Evaluation of Myocardial Energy Parameters during Simulation of Epinephrine Damage under Conditions of Cell Transplantation

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The effects of transplantation of xenogenic neonatal heart cells on energy processes were studied in rat myocardium during the early period of epinephrine injury. Transplantation promoted a less pronounced ATP hydrolys to adenosine monophosphate and inorganic phosphate, a higher level of creatine phosphate, pyruvate, lactate, retention and activation of myocardial enzymes involved in energy metabolism.

Key Words: *myocardium; energy; epinephrine; cell transplantation*

The following effects of cell therapy for acute myocardial injury were reported: stimulation of reparative processes in the myocardium, reduction of the injury, improvement of perfusion and cardiac function, stimulation of angiogenesis [1,3,4,7,9], inhibition of myocardium remodeling [8]. The possibility of cardiomyocyte regeneration is discussed [2,10,11]. We previously showed that cell transplantation limited disorders in metabolic (specifically, enzymatic) processes in the myocardium under conditions of its epinephrine injury [3]. Since the severity of dysmetabolism and changes in cell structure depend on the severity of energy metabolism disorders during the initial period of the pathological process development, we studied the effects of cell therapy on energy processes in the myocardium during the early period of epinephrine injury.

MATERIALS AND METHODS

The experiment was carried out on outbred male rats (200-250 g). Group 1 consisted of normal animals (n=12), their values were considered as basal. Epinephrine stress was induced by a single subcutaneous injection of 0.1% epinephrine (0.2 mg/100 g). Group 2 (control; n=10) were injected with saline (0.5 ml) directly after epinephrine. Group 3 (experiment; n=15) were injected with epinephrine directly followed by a subcutaneous injection of isolated heart cells from a newborn rabbit (500,000 cells/0.5 ml saline). The hearts for the analysis were collected 1 h after the start of the experiment and frozen in liquid nitrogen. The percentage of phosphorus-containing compounds (ATP, ADP, adenosine monosphosphate (AMP), creatine phosphate, inorganic phosphate) was evaluated in specimens of the myocardium by the Varian VXR-500S nuclear magnetic resonance spectrometer. Estimated energy charge (EC) value was used: EC=(2ATP+ADP)/2(ATP+ADP+AMP). The proportion of direct to reverse ADP transformation processes in the adenylate kinase reaction was estimated by the coefficient (K): K=(ATP×AMP)/2ADP [6]. The content of pyruvate [5], lactate, inorganic phosphate was measured. Creatine kinase (CK) activity was measured using standard kits (Biocon). Lactate dehydrogenase-1 (LDH1) was evaluated by its α-hydroxybutyrate dehydrogenase activity using Cormay reagents. Summary activity of ATPases was evaluated by the increment of

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inorganic phosphate. The studies were carried out on a Roki semiautomated biochemical analyzer (Allwex) and SP-16 spectrophotometer.

The results were statistically processed using Statistica software with nonparametric Mann—Whitney *U* test. The differences were considered significant at $p<0.05$.

RESULTS

The content of ATP in the myocardium of animals with simulated epinephrine damage was the same in the control and after cell transplantation and was significantly lower than in intact animals (Fig. 1). Reduced level of ATP in the myocardium after injection of epinephrine indicated active utilization of ATP and a shift of ATP hydrolysis/synthesis balance towards hydrolysis.

