Left ventricular dysfunction and disturbed O2-utilization in stunned myocardium: influence of ischemic preconditioning

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Abstract

Objective: Myocardial dysfunction during postischemic reperfusion is frequently reported only in terms of left ventricular (LV) systolic properties. We additionally assessed diastolic properties, the cardiovascular tone and in particular, the relation between ventricular function and myocardial oxygen consumption. Moreover, these measures are investigated after cardioprotection via ischemic preconditioning (IP). However, this phenomenon is not fully understood, and therefore cardioprotective methods like ischemic preconditioning might provide only insufficient protection. Methods: In a total of 17 isolated rabbit hearts, perfused with an erythrocyte suspension (Hct 30%), we investigated the effect of 20 min low-flow ischemia also on diastolic properties, coronary resistance and cardiac energetics (n = 9). During control and 30 min after the onset of reperfusion, LV systolic function was assessed in terms of aortic flow, dP/dt max and the end-systolic pressure-volume relation (ESPVR). Early relaxation was evaluated via dP/dt min and diastolic properties were assessed via the end-diastolic pressure-volume relation (EDPVR). In addition, coronary resistance (Rcor) and the pressure-volume area (PVA) were calculated. Total oxygen consumption (MVO2) was calculated as well as the contractile efficiency (E = inverse slope of the MVO2-PVA relation). In a second series (n = 8) the effect of ischemic preconditioning (3 min no-flow and 8 min reperfusion before the 20 min low-flow ischemia) was tested. Results: In the first series, systolic function was impaired during reperfusion: aortic flow to 32% of control, dP/dt max to 74% and the slope of ESPVR to 73%. Early relaxation in terms of dP/dt min decreased to 76%. The slope of the EDPVR was steeper in stunned myocardium with an increase of the ventricular stiffness (m increased from 3.2 to 4.1) and with an upward shift of the EDPVR (c from 0.6 to 2.4 mmHg). Coronary resistance was increased (from 0.9 to 1.4 mmHg/ml per min) and PVA was significantly decreased to 68%, whereas MVO2 was not, indicating also a decrease in contractile efficiency E from 28 to 14%. In the second series, recovery of systolic function was significantly improved by IP compared with the first series (aortic flow 56% of preischemic control, dP/dt max to 91% and ESPVR to 78%). LV stiffness m was also slightly increased from 3.1 to 3.9 and again, c was elevated, indicating no beneficial effect for diastolic properties including dP/dt min (77%). But IP improved Rcor significantly (from 0.9 to only 1.0 mmHg/ml per min) and efficiency E to 21% (from 27% during control). Conclusion: Brief episodes of ischemia not only induce systolic but also diastolic and vascular stunning at almost maintained MVO2. The decreased contractile efficiency clearly indicates an impaired O2-utilization of the contractile apparatus. Ischemic preconditioning did not improve diastolic function during reperfusion, but it provided protection with respect to vascular stunning and myocardial energetics.

Keywords: Ischemic preconditioning; Myocardial stunning; Ventricular function; Myocardial energetics

1. Introduction

Myocardial stunning exists as a clinical syndrome and describes a phenomenon of reversible cardiac contractile dysfunction in patients after a transient episode of ischemia and reestablished coronary blood flow [1]. However, myocardial dysfunction is often only described in terms of systolic properties. To yield further information about the nature of myocardial stunning and the possibilities to limit the consequences of stunning, we investigated in isolated, blood-perfused rabbit hearts the phenomenon of stunning on systolic and diastolic properties, as well as on the vascular- and myocardial energetics.

It is widely accepted that the hearts can be protected against the detrimental effects of a prolonged coronary occlusion...
sion by prior exposure to one or more brief coronary occlusion/reperfusion cycles [2,3]. This endogenous mechanism of ischemic preconditioning is manifested for reduction of infarct size and a reduction of arrhythmias, but the role of attenuation reversible postischemic dysfunction, however, remains unclear, because some studies demonstrate no protective effect on postischemic ventricular function [4,5]. Since this endogenous adaptation to ischemia gets more attention in the clinical setting [6,7], the influence of ischemic preconditioning on myocardial stunning was tested on load-sensitive and load-insensitive parameters of ventricular function, vascular tone and myocardial energetics.

2. Material and methods

2.1. Experimental preparation

The experiments were performed on a total of 17 male New Zealand White rabbits with an average age of 6 months and an average weight of 2200 ± 200 g; the rabbits were handled according to the animal welfare regulations of the German federal authorities. The rabbits were anesthetized with intravenous sodium pentobarbital (30 mg/kg). Mechanical ventilation was achieved after tracheotomy. After sternotomy, the hearts were rapidly excised and immediately connected to a modified Langendorff apparatus and perfused with a modified crystalloid Krebs–Henseleit solution containing (in mM): NaCl 119, NaHCO$_3$ 25, KCl 4.7, CaCl$_2$ 1.8, MgCl$_2$ 1.2, EDTA 0.5 and glucose 11. The buffer was equilibrated with 95% O$_2$, 5% CO$_2$ at 37°C, giving a pH of 7.4. Albumin (4 g/100 ml) and bovine erythrocytes were added to obtain a hemoglobin-concentration of 10 g/100 ml. Ca$^{2+}$-concentration was held constant at 2.5 mM.

