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THE ACTION OF ADRENALINE ON MAMMALIAN SKELETAL MUSCLE

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Since the original observations of Gruber (1914) and of Orbeli (1923) the effects of adrenaline and of stimulation of sympathetic nerves upon the contractions of frog's and mammalian muscle have been studied in great detail. Both injection of adrenaline and stimulation of the sympathetic can produce an increase in the peak twitch tension, recorded from muscle excited through its nerve. Bülbring & Burn (1940) found that the best reponse was obtained when adrenaline was injected into perfused fatigued muscles, stimulated through their nerves, and that sometimes the muscle tension was doubled. They found that adrenaline had little effect upon the unfatigued neuromuscular system, and that it produced little or no increase in the tension developed by fully curarized muscle stimulated directly.

Some workers have interpreted the response of the directly stimulated muscle to adrenaline as an indication that the sole action of adrenaline is upon the muscle fibres themselves (Michol, 1930). More recently, however (Bülbring & Burn, 1940; Burn, 1945), stress has been laid upon the disparity between the responses to adrenaline of muscle excited through its nerve and stimulated directly. Adrenaline has also been found to augment the tension increase produced by the action of prostigmine and eserine upon muscle stimulated indirectly. In view of these and other facts, recent workers have tended toward the view that the main site of action of adrenaline is the neuro-muscular junction, where it has been supposed to facilitate transmission from nerve endings to muscle (Bülbring & Burn, 1942; Bülbring, 1946).

The experiments described in this paper are mainly concerned with an examination of the electrical phenomena in muscle and were designed to provide further information about the site of action of adrenaline upon the mammalian neuro-muscular system. Our results have given us reason to believe that the action of adrenaline in the fatigued nerve-muscle preparation is primarily upon the muscle fibre itself, and that effects upon neuro-muscular transmission play only a small part, if any at all, in the enhancement of twitch tension.

METHODS

In the few experiments in which cats were used, they were decerebrated under ether anaesthesia and the tibialis anterior was prepared for recording (Brown, 1938).

Most of the experiments upon which this account is based were made on the isolated phrenic-diaphragm preparation of the rat (Bülbring, 1946), and the original technique was followed closely, except that strips of muscle only 2–3 mm. in breadth were usually employed. Electrical recording necessitated some minor modifications which will be referred to later.

The electrical stimuli applied to nerve and muscle were taken from the secondaries of air-cored transformers through the primary of which a condenser was discharged; the discharge of the condenser was controlled by a soft valve. When two or more stimuli were given the interval between them was controlled either electrically or with a Lucas pendulum.

The precise form of the electrodes used for stimulation varied with the needs of the experiments, but, in all instances, the contact with the tissue was through silver wire. In the experiments on cats, the stimuli were applied through bare silver wires, 1 cm. apart, on either side of the sciatic nerve which was tied proximally. The test shocks, given during the course of a tetanus, were applied through a second pair of electrodes placed 1 cm. distal to those used for the tetanus.

The phrenic nerve was usually excited through two of a number of silver wires 1 mm. apart upon which it rested, just above the surface of the fluid bathing the muscle. In the course of the experiments we found that satisfactory stimulation of the nerve or muscle could be effected beneath the surface of the bath by applying a capillary glass tube which was connected to a suction pump and contained in its tip a fine silver wire.

Records of muscle tension were taken with a flat spring, cantilever myograph in the experiments on cats and with a very light torsion myograph in the experiments on the rat's diaphragm. The small tensions developed by the diaphragm and the rapidity of its contraction made accurate recording of the form of the single twitch a matter of some difficulty.

All tension records were made under isometric conditions, the magnification of the movement being $\times 200$ and the biggest final deflexion some 3 cm. The applied initial tension was approximately half the twitch tension.

The action potentials of the muscle were led into a condenser coupled, push-pull amplifier, the resistance between each input lead and earth being 0·5 MΩ. In all experiments, except those in which single muscle fibres were recorded, the time-constant of the circuit was 75 msec. In the experiments on the cat's tibialis anterior, the records were taken with the cat earthed and belly-tendon leads.