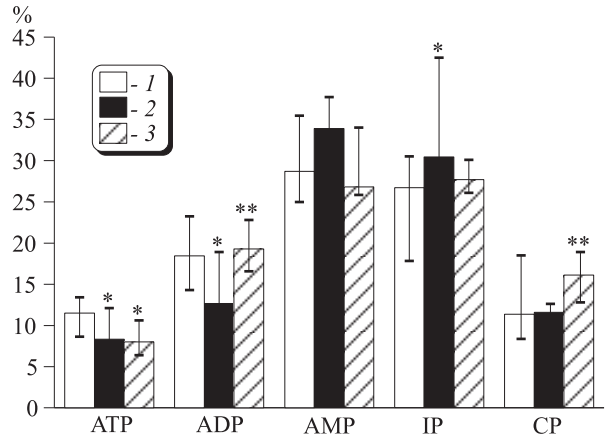

Fig. 1. Dynamics of ATP, ADP, AMP, inorganic phosphate (IP), creatine phosphate (CP) levels in the hearts of experimental rats 1 h after epinephrine injury. Here and in Figs. 2-4: *1*) intact animals; *2*) epinephrine (control); *3*) epinephrine+heart cells (experiment). $p<0.05$ between groups *1 and 2, **2 and 3.

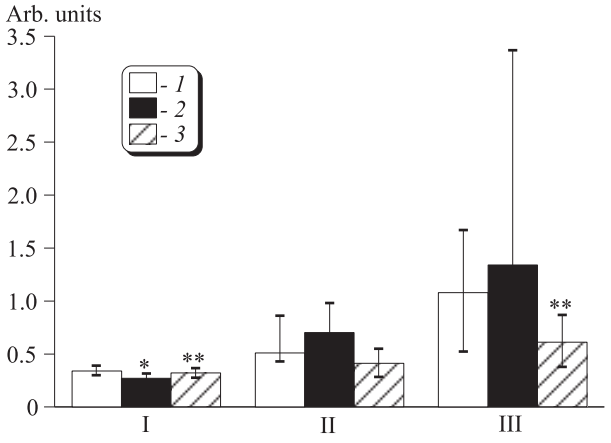

Fig. 2. Dynamics of estimated values characterizing the proportion of activities of energy-producing/energy-utilizing systems in the hearts of experimental rats 1 h after epinephrine injury. I) EC; II) ATP/ADP; III) K.

However, the experimental and control groups differed by the content of ADP and AMP. The percentage of ADP decreased significantly, the percentage of inorganic phosphate increased significantly, and a trend to an increase of AMP percentage in comparison with the basal level was observed in the group without transplantation. On the other hand, the percentage of ADP and AMP and of inorganic phosphate after transplantation did not differ from the initial levels; in other words, the shift towards ATP cleavage to mononucleotides and inorganic phosphate was less significant than in animals receiving no cell transplantation.

The content of creatine phosphate in the myocardium of animals injected with heart cells was higher than in group 2. This indicated the presence of phosphate reserve essential for ATP synthesis and, presumably, was an evidence of less severe disorders in ATP synthesis.

Estimated value of myocardial cell EC was higher in experimental group than in the control and did not differ from the value in intact animals (Fig. 2). Retention of EC value in myocardial cells of animals after transplantation was due to less intensive hydrolysis of adenyl nucleotides (a higher percentage of ADP, lesser percentage of AMP and inorganic phosphate).

Estimation of the ATP/ADP coefficient showed no significant differences between the groups. However, there was a trend to a reduction of the ATP/ADP proportion in experimental group, which was explained by a higher content of ADP.

The proportion of direct to inverse processes of ADP transformation in the myocardum of rats receiving transplantation was significantly lower than in the control group and about the same as in normal animals. Sharp increase of this coefficient in the control group was due to an increase in AMP percentage and a decrease of ADP. These values of the coefficient reflect a more intense cleavage of adenyl nucleotides in the group without transplantation in comparison with the experimental group and a shift of ADP hydrolysis/formation balance towards hydrolysis to AMP and phosphate.

Limitation of energy metabolism disorders after cell transplantation is also confirmed by the levels of lactate and pyruvate (Fig. 3). The lactate/pyruvate ratio was virtually the same in the compared groups, but the concentrations of these substrates in the myocardium was significantly higher in group 3 than in group 2. For example, myocardial pyruvate level in group 3 animals was close to that in intact rats, while in group 2 it was significantly lower. Lactate level was elevated in comparison with intact animals in both groups, but its increase was more intense after cell transplantation.

