THE BIOLOGICAL IMPORTANCE OF AMYLASE TO MUSCULAR ACTIVITY

E. M. Kozhukhar'

From the Department of General and Biological Chemistry of the Kiev Intitute of Physical Culture

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In previous research [1, 2] we have shown that the amylolytic activity of the blood during muscular activity in man is increased $1\frac{1}{2}$ to 3 times, and during athletic contests, by 3 to 6 times or more. In connection with these findings, it was of interest to ascertain the character of the changes in amylase activity of the working muscles themselves.

Taking into consideration the reports in the literature [3, 4] on the amylase activity of the muscles in different conditions, we conducted experiments both on whole muscle and on pounded muscle and muscle extracts, in which the amylase and phosphorylase activity of the muscles were determined simultaneously.

EXPERIMENTAL METHOD

Experiments were carried out on the gastrocnemius muscle of the frog. Contraction of the muscle was caused by the electric current from an induction coil, and lasted 7-8 minutes (voltage in the primary coil 1.7 v, from an accumulator). In the circuit was a metronome with a frequency of 60 per minute. The symmetrically opposite muscle acted as a control.

Since the amylase activity of the muscles at 0° was insignificant without preliminary autolysis, we conducted the investigations at room temperature after autolysis for 25-30 minutes. The muscle preparations were incubated with substrate at a temperature of 38-40°. The experiments with whole muscles were conducted without buffered mixtures, since the pH of the objects examined was optimal for amylase activity.

The incubation medium for the aqueous extracts of pounded muscle were prepared as follows: for amylase: 2 ml of extract, 2 ml of 1 % starch solution, 10 mg NaCl, 6 ml water, pH = 6.9-7.0; for phosphorylase: 2 ml of extract, 1 ml of a 0.1 M solution of NaF, 0.5 ml of an M/3 phosphate buffer, 1 ml adenylic acid, 5 ml of a 1% starch solution, 30 mg $MgSO_4$, pH=7.5. In some experiments 1 ml of 0.05 M phloridzin was added to the incubation mixture to suppress the phosphorylase activity.

As index of amylase activity we used the amount of reducing substances, determined by the Hagedorn-Jensen method [6], and of phosphorylase activity — the fall in the amount of inorganic phosphate determined by the Fiske-Subbarow method [5].

EXPERIMENTAL RESULTS

In the experiments with whole muscles it was found that the decomposition of starch per g of working muscle after incubation for 60 minutes was 1.68 times greater, and after 120 minutes, 2.07 times greater than per g of resting muscle.

Similar results were obtained when the muscle was incubated for 120 minutes in physiological saline (Ringer's solution) and the amylolytic activity of the solutions subsequently determined. The physiological saline in which the working muscle was incubated was 1.65 times more active in decomposing starch than the saline in which the resting muscle was incubated. It was therefore concluded that there was a more intensive transfer of amylase into the medium from the working than from the resting muscle.

This confirmed the findings relating to the increase in amylolytic activity of the frog's blood after work by the hindlimb by 1.42 times, and also to the increase in the amylase activity of the aqueous extract of the pounded working muscles of an amputated limb by 1.76 times, whereas in the corresponding, unamputated limb the increase was only 1.43 times by comparison with the amylase activity of aqueous extracts of pounded resting muscles. The addition of phloridzin had no effect on the results of the experiment.

We investigated the phosphorylase activity of aqueous extracts of amputated or unamputated muscles. The results obtained showed that the phosphorylase activity was increased by muscular activity in an aqueous extract of pounded muscles of an unamputed limb by 1.16 times, and of an amputated limb by 1.38 times, by comparison with controls, and moreover, the addition of phloridzin completely inhibited the phosphorylase activity.

Under the influence of work, the phosphorolytic activity was thus increased also, but to a far less extent than the amylolytic activity.

The findings described thus demostrate a considerable increase in the amylase activity of working muscle, and also a more intensive transfer of amylase from the working muscle into the surrounding medium, presumably connected with the increased permeability of the working muscle by comparison with that at rest.

SUMMARY

The amylolytic activity in the frog's gastrocnemius is much higher after exertion than in a muscle performing no work. With the gastrocnemius at work an intensive transfer of amylase and phosphorylase into the surrounding environment is observed, this depending upon the increased muscle permeability during exertion.

LITERATURE CITED

[1] E. M. Kozhukhar', Ukrain. Biokhim. Zhur., No. 3, 327-336 (1952).

[2] E. M. Kozhukhar', Transactions of the Research Institute of Animal Physiology, No. 7, pp. 227-235. Kiev, 1953 [In Russian].

[3] A. N. Petrova, Biokhimiya, 11, No. 2, 11 (1946).

[4] Z. Augustin, Ztschr. Physiol. Chem., Bd. 255, S. 61 (1938).

[5] C. H. Fiske and Y. Subbarow, J. Biol. Chem. v. 66, p. 375-400 (1925).

[6] H. G. Hagedorn and B. N. Jensen, Biochem. Ztschr., Bd. 135, S. 46-38 (1923).