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THE EFFECTS OF ADRENALINE AND OF SYMPATHETIC STIMULATION ON THE DEMARCATION POTENTIAL OF MAMMALIAN SKELETAL MUSCLE

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The increase in twitch tension which follows a brief tetanus of a muscle has been shown to be associated with a diminution of the demarcation potential of the muscle. A similar increase in twitch tension and a similar reduction in demarcation potential can be produced by arterial injection into a muscle of suitable amounts of potassium chloride (Feng, 1938; Brown & Euler, 1938). The effects were so closely similar indeed, that they suggested that the potentiating action of a tetanus on twitch tension was due to a mobilization of K ions in the muscle.

The effect of adrenaline in increasing the twitch tension of a muscle has been shown to be due to a direct action upon the contractile mechanism of the muscle fibre (Brown, Bülbring & Burns, 1948), and recently Goffart & Brown (1947) have shown that the effectiveness of adrenaline on muscle is inversely proportional to the amount of potassium in the fluid bathing an isolated preparation. They pointed out also that fatigue, as such, is not a necessary condition for a clear potentiating action of adrenaline upon the muscle twitch. There are many superficial similarities between the effect of adrenaline and the post-tetanic potentiation: they are both effects upon the muscle fibre, their time courses are similar and they both appear to be associated with changes in the K^+ of the muscle. It appeared probable therefore, that a study of the effect of adrenaline upon the demarcation potential would be profitable. Adrenaline has been found to produce small but constant changes in it, but in the direction of increase of potential and not, as we had expected, in depolarization.

METHODS

The majority of the experiments were made on cats anaesthetized with chloralose, 0·6 mg./kg.; a few cats were decerebrated under ether. The tibialis muscle was prepared for arterial injection (Brown, 1938) and its nerve supply was severed. For tension recording the limb was held in a myographic stand, sometimes vertically, sometimes horizontally, and the tendon was connected to a flat spring myograph. The electrical records were taken with the limb horizontal and the muscle lying in medicinal liquid paraffin in a bath formed by the skin flaps. We cut the sciatic nerve on one

side in two cats, and in two we removed the abdominal sympathetic chains from the crura of the diaphragm to the 2nd sacral ganglia; the operations were made under nembutal anaesthesia and with aseptic precautions.

The demarcation potential was measured between a burned area at the tendinous end of the muscle and the belly of the muscle 2–3 cm. proximal to it. For electrodes were used wicks soaked in 0·9% sodium chloride solution, or 1% agar in a similar salt solution, in contact with chloride-coated silver wires. The electrodes were connected to the grids of a pair of cathode followers giving an effective input impedence of 90 MΩ. The output of the valves was taken to a moving coil galvanometer suitably damped. In our earlier experiments we read (at ½ min. intervals) the deflexion of the galvanometer on a mm. scale; in the later experiments we recorded the deflexion on bromide paper on a slowly moving drum camera. The normal procedure was to place the recording electrodes close together on the belly of the muscle and to balance any observed p.d. to zero. One electrode was then placed on the burnt region of muscle, and the absolute value of the demarcation potential was measured or recorded at low sensitivity (1–10 mm./mV.). The sensitivity was then increased to 1–10 cm./mV. and the circuit was balanced so that the spot was in the centre of the scale. During each experiment frequent checks were made of the interelectrode potential and of the absolute value of the p.d.

We found it desirable to make an annular burn and to wait about ½ hr. afterwards before beginning recording. Even then drift towards a diminished p.d. often occurred and was a nuisance. Recording was made much easier by immersion of the muscle in paraffin; the wick electrodes made entirely satisfactory contact with the muscle under the paraffin, even when they were frequently moved. The cat and the paraffin bath were kept between 37° and 39° by a source of radiant heat.

L-Adrenaline base (B.D.H.) was dissolved with HCl in saline just before use and diluted to give the appropriate concentration for close arterial injection in a volume of 0·25 c.c. DL-Noradrenaline hydrochloride (I.G. Farbenindustrie) and DL-isopropyl-noradrenaline sulphate (B.W. and Co.) were dissolved in saline and similarly diluted.

