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POSTISCHEMIC LIPID PEROXIDATION AND MYOCARDIAL CONTRACTILITY DEPENDING ON LEVEL OF HYPOTHERMIA PROTECTING THE HEART

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Deep cooling of the myocardium is one of the most important factors for protection of the heart muscle against ischemia during open heart operations [2, 5]. However, hypothermia has a twofold action on cellular structures: on the one hand it lowers the level of metabolism and thereby prevents the development of ischemic lesions connected with a disturbance of energy metabolism [4]; on the other hand, by changing the phase state of lipids, it can induce structural and functional disturbances of the cell membranes. These changes, moreover, only become evident after reperfusion (RP) of the heart [8]. One of the systems maintaining the lipid composition of membranes and its physicochemical parameters within an assigned physiological range is lipid peroxidation (LPO). Accordingly, the aim of the investigation described below was to compare LPO and cardiac contractility during ischemia and RP and their dependence on the level of hypothermia used to protect the myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on isolated hearts of noninbred albino rats weighing 180-220 g, anesthetized with pentobarbital (25 mg/kg). The heart was perfused in the retrograde direction by the Langendorff–Falen method, with standard Krebs–Henseleit solution, aerated with a gas mixture of 95% O_2 and 5% CO_2, at 37°C and pH 7.4. A small latex balloon with constant volume was introduced into the left ventricle. The pressure inside the balloon was recorded by means of a 6MDXIIS pressure transducer. The following parameters of cardiac contractility were calculated from the pressure curve and its first derivative: the developed pressure (P_d) and end-diastolic pressure (EDP). The heart was stopped after 15 min of perfusion by simultaneous compression of the aorta and cooling of the myocardium to 8-12°C in the experiments of series I and to 4-6°C in series II. RP was carried out after 90 min of ischemia. At the 15th minute of perfusion, at the end of the period of ischemia, and after 7 and 90 min of RP the hearts were placed in liquid nitrogen in order to study LPO products. Intact hearts served as the control. The level of conjugated dienes (CD) and trienes (CT) was determined spectrophotometrically at wavelengths of 233 and 275 nm respectively in a solution of lipids, extracted by Folch's method

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TABLE 1. Change in Parameters of Myocardial Contractility Depending on Level of Cooling of the Heart during Ischemia (M ± m)

Series	Level of cooling	Parameter	15 min of perfusion	Period of RP, min	
				7	90
I	8—12 °C	P_d, mm Hg	116±5	112±6,5	83,4±4,4*
		EDP, mm Hg	8,4±0,7	11,8±1,1*	7,6±0,6
II	4—6 °C	P_d, mm Hg	127,0±2,4	60,1±7,2*	71,8±3.3*
		EDP, mm Hg	5,9±0,7	13,7±1,9*	11,8±2,1*

Legend. Asterisk indicates differences compared with 15th minute of perfusion are significant, $p < 0.01$.

TABLE 2. Effect of Different Levels of Hypothermia on State of LPO in Heart Muscle during Ischemia and RP

Series	CD	CT	MDA, optical density units/mg protein	SB, relative units/mg tissue	SOD, conventional units/min/mg protein
	optical density units/mg TL/g tissue				
Control	2,84±0,14	0,70±0,15	0,0302±0,001	0,39±0,33	3,58±0,40
15 min of perfusion	7,19±0,16[a]	1,44±0,16[a]	0,0309±0,002	5,88±0,91	9,23±0,19[a]
1) 90 min of ischemia	5,95±0,17[a]	1,92±0,25[a]	0,037±0,004	8,08±0,57[a]	6,03±0,16[a]
7 min of RP	4,54±0,26[a,b]	1,48±0,21[a]	0,037±0,002	7,64±1,16	4,39±0,23[b]
90 min of RP	1,99±0,18[a,b,c,d]	0,50±0,11[a,b,c,d]	0,034±0,002	5,97±1,75[c,d]	2,54±0,18[a,b,c]
1) 90 min of ischemia	8,12±0,20[a,b]	1,89±0,06[a,b]	0,077±0,006[a,b]	12,60±0,28[a,b]	2,44±0,25[a,b]
7 min of RP	5,28±0,70[a,b,c]	1,49±0,17[a,b]	0,056±0,002[a,b]	14,56±1,50[a,b]	2,99±0,02[a,b]
90 min of RP	2,03±0,02[a,b,c]	0,97±0,06[b,c,d]	0,032±0,004[c,d]	11,80±2,56[a,b]	2,02±0,97[a,b]

Legend. Significance of differences relative to: a) control, b) 15th minute of perfusion, c) ischemia, d) 7th minute of RP ($p < 0.01$).

