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THE INTERRELATION OF PROSTIGMINE, ADRENALINE AND EPHEDRINE IN SKELETAL MUSCLE

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We have recently described experiments made to investigate the transmission of impulses across the synapses of the spinal cord [Bülbring & Burn, 1941]. These experiments were carried out by perfusing the lower half of the spinal cord of a dog with one circulation, and the muscles of the hind-limb by a second circulation. This system enabled substances to be injected into the blood running to the spinal cord without the possibility of them acting on the muscles directly.

In the course of our observations, we discovered that doses of acetylcholine reaching the spinal cord caused a discharge of motor impulses from the cord, and this action was greatly facilitated by the presence of adrenaline. Secondly, when the flexor reflex was elicited by stimulating the posterior tibial nerve and we recorded the contraction of m. tibialis anterior, it was found that the reflex contraction was small, if there was no adrenaline in the blood perfusing the spinal cord; this remained true even when much adrenaline was present in the blood perfusing the hind-limb. However, if adrenaline was added to the blood perfusing the cord, the flexor reflex then became much greater. A third observation concerning adrenaline was also made. When the flexor reflex was recorded, and prostigmine was added to the blood perfusing the cord, the reflex was scarcely affected. This was surprising because when eserine was similarly added, the reflex was greatly increased. We found, however, that when adrenaline was present in the blood perfusing the cord, and prostigmine was injected into it, the injection caused an increase in the flexor reflex similar to the increase caused by eserine.

If these observations are explained as effects of adrenaline on humoral transmission by acetylcholine across the synapse in the cord, then, we argued, a similar effect might be seen in other places where acetylcholine

transmits the impulse. Would it be found that adrenaline affects the action of prostigmine at the neuromuscular junction in the same way as it affects its action in the spinal cord?

Methods

Cats were used, anaesthetized first with ether and then with chloralose. The sciatic nerve was divided high up between the thigh muscles and laid upon silver electrodes shielded in a vulcanite fork. Maximal single shocks were applied to it by a neon lamp circuit at rates varying from 4 per min. upwards. The femur was transfixed near its lower end by a steel rod which was held rigidly between clamps. The portion of the os calcis into which the tendon of Achilles is inserted was detached from the rest of the bone, and connected by a stout copper wire to a tension lever. As a rule these preparations were made in the left leg. The right external iliac artery was divided between ligatures, and a cannula inserted into the central end pointing towards the bifurcation of the aorta. The aorta was ligatured below the origin of the external iliac arteries. When injections were made through the cannula, the injected fluid was then carried to the left leg. Injections were also made intravenously.

Results

Stimulation at 4 per min. The contractions of the gastrocnemius recorded in this way are illustrated in Fig. 1, where it is first of all shown (*a*) that an intravenous injection of 0·04 mg. adrenaline had no appreciable effect on the size of the contractions. In (*b*) the injection of 0·01 mg. prostigmine by the same route also had no effect, but when it was followed by 0·04 mg. adrenaline, this produced a clear increase. The same effect is shown in (*c*), (*d*) and (*e*) at a later stage, when 0·02 mg. prostigmine itself caused an increase, as it is well known to do. The injection of 0·02 mg. adrenaline, which neither before (*c*) nor later (*e*) had an appreciable effect, augmented the contractions when injected at a short interval (5 min.) after the prostigmine.

We observed a similar effect when, instead of prostigmine, we used eserine. For example the injection of 0·05 mg. eserine sulphate caused a small increase of twitch tension, and the injection of 0·01 mg. adrenaline 6 min. later caused a further increase. The effect of adrenaline in augmenting the action of eserine was, however, much less than its effect in augmenting the action of prostigmine. The difference was shown most clearly when adrenaline was given simultaneously. Thus Fig. 2 *a* shows the effect of 0·05 mg. eserine and 0·02 mg. adrenaline, given together, to

be not much greater than the effect of 0·05 mg. eserine alone, which is seen in Fig. 2 *b*; whereas 0·01 mg. prostigmine with 0·02 mg. adrenaline (Fig. 2 *c*) had a much greater effect than 0·01 mg. prostigmine alone

