Moderation of Postischemic Damage to Cardiomyocytic Membranes with Reperfusion Solution

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Isolated perfused hearts of Wistar rats subjected to total ischemia and reperfusion were used to examine the possibility of moderating damage to cardiomyocyte membranes with reperfusion solution containing l-aspartic acid, d-glucose, and d-mannitol. During the first 5 minutes of reperfusion, this solution significantly improved recovery of the pumping and contractile functions of the heart compared to the control and reduced the release of lactate dehydrogenase and systems generating short-living ROS into the effluent. To the end of reperfusion, the content of ATP and phosphocreatine was higher and the loss of total creatine was lower in hearts perfused with the test solution compared to the control. It is hypothesized that better integrity of the myocyte sarcolemma in hearts perfused with the test solution results from better preservation of macroergic phosphates and inhibition of ROS generation in this solution.

Key Words: *cardiac reperfusion; reactive oxygen species; energy metabolism; myocytic membranes*

The development of pharmacological agents protecting the myocardium during ischemia and reperfusion is an urgent problem of modern cardiology. It was recently established that reperfusion solutions should contain membrane stabilizers, metabolic cardiaoprotectors, antioxidants, and in addition, they should possess high buffer capacity to cope with intracellular acidosis [8]. In our previous experiments on rats we showed that intravenous injection of hypocalcium reperfusion solution (RS) containing l-aspartic acid, d-glucose, and d-mannitolol after coronary artery occlusion reduced the size of infarction zone and maintained the level of ATP and total creatinine in the risk area [2]. These results indicate that RS reduced damage to cardiomyocyte membranes in the risk area at the early stage of reperfusion.

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Our aim was to study the effect of RS on ROS production and on the metabolic markers of the damage to cardiomyocytic sarcolemma (loss of total intracellular creatine and release of lactate dehydrogenase, LDH) during reperfusion of isolated rat heart.

MATERIALS AND METHODS

Experiments were carried out on hearts of Wistar rats (body weight 350±6 g) perfused by the method of Neely with oxygenated Krebs solution (95% O_2+5%CO_2, pH 7.4±0.1, 37°C) containing 11 mM glucose [4]. After 15-20-min stabilization of the left ventricular function (constant filling pressure 15 mm Hg and stable left ventricular outflow backpressure 60 mm Hg) referred to as the initial state, the hearts were subjected to 30-min normothermal global ischemia. Then the hearts were retrogradely reperfused at the constant rate of 3.0±0.2 ml/min for 5 min with control Krebs solution or with RS containing (in mM): 144.0 Na^+, 4.7 K^+, 1.2 Ca^{2+},

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1.2 Mg^{2+}, 10.0 trisamine, 20.0 l-aspartic acid, 20.0 d-glucose, 36.0 d-mannitol (pH 7.5±0.1, 22°C). Then the hearts were reperfused for 25 min with Krebs solution by the method of Neely.

The myocardial effluent was collected in successive fractions during the first 10 min after the onset of reperfusion to measure ROS content and activity of LDH. After addition of a spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO) in a final concentration of 100 mM, the specimens were frozen and stored in liquid nitrogen until EPR recording. EPR spectra were recorded on a E-109E X-range spectrometer (Varian) at microwave power and frequency of 10 mW and 9.15 GHz, respectively [2]. LDH activity in effluent specimens was measured on a Yanako UO-2000 spectrophotometer [5].

The hearts in the initial state and after reperfusion were frozen synchronously at the end of diastole [1] in liquid nitrogen to determine ATP, phosphocreatine (Pcr), and creatine. Protein-free extracts were prepared, metabolites were assayed enzymatically, and tissue dry weight was determined. The data were processed statistically using Student's *t* test.

RESULTS

Table 1 shows the mean indexes of contractile and pumping functions of the heart for both groups in the initial state and the effect of RS on the recovery of these parameters 10 and 30 min after the start of reperfusion. RS considerably improved the recovery of cardiac functions at the early stage of reperfusion. This effect persisted for the most indexes until the end of reperfusion.

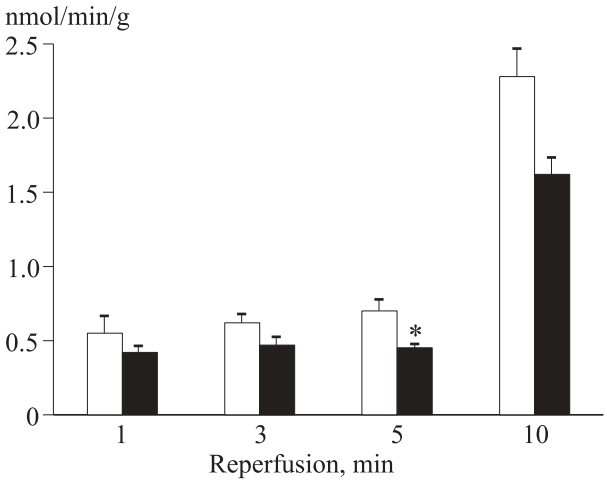

Fig. 1. Effect of RS on the release of ROS generating systems into the myocardial effluent in isolated rat heart during reperfusion. Open bars: control; dark bars: RS. The data are means for 5 experiments. $p^*<0.05$ compared to the control.

