The Relationship between Mitochondrial Respiration and Postischemic Recovery of Isolated Heart Contractility

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Recovery of heart contractility after global normothermic ischemia in New-Zealand rabbits depends on the reperfusion mode and the composition of reperfusion medium and correlates with mitochondrial respiration. Cardiac function can recover also at low ATP concentration (about 1 μmol/g dry tissue).

Key Words: *rabbit heart; ischemia; reperfusion; mitochondrial respiration; high-energy phosphates*

Deep myocardial ischemia reduces tissue content of high-energy phosphates, in particular ATP and creatine phosphate (CP), due to inhibition of oxidative phosphorylation in mitochondria followed by lactate accumulation and enhanced glycogen decomposition. It has been shown that ATP plays an essential role in the development of ischemia-induced myocardial damage [2,4,11]. These studies also demonstrated that myocardial contractility can be restored if the ischemized tissue contains at least 2-3 μmol ATP per gram dry tissue, whereas lower ATP content leads to irreversible changes in cardiomyocytes and loss of contractility [3,7,8]. We assume that under conditions of preserved mitochondrial functions the contractility of myofibrils can be restored even at negligible or zero tissue ATP content. Since tissue ATP content corresponds to the difference between ATP synthesis and degradation, the absence of ATP accumulation during reperfusion may imply its complete utilization. It should be noted that these studies were carried out on the model of regional hypothermic ischemia with cardioprotection. Mitochondrial respiration was assessed on isolated mitochondria. It is known that considerable proportion of the mitochondria is lost during isolation, particularly from damaged tissues, which masks the real picture and the degree of damage [10].

We investigated the relationship between mechanical function of the myocardium and the rate of mitochondrial respiration and ATP content during reperfusion after normothermic global ischemia.

MATERIALS AND METHODS

Hearts isolated from New Zealand rabbits weighing 1.7-2.0 kg were perfused via the aorta with Krebs-Henseleit buffer (Langendorff retrograde perfusion at constant pressure without recirculation) [1]. Before ischemia and during reperfusion the hearts were arrested with St. Thomas's Hospital cardioplegic solution, pH was measured after carbogen saturation (95% O_2, 5% CO_2) at 37°C; 10 mM CP (disodium salt) and 15 mM glutamaic acid (GA, potassium salt) were added to the cardioplegic solution as cardioprotectors (CP-containing solution with 90 mM NaCl). To model global ischemia the perfusate flow was interrupted; hypothermia (22°C) and cardioprotectors were used only during reperfusion with cardioplegic solution.

The hearts were perfused before ischemia (group 1), subjected to 80-min cardioplegic normothermic ischemia (group 2), and reperfused under different

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conditions (groups 3-6): with normothermic and hypothermic (22°C) cardioplegic solution without cardioprotectors (group 3 and 4, respectively), or containing 10 mM CP+15 mM GA (groups 5 and 6, respectively).

At the end of the experiment, tissue samples from the left ventricle endocardium were collected for the mitochondrion respiration assay [13] allowing one to evaluate mitochondrial respiration in 6-8-mg myocardium samples without mitochondria isolation. The reminder tissues were frozen in liquid nitrogen for the high-energy phosphate assay. The contents of CP, total creatine, and adenine nucleotides (ADN) ATP, ADP, and AMP in frozen hearts were measured after extraction with 6% perchloric acid and 10% methanol. ADN were determined by high-performance liquid chromatography.

RESULTS

The recovery of cardiac function after 80-min global ischemia depended on perfusion conditions and the composition of cardioplegic solution (Table 1). The best recovery (33% of the preischemia level) was observed in group 6 (hypothermia+cardioprotectors).

Global normothermic ischemia (80 min, group 2) significantly reduced the rate of mitochondrial respiration to 54% of the control. The maximum respiration rate was observed in group 6 (55% of the control). Hence, the rate of mitochondrial respiration in hearts perfused with cardioprotectors under hypothermic conditions was equal to that in group 2 (ischemia). Of particular interest is weak acceleration of mitochondrial respiration by creatine under conditions of low ADP concentration (100 μM): 37% after ischemia and 26% after reperfusion with a normothermic cardioplegic solution without cardioprotectors. In groups 4, 5, and 6 this acceleration attained 52, 58, and 65%, respectively. No significant differences were noted between the ischemia and reperfusion groups (except group 6) by the V_{max}/V_o parameter (mitochondrial respiratory control analog), while all these groups differed considerably from the control. Table 1 shows that global ischemia was accompanied by complete CP utilization, tissue ATP content being 1.05 μmol/g. Normothermic reperfusion without cardioprotectors led to further decrease in the ATP content. Hypothermia and cardioprotectors (group 4-6) slightly increased the content of ATP and ADP. For instance, the content of ATP in groups 4, 5, and 6 constituted 7, 11, and 13% of control, respectively, whereas the content of CP in these groups recovered more rapidly and attained 44, 53, and 93% of control.