The method of recording from the diaphragm varied with the type of experiment. When the gross action potential of the muscle was required, one amplifier lead was connected to the tendon through a silver wire, and the other to the earthed fluid in the bath. The muscle fibres then projected 2–3 mm. above the surface of the fluid in the bath and were kept moist by spray from the vigorous oxygen supply. This method of recording has the disadvantage that the electrical record shows random fluctuations in amplitude which are due to the formation of bubbles on the surface of the liquid close to the muscle, an irregularity which is not remedied, but is even increased by covering the fluid with a layer of liquid paraffin. Another disadvantage of this method is that the electrical record is taken from only a small fraction of the fibres contributing to the total tension. Yet a further difficulty introduced by recording in this way is that the effective distance between the recording electrodes, and consequently the shape of the action potentials, vary with the initial tension to which the muscle is subjected.

When records were taken from very small fractions of muscle, electrodes were applied to the surface of the muscle which was suspended horizontally. An insulated silver wire, with its end cleaned by a transverse cut, was found to give satisfactory records of the electrical activity of a few motor units.

In order to obtain records from single muscle fibres, pore electrodes were used for stimulating and recording. They were constructed by melting and pulling out a piece of 'Pyrex' tubing containing a length of silver wire. These make a satisfactory joint and enable the end of the glass

tubing to be ground flat until the diameter of the silver core is of the required size. We used electrodes 0·5 mm. in diameter with a silver core of 5 μ cross-section. They were held in micro-manipulators.

In the figures, no absolute values of either the tension developed by the diaphragm or of the recorded action potential are given. The reason for this is that we used strips cut to suit the needs of each particular experiment; the strips varied in breadth from 1 mm. to 1 cm., although, as stated above, 2–3 mm. was the usual width. The tensions recorded were, therefore, widely different from experiment to experiment. For the same reason, the absolute values of the recorded potentials were without significance; not only did the strips vary in size, but the degree of short circuiting by the bath fluid varied from experiment to experiment.

RESULTS

Experiments on the tibialis anterior of the cat

Preliminary experiments showed that the effect of adrenaline upon the tension developed by a fatigued muscle could be demonstrated easily. Maximal stimulation of the sciatic nerve at frequencies of the order of 30/sec. produces a contraction of tibialis, the tension of which falls steeply to about half its initial value in some 3 min., and then shows little decline for 10 min. or more. There is a corresponding change in the peak voltage of the action potential. In testing the effect of adrenaline we generally interrupted the tetanic stimulation for a period of about 2 sec. and interpolated a single maximal stimulus to the nerve, at a time when the muscle was fully relaxed after the end of the tetanic stimulation. This enabled us to maintain a steady level of 'fatigue' and yet to record the uncomplicated response of the muscle to a single nerve volley.

The intravenous injection of 50 μg. of adrenaline during the steady period caused prompt increase of tetanic tension and of the tension produced by the test shock. An increase in twitch tension as great as 40 % may not be accompanied by any change in peak action potential, and we have observed increases in tetanic tension up to 80 %, again without alteration of peak action potential. We have, on occasion, observed small increases in peak action potential running parallel with the increase of tetanic tension, but these were, in any event, irregular and were not observed in the responses to the single volleys. Their significance is discussed later.

These experiments showed that, whatever action adrenaline might have upon fatigued muscle in the intact animal, it was improbable that the recruitment of additional muscle fibres or units played a significant part in the increase of tension. It was, of course, possible that additional fibres were in fact contracting, but that the action potential of all fibres was reduced in such a way as to leave the total potential unaltered. Previous experience of the effects of potassium chloride (cf. Brown & von Euler, 1938) and of quinine upon muscle (Harvey, 1939) has shown that increases in twitch tension may be accompanied by an actual diminution in peak action potential. It was, therefore, obvious that the phenomenon required much more detailed analysis

than could conveniently be made on a large muscle with natural circulation, and we consequently continued the investigation on an isolated mammalian nerve-muscle preparation.

