CONTENT OF FREE NUCLEOTIDES IN MUSCLES AFTER ADMINISTRATION OF ACTH AND HYDROCORTISONE

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Experiments on the gastrocnemius muscles of rabbits showed that transient contraction of the muscle with a frequency of 2 Hz, in response to stimulation of the nerve, is accompanied by a decrease in the creatine phosphate content and an increase in the inorganic phosphorus content. The content of free nucleotides is unchanged. Contraction of the muscle after administration of ACTH was accompanied by decomposition of less creatine phosphate per unit of work than after administration of hydrocortisone or isotonic NaCl solution. The quantity of inorganic phosphate formed was correspondingly smaller.

ACTH and hydrocortisone are known to influence protein, carbohydrate, lipid, mineral, and other types of metabolism in organs and tissues [3, 5, 6, 12-14, 15]. However, the effect of these hormones on energy metabolism in the skeletal muscles in a state of rest and during contractions has been inadequately studied.

In the investigation described below, the effect of ACTH and hydrocortisone was studied on the content of adenine nucleotides, creatine phosphate, and inorganic phosphate in skeletal muscles during physiological rest and contraction.

EXPERIMENTAL METHOD

The sciatic nerve was divided bilaterally in rabbits under urethane anesthesia. Stimulating electrodes were applied to the peripheral end of one of the tibial nerves. The tendo Achillis was connected to a myograph. A copper-constantan thermocouple was inserted into the gastrocnemius muscle to record changes in its temperature [7] which was used as a criterion of the functional state of the organ [10]. The symmetrical gastrocnemius muscle was not stimulated and served as the control. After establishment of the initial temperature level, in the experiments of series I (control) the rabbit received an intramuscular injection of 1 ml isotonic NaCl solution, in the experiments of series II, 2 mg/kg of a hydrocortisone suspension, and in series III, 10 units/kg of ACTH. In all experiments a contraction of the gastrocnemius muscle was evoked 25-30 min after injection of the substances by stimulating the nerve for 5 min with square pulses, 1.5 msec in duration, at a frequency of 2 Hz. Contractions of the muscle were produced under isotonic conditions with a load of 200 g. A time of 25-30 min was chosen because it is at this time after injection of ACTH that clearly defined changes in temperature take place [7]. After the end of stimulation, in the phase of the maximal rise of temperature, both gastrocnemius muscles were simultaneously excised, frozen in liquid nitrogen, and ground into a powder in metal mortars. The creatine phosphate content in the powder was determined by Alekseeva's method [1], inorganic phosphate by Delory's method in Grigor'eva's modification, and adenosine phosphates (ATP, ADP, AMP) by electrophoresis on paper [4, 16]. The nucleotide concentration was determined spectrophotometrically at 260 and 290 nm. The experimental results were analyzed by statistical methods [11].

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TABLE 1. Content of Adenine Nucleotides (in μmoles/g tissue), Creatine Phosphate (in mg% creatinine), and Inorganic Phosphate (in mg%) in Gastrocnemius Muscles of Rabbits at Rest and during Contractions (M±m)

Substance	Isotonic saline		Hydrocortisone		ACTA	
	rest	contraction	rest	contraction	rest	contraction
ATP	3,96±0,29	3,84±0,29	3,38±0,34	3,16±0,27	2,75±0,34	2,82±0,25
ADP	0,55∓0,135	0,41±0,10	0,70±0,19	0,62±0,145	0,75±0,07	0,69±0,064
AMP	0,71±0,05	0,68±0,067	0,60±0,08	0,60±0,10	0,59±0,074	0,63±0,075
AMP_1	Traces	<0,05	Traces	0,20±0,06	Traces	0,17±0,05
Creatine phosphate	235±7,7	89±4	237±7,1	89±6,4	242±12,1	121±10,4
Inorganic phosphate	38±4,1	61,45±7,9	38±6,3	89,9±4	42,4±15,7	65,4±4,3

