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THE ACTION OF ADRENALINE ON THE RATE OF LOSS OF POTASSIUM IONS FROM UNFATIGUED STRIATED MUSCLE

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Small doses of adrenaline cause an increase in the maximal twitch tension of unfatigued mammalian muscle. The effect appears to be closely related to the concentration gradient of potassium ions across the cell membrane, since Goffart & Brown (1947) and Goffart (1947) found that excess of K^+ ions in the fluid bathing the muscle reduces the effect of adrenaline, and that depletion of the intracellular K^+ ions, produced by feeding an animal on a low potassium diet, may reverse the potentiating effect.

The recent finding of Brown, Goffart & Vianna Dias (1950), that adrenaline produces changes in the demarcation potential of muscle, lends further support to the idea that this adrenaline effect is associated with changes in ionic distribution. We have studied the effect of adrenaline on the output of potassium from mammalian muscle in order to obtain more direct evidence of the relationship between potassium and the effect of adrenaline on the twitch tension and demarcation potential of muscle. Experiments were carried out to measure the rate of loss of K^+ ions across the muscle-cell membranes, making use of the radioactive isotope of potassium—^{42}K.

METHODS

Some early experiments were made on the cat's extensor digitorum longus perfused with plasma. This muscle has a theoretical advantage over the more commonly used tibialis anterior preparation, in that both its origin and its insertion are tendinous, and a complete isolation of the muscle is therefore more easily effected. The cat was anaesthetized with chloralose, the leg muscles were dissected, and the isolated extensor digitorum longus was perfused through its arterial system with normal cat's plasma. The muscle was stimulated through the sciatic nerve in the thigh with maximal shocks of 0·2 msec. duration at a frequency of 1 in 10 sec. throughout the experiment. The effluent from the veins was collected and measured. The cat either received an intraperitoneal injection of a 1 % solution of ^{42}KCl some 3–4 hr. before the experiment began, or else a more dilute solution of ^{42}KCl in plasma was perfused through the muscle for about 30–60 min.

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in the first part of the experiment. In both cases the muscle was thus 'loaded' with radioactive potassium before the experiment proper began. In experiments in which the loading was carried out by intraperitoneal injection of the cat, the dose given on the first day was 25 ml. of a 1% solution of irradiated KCl; the ratio of ^{42}KCl to ^{39}KCl in the solution is not known. The samples of venous effluent collected from the muscle contained an amount of ^{42}K which could be measured by counting the β radiations in a Geiger-Müller fluid counter; and the extent to which the muscle was 'loaded' influenced only the total count and not the relative result of the experiment.

Because of the difficulties in controlling accurately small changes in temperature and rate of flow of the perfusion fluid, which influence greatly the potassium output, the method was later abandoned in favour of one based upon that described for nerve by Keynes (1948), using an isolated strip of rat's diaphragm.

The experiments on the rat's diaphragm were designed to record the radioactivity present in the muscle cells rather than in the perfusate leaving a muscle. Keynes (1949) has shown that the radioactivity in the extracellular fluid is rapidly washed out in the first few samples of effluent, and we were able to confirm this. It had been shown by Noonan, Fenn & Haege (1941) that the diaphragm of the rat is 'loaded' very rapidly after an intraperitoneal injection of ^{42}KCl. We found that after such an injection of 5 ml. of a 1% solution, a convenient concentration in the diaphragm was reached in about 1–2 hr. A rat was killed by a blow on the head, the thorax opened and a strip of diaphragm was carefully removed so that one end of the strip was left attached to the rib margin and the other consisted of the central tendinous part of the muscle. The strip was removed by two single parallel cuts from rib margin inwards, so that the fibres of the muscle, which run in this direction, were not cut transversely. This is very important since there may be considerable K^+ loss from damaged or transected fibres. The strip of diaphragm was stretched out and pinned in a trough cut in a Perspex chamber. A thin mica window was set in the base of this trough, so that about two-thirds of the diaphragm strip lay above it, and the chamber fixed so that the window lay immediately above a Geiger-Müller tube. Throughout the experiment the muscle was washed with a potassium-free Tyrode solution, the rate of flow being about 80 ml./min. in most experiments. The fluid irrigating the muscle could be drawn from one of two 2 l. reservoirs heated in a water-bath at 41° C. so that the fluid in the trough was 38° C. In one of these reservoirs the drugs to be tested were dissolved and the muscle was irrigated for varying periods with this fluid. For the control experiments the second reservoir contained no drug, but the fluid was drawn from it for a given period in the usual way. The muscle strip remained in position throughout the experiment and one-minute counts could be made easily each alternate minute. The usual level of count was about 3000–6000/min. Experiments have been carried out using L-adrenaline hydrochloride in a concentration of 1 in 100,000, DL-noradrenaline hydrochloride 1 in 100,000 and DL-*iso*propyl-noradrenaline sulphate 1 in 20,000.