Experiments on the isolated diaphragm

The isolated phrenic-diaphragm preparation is more susceptible to fatigue than one with natural circulation; nevertheless, the threshold of its nerve, its nerve action potential, and, indeed, the action potential of the muscle show little change in the course of a day's experiment if stimulation is not maintained for long periods. We have found that stimulation of the nerve with single maximal shocks at a frequency of 2/sec. for some minutes is sufficient for the demonstration of the action of adrenaline. The immediate consequence

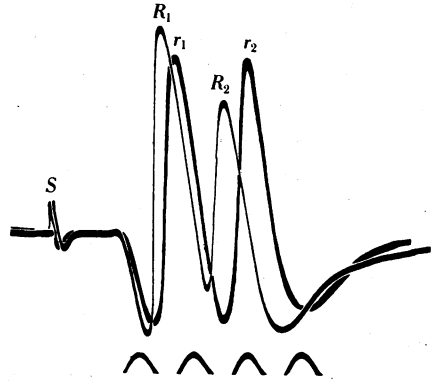

Fig. 1. Rat diaphragm. Action potential recorded from two points of surface, two records being superimposed. *s*, stimulus artefacts. R_1, R_2, responses of fresh preparation to single maximal nerve volley; r_1, r_2, responses of same muscle after 12 min. maximal stimulation of nerve at 2/sec. Time, 1000 cyc./sec. Photograph retouched.

of repeated stimulation of the nerve with supramaximal shocks at frequencies of 2/sec. is a steady decline in peak tension, and a parallel decline in peak action potential; at the same time, however, the action potential lasts longer. In other words, the action potential, recorded monophasically, becomes smaller in height and broader across the base.

The cause of this broadening appears to be an increase in the temporal scatter of the responses of the constituent fibres of the muscle. We were able to demonstrate this by applying two pore electrodes to points approximately 6 mm. apart on the surface of the diaphragm and feeding the combined action potentials into the same amplifier channel. By placing the electrodes so that one lay near the anatomical entry of the nerve and the other some distance from it we were able to obtain two discrete spikes with very different latencies. Fatigue of the preparation caused a clear increase in the temporal dispersion of the responses (Fig. 1).

Effect of adrenaline on the response to single maximal nerve volleys. The effect of the addition of adrenaline to the bath in doses of 1–2 μg./c.c. Tyrode's solution is an increase in the peak twitch tension, without any consistent change in the peak action potential. There is, on the other hand, a further broadening of the base of the action potential which runs *pari passu* with the change in tension.

The results of a typical experiment are shown graphically in Fig. 2 and examples of the changes in action potential and tension are given in Fig. 3.

It will be observed that the tension records in Fig. 3 suggest that the broadening of the action potential is associated not only with an increase in peak twitch tension, but also with an increased duration of the twitch. Tension records taken at higher speeds have, on occasion, revealed an unmistakable slowing of the tension wave, but this has always been small, and we have not been able to demonstrate it regularly with the methods of recording available.

Effect of adrenaline on the response to two nerve volleys. In these experiments, the nerve was excited with two supra-maximal shocks at various intervals, and the mechanical and electrical responses were recorded. Continued stimulation of the nerve with single and paired stimuli, repeated at 1–2 sec. intervals, had little effect upon the absolute refractory period of the system or upon the recovery curve of the second response. The absolute refractory period of the preparation stimulated through a single electrode 1 cm. from the muscle was about 0·75 msec. at 37° C., and recovery of the second response was complete in 5 msec.

The administration of adrenaline in doses sufficient to cause a clear increase in the twitch tension and in the duration of the first action potential was without effect upon either the absolute refractory period or the recovery curve. Although the peak tension resulting from a pair of stimuli, separated by a small time interval (2–10 msec.), was increased by adrenaline, the contribution of the second response of the muscle to the total tension was unaltered. If, for example, the tension developed by the fatigued muscle in response to a single stimulus was 1 g. and after adrenaline was 1·25 g. we found that the tension for a pair of stimuli 4 msec. apart was increased by adrenaline from 2·5 to 2·75 g. (cf. Fig. 2).

It was clear, in view of the constant broadening of the action potential produced by adrenaline, that more information was needed about the effect of adrenaline on the directly excited muscle and upon smaller contractile units than we had been using.

