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Histoenzymological Characteristics of the Contractile Myocardium in Experimental Stenosis of the Aorta

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Contractile cardiomyocyte metabolism was studied by histochemical methods in experimental stenosis of the aorta complicated and not by heart failure. Acceleration of the citric acid cycle, more intense oxidation of free fatty acids and their metabolites, glycolysis intensification, and higher activity of shuttle mechanisms were found in the contractile cardiomyocytes in stenosis of the aorta not complicated by heart failure. The presence of these metabolic shifts in the myocardium of all studied compartments suggests their association with not only more intense heart work, but also with the effects of total systems neurohumoral factors. Comparative study of myocardial metabolism in two variants of experimental stenosis of the aorta has revealed changes prognostically unfavorable for the development of heart failure. These changes include exhaustion of glycogen reserve, glycolysis inhibition, and metabolism shift towards biosynthetic processes. These data indicate an important role of glycolysis in support of myocardial contractile function during the acute phase of pressure overloading of the heart.

Key Words: *histoenzymology; cardiomyocytes; afterload; heart failure*

The contractile function of the myocardium and its metabolism are closely related. Metabolic changes in the contractile cardiomyocytes underlie heart adaptation to increase of hemodynamic loading and to heart failure [5,6]. Experimental stenosis of the aorta traditionally serves as the basic model for studies of the mechanisms of compensatory hyperfunction of the heart. Studies of the myocardium of not only hemodynamically overloaded left ventricle (LV), but also of right ventricle (RV) and interventricular septum (IVS) are an important methodological approach to these studies, due to which it is possible to differentiate the changes emerging as a result of hyperfunction from changes caused by total systems factors (common for all compartments of the heart). Comparative study of these processes under conditions of experimental stenosis of the aorta complicated or not by heart failure is particularly interesting, as it demonstrates the prognostically unfavorable changes associated with the development of heart failure.

We studied by histochemical methods the metabolism of contractile cardiomyocytes in LV, RV, and IVS in experimental stenosis of the aorta complicated and not by heart failure.

MATERIALS AND METHODS

The study was carried out on guinea pigs (500-700 g) under conditions of open chest with artificial ventilation of the lungs. Cardiovascular function was evaluated by recording ECG, pressure in the aorta, LV, and

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RV, and intraventricular pressure first derivative (dP/dt). The parameters were recorded and processed on a Mingograf-82 PC and polycardiograph programmed complex. The afterload was increased by ligation of the ascending aorta, which was paralleled by a 100% elevation of systolic pressure in LV in comparison with the initial level. The duration of aorta stenosis was 30 min.

The animals were divided into 3 groups. Group 1 (n=8) were controls, in which all instrumental and surgical interventions, including forced ventilation of the lungs, chest opening, and catheterization of the ventricles, were carried out, but no stenosis of the aorta was created. In group 2 (n=15), stenosis of the aorta was not aggravated by the development of irreversible heart failure. In group 3 (n=3), stenosis of the aorta was aggravated by the development of heart failure with lethal outcome within the first 30 min.

Immediately after the experiment the hearts were isolated and dissected longitudinally into halves, one half was used for histological and the other for histoenzymological studies. The material for histological studies was fixed in 10% neutral formalin in Lilly buffer and embedded in paraffin. Longitudinal serial sections (5 μ) were stained with hematoxylin and eosin and Schiff's reagent after Mac–Manus with amylase control. Glycogen content in contractile cardiomyocytes was evaluated in preparations stained with Schiff's reagent. The material for histoenzymological studies was frozen in petroleum ether cooled by dry ice and serial cryostat sections (10 μ) were prepared. Activities of succinate dehydrogenase (SDH), isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH), α-glycerophosphate dehydrogenase (α-GPDH), lactate dehydrogenase (LDH), β-hydroxybutyrate dehydrogenase (β-HOBDH), NAD-diaphorase, and NADP-diaphorase were detected routinely using nitroblue tetrazolium [4] and evaluated by a 4-point scale based on two criteria: formazan quantity and density [1]. In addition, enzyme activities were evaluated using MECOS-C television image analyzer.

The data were processed using Student's t test.

RESULTS

The histoenzymological profile of contractile cardiomyocytes in the control reflected metabolic heterogeneity characteristic of the myocardium. The highest activity was shown by SDH, LDH, and NAD-diaphorase, lower activities by ICDH, MDH, and NADP-diaphorase, and still lower activities by α-GPDH and β-HOBDH. The ratio of NAD-diaphorase to NADP-diaphorase activities reflecting the balance of catabolic and anabolic processes in the cell [2,3,6] was 1.8/1.0 in LV, 2.1/1.0 in RV, and 1.8/1.0 in IVS, which attests to predominance of energy-producing processes over biosynthetic ones in the myocardium. The levels of SDH, MDH, α-GPDH, LDH, and NAD-diaphorase activities in the myocardium of all the studied heart compartments were similar. Activities of ICDH, β-HOBDH, and NADP-diaphorase in LV and IVS were higher than in RV. This indicated higher velocity of metabolic reactions in LV and IVS cardiomyocytes and correlated with higher levels of their contractile activities in comparison with RV cardiomyocytes.

