muscle to nerve stimulation were found to be directly proportional to their inhibiting actions on cholinesterase.

6. Veratrine causes a potentiation of the muscle response to nerve stimulation, but, unlike the inhibitors of cholinesterase, its activity is not altered by anæsthetics, denervation or curarization of the muscle.

7. These facts offer further evidence in favour of the chemical transmission of excitation from nerve to voluntary muscle in the mammal.

We wish to express our thanks to Sir Henry Dale for the help and advice which he has given us in this investigation.

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