Effect of adrenaline on muscle excited directly. Preparations of the diaphragm were suspended in the bath and curarine chloride (King, 1935) was added until neuro-muscular transmission was blocked. In the course of the experiment, the completeness of the block was checked by nerve stimulation. The muscle was excited by stimuli from an insulated silver wire applied to its surface

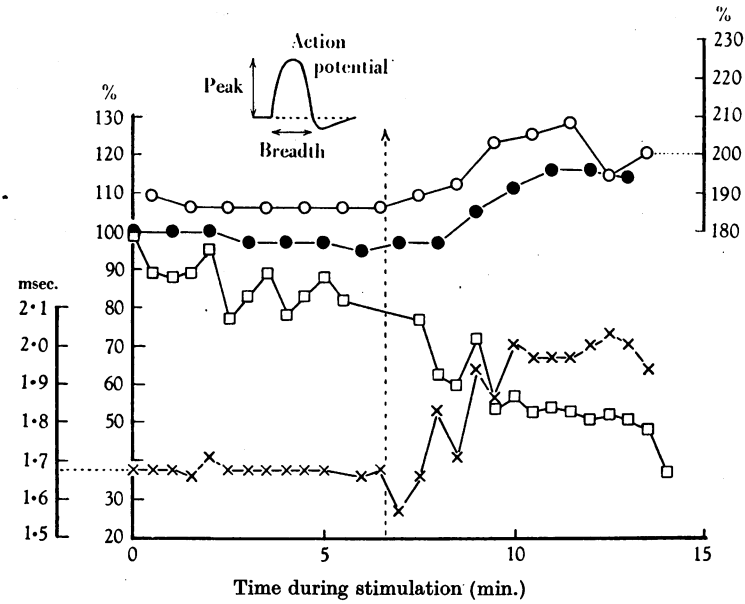

Fig. 2. Rat diaphragm. Responses to maximal nerve volleys at 30/min. o—o tension response to two volleys at 2 msec. interval as percentage single response at start. •—• tension response to single volley as percentage at start. □—□ peak action potential as percentage at start. × — × breadth of action potential in msec. At arrow adrenaline to give a concentration of 1 μg./c.c.

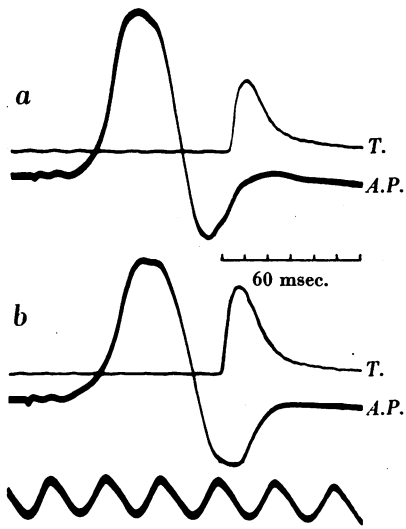

Fig. 3. Rat diaphragm. Action potential (A.P.) and tension (T.) in response to single maximal nerve volleys. Fatigued preparation, (*a*) before and (*b*) 3½ min. after adrenaline 2 μg./c.c. Time, top, 10 msec.—applies to tension. Time, bottom, 1000 cyc./sec.—applies to action potential.

beneath the liquid of the bath, and electrical and mechanical records were taken. The stimuli were made as strong as the apparatus could supply, but it must be emphasized that they could not be in any sense maximal. The reason for this is that the fibres of the diaphragm are not more than 5 mm. in length, and any stimulus applied to one group excites that and not adjacent fibres.

Sufficiently reliable records were obtained, however, to make it clear that adrenaline produces, in directly excited muscle, a broadening of the action potential as great as that in muscle excited through the nerve (Fig. 4). The broadening is associated with an increase in the time from stimulus to peak potential.

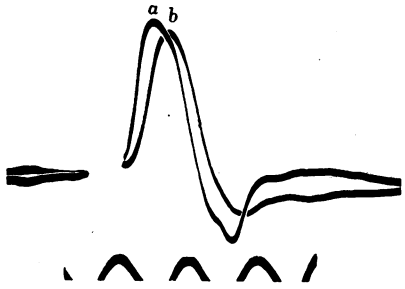

Fig. 4.

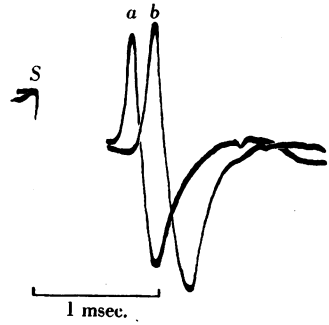

Fig. 5.

Fig. 4. Rat diaphragm. Action potential of fully curarized preparation, stimulated directly. Superimposed records (*a*) before and (*b*) 1½ min. after adrenaline 2 μg./c.c. Time 1000 cyc./sec. The stimulus artefact has been removed.