RESULTS

Effect of adrenaline on twitch tension

Since relatively little is known about the effects of adrenaline on unfatigued mammalian muscle, we made a preliminary study of the effects of arterial and intravenous injection of adrenaline on the tibialis anterior muscle.

The first administration in an experiment of 0·5–20 μg. adrenaline in 0·25 c.c. saline invariably produced an increase in the tension evoked by single maximal motor nerve volleys at 10 sec. intervals. The increase in tension varied between 3 and 15%, but occurred regularly without the necessity of fatiguing the muscle. Subsequent injections, made within an hour of each other, were progressively less effective, the 3rd and 4th generally failing to have any noticeable action. The onset of the increase in tension is gradual, the peak of the potentiation being reached in 2–5 min. Thence the tension declines until the potentiation is over in 15–20 min. (Fig. 1). Thereafter there may be some decline in tension, but it is difficult to distinguish this from the normal decay of the preparation. With these doses, and with this frequency of stimulation, we did not observe the immediate depression of the twitch such as is described by Luco (1939) and by Bülbring & Burn (1939), under rather different experimental conditions.

The time course of the potentiation is not related to that of the general vascular effects of the adrenaline, as judged by the changes in arterial blood

pressure. The peak of the rise in blood pressure is attained before the peak tension, and the blood pressure has returned to its previous level before the

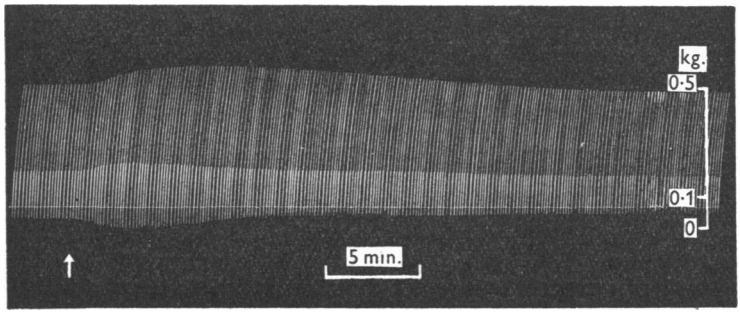

Fig. 1. Cat, 3·7 kg. chloralose. Contraction of tibialis anterior in response to single maximal nerve volleys at 6/min. At arrow, close arterial injection of 5 μg. adrenaline. (The record was made with a weighted recording lever to produce an 'overthrow'.)

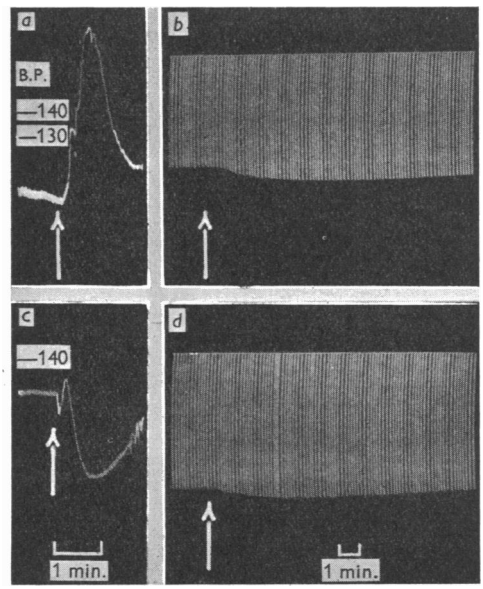

Fig. 2. Cat, 2·4 kg. chloralose. *a* and *c*, arterial blood pressure; *b* and *d*, contractions (downwards) of tibialis with single maximal nerve volleys at 6/min. At arrows, arterial injection of 5 μg. adrenaline. Between *b* and *c* intravenous injection of 7·5 mg. ergotamine tartrate. The blood pressure and muscle records were taken simultaneously, but at different speeds of recording surface.

potentiation is maximal. Abolition or reversal of the blood pressure changes by the administration of ergotoxine, ergotamine or dibenamine does not significantly alter the effect of the adrenaline on muscular tension (Fig. 2). Exact

comparison of the effects of adrenaline before and after ergotoxine or other substances, is made difficult by the tachyphylaxis which is such a characteristic feature of the action of adrenaline on muscle tension.