Thus, our findings suggest that the recovery of cardiac function after 80-min global normothermic ischemia occurs despite complete CP exhaustion and low ATP content (about 1 μmol/g). The rate of mitochondrial respiration was about 54% in all experimental groups. Decreased activation of mitochondrial respiration with creatine indicate disturbances in the oxidative phosphorylation-coupled creatine kinase mitochondrial system during ischemia. This probably results from ischemia-induced changes in the ionic and osmotic homeostasis in cardiomyocytes, in particular, accumulation of inorganic phosphate which stimulates the dissociation of creatine kinase from the mitochondrial membrane.

Another possible mechanism of ischemia-induced damage to the mitochondrial creatine kinase system is the inhibition of ADP-ATP translocase by acyl-CoA accumulated during ischemia [14]. Normothermic reperfusion without cardioprotectors did not restore cardiac function. This was accompanied by significant inhibition of mitochondrial respiration and a decrease in ATP and ADN contents. It can be

TABLE 1. Mitochondrial Respiration Rate, High-Energy Phosphates, and Work of Isolated Rabbit Heart ($M \pm m$)

Group (n=8)	Heart work, mm Hg/sec[1]	V_O	V_{CP}	V_{max}	ATP	CP	ADP
		ng-atom O/min/mg dry tissue			μmol/g dry tissue		
1	273.9±11.1	3.55±0.24	13.46±0.52	25.85±0.73	18.38±0.98	26.02±2.07	23.36±1.30
2	0	2.35±0.27	6.32±0.48	11.25±1.36	1.05±0.22	0	5.29±.44
3	0	1.56±0.14	3.73±0.36	9.07±0.42	0.88±0.12	0	2.19±0.16
4	19.3±5.2**	1.66±0.18	4.45±0.21	10.15±0.97	1.21±0.12	11.50±1.95	2.48±0.08
5	34.8±2.6**	1.92±0.19	5.72±0.39	10.97±0.71*	2.10±0.72	13.81±3.23	3.84±0.67
6	80.3±14.0**	2.25±0.60	6.62±1.51*	14.21±0.77**	2.33±0.46*	24.25±0.44**	4.11±0.47*

Note. [1]Product of developed pressure and heart rate. Mitochondrial respiration rate was measured after addition of substrates (5 mM glutamate and 2 mM malate) to the medium (V_O), in the presence of 100 μM ADP (V_{ADP}) after addition of 20 mM creatine (V_{CP}), in the presence of high (1 mM) concentration of ADP (V_{max}), in the presence of 35 μM carboxyatractyloside, ATP-ADP translocase inhibitor. *p<0.01, **p<0.001 compared with group 3.

hypothesized that these reperfusion conditions had an adverse effect on ischemic myocardium and aggravated cardiomyocyte damage resulting from calcium and oxygen paradoxes [6,12]. The inhibition of mitochondrial respiration and oxidative phosphorylation probably results from the disturbances in electromechanical coupling accompanied by heart contracture due to impaired Ca^{2+} homeostasis, exhaustion of cell substrates for the high-energy phosphate synthesis, and disruption of mitochondrial membranes.

Hypothermic reperfusion with cardioplegic solution containing 10 mM CP and 15 mM GA promoted the recovery of mechanical function of the heart and mitochondrial respiration. Numerous studies over the last two decades demonstrated a protective effect of exogenous CP and GA on ischemic myocardium [5,9]. When hypothermia and cardioprotectors were used, both the relative and absolute rates of mitochondrial respiration increased (groups 4-6) and attained the maximum (55%) if the factors were applied simultaneously. The recovery of heart contractility directly correlated (r=0.96) with the rate of mitochondrial respiration under different reperfusion conditions (Fig. 1). It should be noted that in our experiments the recovery of mechanical function and mitochondrial respiration was accompanied by a minor increase in the ATP content (maximally 2.3 μmol/g, i.e., 13% of control) probably due to its rapid utilization in myofibril contraction cycle and other ATP-dependent processes. Our findings suggest that mitochondrial respiration plays a key role in postischemic recovery of the heart contractility. The content of ATP cannot serve as an indicator of the irreversibility of the ischemia-induced damage.

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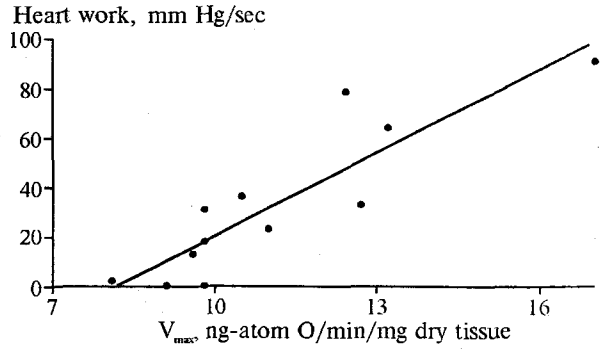

Fig. 1. Correlation between maximum mitochondrial respiration rate and cardiac function recovery.

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