Fig. 5. Rat diaphragm. Action potential of single muscle fibre, fully curarized, stimulated directly. Superimposed records (*a*) before and (*b*) after adrenaline 2 μg./c.c. The stimulus artefact beginning at *S* has been omitted. Amplifier time constant 7·5 msec. Time 1 msec.

Experiments with single muscle fibres. The experiments on directly excited muscle provided evidence that adrenaline altered the spread of the excitation wave in the individual muscle fibre, and only experiments with single muscle fibres could give direct proof of this hypothesis. The method of stimulation and recording has already been described, but attention should be drawn to the method adopted to ensure excitation and recording of the same single fibre. The electrodes were alined by adjusting them so that the smallest effective stimulus gave the greatest response. We have assumed that when a continuous variation of stimulus strength gave an all-or-nothing electrical response, the response was that of one muscle fibre only. It is easy to avoid exciting the fibre through its nerve terminal, since, when this occurs, the electrical records show the 'immediate' response of the single fibre in question, followed 1 or 2 msec. later by the response of the remainder of the fibres in the motor unit. We have checked a number of our preparations by giving a dose of curarine, sufficient to produce complete curarization, but have invariably found that this left the records of action potential unaltered.

The action potentials recorded were potential differences between that part of the fibre beneath the silver electrode and the earthed fluid in which the whole diaphragm was bathed. Since the end of the silver electrode was insulated from the surrounding fluid by the glass sheath in which it was fused, the action potentials recorded were triphasic. Whatever interpretation is given to the triphasic record, it shows clearly that adrenaline delays the spread of the excitation wave (Fig. 5).

The effect of temperature. The foregoing experiments made it clear that the major effect of adrenaline was upon the speed of conduction of the excitation wave, and we consequently sought another means of slowing the wave and of studying its effect upon the tension.

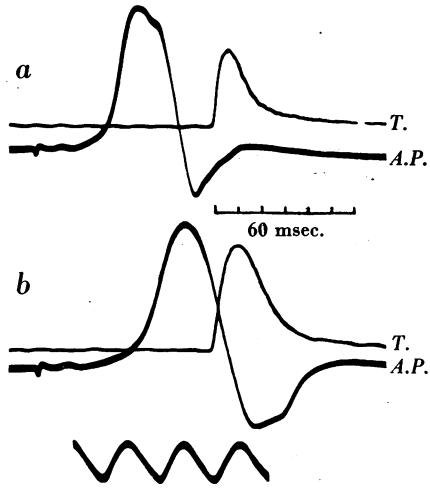

Fig. 6. Rat diaphragm. Action potential (A.P.) and tension (T.) in response to single maximal nerve volleys. Fatigued preparation, (*a*) at 37·8° C. and (*b*) at 31·5° C. Time, top, 10 msec.—applies to tension; bottom, 1000 cyc./sec.—applies to action potential.

We found that cooling the muscle produced a slowing of the electrical excitation wave and a corresponding increase in duration and tension of the mechanical response. Fig. 6 should be compared with Fig. 3, when the general similarity of the effects becomes obvious. It will be noted that they differ in one important respect: the latent period of the electrical response is unaffected by adrenaline, whereas in the 'cold' record it is increased in duration.

The experiments which we have described were designed to reveal the site of action of adrenaline upon the neuro-muscular system. Their results gave no evidence that adrenaline affects neuro-muscular transmission, but did point to a direct action upon the muscle fibres themselves. If we assumed this to be the only action of adrenaline, we still were without an explanation of those particular circumstances in which adrenaline increases twitch tension. The

experiments described below were designed to test whether the reported actions of adrenaline could be explained in terms of direct action upon the muscle fibres alone.