Effect of adrenaline on demarcation potential

The arterial injection of 10 μg. adrenaline into a muscle causes an increase in the demarcation potential which in degree and time course is very like the increase in tension produced by a similar injection. Fig. 3 shows such an effect; in this experiment the demarcation potential was quite steady before the injection,

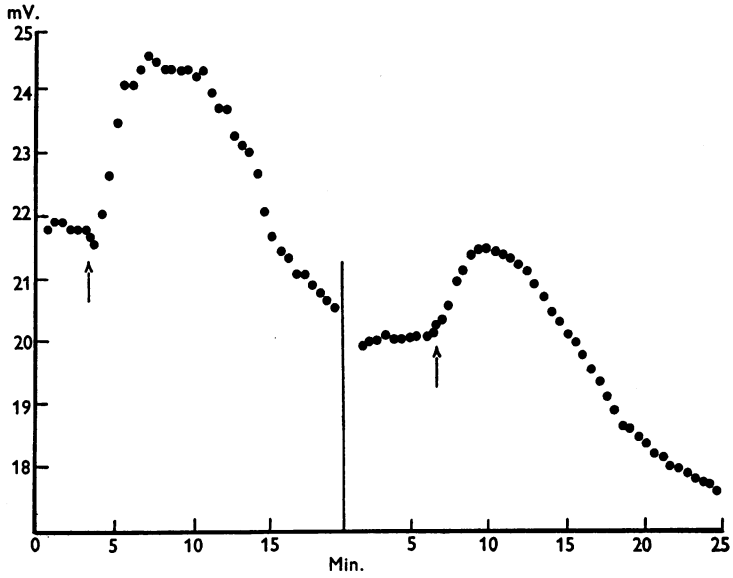

Fig. 3. Cat, 3 kg. nembutal. Demarcation potential of tibialis. Effect of close arterial injection of 10 μg. adrenaline at arrows. An interval of 40 min. elapsed between the two records.

but showed a decline after crossing its original base-line. This has been a constant phenomenon. We have, unfortunately, been unable accurately to correlate the two phases of the changes in polarization with the changes in twitch tension by recording the two phenomena simultaneously, since the electrodes are often dislodged by the movement of the stimulated muscle and observation of the demarcation potential, at the high sensitivity needed to detect the changes in polarization, can seldom be maintained for long enough to follow the whole course of the phenomenon.

It appears to us that there is a reasonably close correlation between the time to maximum polarization and the time to the peak of the increase in twitch tension. Thus the peak of the increase in potential was reached in 2–3 min. in a number of experiments, figures which correspond closely enough with those for the changes in twitch tension. It is difficult to measure the end of the effect

on tension, although we have generally assumed it to be over in about 15 min., a time which again corresponds with estimates of the end of the period of polarization; but exact determination of this point is made even more difficult by the onset of the decline of demarcation potential. We have been able, in a few experiments, to follow the whole course of the changes, and an example is given in Fig. 4. If allowance is made for probable direction of drift, the polarization appears to be over in this instance in 5 min. and the depolarization to have ceased in some 13 min., and this general ratio of duration has been evident in all

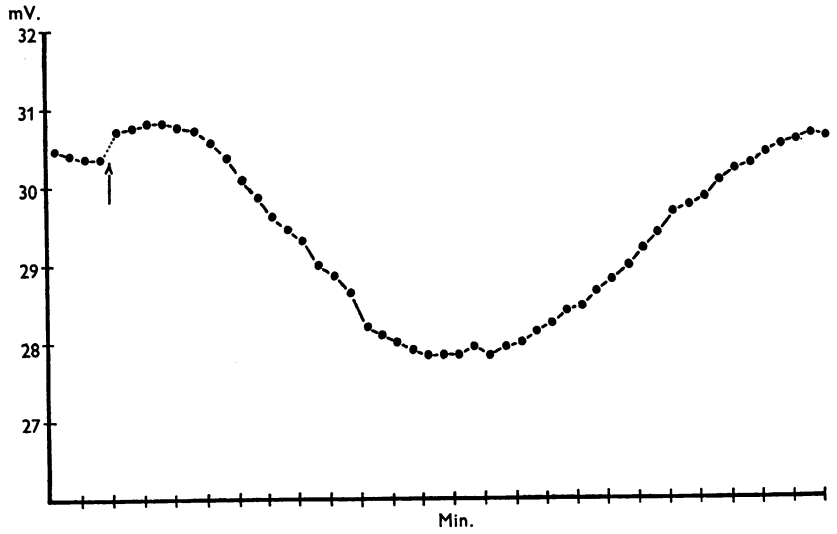

Fig. 4. Cat, 1·7 kg. chloralose. Demarcation potential of tibialis. At arrow close arterial injection of 10 μg. adrenaline.

the experiments in which the stability of the base-line has enabled us to assess it. We can, at all events, say that the polarization is followed by a depolarization of longer duration and greater extent.

