ACETYLPHOSPHATASE AND PHOSPHOPROTEINPHOSPHATASE IN MAMMALIAN MUSCLES

V. V. Ershov

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Phosphoproteinphosphatase activity is always higher in intensively working mammalian muscles (myocardium, red muscles) than in muscles working at lower intensity (white muscles). Acetylphosphatase activity in heart muscle is always 33-50% below that in skeletal muscles. Training significantly increases phosphoproteinphosphatase activity but has no effect on acetylphosphatase activity.

Acetylphosphatase (acylphosphate phosphohydrolase, 3.6.1.7) and phosphoproteinphosphatase (phosphoprotein phosphohydrolase, 3.1.3.16) of muscles have not been investigated from the comparative biochemical standpoint. However, the natural substrates of these enzymes (acetylphosphate and the tissue phosphoproteins) are intensively metabolized in animal tissues [1, 3, 5, 6].

Acetylphosphatase (ACP) is widely distributed in animal tissue [12], and it hydrolyzes acetylphosphate and other acyl phosphates very rapidly [9].

Two types of phosphoproteinphosphatase (PPP) evidently exist in animal tissues. One PPP, stable and of low specificity, is present mainly in the spleen and liver, while the other, labile and specific, is found in the liver and spleen, and also in the kidneys, brain, and other organs and tissues. The more widespread specific phosphatase evidently is concerned with rapid phosphoprotein metabolism [11].

Muscle PPP has lower activity than that in other organs and tissues [8]. Mn^{++} and Mg^{++} ions (0.01 M) activate muscle PPP, but ascorbic acid and cysteine (0.001 mole) usually used to activate PPP from the liver and spleen have no effect on its activity [7].

The object of this investigation was to determine ACP and PPP activities in various mammalian muscles and to investigate the effect of training on their enzyme activity.

EXPERIMENTAL METHOD

Activity of the enzymes was determined in the heart muscle, the muscles of mastication, and muscles of the hind limbs (biceps) of adult animals: dogs, rabbits, guinea pigs, rats, susliks.

The biceps femoris of the rabbits was trained unilaterally by application of an interrupted tetanizing current (50 Hz, 60 volleys/min). The training load was increased gradually to reach a maximum on the 10th day: 3 sessions, each 5 min in duration, with two intervals of 5 min. The duration of training was 35-40 days. Two days before the tests were carried out the training was discontinued. The muscles were taken from the rabbits under deep nembutal anesthesia, and from the other animals under ether anesthesia.

Weighed samples (1 g) of the muscles were ground with powdered glass in porcelain mortars in the cold (0°), and the enzymes were extracted with 0.1 M acetate buffer (10 ml, pH 6.0) for 30 min. The ex-

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TABLE 1. PPP Activity in Mammalian Muscles (in μmoles inorganic phosphate hydrolyzed by 1 g muscle during incubation for 15 min)

Species of animal	Heart muscle	Muscle of mastication	Limb muscles	Number of experiments
Rabbit	4.2 ± 0.34	5.4 ± 0.39	3.3 ± 0.22	8
Guinea pig	8.0 ± 0.55	6.7 ± 0.31	4.1 ± 0.33	6
Rat	7.5 ± 0.37	5.5 ± 0.31	5.0 ± 0.16	6
Dog	5.3 ± 0.35	3.0 ± 0.44	5.0 ± 0.61	6
Suslik	7.2 ± 0.51	6.5 ± 0.28	6.0 ± 0.41	6

TABLE 2. ACP Activity in Mammalian Muscles (μmoles acetylphosphate hydrolyzed by 1 g muscle in 15 min)

Species of animal	Heart muscle	Muscle of mastication	Limb muscles	Number of experimens
Rabbit	294 ± 20	630 ± 30	720 ± 48	8
Guinea pig	112 ± 7	115 ± 15	115 ± 13	6
Rat	595 ± 27	825 ± 35	880 ± 45	6
Dog	286 ± 50	620 ± 44	685 ± 28	6
Suslik	364 ± 24	566 ± 44	640 ± 41	6

tract was obtained by centrifuging the homogenate for 10 min at 600 g, and it was used to determine the enzyme activity (1 ml per sample). PPP activity was determined by the method of Feinstein and Volk [8], using 3% casein solution (3.5 ml) as substrate and 0.005 M $MgCl_2$ as activator. Incubation took place for 30 min at 38°C and pH 6.0. The reaction was stopped by addition of 3 ml 30% TCA. The inorganic phosphate concentration in the samples was determined in the samples after filtration.

ACP activity was determined by Koshland's method [9]: substrate 0.006 M acetylphosphate solution, incubation time 10 min, pH 5.4. Activity was determined from the decrease in acetylphosphate concentration under the influence of muscle extracts. The acetylphosphate concentration was determined by the method of Lipmann and Tuttle [10].

Activity of the enzyme was calculated from the amount of substrate converted by 1 g muscle during incubation for 15 min at 38°C. The results were analyzed by statistical methods.

EXPERIMENTAL RESULTS

The results of the investigation of PPP activity are shown in Table 1.

Activity of the enzyme in the continuously working heart muscle was always higher than in the skeletal muscles. In the red muscles of mastication of rodents (rabbit and guinea pig) activity was higher than in the white muscles of the hind limbs. In the mixed (red and white) muscles of the limbs of the laboratory rat and red muscles of the suslik's limbs, PPP activity was not significantly different from that in the muscles of mastication. Activity of the enzyme in the muscles of mastication in dogs was lower than in the limb muscles.

The results of investigation of ACP activity are given in Table 2.

The muscle ACP activity was very high, yielding up to 880 μmoles of converted substrate. The activity was highest in muscles of the laboratory rat and lowest in the muscles of the guinea pig. ACP activity in the heart muscle was always 33-50% below that of the skeletal muscles. Slight yet significant differences in enzyme activity were found between the red (muscles of mastication) and white (biceps) skeletal muscles of the rabbit ($P = 0.05$). No significant differences in ACP activity were found in the various skeletal muscles of the other animals investigated. ACP activity in the muscles of the guinea pig was unusually low compared with other mammals.

Since muscles with different work loads differed in their PPP and ACP activity, it was interesting to study the effect of training on the activity of the muscle enzymes. Experiments showed that under the influence of training by an interrupted tetanizing current, the PPP activity rose significantly (from 3.6 ± 0.21 to 4.8 ± 0.44 μmoles/g muscle/15 min). However, training had no significant effect on the muscle ACP activity. The investigation thus showed that the PPP activity is directly dependent on the work load of the muscle: in intensively working muscles the activity of this enzyme is higher than in muscles working less intensively. These results confirm those obtained by Parsadanyan [4] for rabbit cardiac and skeletal muscles.

Changes in PPP activity under the influence of training are in line with the previously established rule [2] that training increases those indices which are predominant in red muscles.

Activity of muscle ACP is inversely proportional to the probable work load of the muscle tested. However, this rule is not universal.

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