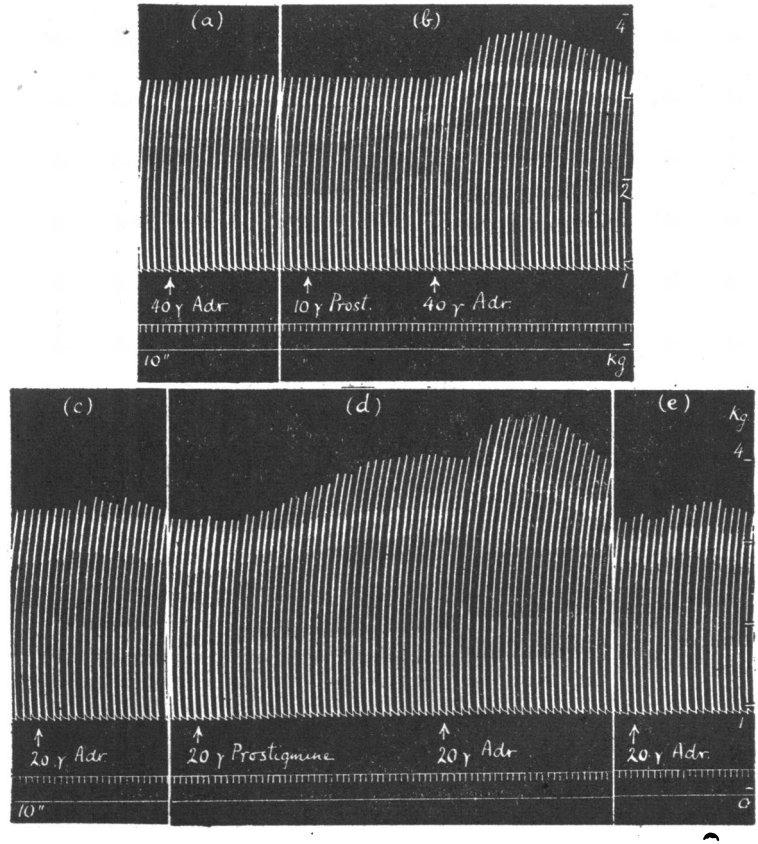

Fig. 1. Cat anaesthetized with chloralose. Contractions of gastrocnemius muscle stimulated by single maximal shocks applied to the sciatic nerve 5 times per min. (*a*) Shows the effect of an intravenous injection of 40 μg. adrenaline before and (*b*) 4 min. after a dose of 10 μg. prostigmine. (*c*) Shows a slight augmentation of muscular contractions by 20 μg. adrenaline. (*d*) Shows a slow prolonged augmentation caused by 20 μg. prostigmine; on top of this augmentation a further increase was produced by injecting 20 μg. adrenaline. (*e*) Effect of adrenaline 15 min. later.

(Fig. 2 *d*). It should be noted that in all experiments in which eserine and prostigmine were compared, a similar increase in tension was caused by doses of prostigmine and eserine in the ratio 1 : 5, when the doses were given without adrenaline.

The effect of adrenaline on nicotine was also examined. The injection of 1 mg. nicotine tartrate (see Fig. 3 *a*) slightly depressed the tension evoked by a single shock, and a further slight depression was seen when 0·02 mg. adrenaline was injected 1½ min. later. There was no striking change in the nicotine effect. If, however, the nicotine and adrenaline were injected together (Fig. 3 *b*), the depression was much greater.