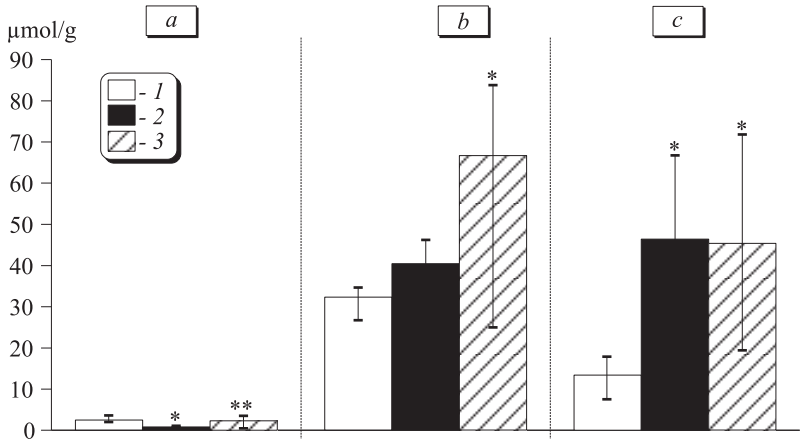

Fig. 3. Dynamics of pyruvate and lactate content in the rat hearts 1 h after epinephrine damage. *a*) pyruvate; *b*) lactate; *c*) lactate/pyruvate. Ordinate: median and quartiles.

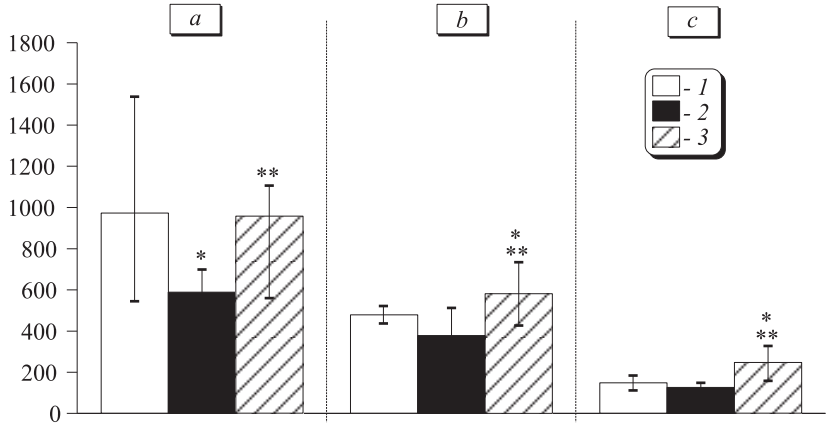

Fig. 4. Activities of CK (*a*; μmol/g×min), LDH1 (*b*; μmol/g×min), summary ATPase (*c*; μmol P/g×h) in the hearts of rats 1 h after epinephrine injury.

These data indicate that aerobic synthesis of ATP in the myocardium of group 3 rats was less impaired and anaerobic synthesis of ATP was stimulated better than in the myocardium of controls.

The dynamics of enzyme activities is in line with this conclusion. As was previously shown, intensive hypoxia develops during the first hour of epinephrine injury [3]. Activities of myocardial enzymes decrease significantly during this period. In the present study the activities of the studied enzymes (LDH1, CK, summary ATPase) in the myocardium of experimental animals were higher by the end of the first hour of experiment than in the controls (Fig. 4). Activity of CK approached the value in normal rats; activities of LDH1 and ATPase tended to increase and surpass the values in intact animals. Retention of enzyme activities and their increase indicate the intactness and activity of the processes in which these enzymes are involved.

Hence, transplantation of neonatal xenogenic heart cells used under conditions of experimental epinephrine injury promoted limitation of energy metabolism disorders in myocardial cells. Less pronounced impairment of energy metabolism was associated with retention and adaptive activation of the enzymes involved in the formation, transport, and activation of ATP.

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