Artificial desynchronization of the fibre responses in the rat's diaphragm

We sought some feature common to fresh muscle excited through its nerve and to muscle excited directly, because these were the outstanding circumstances in which adrenaline was reported to have little effect upon twitch tension. The degree of synchronization between the contracting fibres appeared a promising line of approach, since we already had evidence that fatigue caused a considerable increase in asynchrony of the fibre responses, when the muscle was excited through its nerve. Assuming perfect synchronization of contraction, a delay in relaxation or an increase in the duration of the contraction of the component units could occur without any effect upon the peak twitch tension. With less than perfect synchronization, the peak tension is the sum of the many different instantaneous tensions reached by the component fibres of the muscle in their various stages of contraction or relaxation. In these circumstances an increase in the duration of any phase of the contraction or relaxation process, without alteration of the peak tension attained by an individual unit, must increase the gross peak twitch tension, provided that the duration of tension in the individual unit is of the same order as the degree of desynchronization between units. Desynchronization can, therefore, only account for the observed facts if the peak tension in the single muscle fibre lasts no longer than 2 or 3 msec., since the observed desynchronization, with the degrees of fatigue we have used, has never exceeded 1 msec. In our experiments adrenaline has never increased the observed duration of the action potential by more than 0·5 msec., and the time to the peak of the twitch has never been observed to increase beyond 2 msec., and only in a few experiments was it demonstrable at all.

It seemed possible, nevertheless, that an artificial desynchronization produced in a fresh muscle might reveal an augmentation of peak tension by adrenaline which was not present when the nerve was excited maximally in the usual way. It also suggested an explanation of the puzzling phenomenon of adrenaline affecting the response to submaximal, but not to maximal nerve volleys in the unfatigued muscle (Bülbring, 1946); submaximal might well be expected to initiate a less synchronous volley than maximal stimuli (cf. Blair & Erlanger, 1935). Bülbring's observations were made by testing the effect of adrenaline first on a series of submaximal twitches, and then on a series of maximal twitches; she found that adrenaline increased the tension of the submaximal twitches but not that of the maximal. We excited the nerve alternately with submaximal square waves, 2·13 msec. in duration, and by maximal induction shocks from our usual stimulator. The long duration of the submaximal stimuli was chosen to imitate those used in Bülbring's experiments.

The effect of adrenaline upon tension was found to be identical in both responses, whether its access to the nerve was excluded or not: a surprising finding in view of Bülbring & Whitteridge's (1941) observation of the effect of adrenaline on the excitability of nerves in the whole animal.

A second method which we adopted was to prepare a diaphragm and cut half way across the nerve trunk some 2 cm. from the muscle. Stimulating electrodes were applied on either side of the cut and close to it. The nerve was excited alternately with a maximal single shock through the electrode nearer the muscle and with maximal paired shocks separated by 0·5 msec. through both electrodes, the point further from the muscle being excited first. With this method, the greatest separation between the volleys is limited by the refractory period of the nerve, usually about 0·75 msec. In order to obtain a greater separation, attempts to split the nerve longitudinally having failed, we adopted the device of making two preparations and arranging them to pull in series, the tendon of one being attached to the costal margin of the one above it. The muscles could then be excited either independently, simultaneously or at any chosen interval.

Artificial desynchronization by all these expedients failed to increase the effectiveness of adrenaline. Our failure to reproduce this effect of fatigue does not necessarily exclude desynchronization as at least one factor contributing to the enhancement of the effect of adrenaline, since the desynchronization which we have observed is almost certainly between the fibres of a motor unit, and that which we produced artificially was of necessity between many motor units. But it seemed improbable that the effect of desynchronization between the responses of fibres in one motor unit could not in part be demonstrated by a desynchronization imposed between one motor unit and the next, many of whose muscle fibres must be intimately mixed.

Action of adrenaline upon the rat's diaphragm stimulated alternately through its nerve and directly

These findings made it seem probable that, despite earlier accounts, contractions of the rat's diaphragm produced by excitation of the nerve and by direct stimulation of the muscle would be equally affected by adrenaline. In order to make a satisfactory comparison between the responses from these two different methods of stimulation it was essential that the direct stimulus should be maximal when the muscle was submerged in Tyrode's solution. Maximal tension responses to direct stimulation were obtained by enclosing a strip of muscle in a short length of 3–5 mm. bore glass tube, open at both ends. The glass tube contained annular silver electrodes at opposite ends of the muscle strip for direct stimulation, and the nerve was led out to the electrodes for indirect stimulation through a small hole in the glass tube. A square wave of about 100 V. and 1 msec. duration applied to the ring electrodes was found

adequate for direct stimulation. Stimuli lasting more than 1·5 msec. are liable to produce double responses in the fresh muscle. In all instances the adequacy of the direct stimulus was tested at the end of the experiment with complete curarization.