[10], in methane and hexane, the concentration of malonic dialdehyde (MDA) spectrophotometrically by the reaction with thiobarbituric acid at 535 nm [11], and the content of Schiff's bases (SB) by measuring the intensity of fluorescence of lipid extracts in chloroform [6]. Activity of the enzyme superoxide dismutase (SOD) was detected by the method in [9]. The results were subjected to statistical analysis by the usual methods, using Student's test.

EXPERIMENTAL RESULTS

Restoration of cardiac activity after hypothermic ischemia differed in the two series. In the series with cooling of the myocardium to 8-12°C rhythmic contractions appeared as early as after 71 ± 1.9 sec of RP. The value of P_d at the 7th minute of RP was close to its value before ischemia, and only after 90 min of RP was its value reduced by 30% (Table 1). The increase in EDP by 40% at the beginning of RP compared with the initial level indicated the development of a mild degree of reperfusion contracture of the myofibrils. However, by the 15th minute of RP, EDP was restored to normal, and by the 90th minute of the postischemic period it was actually reduced a little.

Restoration of electrical activity of the heart in the series with cooling of the myocardium to 4-6°C took place through fibrillation. Spontaneous contractions appeared after 235 ± 29 sec of RP. P_d at the 7th minute of RP was 53% lower than initially, but the EDP level was 2-2.5 times higher. These changes, moreover, continued throughout the period of RP (Table 1) indicating the formation of persistent myofibrillar contracture and inhibition of myocardial contractility in the hearts of this series.

The study of LPO products in the myocardium showed that perfusion for 15 min led to elevation of the CD and CT levels (Table 2), which was connected with the response of the cardiomyocytes to this process. There was a simultaneous compensatory increase in SOD activity, which probably prevented the further development of the LPO process, for concentrations of MDA and SB at this stage did not change significantly. The state of LPO during ischemia depended on the level of myocardial cooling. During hypothermia to 8-12°C a small fall was observed in the CD level, with an increase on average by 30% in the CT, MDA, and SB levels compared with the 15th minute of perfusion. SOD activity was reduced, although it still remained 1.7 times higher than in the control series.

Cooling the heart muscle to 4-6°C, despite a marked decrease in the velocity of metabolism and oxygen consumption [4], led to a marked increase in the content of LPO products in the myocardial tissue compared with the preischemic level. Concentrations of CD and CT rose by 12 and 31%, and those of MDA and SB rose by 2.5 and 2.1 times respectively. One cause of intensification of LPO in this series was reduction of SOD activity by almost 3.8 times compared with that at the 15th minute of perfusion and by 17% compared with the control series.

RP and reoxygenation for 7 min in series I caused no change in the content of LPO products in the myocardium relative to the period of ischemia. Only a decrease in SOD activity was observed. In series II no change likewise was observed in the concentration of LPO products, although their level was rather higher than in series I. SOD activity did not differ significantly from that during ischemia.

After 90 min of RP the concentration of LPO products and SOD activity in both series did not differ from those in the control series. Only a high concentration of SB in the series with hypothermia to 4-6°C was determined.

It can thus be concluded from these results that the state of LPO during ischemia depends on the level of myocardial hypothermia. Cooling to 8-12°C, for instance, causes no significant changes in the LPO/antioxidants system, thereby preventing the development of reperfusion damage when cardiac activity is restored. Lowering the temperature to 4-6°C leads to activation of LPO, which is probably connected with a change in the phase state of membrane lipids [3], and the presence of free iron ions [7] at that temperature. The rise of the MDA and SB levels accompanied by depressed SOD activity also are evidence of exhaustion of the antioxidant system of the cells or disturbance of enzyme activity at low temperatures [1]. During RP an increased concentration of LPO products, especially end products (MDA and SB) is preserved, whereas SOD activity is low, and this is one factor in the disturbance of myocardial contractility.

It thus follows from the above account that cooling heart muscle to 8-12°C is the optimal method of preventing intensification of LPO in the myocardium during ischemia and subsequent RP, and in turn this promotes rapid and full recovery of cardiac contractility.

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