EXPERIMENTAL RESULTS AND DISCUSSION

In the control series of experiments, no significant changes in temperature were observed after injection of isotonic NaCl solution. Stimulation of the muscle for 5 min led to an increase in temperature of 1.17±0.19°C. The amplitude of the contraction by the end of stimulation was reduced by 28±5.5%. The amount of work done by the muscle, calculated from the ergogram, was 0.085±0.019 Kg/m. The concentration of creatine phosphate in the contracting muscle fell sharply, while the concentration of inorganic phosphate rose. The content of adenosine phosphate was unchanged (Table 1). On electrophoretic fractionation of the adenine nucleotides in homogenates of resting muscles three fractions were found (corresponding to AMP, ADP, and ATP by their arrangement relative to the cathode). In contracting muscles, on the other hand, four fractions were isolated, for the nucleoside monophosphate consisted of two fractions. Subdivision of AMP into two fractions has been described previously [2]. The additional fraction was described conventionally in AMP_1. It is comparatively strongly fluorescent but adsorbs weakly in the UV region. The same fraction was found on stimulation of the muscles in the subsequent series of experiments (Table 1). Comparison of the position of this fraction after electrophoresis relative to ATP, ADP, and AMP with the results of chromatographic analysis of the content of adenosine phosphates [9] suggests that it is inosinemonophosphate.

During the first 25-30 min after injection of hydrocortisone the temperature of the muscle remained substantially unchanged. After contraction of the muscle it rose by 1.54-0.21°C, the amplitude of the contraction fell towards the end of stimulation by 23±5.6%, and the work done by the muscle was 0.056±0.006 kg/m ($P < 0.01$).

A decrease in the temperature of the muscle by 1.35±0.2° ($P < 0.01$) was observed 25-30 min after injection of ACTH. A decrease in temperature, a decrease in blood flow, and a decrease in oxygen tension in the muscles under these conditions were observed previously [7]. Stimulation of the muscle against the background of ACTH led to an increase in temperature of 1.42±0.17°C. The amplitude of the contractions decreased by 23±5.6% ($P < 0.01$), i.e., to the same extent as in the previous series of experiment. The work done by the muscles in the experiments with ACTH was 0.147±0.03 kg-m.

It is clear from the results in Table 1 that the ATP content in the muscles in a resting state was lower after injection of ACTH than in the control experiments, while the contents of ADP and AMP were the same in all three series of experiments.

A correlation was found between the amount of work done and the degree of increase in temperature of the muscle during contraction. In the experiments of series I the coefficient of correlation was 0.88 ($P < 0.05$), in series II 0.76 ($P < 0.05$), and in III 0.94 ($P < 0.001$). A definite correlation also was observed between the content of creatine phosphate split during contraction and the amount of work done. In the experiments of series I the coefficient of correlation between these indices was 0.87 ($P < 0.05$), in II 0.91 ($P < 0.05$), and in III 0.87 ($P < 0.05$). This fact is in agreement with results showing that the breakdown of creatine phosphate is dependent on the size of the working load [2].

The ratio between the quantity of creatine phosphate split during contraction of the muscle and the amount of work done was 1717.6 mg%/kg-m in the experiments of group I (control), 2642.8 mg%/kg-m in

series II, and 823.1 mg%/kg-m in III. Hence, during contraction of the muscle 25-30 min after injection of ACTH, 2.08 times less creatine phosphate was decomposed than in the control experiments and 3.2 times less than during contraction of the muscle after injection of hydrocortisone. During contraction of the muscle after injection of hydrocortisone, 1.5 times more creatine phosphate was decomposed than in the control experiments.

Injection of ACTH reduced the decomposition of creatine phosphate during contraction of the muscle and led to the accumulation of inorganic phosphorus. Conversely, after injection of hydrocortisone, the breakdown of creatine phosphate, expressed per unit of work, on the other hand, was increased.

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