We found that when a freshly excised muscle is fatigued by maximal stimuli to the nerve at 1–2/sec., there is a parallel decline in the twitch tension resulting from excitation of the nerve and in the tension produced by direct stimulation of the muscle. Adrenaline in doses of 1–2 μg./c.c. bath fluid affects both responses equally. The results of a typical experiment are plotted in Fig. 7. It is of considerable interest that these experiments provided no evidence of neuromuscular block.

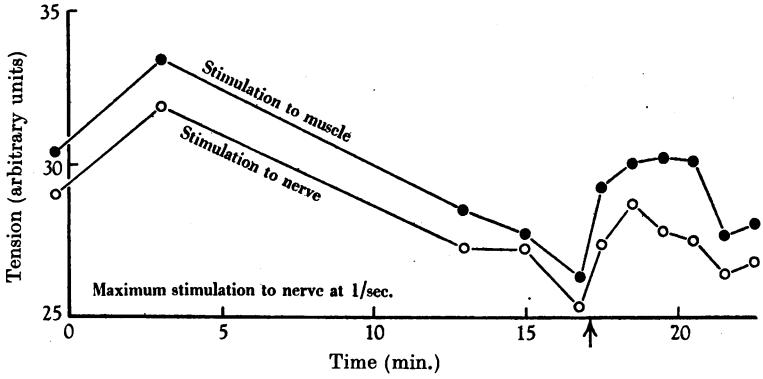

Fig. 7. Rat diaphragm. Tension response to •—• maximal direct stimulation of muscle and to o—o maximal nerve volleys, sampled during continuous stimulation of the nerve at 1/sec. At arrow, adrenaline 2·5 μg./c.c.

The action of adrenaline on partially curarized muscle

Although the preceding experiments showed that adrenaline could produce considerable increases in the twitch tensions of muscles in which no failure of neuro-muscular transmission was demonstrable, it still seemed possible that adrenaline might facilitate transmission when this failed. With this point in mind we examined the action of adrenaline upon the partially curarized isolated rat's diaphragm excited through its nerve, its action potential and tension being recorded simultaneously. Fig. 8 shows the results of an experiment in which a reasonably steady level of partial curarization was attained. The effect upon twitch tension is quite dramatic, but it is accompanied by an increase in peak action potential which is less in degree and duration. We also adopted the more direct technique of recording the tension response of the partially curarized muscle, excited with stimuli applied alternately directly and to the nerve. The results of a typical experiment are shown in Fig. 9. The

responses to the direct stimulation show a steady decline as the muscle fatigues, and those to the nerve volleys a similar decline, the origin of which may be muscular fatigue or a change in the degree of curarization. The administration of adrenaline causes an increase in tension, the absolute amount of which is equal in both curves.

Neither of these experiments provided completely convincing evidence that adrenaline is without any effect upon neuromuscular transmission depressed with curarine. In the first, there is a small increase in the peak action potential during the early part of the increase in tension. In our opinion this transient increase of peak action potential does not necessarily indicate that there has

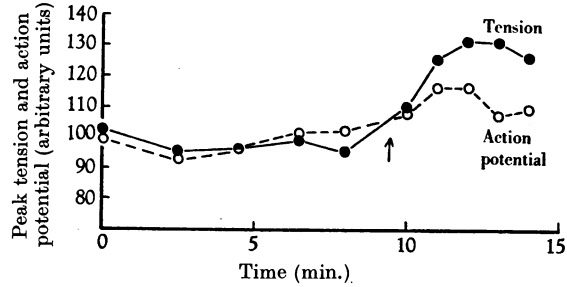

Fig. 8. Rat diaphragm. Action potential o—o and tension •—• of partially (40 %) curarized muscle, responding to maximal nerve volleys 12/min. At arrow, adrenaline 2·5 μg./c.c.