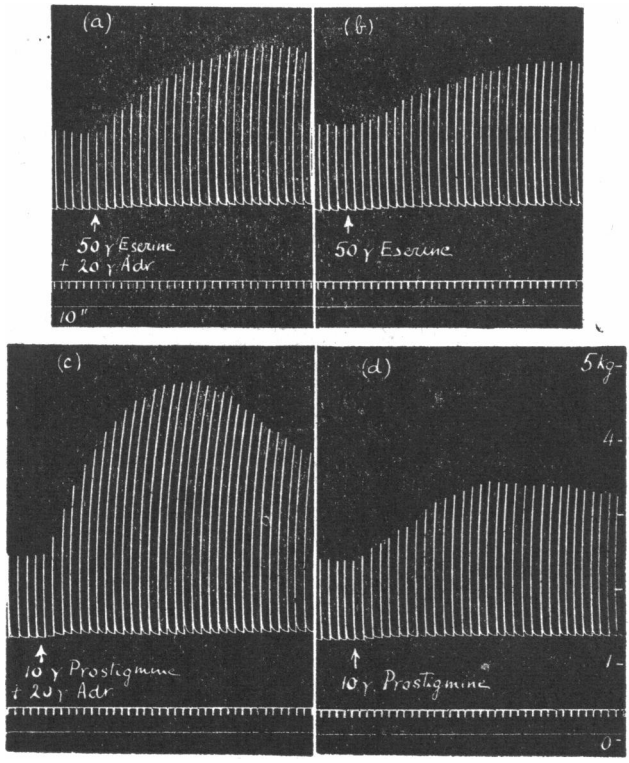

Fig. 2. Records as before. (*a*) Effect of simultaneous intra-arterial injection of 50 μg. eserine and 20 μg. adrenaline. (*b*) Effect of injecting eserine only. (*c*) Effect of simultaneous intra-arterial injection of 10 μg. prostigmine and 20 μg. adrenaline. (*d*) Effect of injecting prostigmine only.

Single shocks at higher rates. The foregoing observations were made when single shocks were applied to the sciatic nerve at rates from 4 to 6 times per min. When higher rates, from 15 to 45 per min., were used, a different action of adrenaline was seen, which is shown in Fig. 4, taken from an experiment in which the rate of stimulation was 15 per

min. In (*a*) the injections of 0·02 mg. adrenaline and of 0·02 mg. prostigmine are seen to have little, if any, effect when given separately. In (*b*) the simultaneous injection of both substances produced a sharp rise soon interrupted by a depression. In (*c*) the adrenaline injection was given later than the prostigmine injection which by this time produced on its own account an increase of nearly 50 % in the tension. When, in Fig. 4 *c*, adrenaline was injected, a clearly defined depression of the prostigmine effect was recorded, so that at this higher rate of stimulation the adrenaline effect was reversed. The rate of stimulation was then reduced to 4 per min., and the injections were made again. Fig. 4 *d* shows that the augmenting action of prostigmine was still greater, but that the depressant effect of adrenaline was no longer present.

When larger doses of prostigmine, from 0·05 to 0·1 mg., were injected during stimulation at 15 per min., the tension was diminished, as shown in Fig. 5. Adrenaline injected before this diminution had disappeared caused a further diminution, although the same dose injected before the prostigmine caused a small increase, which was probably due to the persistence of prostigmine injected earlier.

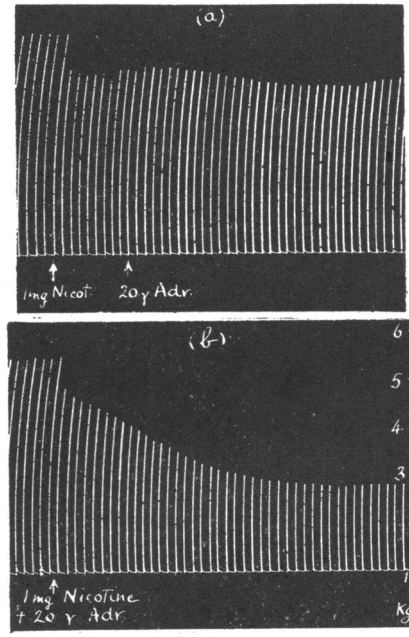

Fig. 3. Records as before. (*a*) Effect of intra-arterial injection of 1 mg. nicotine followed by 20 μg. adrenaline. (*b*) Effect of simultaneous injection of these doses of nicotine and adrenaline.

Effects during an interrupted tetanus. The effect of adrenaline in increasing the tension developed by fatigued muscle has been known since it was described by Gruber [1914]. The evidence [e.g. Bülbring & Burn, 1940] is mainly in favour of the view that this action is due to improved neuromuscular transmission, and therefore we made experiments to observe the effect of adrenaline on fatigued muscle in the presence of prostigmine. We perfused the hind-leg of a dog with defibrinated blood, stimulated the sciatic nerve with condenser discharges at the rate of 400 per sec. applied 120 times per min. for a duration of 0·02 sec. The contractions of the gastrocnemius muscle were recorded by

a tension lever. When the stimulation had been applied for several minutes the contractions, which had diminished rapidly at first, remained at a uniform height, and the injection of 0·008 mg. adrenaline into the