RESULTS

In the perfusion experiments on the extensor digitorum longus of the cat, the amount of ^{42}K in the effluent reflects directly the amount of ^{42}K lost from the muscle. Generally there was a very large loss of ^{42}K immediately the perfusion was started—probably derived mainly from the intercellular spaces. This was followed by a fairly steady loss of ^{42}K, which we consider is derived from the muscle cells themselves. Fig. 1 shows typical results. The lower record was from a control experiment in which, at the arrow, a saline solution was injected into the perfusion plasma in the arterial cannula. The upper record was from an experiment in which 10 μg. of DL-*iso*propyl noradrenaline was injected at the arrow. The ordinate is the concentration of ^{42}KCl in the venous effluent from

the muscle; in the control, this remained fairly constant throughout the experiment. The effect of *iso*propylnoradrenaline was to cause a fall in concentration lasting for some 10–12 min., followed by a rise in concentration above the original value. The total amounts of ^{42}K lost were known but, owing to changes in the rate of perfusion, the figures for concentration provide a more correct picture of the changes which occur. DL-*Iso*propylnoradrenaline was used in these experiments in place of adrenaline, since the latter was found to produce such a vaso-constriction that the flow of perfusion fluid ceased alto-

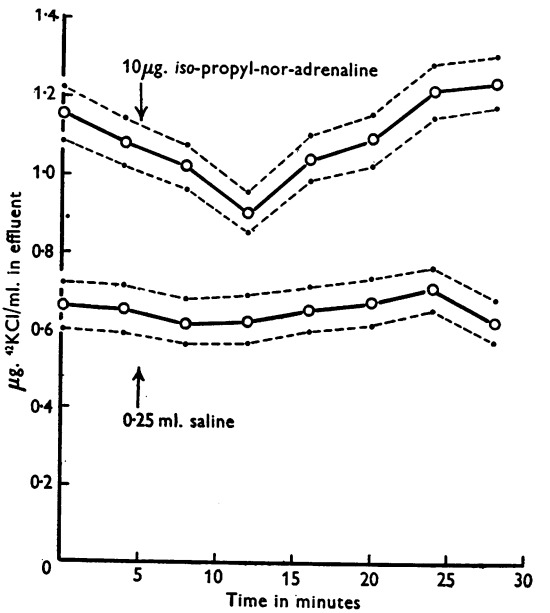

Fig. 1. Effect of *iso*propylnoradrenaline on the rate of loss of ^{42}K from extensor digitorum longus of the cat (previously 'loaded' with ^{42}K) perfused with cat's plasma. The dotted lines represent the limits of error of the estimations ($P = 0{\cdot}05$).

gether for several minutes. DL-*Iso*propylnoradrenaline has the same effect as adrenaline on the maximal twitch tension of the unfatigued mammalian muscle (Goffart, 1949), but has no vaso-constrictor action. We were able to reproduce this result in several but not in all experiments. This was probably due to irregularities produced by slight changes in the temperature and rate of flow of the perfusion fluid, which we were unable to control adequately.

The results on strips of rat diaphragm were remarkably constant and reproducible. In these experiments the amount of ^{42}K in the muscle itself is measured, and thus an increased loss of ^{42}K is reflected by an accelerated decrease in the amount of ^{42}K remaining in the muscle. Fig. 2 shows a control experiment in which the reservoirs were switched over during the period indicated, both reservoirs containing normal potassium-free Tyrode's solution.

