HISTOCHEMICAL INVESTIGATION OF SOME INDICES OF ENERGY METABOLISM IN THE RAT HEART AFTER ACUTE FATIGUE

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The effect of a single period of physical exertion on certain enzymes of energy metabolism in the rat heart was studied. Qualitative and quantitative histochemical methods showed that after the rats had been swimming for 3 h at 28-30°C the activity of glycogen phosphorylase A and B and of glucose-6-phosphate, cytoplasmic α-glycerophosphate, lactate, succinate, and NAD · H_2 dehydrogenases was lowered while the activity of mitochondrial α-glycerophosphate dehydrogenase was raised. An increase in glycogen phosphorylase activity and glucose-6-phosphate and cytoplasmic α-glycerophosphate dehydrogenase activity was observed 3 h after swimming. NAD · H_2 and lactate dehydrogenase activity remained unchanged, while mitochondrial α-glycerophosphate and succinate dehydrogenase activity continued to fall. The possible mechanisms of these changes are discussed.

The study of metabolism in the heart muscle under different functional states is an important problem in current cardiology, for many diseases are accompanied by changes in the load on the myocardium.

It is now generally accepted that the leading energy-forming process in the myocardium of mammals under normal conditions is the Krebs cycle, the substrates for which are provided chiefly by β-oxidation of fatty acids and anaerobic breakdown of carbohydrates [2, 10, 12, 18]. Competitive relationships constantly exist between these processes, and they are determined by the state of the body itself, the activity and accessibility of the substrates and enzymes of these metabolic pathways, and various external factors. It is generally considered that substrates from the system of β-oxidation of fatty acids account for 60% of the total, while those of glycolysis and glycogenolysis account for up to 30% of the total substrates for the Krebs cycle [12, 18]. However, according to data in the literature, in some pathological states (hypoxia, myocardial lesions, physical exertion, and so on) this ratio changes in favor of activation of glycolysis and glycogenolysis [12, 18].

The object of the present investigation was the histochemical study of certain enzymes of glycolysis and glycogenolysis and of the Krebs cycle in the mammalian myocardium after acute fatigue. The following enzymes were studied: glycogen A and B phosphorylase – the enzyme responsible for the initial stages of glycogenolysis; glucose-6-phosphate dehydrogenase (G6PDH), participating in the first phase of the pentose cycle; α-glycerophosphate dehydrogenase (αGPDH) – mitochondrial and cytoplasmic – an indicator of phosphate oxidation in the mitochondria and in the cytoplasm; lactate dehydrogenase (LDH), characterizing the conversion of lactate into pyruvate; succinate dehydrogenase (SDH), an indicator of the Krebs cycle; and reduced nicotinamide – adenine dinucleotide dehydrogenase (NAD · H_2DH) – an indicator of terminal oxidation.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180–200 g. Fatigue was produced by swimming in a bath for 3 h at 28-30°C. The animals were decapitated immediately after swimming and

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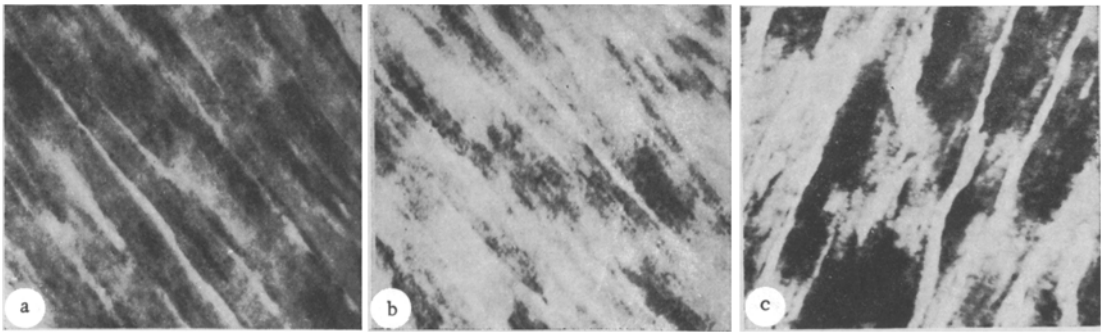

Fig. 1. Total glycogen phosphorylase in the left ventricle of the rat heart: a) intact rat; b) immediately after swimming for 3 h; c) 3 h after swimming for 3 h. Takeuchi's method, 200×.