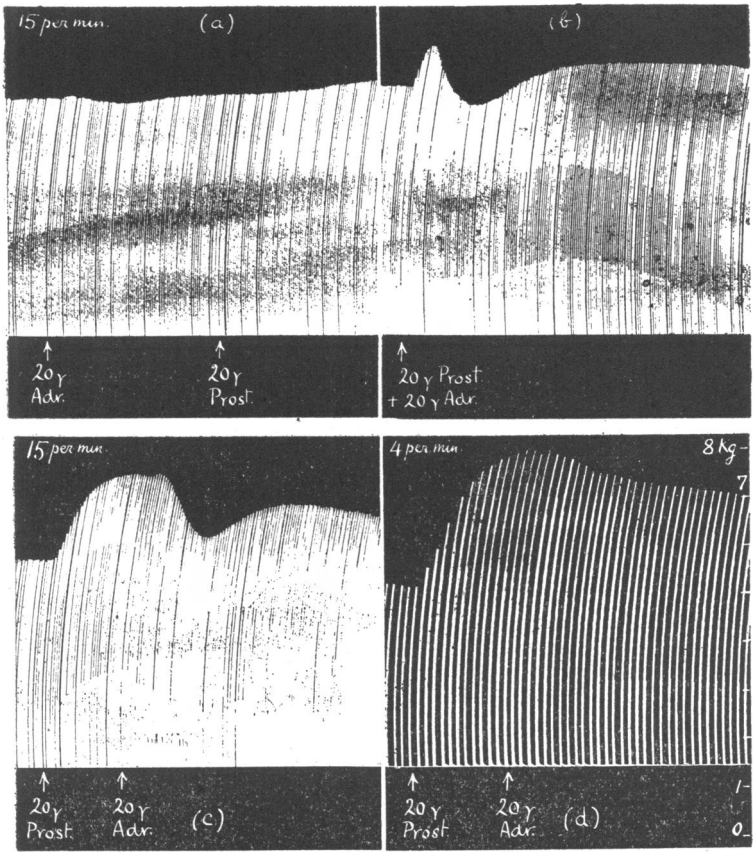

Fig. 4. Records as before. (*a*) Fifteen maximal shocks per min. Very slight changes in the size of muscle contractions after injections of 20 μg. adrenaline and 20 μg. prostigmine. (*b*) Sharp rise interrupted by depression after simultaneous injection of prostigmine and adrenaline. (*c*) Analysis of the effect observed in (*b*) by injecting the adrenaline after the prostigmine. (*d*) Same sequence of injections at slower rate of stimulation when adrenaline caused no depression.

arterial cannula now caused the increase shown in Fig. 6 *a*. In Fig. 6 *b* is shown the effect of injecting 0·02 mg. prostigmine, which diminished the contractions. In Fig. 6 *c* the adrenaline injection was repeated and caused a diminution. The conditions here were, however, different from the conditions when the nerve was stimulated with single shocks at

15–45 per min. With that stimulation, whether prostigmine increased (Fig. 4 *c*) or decreased (Fig. 5) the contractions, a subsequent dose of

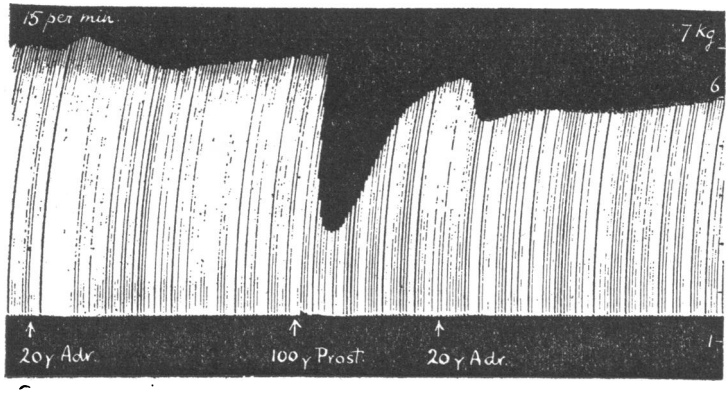

Fig. 5. Records as before. Fifteen maximal shocks per minute. (Two doses of 0·1 mg. prostigmine had been injected 1 hr. before.) The injection of 20 μg. adrenaline caused a small increase of muscle contractions, 100 μg. prostigmine depressed them, and, after this, 20 μg. adrenaline caused a further depression.

