Original

Myocardial Oxygen Consumption and Mitochondrial Membrane Potential in Post-Ischemic Myocardium

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Abstract : Myocardial oxygen consumption (MVO_2) may be disproportionately high relative to contractile function in post-ischemic, reperfused myocardium. This study investigated the mechanism of dissociation between MVO_2 and contractile function in post-ischemic, reperfused myocardium using isolated rat hearts. Mitochondrial dysfunction secondary to increased calcium uptake has been implicated as an important mediator of reperfusion injury in the heart. In post-ischemic, isovolumic, antegrade-perfused rat hearts, MVO_2 rate and contractile function were studied in relation to mitochondrial function. Left ventricular pressure, coronary blood flow, and MVO_2 were determined. Mitochondrial respiration and membrane potential were measured by polarography and flow cytometry. To examine the role of mitochondrial calcium uptake in ischemic reperfusion injury, isolated rat hearts perfused with ruthenium red (an inhibitor of calcium uptake) were compared to control perfused hearts. Hearts were subjected to 60 minutes of no-flow ischemia, followed by 60 minutes of reperfusion. At 15 minutes after the onset of reperfusion, there was poor recovery of left ventricular pressure to 54% of the control level, but MVO_2 increased to 149% of the control. Addition of 2.5 μM ruthenium red to the perfusate resulted in a decrease in MVO_2 . The oxygen consumption rate in state 3 mitochondria decreased similarly following reperfusion both in control and in ruthenium red hearts. The mitochondrial membrane potential was reduced to 89% (logarithmic scale) after 15 minutes of reperfusion. This data suggests that the dissociation between MVO_2 and contractile function following early reperfusion is partly caused by the repair of intracellular damage resulting from reduction of the mitochondrial membrane potential.

Key words : reperfusion injury, calcium overload, myocardial oxygen consumption, mitochondrial membrane potential

Introduction

In normal myocardium, there is a close relationship between contractile function and the oxidative metabolic rate^{1,2)}. Recently several reports have indicated that this relationship may be altered after post-ischemic reperfusion³⁻⁵⁾. The mechanisms responsible for the

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dissociation between myocardial oxygen consumption (MVO_2) and contractile function in the early period after reperfusion are poorly understood. Periods of prolonged myocardial ischemia result in irreversible tissue damage and abnormalities of metabolic and mechanical function^{5,6}. An important component of this injury may occur at the time of reperfusion and not during ischemia itself^{5,6}. It seems possible that this reperfusion injury is brought about by mitochondrial calcium loading during reperfusion. Mitochondrial calcium loading causes abnormalities of mitochondrial function and mitochondrial damage may lead to uncoupling of oxidative phosphorylation⁷. These process abnormalities include changes in transmembrane ion transport⁸ and futile metabolic cycling⁹. Elevation of the cytosolic calcium concentration activates calcium transport systems at the inner mitochondrial membrane and at the sarcoplasmic reticulum, which may contribute to enhanced energy expenditure after reperfusion⁸. Ruthenium red is a polysaccharide stain that inhibits mitochondrial calcium accumulation without any changes in the cytosolic calcium concentration. The present study was designed to investigate the mechanism of dissociation between MVO_2 and contractile function in relation to mitochondrial calcium accumulation by using ruthenium red perfusion after reperfusion in rat hearts.