We have no reason to believe that the polarization is an accidental effect, since it occurs with regularity after both arterial and intravenous injection of adrenaline. Mechanical disturbances of any sort are, of course, liable to upset the recording, but we have only occasionally observed changes in the direction of increased polarization after a control, saline injection, and these have always been in shape quite unlike those after adrenaline. We have similarly obtained negative controls with the injection of saline solutions at a variety of temperatures from room temperature to 40° C.

The electrical changes produced in muscle, like the mechanical changes, diminish with repeated doses of adrenaline. Thus, in a number of experiments, first doses have produced increases in demarcation potential of 5–10% and

second doses increases of 3–5%; third doses have usually been much less effective.

Denervated muscle. Adrenaline produces in muscle, after degeneration of its nerve supply, electrical changes indistinguishable from those seen in normal muscle. Fig. 5 shows the effect of 10 μg. of adrenaline in the normal leg and in a muscle 7 days after section of the sciatic nerve.

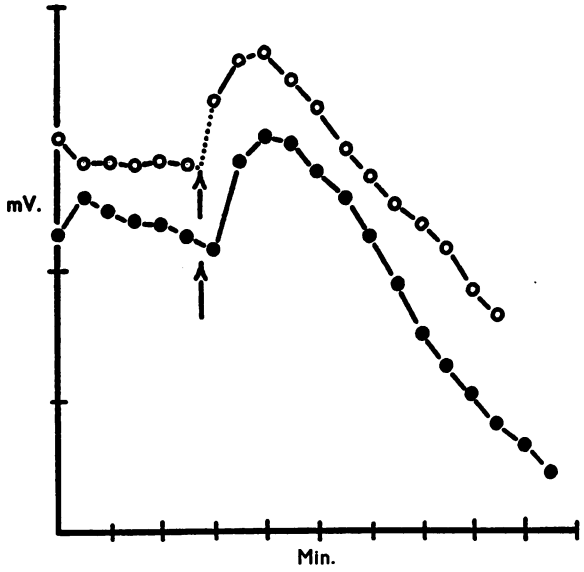

Fig. 5. Cat, 2·1 kg. chloralose. Demarcation potential of both tibialis muscles (absolute value not determined). Upper record normal muscle, lower record from muscle denervated 7 days before. At arrow, close arterial injection of 10 μg. adrenaline.

Neuromuscular block. The production of complete neuromuscular block by the arterial injection of some 200 μg. of D-tubocurarine chloride or of 20 μg. decamethonium iodide did not diminish the effectiveness of adrenaline in increasing the demarcation potential. After decamethonium indeed, which itself depolarizes muscle (Brown, Paton & Vianna Dias, 1949), the effect of adrenaline appears to be greater.

Effect of sympathectomy. In two cats we removed the abdominal sympathetic chains 2 weeks before examining the effects of adrenaline. Sympathectomy in no way alters the effect of adrenaline on demarcation potential.

Effect of other sympathomimetic amines

Both arterial and intravenous injection of DL-noradrenaline produce effects on tension which are generally comparable, but often less than those produced by adrenaline (cf. West & Zaimis, 1949). The effect on demarcation potential does not differ from that produced by adrenaline. Isopropyl-noradrenaline was

a substance of special interest to us, since its only known effects on smooth muscle are inhibitory; it causes the characteristic increase in twitch tension and resting potential. It differs from the other substances which we have used in producing, on occasion, much longer lasting polarization, and this again runs parallel with the changes in twitch tension which can, apparently, persist much longer than they do with other sympathomimetic substances.