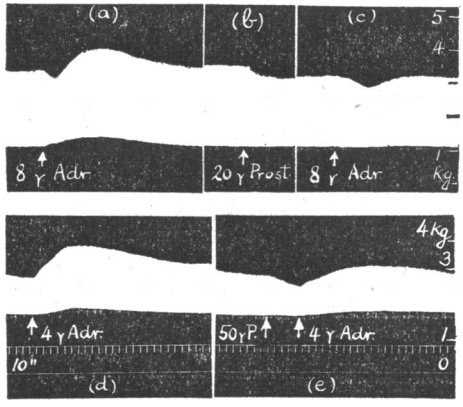

Fig. 6. Dog. Perfused hind-leg. 120 tetani per min.; muscle fatigue. (*a*) Injection of 8 μg. adrenaline into arterial cannula caused, after a small initial diminution, augmentation of muscle contractions of 1 kg. (*b*) 20 μg. prostigmine was given, after which (*c*) adrenaline caused depression only. (*d*) (from another experiment) shows the big increase of muscle tension after the injection of 4 μg. adrenaline and (*e*) a much smaller effect after 50 μg. prostigmine had been given.

adrenaline only depressed them. With interrupted tetani, adrenaline at an early stage after prostigmine was still able to cause an increase as shown in Fig. 6 *e*, though a smaller increase than before (Fig. 6 *d*).

The action of ephedrine. Since ephedrine has been used in the treatment of myasthenia gravis, both alone [Edgeworth, 1930] and in conjunction with prostigmine [Viets & Schwab, 1939; Schlezinger, 1940], we were anxious to see if ephedrine augmented the action of prostigmine as did adrenaline. We injected ephedrine during the increased tension

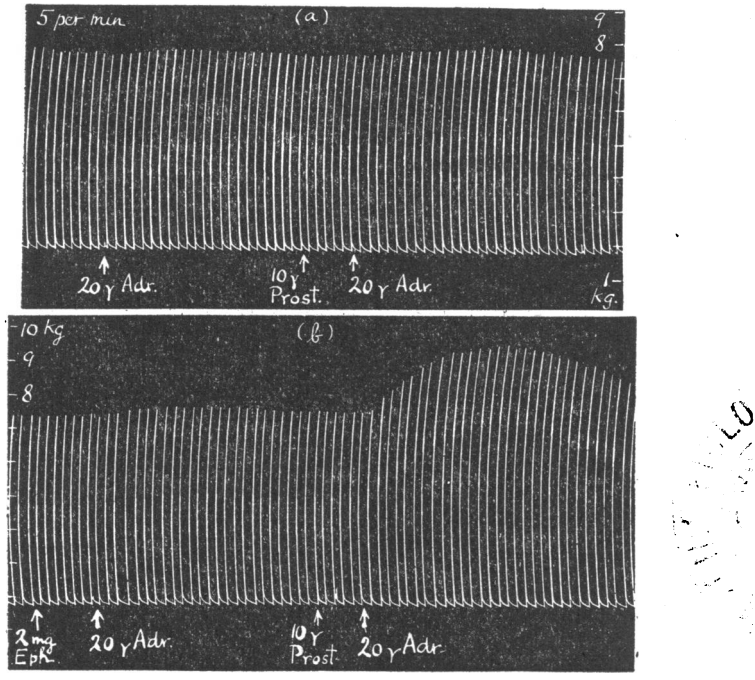

Fig. 7. Cat, chloralose. Reponse of gastrocnemius to maximal shocks to sciatic nerve 5 per min. (*a*) Shows very slight changes in the size of muscular contractions due to adrenaline injected before or after prostigmine. (*b*) Shows that 2 mg. ephedrine did not affect the action of adrenaline or prostigmine itself, but after prostigmine adrenaline now caused an augmentation.

produced by prostigmine when the rate of stimulation was 4 per min., but observed no augmentation, although adrenaline injected 3 min. later caused the usual increase. It seemed that ephedrine had no direct relation to the action of prostigmine. We then found that after the injection of ephedrine, adrenaline was more effective in augmenting the action of prostigmine than before. An example of this is given in Fig. 7. In (*a*) the injection of 0·02 mg. adrenaline, of 0·01 mg. prostigmine and of 0·02 mg. adrenaline 1 min. later were alike without effect. In (*b*) 2 mg. ephedrine

hydrochloride were first injected. This had no effect itself, nor on the subsequent injection of 0·02 mg. adrenaline. It did not affect the response to 0·01 mg. prostigmine. The response to 0·02 mg. adrenaline injected after the prostigmine was, however, greatly increased by the injection of ephedrine.