Materials and Methods

Perfusion protocol

Male Wistar rats, weighing 250 to 400 g, were anesthetized by intraperitoneal injection of pentobarbital. The hearts were excised and perfused in the Langendorff mode at an aortic pressure of 100 cmH₂O with Krebs-Henseleit cycle which was equilibrated with a mixture of O₂ (95%) and CO₂ (5 %) at a temperature of 37°C and a pH of 7.4. A compliant latex balloon was inserted through the left atrium into the left ventricle to monitor myocardial function. The volume in the balloon was adjusted to 50 μl . Hearts were paced at 250 beats $\cdot$ min⁻¹. There were three phases of perfusion in this experiment. The first phase consisted of a 45 minutes control period allowing for experimental setup and acquisition of baseline measurements. The second phase consisted of 60 minutes of global myocardial ischemia, during which time all perfusate flow to the heart was stopped. The third phase of this study consisted of 60 minutes of reperfusion during which sequential collections of pulmonary artery effluent were done to determine myocardial coronary flow. The hearts were weighed at the end of the experiment. MVO_2 was calculated by multiplying the coronary arteriovenous difference in the oxygen content by the myocardial blood flow. Ruthenium red was added to the perfusate at a final concentration of 2.5 μM from the beginning of the first phase to the third phase.

Mitochondrial respiration

Mitochondria were prepared by a modification of the method of Sordahl and Stewart¹⁰. The protein content was determined by Lowry's method¹¹ using BSA as a standard. Mitochondrial respiratory activity was determined with an oxygen electrode. The reaction medium contained 0.3 M mannitol, 5 mM MgCl₂, 30 mM KCl, and 10 mM KH₂PO₄ (pH 7.2) at 30°C, and succinate was used as a substrate.

Measurement of mitochondrial membrane potential

Mitochondrial membrane potential was measured according to Ronald's method, which

was quoted by Emaus¹²⁾, by flow-cytometry using rhodamine 123. For flow-cytometric studies, mitochondria were suspended in a medium containing 0.3 M mannitol, 10 mM KCl, 5 mM MgCl₂ and 10 mM KH₂PO₄ at pH 7.2, as well as 1 mg · ml⁻¹ BSA where indicated. Mitochondria (125 µg protein · ml⁻¹) were analyzed for their natural fluorescence and for their fluorescence after staining with aqueous rhodamine 123 (Sigma). Rhodamine 123 fluorescence was measured using an excitation wavelength of 488 nm and 400 mW, while the final dye concentration was 5 nM. All measurements were made after a 30-minute incubation period in the presence of the dye and 10000 mitochondria particles were assessed in each analysis. The potential was shown as fluorescence on a logarithmic scale. The mitochondrial membrane potential was expressed as the ratio of the fluorescence value of perfused heart mitochondria divided by the control value.

Statistical analysis

Results are expressed as the mean ± S.D. Statistical significance was evaluated by Student's t-test for paired or unpaired data and P values (two-tailed) were calculated. P < 0.05 was considered to be significant.

Results

Hemodynamics

Fig. 1A and 1B show myocardial oxygen consumption (MVO₂) and left ventricular systolic pressure (LVSP) in the control and the ruthenium red group. Values in the control group and ruthenium red group before ischemia were: MVO₂ 1151 ± 522, 842 ± 366 mmHg · ml min⁻¹ g⁻¹; LVSP 13.5 ± 3.5 versus 26.5 ± 6.9 mmHg; P = 0.0048; P = 0.25. In the control group at 10 minutes after beginning reperfusion, MVO₂ increased to 149% of the preischemic value (1151 ± 522 versus 1715 ± 325 mmHg · ml min⁻¹ g⁻¹; P = 0.048), although contractile function continued to decrease to 54% (LVSP 13.5 ± 3.5 versus 7.3 ± 4.2 mmHg; P = 0.007), indicating dissociation between MVO₂ and contractility. In contrast, in the ruthenium red group, there was no increase in MVO₂ as the contractile function decreased to 56% (LVSP 26.5 ± 6.8 versus 16.2 ± 11.3 mmHg; P = 0.016) of the preischemia value.

Fig. 2 shows changes in myocardial contractility after ischemia and reperfusion. These changes were similar in both groups with contractility quickly dropping to zero when the heart became ischemic, and then returning to the baseline after reperfusion.

