Energy Metabolism Disturbances Induced by Multiple Ischemic Preconditioning Worsen the Recovery of Cardiac Function in Reperfusion

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Single ischemic preconditioning improves functional recovery of isolated rat heart subjected to global ischemia and reperfusion. Two preconditioning cycles abolish this protective effect, while 4 cycles impair functional recovery of the heart. These phenomena are due to the fact that repetitive ischemic preconditioning reduces the content of phosphocreatine and adenine nucleotides in the heart before long-term ischemia, which prevents the recovery of energy metabolism and induces additional damage to cardiomyocyte membranes during reperfusion.

Key Words: *rat heart; preconditioning; pump function; energy metabolism*

Repeated ischemia and reperfusion episodes protect the heart against prolonged ischemic and reperfusion stress. This so-called ischemic preconditioning (IP) is related to a decrease in the rate of phosphocreatine (PCr) and ATP utilization and glycogenolysis during long-term ischemia. This inhibits accumulation of catabolites and the development of acidosis in ischemized tissue and promotes recovery of oxidative respiration and cardiac function during subsequent reperfusion [7]. Most studies do not compare metabolic and functional effects of single and repeated cycles of IP. It was reported that compared to single IP, multiple IP does not improve myocardial protection [1,4,8] and even abolishes its beneficial effect [9,10]. This hinders the study of IP mechanisms and its use in clinical practice. The aim of the present study was to compare the effects of single and multiple IP on functional and metabolic parameters of isolated working heart subjected to global ischemia. The study is focused on the assessment of cardiac function during reperfusion and determination of adenine nucleotides (AN), PCr and creatine (Cr) during IP and after reperfusion.

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MATERIALS AND METHODS

Hearts were isolated under urethane narcosis (1.6-2.0 g/kg) from Wistar rats weighing 250-300 g. The hearts were perfused by the method of R. Neely against constant filling and resistance pressures of 20 and 90 cm H_2O, respectively, with a Krebs—Henseleit bicarbonate buffer containing (in mM): 118 NaCl, 4.7 KCl, 3.0 $CaCl_2$, 0.5 Na_2-EDTA, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25.0 $NaHCO_3$, and 11.0 glucose, pH 7.4± 0.1 at 37°C and oxygenated with 95% O_2 and 5% CO_2. Left ventricle pressure, coronary flow, and aortal pressure and output were recorded. Volume work was calculated as the product of cardiac output and mean aortal pressure; contractile function was calculated as the product of developed pressure of the left ventricle and heart rate.

In the control series the hearts were perfused for 50 min in a working regime for recording the initial parameters and then subjected to 15-min global ischemia and 30-min reperfusion. The 50-min perfusion period was replaced with 3 types of IP. In the IP-1

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TABLE 1. Pre- and Postischemic Parameters of Cardiac Function (*M±m*, *n*=14)

Parameters	Control	IP-1	IP-2	IP-4
Left ventricular systolic pressure, mm Hg	148±1/80±6	133±3*/91±5	120±4*/88±6	92±5*/27±7**
Left ventricular end-diastolic pressure, mm Hg	3±0/14±1	8±1*/12±1	8±1*/14±1	17±2*/15±1
Heart rate, min^{-1}	275±4/260±18	287±5/293±12	286±5/270±17	267±16/236±31
Coronary flow, ml/min	28±1/15±2	31±1/18±1	29±1/16±1	26±2/13±2
Cardiac output, ml/min	69±2/6±2	55±2*/17±2**	46±2*/7±2	24±5*/1±1**
Contractile function, $\times10^{-2}$, mm Hg/min	399±7/172±25	359±12*/232±21	320±14*/200±26	200±20*/28±18**
Volume work, $\times10^{-2}$, mm Hg×ml/min	53±2/5±2	42±2*/13±2**	35±2*/5±2	18±4*/2±1**

Note. Numerator: preischemic values (after IP for the IP-1, IP-2, and IP-4 groups), denominator: values after reperfusion. $p<0.05$ in comparison with the control: *before ischemia, **after reperfusion.

group the hearts were subjected to a 5-min global ischemia followed by a 5-min reperfusion before a long-term ischemia period. In the IP-2 and IP-4 groups the period of long-term ischemia was preceded by 2 and 4 ischemia-reperfusion cycles (5 min of each mode), respectively. After preliminary perfusion, IP, and reperfusion, the hearts were frozen in liquid nitrogen for the metabolite assay.