EPR spectra revealed the appearance of four equidistant narrow peaks with the intensity ratio of 1:2:2:1 corresponding to a spin adduct DMPO-OH formed during interaction of DMPO molecules with extremely toxic short-living hydroxyl radicals. DMPO-OH can be formed due to spontaneous decay of unstable adduct DMPO-OOH resulting from interaction of DMPO and superoxide radicals [7]. The release of systems producing ROS in hearts protected with RS was insignificantly lower than in control hearts during the whole early reperfusion period (Fig. 1) and significantly lower during the 5th minute after the start of reperfusion (Fig. 1). The output intensity was expressed as the product of DMPO-OH concentration in the effluent by the coronary flow per wet heart weight.

TABLE 1. Effect of RS on Recovery of Contractile and Pumping Functions of Isolated Rat Hearts during Reperfusion (*M*±*m*, *n*=20)

Index		Initial values, abs. units	Reperfusion (% initial value)			
			10 min		30 min	
			control	RS	control	RS
Blood pressure, mm Hg	systolic	109±1	44±1	86±1***	65±1	77±1***
	diastolic	-5±1	445±16	230±8***	365±19	301±11**
	developed	113±1	28±1	82±1***	53±1	69±1***
HR, min^{-1}		278±2	62±2	103±2***	83±3	85±2
Contractile function intensity, mm Hg/min		30 266±396	16±1	88±3***	49±2	60±2***
Coronary flow, min/ml		15±1	96±2	100±1*	89±2	90±2
Cardiac output		43±1	0	78±1***	34±5	63±1**

Note. Contractile function intensity is the product of HR by the developed pressure *p<0.05, **p<0.01, ***p<0.001 compared to the control.

TABLE 2. Effect of RS on LDH Release from Isolated Rat Heart into Myocardial Effluent during Early Reperfusion Stage (U/g dry weight, *M*±*m*, *n*=10)

Index	Control	RS
First 5 min of reperfusion	41.43±4.20	29.18±3.80*
The next 5 min of reperfusion	63.60±11.40	56.71±9.4
Total release during 10 min reperfusion	105.03±12.15	85.88±10.14

Note. *$p<0.05$ compared to the control.

TABLE 3. Effect of RS on Metabolite Content in Isolated Rat Heart on Reperfusion Minute 30 (μmol/g dry weight, *M*±*m*, *n*=10)

Index	Initially	Control	RS
ATP	23.93±1.84	12.65±1.59*	14.93±0.98*
Phosphocreatine	24.30±3.00	18.37±1.65	22.61±1.37+
Creatine	35.49±2.13	29.08±1.79*	32.92±2.22*
Total creatine	59.46±2.94	47.45±0.75*	55.52±2.89+

Note. $p<0.05$ compared to *initial and +control data.

The release of LDH from the postischemic heart into RS during the first 10 min reperfusion was lower than in the control due to significantly lower release of this enzyme during the first 5 min of reperfusion (Table 2). Coronary flow during the first 5 min of retrograde reperfusion was 3.0±0.2 ml/min in both groups, but during the following reperfusion this parameter recovered more efficiently under the effect of RS. For example, from minute 6 through 10, the mean coronary flow in hearts reperfused with RS was 19.5±1.2 ml/min *vs.* 7.7±0.3 ml/min in control hearts during the same period ($p<0.01$). At the same time, the release of LDH from postischemic myocytes did not increase under conditions of 2.5-fold increased coronary flow, while the production of ROS in the effluent even insignificantly decreased (Fig. 1), which indicated less pronounced damage to myocyte membranes in hearts perfused with RS.

To the end of reperfusion, the contents of ATP and phosphocreatine in the hearts reperfused with RS were significantly higher than in the control (Table 3), which attested to better recovery of aerobic metabolism. Since sarcolemma of intact myocytes is impermeable for phosphocreatine and creatine, the intracellular content of total creatine can serve as the index of reperfusion damage to membrane [6]. To the end of reperfusion, the loss of total creatine was 20.2±1.3 ($p<0.05$) and 6.6±4.8% of its initial level in the control and experimental (RS-protected) hearts, respectively. This phenomenon suggests better integrity of the sarcolemma in the experimental hearts, which agrees with lower release of LDH into the myocardial effluent during the early stage of reperfusion (Table 2).

Thus, better recovery of the contractile and pumping functions of isolated heart subjected to total ischemia and perfused with RS can directly result from moderation of reperfusion damage to the myocytes. The cardioprotective effect of RS results from its antioxidant properties and maintenance of higher level of intracellular macroergic phosphates, which provides better integrity of cardiomyocyte membranes.

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