In our experimental model, the contraction was isovolumic and the beating rate was fixed by electrical pacing, thus the ratio of MVO₂ divided by left ventricular systolic pressure (LVSP) is assumed to indicate the efficiency of the mechanical utilization of respiratory energy. Fig. 3 shows the changes in MVO₂/LVSP as an index of contractile efficiency during ischemia-reperfusion. In the control group, MVO₂/LVSP increased significantly to 203% of the preischemic level (from 141 ± 17 to 286 ± 78 ml min⁻¹ g⁻¹; P = 0.035) at 10 minutes after reperfusion, indicating a decrease in contractile efficiency, and after 30 minutes of reperfusion returned to the baseline. In the ruthenium red group, MVO₂/LVSP decreased to 69% (28.5 ± 8.9 to 19.7 ± 17.8 min⁻¹ g⁻¹; P = 0.035) 10 minutes after reperfusion and gradually increased during reperfusion.

Coronary flow during the preischemic and reperfusion periods in the control and the ruthenium red treated group is shown in Fig. 4. In the control group, coronary flow was transiently increased after 10 minutes of reperfusion (3.4 ± 1.2 to 4.2 ± 1.3 ml min⁻¹ g⁻¹;

(A) Myocardial oxygen consumption (MVO_2) and left ventricular systolic pressure (LVSP) versus time.

(B) MVO_2 and LVSP in ruthenium red treated group versus time.

* $P<0.05$ versus preischemia ** $P<0.01$ versus preischemia

Fig. 1.

$P=0.006$), indicating reactive hyperemia and returned to the preischemic baseline level. In the ruthenium red group, coronary flow significantly decreased after the beginning of reperfusion, indicating no reactive hyperemia (2.3 ± 1.1 to 1.3 ± 1.1 $\text{ml min}^{-1} \text{g}^{-1}$; $P=0.024$).

mmHg/sec

Fig. 2. Myocardial contractility

Contractility was determined by the peak positive time derivative of developed pressure (dp/dt) versus time.

Fig. 3. Contractile efficiency

Myocardial oxygen consumption (MVO₂)/Left ventricular systolic pressure (LVSP) versus time. *P<0.05

Oxygen consumption rate in mitochondria

The mitochondrial oxygen consumption rate in state 3 is shown in Fig. 5. The values of the oxygen consumption rate in state 3 were 319 ± 62 and 356 ± 84 $\mu\text{atoms O mg protein}^{-1}$

Fig. 4. Coronary flow rate ($\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) versus time.
*P<0.05

$\text{m} \mu \text{ Atoms O/mg protein/min}$

Fig. 5. Oxygen consumption rate in state 3 mitochondria.

min^{-1} ($P=0.520$), in the control group and the ruthenium red group, respectively. After 30 minutes of reperfusion following 60 minutes of ischemia, the mitochondrial respiration rate was decreased to 50% (319 ± 62 to $161 \pm 52 \text{ m} \mu \text{ atoms O mg protein}^{-1} \text{ min}^{-1}$; $P=0.0042$)

Fig. 6. Mitochondrial membrane potential (MMP) versus time. MMP was expressed as the ratio of the fluorescence value of perfused heart mitochondria divided by the control value on a logarithmic scale. *P<0.05

and 68% (356 ± 84 to 153 ± 12 m μ atoms O mg protein⁻¹ min⁻¹; P=0.002) in state 3, in the control and the ruthenium red group, respectively. No significant difference was observed between the two groups.

Mitochondrial membrane potential

The mitochondrial membrane potential during the preischemic period and after reperfusion in both the control and the ruthenium red group is shown in Fig. 6. In the control group, the mitochondrial membrane potential was reduced to 89% of the control value (1.074 ± 0.070 to 0.952 ± 0.038 ; P=0.039) at 15 minutes of reperfusion and returned to the preischemic baseline level. In contrast, in the ruthenium red group, no reduction of the mitochondrial membrane potential was observed after reperfusion (0.945 ± 0.109 to 0.958 ± 0.066 ; P=0.877).