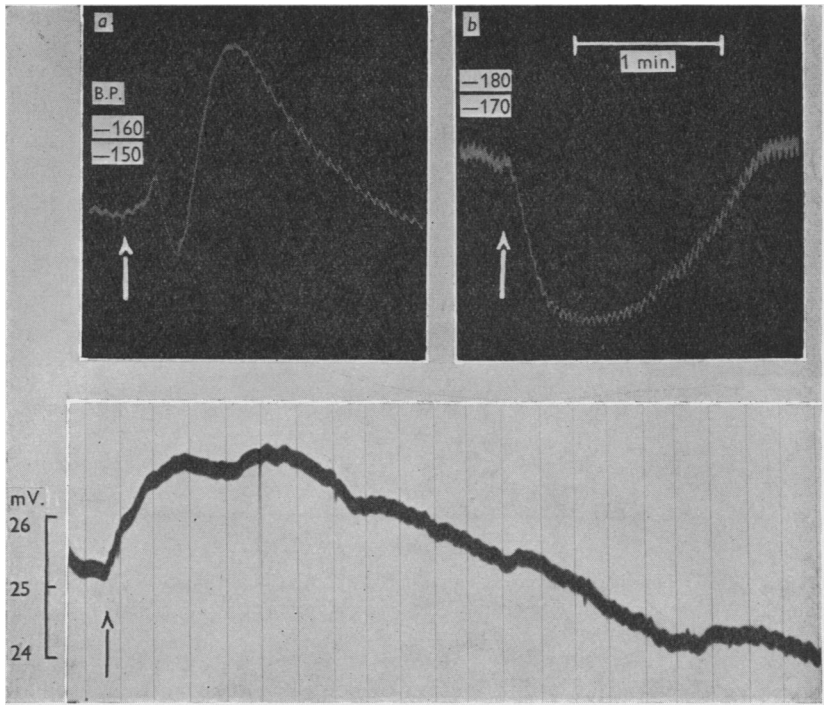

Fig. 6. Cat, 2·4 kg. chloralose. Effect of close arterial injection of 10 μg. adrenaline on blood pressure (*a*) before and (*b*) after administration of 7·0 mg. ergotamine. The lower record is of the demarcation potential of tibialis and corresponds to upper record (*b*). Time marks, 1 min.

Effect of antagonistics to adrenaline

Dibenamine, ergotoxine and ergotamine, which reverse the vaso-pressor action of adrenaline leave unchanged the effects of adrenaline on the demarcation potential (Fig. 6), just as they do not alter the effect on twitch tension (Fig. 2).

Effect of other substances

Brown & Euler have already illustrated the effect of KCl on twitch tension and demarcation potential, but we have included an illustration (Fig. 7) of the effect on demarcation potential of an injection of KCl taken under conditions identical with those of the adrenaline experiments. The initial, profound

depolarization is synchronous with the tetanic contraction which arterial KCl usually produces; the subsequent, lasting depolarization is associated with the increase of twitch tension.

Quinine. There is some similarity between the effects of quinine and of adrenaline on muscle, in that both appear to evoke an increase in twitch tension