TABLE 1. Dehydrogenase Activity in Rat Myocardium (in μg formazan/μg protein per section per minute), M ± m

Enzyme	Normal	Immediately after swimming for 3 h	P_{1-2}	3 h after swimming for 3 h	P_{2-3}
G6PDH	43±0,7	35±0,9	<0,001	44±1,9	<0,001
Total α GPDH	67±2	56±1,1	<0,001	55±3	>0,001
Mitochondrial α GPDH	39±2,5	48±1,1	<0,001	36±2,5	<0,001
Cytoplasmic α GPDH	28±0,5	8±0,009	<0,001	19±0,6	<0,001
LDH	135±1,6	117±3,2	<0,001	117±2,9	
SDH	148±4	127±1,9	<0,001	112±3,1	<0,001
NAD· H_2DH	130±3,6	98±1,3	<0,001	104±3,9	0,05<P<0,1

3 h later. At each time nine animals were sacrificed. The hearts of nine intact animals were used as the control. Sections were cut in a cryostat to a thickness of 7 μ for the detection of phosphorylase and 20 μ for quantitative histochemistry. Total phosphorylase was determined by Takeuchi's method [19]. By excluding AMP from the incubation medium, phosphorylase A was detected. The difference between these two reactions gave the content of phosphorylase B. Quantitative histochemical investigation of the dehydrogenases was carried out by the method of Nartsissov and co-workers using the media of Hess, Scarpelli, Pearse (G6PDH, total αGPDH, and LDH) and of Quaglino and Hayhoe (mitochondrial αGPDH, SDH, and NAD · H_2DH).

The numerical results were subjected to statistical analysis by the usual methods.

EXPERIMENTAL RESULTS

According to data in the literature [15, 21] glycogen phosphorylase in the mammalian heart exists in two forms: active phosphorylase A and inactive B, which is activated in the presence of AMP and converted into A. Although glycogen phosphorylase can participate in glycogen breakdown and synthesis, in the mammalian heart this enzyme is considered to be responsible for glycogen breakdown, whereas glycogen synthesis takes place by means of uridine nucleotides without the participation of phosphorylase [15, 21]. In microscopic sections phosphorylase activity was revealed as glycogen granules of different sizes, arranged diffusely in the myocytes or repeating the cross-striation of the myofibrils. Differences in the activity of the enzyme in individual myocytes in the different chambers of the heart and layers of the myocardium was observed, creating a picture of mosaicism in its topography.

Alongside myocytes almost devoid of activity there were fibers with large numbers of glycogen granules (Fig. 1a).

Moderate phosphorylase B activity, slightly higher than phosphorylase A activity, was observed in the heart of the control animals, and in the ventricles the activity of both phosphorylases was rather higher than in the atria, while in the right ventricle it was higher than in the left. The activity was maximal in the subendocardial zones, especially of the right ventricle, and in the ventricular septum nearer to the base of the heart. The localization of phosphorylase corresponded to the distribution of glycogen, in agreement with data in the literature [6, 16]. Immediately after swimming for 3 h there was a small decrease in the activity of both forms of phosphorylase (Fig. 1b), but phosphorylase B still continued to be slightly predominant,

although at the same time the activity of the enzyme was redistributed. There were fewer myocytes with high activity of the enzyme but a sharp increase in the phosphorylase A and, in particular, phosphorylase B activity around the vessels. In the subendocardial zones and, to a lesser degree, in the ventricular septum the activity of both forms of phosphorylase remained high, just as in the normal heart.

The high activity of both forms of the enzyme in the right atrium and their lower activity around the vessels than at the previous time will be noted.

By comparison of these results with the well-known fact that the glycogen content in the rat heart falls after a single exposure to physical exertion (swimming) [3, 4, 7, 11, 13], it can be postulated that a single period of physical exertion leds to activation of glycogenolysis and to a decrease in the quantity of glycogen. After a rest for 3 h the glycogen content and phosphorylase activity were restored to or above their initial level.

No information on quantitative histochemical investigation of dehydrogenases in the mammalian heart after exertion could be found in the literature. The existing biochemical and solitary histochemical investigations [8, 9, 20] have yielded conflicting results and deal mainly with the study of the enzyme content after training [1, 14, 17].

It will be clear from Table 1 that the most active enzyme in the rat myocardium is SDH. Next follow LDH, NAD · H_2DH, mitochondrial αGPDH, cytoplasmic αGPDH, and G6PDH. Consequently, the predominant energy-producing process in the rat heart must be the Krebs cycle, in agreement with data in the literature [10, 12, 18].

Immediately after exertion there was a decrease in the activity of G6PDH, cytoplasmic αGPDH, LDH, SDH, and NAD · H_2DH and an increase in the activity of mitochondrial αGPDH. Presumably, therefore, the increased glycogenolysis necessary for the work of the myocardium under the condition of an increased functional load led to exhaustion of the activity of certain enzymes of glycogenolysis and the Krebs cycle. The increase in mitochondrial αGPDH activity may indicate an increase in the metabolism of triose phosphates, the source of which could be lipids as well as carbohydrates. The decrease in SDH and NAD · H_2DH activity could be connected with changes in the structure of the mitochondria [7]. The changes described are partly reversible, for 3 h after exertion a tendency was observed for the activity of G6PDH and cytoplasmic αGPDH to rise, possible evidence of restoration of normal carbohydrate metabolism. A rest of 3 h, however, was not long enough to allow recovery of the activity of the mitochondrial enzymes and LDH.

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