Discussion

Under physiological conditions, myocardial oxygen demand and supply are well balanced. However many investigators have demonstrated the dissociation between MVO₂ and contractile function in post-ischemic reversibly injured tissue such as stunned myocardium¹⁻⁴⁾. In isolated re-perfused heart, relatively high myocardial oxygen consumption is associated with depression in regional contractility in irreversibly injured myocardium after prolonged ischemia. Benzi and Lerch¹³⁾ showed dissociation between MVO₂ and contractility after 60 minutes of ischemia in re-perfused rat hearts. Our study also demonstrated increased MVO₂ while contractile function was decreased in the early phase of reperfusion after 60 minutes of ischemia. This dissociation means that postischemic myocardial oxygen utilization may be increased above physiological levels, indicating a decrease in contractile efficiency. Possible explanations for this phenomenon include uncoupling of oxidative phosphorylation,

resulting in inefficient mitochondrial ATP production, or an increase in the cellular energy requirement for processes other than contraction.

In a previous study the mitochondrial respiratory function was unchanged in stunned myocardium¹⁴⁾, thus a relatively high MVO₂ in the post-ischemic tissue has not been considered in relation to mitochondrial respiration uncoupling. In contrast, our results demonstrated a significant decrease in the mitochondrial respiration rate in the reperfused heart. Although in reperfused heart treated with ruthenium red, the mitochondrial respiration rate decreased while the MVO₂ did not increase, we can postulate that the dissociation of MVO₂ and contractile function in ischemic injured tissue is not secondary to mitochondrial uncoupling. An increase in non-contractile energy requirements seems to be more likely, perhaps related to an increase in ion pumping (i.e. extrusion of calcium from mitochondria in calcium overloaded myocytes) or an increase in the activity of calcium ATPase to maintain normal sequestration of calcium by the sarcoplasmic reticulum¹⁵⁾.

It is widely accepted that calcium contributes to cell injury caused by ischemia and reperfusion. Calcium influx results in the reduction of mitochondrial membrane potential, severely compromising mitochondrial function in the myocardium and triggers apoptotic events leading to cell demise¹⁶⁾. From a mitochondrial point of view, the first response is to maintain the mitochondrial membrane potential by using ATP¹⁷⁾. Murata et al¹⁸⁾ demonstrated that attenuation of mitochondrial calcium overload, as a consequence of partial mitochondrial membrane depolarization by mitochondrial ATP-sensitive potassium channels, underlies cardioprotection. Carafoli¹⁹⁾ has estimated that an increase of cytosolic calcium concentration from 1 to 10 μ M is associated with a more than 20-fold increase of the total mitochondrial calcium uptake per unit of time, whereas the corresponding value for the sarcoplasmic reticulum is less than doubled.

Ruthenium red is a hexavalent polysaccharide stain, which inhibits mitochondrial calcium transport without elevating of intracellular calcium⁵⁾, although the mechanism is unclear²⁰⁾. There was no dissociation between myocardial oxygen consumption and contractile function and no decrease in the mitochondrial membrane potential during early reperfusion in the group with ruthenium red. This suggests the dissociation of MVO₂ and contractility, at least partially, is caused by the accumulation of calcium in the mitochondria. Calcium loading into mitochondria may result in waste respiratory energy by forcing the mitochondrial membrane potential to release calcium to the matrix^{18, 21)}.

In conclusion, in this study the postischemic heart showed dissociation between an increase in MVO₂ and a decrease in contractile function, and a decrease in the mitochondrial membrane potential in early reperfusion. Ruthenium red prevented the dissociation and the decrease in the mitochondrial membrane potential in the early stage after reperfusion. The dissociation is due, at least partially, to the accumulation of calcium in the mitochondria. The increase in MVO₂ is caused by increased ion pumping resulting in the extrusion of calcium from mitochondria in the postischemic myocardium.

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