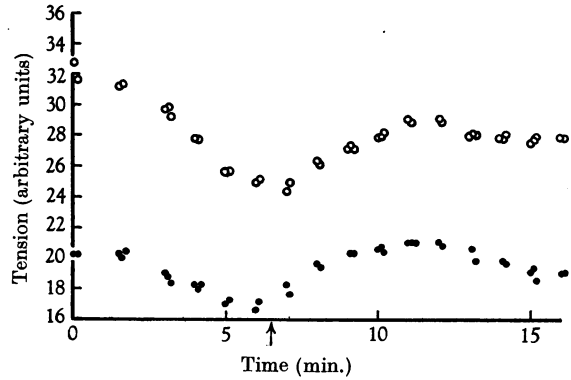

Fig. 9. Rat diaphragm. Tension responses of partially curarized muscle to maximal direct stimulation (o) and to maximal nerve volleys (•). At arrow, adrenaline 2 μg./c.c. Sampled during continuous stimulation at 12/min.

been an increase in the number of muscle fibres contributing to the total action potential; like the peak tension, the peak action potential of the whole muscle can be increased by a prolongation, after adrenaline, of the action potential of the single component muscle fibres, if these are firing asynchronously. In the second experiment, there is a change in the ratio of the tension developed from the direct stimulus to that from the nerve volley, but the degree of

decurarization in terms of relief of neuromuscular block is evidently very small in extent and duration in comparison with the total increase in tension.

The success of experiments of this nature depends upon the maintenance of a steady level of curarization over a considerable period of time. We only partially achieved this, and slow changes in the depth of curarization were taking place in both the experiments we illustrate. We have, in fact, found the maintenance of a steady depression with curarine to be a matter of great difficulty with this preparation, largely because of the slowness of action of the drug and the dependence of its effect upon the precise frequency of the stimuli used either for testing or for fatiguing the tissue.

DISCUSSION

The experiments which we have described on the tibialis anterior of the cat suggested that the characteristic effect of adrenaline in increasing the twitch tension of fatigued skeletal muscle could not be explained on the ground that the adrenaline caused an increase in the number of muscle fibres responding to the nerve volley. A more detailed examination of the effect on the phrenic-diaphragm preparation not only failed to demonstrate any action of adrenaline on neuro-muscular transmission, but has shown that adrenaline can augment twitch tension under circumstances in which no failure of neuromuscular transmission could be demonstrated. The experiments in which the effect of adrenaline was tested upon a partial neuromuscular block produced by curarine failed to give an entirely unequivocal answer for reasons which we have considered above. They made it clear, however, that whatever the effect of adrenaline upon neuromuscular block, it is small compared with its direct action upon the muscle fibre.

Of the precise mechanism whereby adrenaline exerts its action on muscle we have no knowledge. We are, in general, in agreement with the statement made by previous workers that some degree of fatigue is necessary before adrenaline can act, but there were certain irregularities in the behaviour of our preparations which make us uncertain that fatigue is the whole answer to the problem. Adrenaline is known to cause a mobilization of K^+ from animal tissues (cf. D'Silva, 1937; Hebb & Nimmo Smith, 1946), and K^+ has striking effects on both muscle tension and action potential (Brown & von Euler, 1938). The recent observation by Goffart & Brown (1947) that the effectiveness of adrenaline on the isolated diaphragm preparation is a function of K^+ content of the bath fluid suggests that adrenaline may act by causing changes in the distribution of K^+ in the muscle.

The interpretation which we have given to our results differs from the earlier conclusions of Bülbring & Burn (1940). It is difficult, however, to relate our experiments, mainly on isolated tissues, to theirs on intact and perfused muscles excited with brief, tetanic and probably submaximal nerve volleys.

Many indeed of the discrepancies between our findings and those of Bülbring & Burn could be explained by assuming that, in their experiments, adrenaline had increased the plasma [K^+] and thereby evoked changes in the threshold of excitable tissues.

SUMMARY

1. In order to determine the site of action of adrenaline upon skeletal muscle, its effects have been examined on the tibialis anterior of the decerebrate cat and on the phrenic-nerve-diaphragm preparation of the rat.

2. Although adrenaline causes an increase in the tension response to maximal motor nerve volleys of both preparations, the peak action potential is not increased.

3. Adrenaline causes an increase in the duration of the action potential which runs parallel with the increase in tension.

4. The increase in duration of the gross action potential is due to a retardation of the spread of excitation wave along the individual muscle fibres.

5. No evidence has been obtained that adrenaline owes its effect to the relief of neuromuscular block.

6. The effects of adrenaline on the tension response of an excited muscle appear to be due to a direct action upon the tension developed in the individual fibre.

7. Fatigue increases the temporal dispersion in the response of a muscle to motor nerve volleys, but this cannot be shown to account for the greater effect of adrenaline in fatigued muscle.

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