The content of ^{42}KCl in the muscle declined throughout in a remarkably regular fashion, and this fall was in no way affected by changing the source of the perfusion fluid, even though this procedure caused slight changes in temperature and rate of flow. The potassium loss was so steady that variations in the rate of

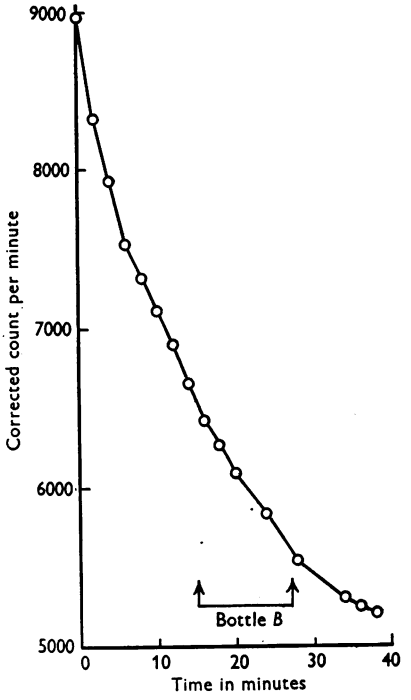

Fig. 2.

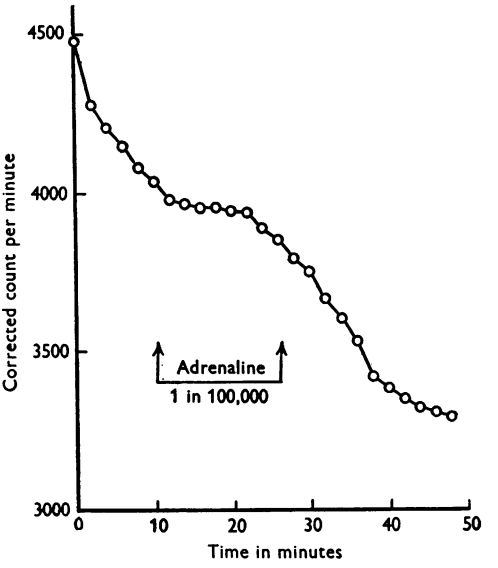

Fig. 3.

Fig. 2. Rate of loss of ^{42}K from diaphragm of rat (previously 'loaded' with ^{42}K) irrigated with potassium-free Tyrode solution.

Fig. 3. Effect of adrenaline on the rate of loss of ^{42}K from diaphragm of rat.

loss of the order of 5% from the normal curve were easily detectable. We have not attempted to calculate the absolute amounts of ^{42}K being lost from the muscle, since we were interested only in the relative rate of loss after exposure to adrenaline.

Fig. 3 illustrates an experiment in which the muscle was irrigated with potassium-free Tyrode solution, containing adrenaline 1 in 100,000, for 16 min. There was, during the first 12 min. of this period, a marked decrease in the rate of loss of potassium from the muscle; this was followed by a period in which the rate of loss of potassium was accelerated. This result was repeatedly obtained, but the effects were not always so prominent. The second phase, the increased rate of loss of potassium, was not dependent upon removal of the adrenaline, since it started before the adrenaline was removed. In other experiments, we

have maintained the adrenaline for longer periods without in any way affecting the time course of the biphasic action.

Similar results were obtained using DL-noradrenaline and *iso*propylnoradrenaline. In a few experiments the usual concentration of Ca^{++} ions in the irrigating fluid (0·02% Ca chloride) was doubled. Such an increase of extracellular Ca^{++} ions is known to potentiate the action of adrenaline on the maximal twitch tension of the rat diaphragm (Goffart, 1949). It did not influence the effect of adrenaline on the rate of loss of K^+ ions from the muscle cell.

DISCUSSION

The results of our experiments on the isolated rat diaphragm and on the perfused cat's extensor digitorum longus show that adrenaline, noradrenaline and *iso*propylnoradrenaline affect the loss of potassium ions from the muscles in the same way; there is first a decreased rate of loss of potassium lasting 10–15 min., followed by an increased rate of loss. We are dealing, in these experiments, with a real 'loss' and not with an increased exchange of K^+ ions, since the rat diaphragm experiments were carried out in a potassium-free solution. Despite the usual assumption to the contrary, there is some evidence (Urey, 1938) suggesting that the degree of hydration of potassium ions is influenced by their nuclear constitution. The degree of hydration of an ion will affect its permeability, and it is therefore justifiable to refer our observations directly only to the unphysiological ^{42}K ion. Nevertheless, indirect reference to the physiological ^{39}K ion is, in our opinion, justifiable in the circumstances, since the precise effect, if any, of the differences in permeability is unknown, and since the results which we have obtained with ^{42}KCl accord so well with the results obtained with ^{39}KCl by Brown *et al.* (1950) on the demarcation potential.