Histoenzymological changes in contractile cardiomyocytes in stenosis of the aorta without heart failure (Fig. 1) corresponded to the data on myocardial metabolism during intensive heart work [3,5,6]. The increase in α-GPDH activity indicated intensification of glycolysis in cardiomyocytes. Activity of LDH decreased, indicating inhibition of reactions catalyzed by this enzyme. This, in turn, confirmed that, first, pyruvate metabolism was shifted towards its involvement in the citric acid cycle and, second, that lactate utilization by the myocardium decreased. Suppression of lactate utilization by noncarbonic substrates (free fatty acids and ketone bodies) competing with it for oxygen consumed by the myocardium was a known fact [2,3,6,11]. Intensification of heart work was associated with increased production of free fatty acids and ketone bodies, their contribution to oxidative metabolism of the myocardium increased [3,6]. In our study, the increase in β-HOBDH activity indicated intensification of noncarbonic substrate oxidation. Glycolysis activation together with more intensive oxidation of free fatty acids and their metabolites led to enhanced acetyl coenzyme A formation and stimulation of the citric acid cycle [2,3,6,11]. The increase of ICDH activity (one of the key enzymes of Krebs' cycle) [3] indicated acceleration of the Krebs cycle in our study. It was found that SDH activity in contractile cardiomyocytes decreased, presumably because acceleration of the citric acid cycle could be paralleled by its shunting at the α-ketoglutarate level, as a result of which SDH- and MDH-catalyzed reactions were blocked [6]. Hence, the increase of MDH activity in contractile cardiomyocytes was presumably caused by not only Krebs' cycle intensification, but largely by more intense work of the shuttle mechanisms. This hypothesis was based on the fact that more rapid glycolysis, found in our experiments, led to accumulation of NADH in the cytoplasm and was paralleled by stimulation of the shuttle mechanisms transmitting reduced equivalents from extramitochondrial NADH molecules to electron-transporting chain [2,3,6,11].

Hence, acceleration of citric acid cycle, more intense oxidation of free fatty acids and their metabolites, intensification of glycolysis, and stimulation of the shuttle mechanisms were observed in contractile

cardiomyocytes in stenosis of the aorta without heart failure. All these metabolic shifts in the myocardium of all studied compartments are related to not only more intense heart work, but also to the effects of systemic neurohumoral factors, primarily the sympathoadrenal system, increase of its activity in experimental stenosis of the aorta was demonstrated previously [9].

Stenosis of the aorta aggravated by heart failure (Fig. 2) was associated with increase in ICDH and MDH activities and decrease in SDH activity in contractile cardiomyocytes; these events indicate acceleration and shortening of the citric acid cycle and suggest activation of the shuttle mechanism. Acceleration of Krebs' cycle during more intense heart work was paralleled by an increase in absorption and oxidation of free fatty acids and their metabolites (which proved to be more active in their competition with carbonic substrates for oxygen absorbed by the myocardium), this resulting in glycolysis inhibition [3,6]. In our study this was seen from increased β-HOBDH activity and decreased LDH and α-GPDH activities. Glycogen content dropped in the contractile cardiomyocytes of all the studied heart compartments. Glycolysis inhibition and exhaustion of glycogen reserves played an important role in disorders of myocardial contractions and relaxation and could lead to heart work depression [5,6]. In addition, aggravated stenosis of the aorta was associated with reduction of NAD-diaphorase activity and increase of NADP-diaphorase activity, as a result of which their proportion decreased in comparison with the control and reached 1.5/1.0 in LV, 1.3/1.0 in RV, and 1.4/1.0 in IVS. Redistribution of the energy reserves in cardiomyocytes towards biosynthesis paralleled by lower expenditure for external work deteriorated metabolic provision of the contractile function and promoted the development of heart failure. Activation of regeneratory processes could be a result of myo-