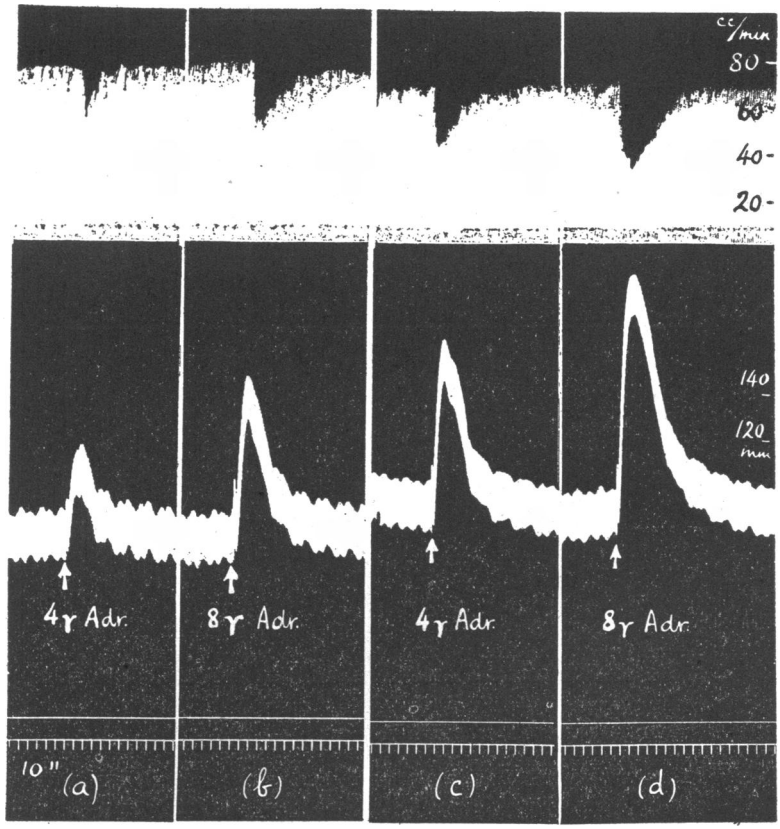

Fig. 8. Dog, hind-leg perfusion. Top: Venous outflow, bottom arterial pressure. (*a*) and (*b*) show vascular effect of 4 and 8 μg. adrenaline before and (*c*) and (*d*) after 20 μg. prostigmine.

Of other sympathomimetic substances we tested dihydroxyphenylethylamine and also *l*-meta-sympatol. This substance is identical with adrenaline save for the absence of the OH group in the para position in the ring. The former substance was inactive, while sympatol had the same augmentor action as adrenaline when given in a dose 8–10 times as great.

Adrenaline and prostigmine on the blood vessels. In the course of these experiments we have observed an effect of prostigmine on the vasoconstrictor action of adrenaline which is relevant to this paper because it indicates that these two substances affect one another directly. Mendez & Ravin [1941] have investigated the action of prostigmine on the circulation, and have observed that prostigmine causes a rise in blood pressure, well shown after atropine. They conclude that constriction of some portions of the peripheral vascular system must be partly responsible for this effect. We have observed that prostigmine increased the tone of the vessels of the dog's hind-leg perfused with defibrinated blood, and increased the constrictor action of adrenaline. Fig. 8 *a*, *b* show the constriction caused by 0·004 and 0·008 mg. adrenaline before the injection of 0·02 mg. prostigmine. Shortly after, the arterial tone rose and the effect of these doses of adrenaline was approximately doubled (Fig. 8 *c*, *d*).

Discussion

In the foregoing account we have described that if, in a cat under chloralose, the sciatic nerve is stimulated at a slow rate, such as 4 times per min., with maximal single shocks, the twitch tension is augmented by adrenaline if a dose of prostigmine has been injected a few minutes before. The same phenomenon is seen after a dose of eserine, though the effect is smaller. If higher rates of stimulation, 15–45 per min., are employed, the increased twitch tension produced by the injection of prostigmine is diminished by the injection of adrenaline. It is well known that if the muscle is tetanized to fatigue, the tension is augmented by adrenaline; we now find that if prostigmine is injected first, the adrenaline augmentation is diminished or abolished.