The experiments were designed to find out if the changes in demarcation potential and in maximal twitch tension produced by adrenaline could be related to movements of K^+ ions across the muscle cell membranes. It is, therefore, interesting to compare these three effects of adrenaline. This is done diagrammatically in Fig. 4, which shows that all three effects are biphasic and follow approximately the same time course. This suggests strongly that they are interdependent.

In view of this interdependence it seems unlikely that the increased loss of K^+ ions is derived from any source—e.g. blood vessels or nerves—other than the muscle cells themselves.

In the absence of any proper insight into the nature of the fundamental relationship between changes in the properties of the cell membranes and the mechanical properties of the muscle, it is difficult to make any useful suggestion about the nature of the interdependence of these three effects of adrenaline. It is not possible to conclude that the changes in the rate of potassium loss are

directly responsible for the other two effects, although it would be expected that a change in the rate of potassium loss would be accompanied by a change in demarcation potential. The demarcation potential changes and the changes in rate of potassium loss might both be the result of an alteration in the membrane resistance; although a change in the rate of potassium loss is not necessarily accompanied by a change in membrane resistance. Since, however, it is

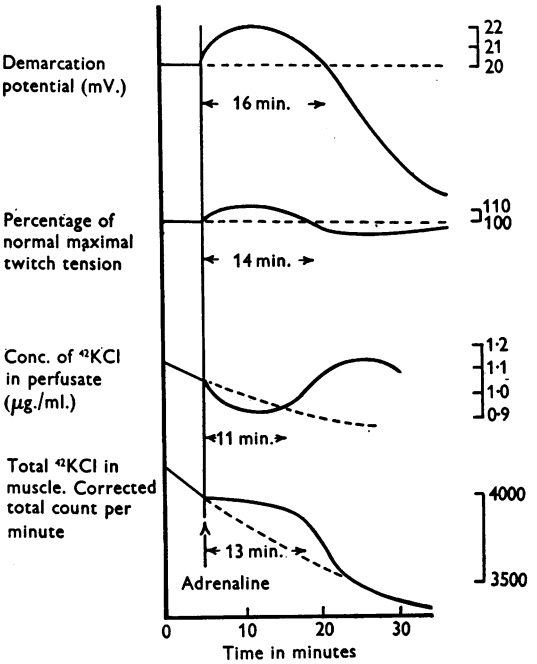

Fig. 4. Diagrammatic comparison of effects of adrenaline on rate of loss of ^{42}K, maximal twitch tension, and demarcation potential of muscle. Calibrations for each curve are taken from the figures for a typical experiment of each type. Dotted lines represent the course of events in control experiments where no adrenaline is given.

not known if there is any link between changes in membrane resistance and in twitch tension, the dependence of the third effect of adrenaline still remains unexplained. For this reason it would be necessary to assume that adrenaline produces, on some other property of the muscle, a change which in turn causes alterations in both membrane resistance and twitch tension; without further knowledge, therefore, this line of reasoning cannot provide a satisfactory hypothesis.

The only justifiable conclusion meantime is that the three interdependent effects are consequential upon a single initial change in equilibrium.

SUMMARY

1. We have studied the effect of adrenaline and of other sympathomimetic amines on the loss of ^{42}K from the isolated resting rat's diaphragm continuously irrigated with potassium-free Tyrode's solution.

2. Adrenaline, noradrenaline and *iso*propylnoradrenaline, added to the irrigating fluid, each cause first a decrease and then an increase in the rate of potassium loss from the muscle.

3. An increase in the calcium concentration of the irrigating fluid does not potentiate this effect of adrenaline.

4. Adrenaline has the same biphasic effect on the rate of potassium loss from the cat's stimulated extensor digitorum longus muscle perfused with plasma through its artery. Consistent results, however, were not regularly obtained.

5. The changes in the rate of potassium loss produced by adrenaline resemble, both in their biphasic shape and in their time course, those produced by adrenaline on the demarcation potential and twitch tension of skeletal muscle.

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