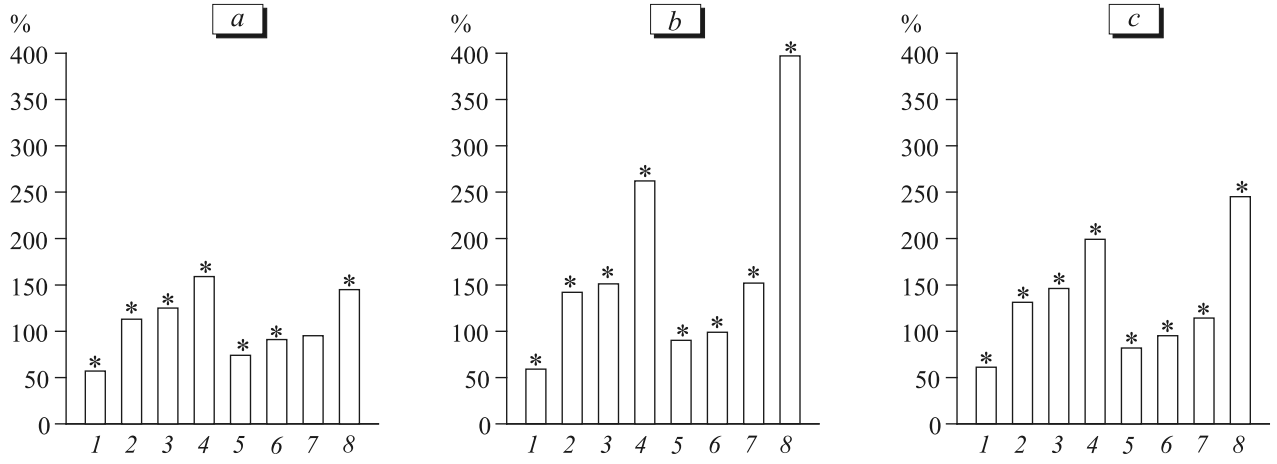

Fig. 1. Histoenzymological profiles of contractile cardiomyocytes in LV (*a*), RV (*b*), and IVS (*c*) in experimental stenosis of the aorta without heart failure. Here and in Fig. 2: ordinate: enzyme activities (control: 100%). *1*) SDH; *2*) ICDH; *3*) MDH; *4*) α-GPDH; *5*) LDH; *6*) NAD-diaphorase; *7*) NADP-diaphorase; *8*) β-HOBDH. **p*<0.05 in comparison with the control.

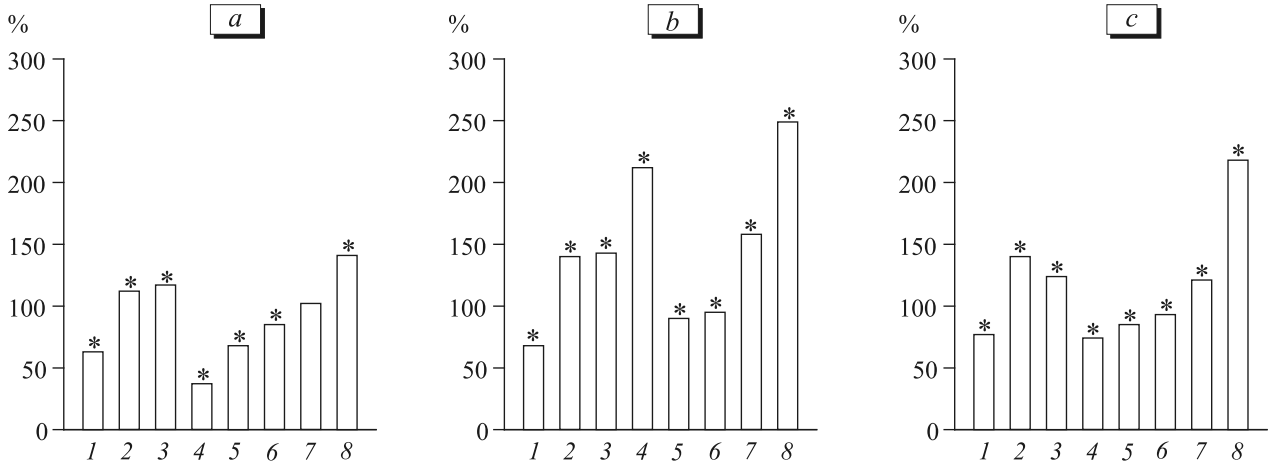

Fig. 2. Histoenzymological profiles of contractile cardiomyocytes in LV (*a*), RV (*b*), and IVS (*c*) in experimental stenosis of the aorta complicated by heart failure.

cardial hyperfunction [5] or reaction to deep injuries in its structure [7]. The reactive variant was confirmed by the results of a previous study [10] which found the most significant (by volume and severity) cardiomyocyte injuries in stenosis of the aorta aggravated by heart failure. Desymapthization of the myocardium and predominance of the adrenal component in the structure of the sympathoadrenal effects on the heart could promote the development of structural and metabolic changes in contractile cardiomyocytes under conditions of complicated stenosis of the aorta [9].

Comparative analysis of myocardial metabolism in two variants of experimental stenosis of the aorta demonstrated changes prognostically unfavorable for heart failure. These are exhaustion of glycogen reserve, glycolysis inhibition, and metabolic shift towards biosynthetic processes. We found similar changes in the contractile cardiomyocyte metabolism in experimental massive pulmonary embolism with heart failure [8]. The results of the present study and previous research indicated that increased afterload of one of the heart ventricles under conditions of different hemodynamic situations were associated with a stereotypical complex of metabolic changes in the contractile cardiomyocytes, which could lead to the development of heart failure. The data indicated the important contribution of glycolysis to provision of the myocardial contractile function during the acute phase of pressure overloading of the heart.

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