Procedures of homogenization, protein precipitation with cold 6% $HClO_4$, and determination of tissue dry weight were described previously [6]. The total pools of AN (ΣAN=ATP+ADP+AMP) and creatine (ΣCr=PCr+Cr) in neutralized tissue extracts were measured by enzymatic methods [2].

RESULTS

Initial parameters of contractile and pump function of all preconditioned hearts did not differ from the control. Repeated IP cycles gradually decreased left ventricular systolic pressure, aortic output, and increased end-diastolic pressure before long-term ischemia, but had no effect on heart rate and cardiac output (Table 1). These changes resulted in a progressive preischemic impairment of contractile and pump function in IP-1, IP-2, and IP-4 groups, but only in the IP-1 group the recovery of contractile and pump functions surpassed that in the control group. Two IP cycles did not improve functional recovery of the heart in comparison with the control, while the 4-cycle protocol (IP-4 group) markedly impaired the recovery of all functional parameters by the end of reperfusion, except of coronary flow, which remained unchanged.

Single IP had no effect on the content of AN, PCr, and Cr in the heart prior to long-term ischemia (Table 2). Repeated IP led to increased ATP and ADP losses, which reduced ΣAN by 14±1 and 24±2% ($p<0.05$) compared with the baseline (preischemic) values in the IP-2 and IP-4 groups, respectively, and to a decrease in the content of PCr but not Cr. The contents of ATP, ΣAN, PCr, and Cr in the hearts of the IP-1 group at the end of reperfusion surpassed the control values, while the energetic state of the IP-2 group hearts did not differ from the control. The lowest parameters of energy metabolism after reper-

TABLE 2. Content of AN, PCr, and Cr, mmol/g Wet Tissue (*M±m*, *n*=6)

Experimental conditions	ATP	ADP	AMP	ΣAN	PCr	Cr	ΣCr
Initial	21.16±1.03	2.48±0.21	0.96±0.08	24.63±1.08	26.17±1.53	36.16±2.53	62.35±2.89
After IP							
IP-1	20.01±1.14	2.59±0.19	1.08±0.09	23.80±1.20	31.40±2.30	32.76±2.19	63.98±2.42
IP-2	17.53±0.89*	2.33±0.26	1.12±0.09	20.75±1.09	23.64±1.87	34.36±2.13	58.41±2.33
IP-4	16.52±0.78*	1.81±0.16*	0.86±0.08	19.11±0.08*	21.37±1.54*	33.98±2.57	56.70±2.15
After reperfusion							
Control	7.70±0.78*	1.63±0.20*	0.75±0.07	10.12±0.81*	15.27±1.04*	24.59±1.48*	39.90±1.39*
IP-1	11.08±0.82*+	1.74±0.22*	0.88±0.09	13.73±0.87*+	19.66±0.95*+	27.72±2.49*	47.25±2.05*+
IP-2	8.49±0.65*°	1.53±0.18*	0.74±0.07	10.79±0.71*°	18.29±1.08*	23.42±1.95*	41.75±1.80*
IP-4	5.76±0.64*°	1.48±0.16*	0.82±0.13	8.08±0.61*°	14.11±1.23*°	20.14±1.78*°	34.08±1.69*+°

Note. $p<0.05$: *compared with the initial value, +compared with the control after reperfusion, °compared with IP after reperfusion.

fusion were observed in the IP-4 group. The content of ΣCr in this group was decreased in comparison with the control, implying a greater damage to myocyte membranes [5], the content of ATP, ΣAN, PCr, and Cr being reliably lower than in the IP-1 group.

Recovery of the volume cardiac work in all groups strongly correlated with the myocardial content of ΣAN and ΣCr (r=0.92 and 0.94, respectively, p<0.05), while the content of ΣAN in hearts subjected to single and repeated IP after reperfusion correlated with the content of ATP and ΣAN after preconditioning.

These findings suggest that disturbances in energy metabolism induced by repeated IP cycles are responsible for the development of heart dysfunction prior to long-term ischemia. The subsequent reperfusion aggravates damage to cell membranes and worsens the recovery of aerobic metabolism, which strictly depends on energy supply of myofibrillae [3]. Thus, all conclusions on the potential advantages of repeated IP over single IP cycle can be made on the basis of adequate comparison of metabolic and functional responses of the heart.

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