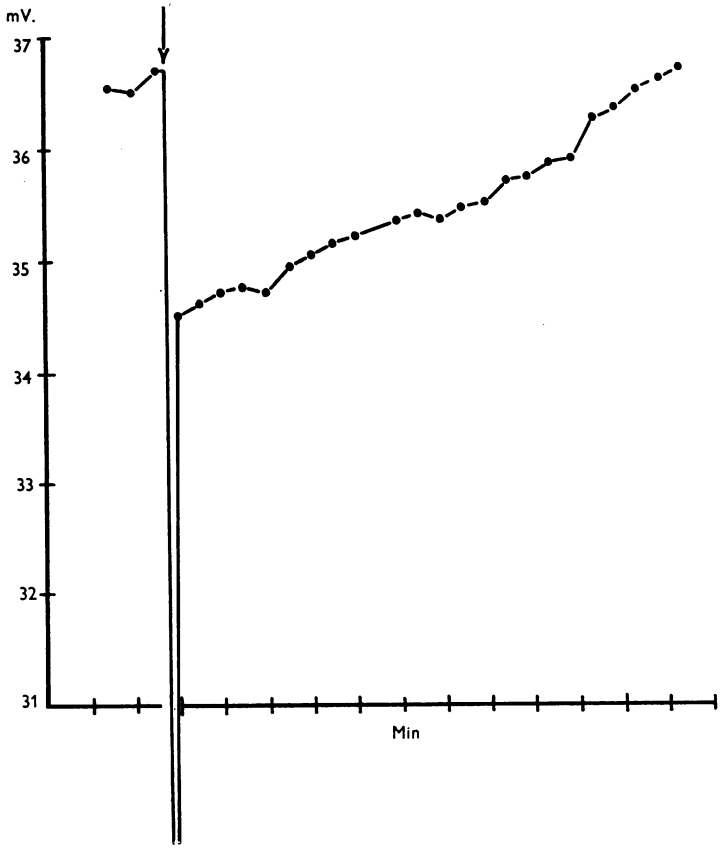

Fig. 7. Same experiment as Fig. 3. At arrow, close arterial injection of 2·5 mg. KCl.

by a slowing of the propagation of the excitation wave (cf. Harvey, 1939; Brown *et al.*, 1948). We thought it desirable, therefore, to investigate again the effect of quinine on demarcation potential. We were able to confirm Harvey's finding that quinine given arterially in doses of 1 mg. is without effect on the resting potential of the muscle.

Pituitary (posterior lobe) extract. Pituitary extract can produce an increase in the twitch tension of a fatigued muscle (Bülbring & Burn, 1940), and it causes vascular changes in a muscle which might be like those produced by adrenaline.

It has, however, no effect on demarcation potential when injected arterially in a dose of 1 i.u. diluted in 0·25 c.c. of saline. This solution so injected had a pH of 4·50, and it is interesting that such an acid solution itself had so little effect.

Effect of stimulation of sympathetic chain

The effects of the sympathomimetic amines on demarcation potential can be closely imitated by stimulation of the abdominal sympathetic chain. Fig. 8 shows an example of such an experiment. After preparation of the muscle, the spinal canal was opened and the dorsal and ventral lumbar and sacral roots

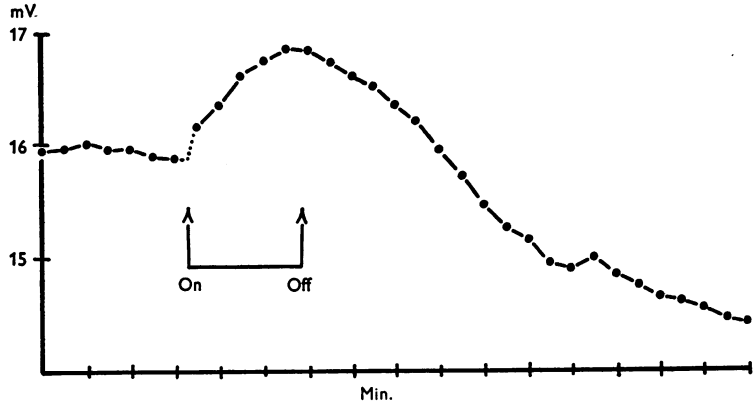

Fig. 8. Cat, 2·1 kg. chloralose. Demarcation potential of tibialis. Between arrows, stimulation of abdominal sympathetic chain.

were cut close to the spinal cord. The ipsilateral sympathetic chain was freed just below the diaphragm and was stimulated at 76/sec. with pulses of 3 V. and 1 msec. duration. Stimulation of the contralateral chain was without effect.

DISCUSSION

Our experiments have shown that adrenaline, noradrenaline and isopropylnoradrenaline all produce characteristic changes in the demarcation potential of the tibialis anterior of the cat. The initial change, which appears to run parallel with the increase in twitch tension of the excited muscle, is in the direction of an increase of the potential. We had expected, by analogy with the effects of injected KCl, that any change we might detect would be in the opposite direction. After adrenaline a depolarization does, in fact, occur, but at a time when the tension is declining after having reached its peak value. Feng and Brown & Euler ascribed the changes which occur in the post-tetanic potentiation to changes in the distribution of K ions because they were able to imitate both the post-tetanic increase in tension and the post-tetanic fall in demarcation potential by the arterial injection of KCl solutions. There is reason to believe, as we have pointed out earlier, that the effect of adrenaline is also associated

with movements of K ions, but our present experiments suggest that the changes produced by adrenaline are more complex than the increased exchange and loss of K^+ which occurs when a muscle is tetanized. Experiments now in progress by one of us (M.G.) and Dr W. L. M. Perry, using labelled K^+, suggest that there is a diminished exchange of K^+ during the polarization with adrenaline and an increase in K^+ exchange during the depolarization.

