EFFECT OF STIMULATING A SYMPATHETIC NERVE ON THE IONIC CONSTITUTION OF FROG SKELETAL MUSCLE PERFUSATE

S.A. Oreshkevich

From the Pharmacological Laboratory (Head – Professor N.P. Sinitsyn) S.M. Kirov Gor'kii Medical Institute

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In spite of the demonstration by L.A. Orbeli of the trophic adaptive influence exerted by sympathetic nerves on frog skeletal muscle, until now, the nature of the substances exerting this effect on fatigued muscle has not yet been established.

Many suggestions have been put forward [3, 5, 6, 7, 8, 10, 14, 15].

The results obtained in our laboratory indicate that stimulation of a sympathetic nerve leads to the production, in frog skeletal muscle perfusate, of active substances which increase the electrical conductivity and acidity of the perfusate, as well as the working capacity of the fatigued muscle.

Changes in electrical conductivity of aqueous solutions may be brought about by changes in electrolyte concentration. The explanation of the nature of the change in the electrical conductivity of the perfusate following sympathetic nerve stimulation is of considerable interest. The present work consists of a study of the effect of sympathetic nerve stimulation on the ionic content of skeletal muscle perfusate. At the same time, an attempt has been made to detect, in the perfusate, ions which may exert an effect on muscle similar to that of sympathetic nerve stimulation, and to exclude the effects of protein and other organic compounds.

METHODS

The perfusate from the hind feet of the frog was collected before the muscle was made to do work, during the working period, during the fatigued state, and during sympathetic nerve stimulation.

Separate portions of the perfusate were evaporated to dryness, heated in an oven at 300-400°, and after cooling, were dissolved in distilled water, after with the volume was made up to that of the original perfusate. Ringer solution, treated in the same way, was used as control.

The perfusate was tested on the frog heart, isolated by Straub's method.

In 20 experiments, 118 portions of perfusate and of Ringer's solution were examined.

RESULTS

In most experiments, the contractions of the heart after bathing in the perfusate taken from the working muscle were of greater amplitude than those of the heart treated with the perfusate from the resting muscle.

In 85% of the cases, a frog heart treated with perfusate from a fatigued muscle gave contractions of smaller amplitude than did one treated with a perfusate from a working muscle. In 85% of the cases treated with a perfusate from a frog to which sympathetic stimulation had been applied, there was a marked increase in cardiac contraction, while in the remaining 15% of the cases, no definite result was obtained.

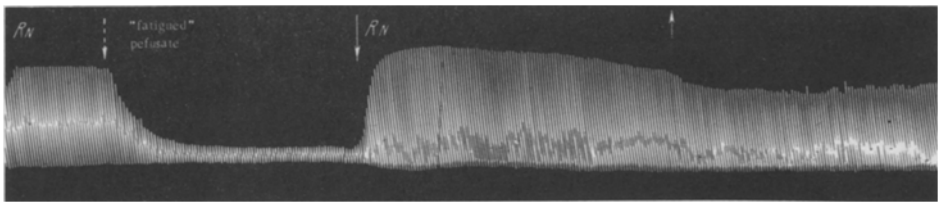

Fig. 1. Effect of the perfusate from a fatigued muscle (↓) and of a "sympathetic" perfusate after heating (↑), and of normal Ringer's solution (↓) on the isolated frog heart.

Fig. 2. ↑ Shows effect of adding solution of dried extract of "sympathetic" perfusate; on the left is shown the effect of adding an extract of Ringer's solution treated in the same way; ↓) shows the effect of adding normal Ringer's solution. Isolated frog heart.

Pure Ringer solution, after treatment, showed the same effect as did untreated Ringer, while treated perfusate taken after sympathetic stimulation again produced a positive inotropic action.

It was necessary to find what ions are concerned in reducing fatigue in skeletal muscle on sympathetic nerve stimulation, and what ions applied to the isolated frog heart can reproduce the effect of the treated perfusate obtained after sympathetic stimulation.

Potassium ions are known to exert a negative inotropic action. Therefore, the positive inotropic effect on the isolated heart can certainly not be due to the action of K' ions.

Additional evidence against potassium ions being involved is afforded by the vascular reaction occurring during sympathetic nerve stimulation, when the number of drops per minute of perfusate is reduced. Many experiments were carried out to test the effect of Na', Ca", and Mg" ions on the frog Straub heart preparation, by applying solutions of $CaCl_2$, NaCl, $MgCl_2$ and $MgSO_4$. Various concentrations of the ions in Ringers solution were tried.

On comparing the effects of these solutions, in concentrations which increase the electrical conductivity of the solution by approximately the same amount as did the perfusate obtained after sympathetic nerve stimulation, it was found that only the Ca" ion gave the same effect.

Sympathetic nerve stimulation caused substances to appear in the frog skeletal muscle perfusate which increased the working capacity of fatigued muscle, increased the electrical conductivity of the perfusate, and which, when tested on an isolated frog heart, caused a positive inotropic effect.

Treatment of this perfusate caused no change in the extent of its action on the heart.

Many authors, using many different methods on other preparations, have come to a similar conclusion.

The action of acetycholine liberated by stimulation of the nerve is associated with the liberation of calcium ions, and, in this reaction, it is possible that acetic acid formed during the hydrolysis of acetylcholine under the influence of cholinesterase may be involved [6, 7].

There are some indications that potassium reduces fatigue, i.e., increases the contractions of fatigued muscle [2].

It has been shown that calcium ions sensitize myosin to the action of adenosinetriphosphate [15].

On increasing the concentration of calcium chloride in this isolated salivary gland, an increase in the secretion is found which is similar to that occurring on stimulation of the sympathetic nerve [11].

It may, incidentally, be pointed out that if the frog suffers from any sort of pathological condition (renal adhesions, hemorrhage, cardiac changes, etc.), stimulation of the sympathetic nerve either causes no change in the electrical conductivity or in the pH of the perfusate, or else makes it more alkaline and reduces the conductivity.

Evidently, the effect of sympathetic nerve stimulation depends on the general condition of the animal.

SUMMARY

The author investigated the nature of the substances which influenced the skeletal muscle in stimulation of the sympathetic nerve. It was established that by stimulating the sympathetic nervous system, active substances appear in the perfusate of the frog's skeletal muscle. Evaporation of the perfusate, with subsequent calcination of the dry residue and its disolution in distilled water, does not remove the positive effect exerted on the frog's heart. Of the ions tested (Na^{+}, Mg^{++}, and Ca^{++}), the picture analogous to the action of the "sympathetic" perfusate is obtained only in the case of Ca^{++}.

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