We think these observations are best explained by reference to the observation made by Dale & Gaddum [1930] that when a strip of the denervated diaphragm of a kitten was suspended in a bath, the addition of acetylcholine caused a contraction, but that in the presence of adrenaline, which by itself had no effect, this action of acetylcholine was greatly enhanced. This observation leaves no doubt that adrenaline potentiates the action of acetylcholine in skeletal muscle, and does so by a mechanism which is independent of the circulation. Vascular effects are not concerned.

There are now two conditions known in which adrenaline augments the contraction of skeletal muscle in the body. The first is during fatigue, in response to tetanic stimuli applied to the nerve, as demonstrated by Gruber [1914]. The action of adrenaline is almost certainly the same as the action of the sympathetic nerves described by Orbeli [1923]. The

second is in unfatigued muscle when prostigmine has been injected a short time previously. What is the common feature of these two conditions? It is that in both a larger amount of acetylcholine than usual appears at the motor end plates. In the fatigued muscle this comes from the very frequent stimuli reaching the nerve ending, and liberating the acetylcholine; in muscle into which prostigmine has been injected, stimulated at infrequent intervals, the acetylcholine accumulates because the prostigmine prevents its rapid destruction. We suggest that adrenaline increases the height of muscle contraction in both conditions by potentiating the effect of this increased amount of acetylcholine.

In the fatigued muscle the potentiating effect of adrenaline is diminished, then abolished, and finally converted to a depression by the injection of prostigmine. In the infrequently stimulated muscle treated with prostigmine, the potentiating effect of adrenaline is converted into a depression by increasing the rate of stimulation. That is to say, in both conditions, when too much acetylcholine is produced, the effect of adrenaline is again to intensify its action and to produce the effect of excess, which is depression.

While recognizing that adrenaline does potentiate the action of acetylcholine, and that this fact may explain all the phenomena, we think that the possibility that adrenaline modifies the action of prostigmine should be borne in mind. A direct relation between adrenaline and prostigmine is suggested by several facts. Both prostigmine and eserine increase the tension developed in the muscle in response to single shocks applied to the nerve; yet adrenaline augments the effect of prostigmine very much more than it augments that of eserine. It was, indeed, a difference of this kind in the spinal cord that led to these experiments, for while eserine readily increased the flexor reflex, prostigmine had this effect only in the presence of adrenaline. On the blood vessels, where acetylcholine is certainly not involved, prostigmine causes some vasoconstriction and augments the constrictor action of adrenaline. It seems clear that the interaction of these two substances is not confined to the potentiation by adrenaline of the acetylcholine protected by prostigmine, but that adrenaline somehow increases the action of prostigmine itself.

Our observations on ephedrine show that this substance has no action like that of adrenaline in relation to prostigmine, and does not augment the muscle tension in the presence of prostigmine. The effect of adrenaline on this tension is, however, greater after ephedrine has been injected, presumably because the rate of adrenaline destruction is then slower.

In these observations we have a possible explanation of the finding

of Edgeworth [1930] that ephedrine benefits sufferers from myasthenia gravis. If adrenaline plays a part in normal muscle contraction (and our observations make it more likely that it does), ephedrine will benefit sufferers from myasthenia gravis by prolonging the effect of adrenaline naturally produced in the body, either from the suprarenal medulla or at sympathetic nerve endings.

The use of ephedrine in myasthenia has been obscured by Walker's discovery of the value of prostigmine [1935], but recently Viets & Schwab [1939] and Schlezinger [1940] have stated that it is better to give ephedrine in addition to prostigmine than to give prostigmine alone. Our observations agree very well with this clinical experience.

SUMMARY

1. If contractions of the gastrocnemius of a cat are elicited 4–6 times per min. by single maximal shocks applied to the nerve, the increase of tension produced by the injection of prostigmine is very much greater if adrenaline is given together with prostigmine.

2. The increase due to adrenaline is also evident if the adrenaline is given some minutes after the prostigmine.

3. Adrenaline augments the effect of eserine much less than it augments the effect of prostigmine.

4. When the rate of stimulation is raised to 15–45 per min., prostigmine increases the tension, but adrenaline then decreases it.

5. Ephedrine has no action like that of adrenaline in relation to prostigmine; but after the injection of ephedrine, adrenaline has a greater effect than before.

6. The relation of these and other observations to the Orbeli phenomenon and to myasthenia gravis is discussed.

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