We have much evidence to exclude the participation of vascular effects in the causation of the potential changes. In the first place there is no correlation between the vasoconstriction produced by adrenaline and the potential changes, they occur equally well when the vascular effects are reversed by ergotoxine and dibenamine, and, finally, they are equally well produced by isopropyl-noradrenaline which has only vasodilator actions. Complete occlusion of the circulation of the legs for 3 min. by clamping the aorta does not, strangely enough, affect the demarcation potential.

We have made a number of experiments on the isolated sartorius muscle of the frog, but we have never observed any effect with dilutions of adrenaline from $1:10^6$ to $1:10^{12}$. Many authors have pointed out that no augmentation of twitch tension can be observed with adrenaline in unfatigued frog's muscle and our observations are in keeping with this.

The characteristic effect which we have observed with adrenaline can be equally well produced by two other sympathomimetic amines, noradrenaline and isopropyl-noradrenaline which have actions on smooth muscle differing widely in degree and sign. Their action on striated muscle is, moreover, unaffected by those drugs which suppress or reverse their effects on smooth muscle. There seems, therefore, to be no relation between the effect of adrenaline on the contractile process of striped muscle and its effect on specific receptors in smooth muscle related to the terminations of nerves.

Brown *et al.* (1948) concluded that the augmentation by adrenaline of the twitch tension of fatigued muscle was due to a direct effect of adrenaline upon the muscle fibre. They also decided that in isolated muscle the 'decurarizing' effect of adrenaline could be entirely accounted for by an increase in fibre tension and was not attributable to recruitment of additional fibres. Maddock, Rankin & Youmans (1948) have shown that a dose of adrenaline given intravenously in the dog can reduce a partial curarization of the tibialis muscle, and that this effect is abolished by dibenamine; we have been able to confirm this in the cat. It appears, therefore, that if adrenaline has, in fact, an effect upon neuromuscular transmission, it is different from its direct effect on the muscle fibre in its sensitivity to dibenamine. Dibenamine has been shown to diminish the liberation of K^+ from the liver by adrenaline (West, 1949), and this gives further support to the suggestion made by Brown *et al.* that some of the effects of adrenaline in the whole animal might be ascribed to the liberation of K^+ from tissues other than the muscle.

The amounts of adrenaline which we have used have been greatly in excess of any likely to be present in the blood stream during normal life. But the fact that the effect on demarcation potential can be reproduced by stimulation of the sympathetic supply to the lower limbs suggests that similar changes can occur in normal muscle.

Our experiments have given us no clue as to the nature of the fundamental change underlying the changes in the potential. We know that adrenaline can cause an increase in twitch tension of the individual fibre and that this is accompanied by, or due to, a retardation in the progression of the excitation wave; it is perhaps not surprising that such changes should be accompanied by changes in the resting potential of the muscle membranes.

SUMMARY

1. Close arterial injection of 0·5–20 μg. of adrenaline into the tibialis anterior muscle of the cat causes an increase of 2–15% in the tension response to single maximal nerve volleys. The effect is independent of muscular fatigue.

2. A similar arterial or intravenous injection of adrenaline causes an increase up to 10% in the demarcation potential of the muscle which follows approximately the increase in twitch tension.

3. The increase in demarcation potential is followed by a depolarization of greater magnitude and longer duration.

4. These effects are produced also by DL-noradrenaline and by DL-isopropylnoradrenaline, and they are unaffected by doses of ergotoxine or dibenamine sufficient to reverse the vasopressor actions.

5. These effects of sympathomimetic amines can be observed in denervated muscle and in muscle in which complete neuromuscular block has been produced by D-tubocurarine chloride or by decamethonium iodide.

6. Stimulation of the abdominal sympathetic chain produces similar changes in the demarcation potential.

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