A buffer-filled latex balloon was inserted into the left ventricular cavity via the left atrium. The balloon was connected to a ‘systemic’ circuit that contained two artificial valves, and via an ultrasonic flow probe (T 206, Transonic Systems) aortic flow was assessed. Aortic pressure (= afterload) was measured with a pressure transducer (P 23 II, Statham), the circuit permitted changes in afterload in preload without alteration of coronary perfusion pressure. A 3F microtip manometer (SPR-249, Millar), inserted into the balloon, measured left ventricular pressure. For measurement of left ventricular dimensions, sonomicrometry was employed (system 6, Triton) using two ultrasonic crystals, glued to either side of the balloon. Different balloon sizes were used depending on the heart size.

Coronary venous flow was drained via the right ventricle and the pulmonary artery to measure total coronary flow via an ultrasonic flow probe. The difference in arterio-venous oxygen content was continuously measured using absorption spectrophotometry (AVOX systems).

2.2. Experimental protocols

Control conditions, at a perfusion pressure of 80 mmHg, were recorded after stabilization of left ventricular function. The hearts were randomly assigned to one of the two series. After instrumentation and 15 min of stabilization, the hearts of the first series (n = 9) underwent 20 min of normothermic or hyperthermic (n = 8) were subjected to 20 min of low-flow ischemia followed by 30 min of reperfusion. In group 2, the hearts were preconditioned before low-flow ischemia.

2.3. Data acquisition

The following variables were continuously registered on a forced ink chart recorder (type 481, Brush): aortic flow, coronary flow, left ventricular pressure, LV inner diameter, wall thickness, the difference in arteriovenous oxygen content. Heart rate and LV dP/dt were derived from the pressure signal. At steady state conditions after five to seven preload alterations, the variables were simultaneously stored digitally for later analysis at a sampling rate of 300 Hz.

2.4. Calculations and statistical analysis

Hemodynamic data were computer-assisted analyzed with six to eight consecutive beats being averaged. The end-systolic pressure-volume relationship, the pressure-volume area (PVA, Fig. 2), the ventricular stiffness (monoexponential fitting the end-diastolic pressure-volume relation) were calculated with the help of a custom-made computer program (EASYDAT) and, if appropriate, using equations suggested by Mirsky [8]. The end-diastolic pressure-volume relationship is approximated by the equation

$$LVP_{ed} = c \cdot \exp(m \cdot LVV_{ed})$$

where $LVP_{ed}$ is LV diastolic pressure, $LVV_{ed}$ is intraventricular end-diastolic volume, $c$ equals the $LVP_{ed}$-axis intercept and $m$ equals LV stiffness. The LV end-diastolic pressure-volume relationship is not strictly exponential, particularly if examined over a wide volume-range. However, over the range of volumes and pressures used in this study, this relation may be used to estimate $m$. The slope of the ESPVR ($E_{max}$) was calculated as suggested by Suga and coworkers.
Coronary flow was normalized to 100 g wet weight. Myocardial oxygen consumption was calculated according to the Fick principle from normalized coronary flow and the difference in arterio-venous oxygen content.

The contractile efficiency was determined as the inverse slope of the MVO$_2$-pressure-volume area relationship, and the MVO$_2$ for the unloaded contraction was assessed as the intercept of the MVO$_2$-pressure-volume area relationship with the MVO$_2$ axis (Fig. 2).

Data are expressed as means ± SD. Statistical analysis was performed with a statistical software package (Systat). ANOVA for repeated measurements was used to test differences in hemodynamic variables within any given group. If significant overall effects were encountered, further analysis was performed via the Bonferroni correction. Differences were considered to be significant with $P < 0.05$.

3. Results

There were no statistically significant differences in baseline left-ventricular performance concerning heart rate, mean aortic pressure, left ventricular end-diastolic (LVP$_{ed}$) and peak pressure (LVP$_{max}$), left ventricular dP/dt$_{max}$ and dP/dt$_{min}$, aortic and coronary flow between the two groups.

Systolic function parameter as LVP$_{max}$, dP/dt$_{max}$, aortic flow and the slope of the ESPVR were significantly decreased during reperfusion after 20 min low-flow ischemia (see Fig. 3). After ischemic preconditioning (IP, group 2) systolic function was less decreased compared with the untreated hearts. LVP$_{max}$ was decreased by 44% in the untreated hearts (group 1) and by 36% in the IP-group, dP/dt$_{max}$ by 27 and 9%, aortic flow by 68 and 44%, the slope of ESPVR by 27 and 21%, respectively. Heart rate was comparable for both groups and was nearly unchanged after ischemia.

Diastolic function in terms of dP/dt$_{min}$, an index for early relaxation, showed for both groups a comparable decline (in group 1, 24% and group 2, 23%; Fig. 4). The end-diastolic pressure-volume relations (EDPVR, Fig. 5) showed an upward shift after ischemia for both groups. The upward shift, calculated by the index c, was significant (see Table 1), but did not show any differences between the two groups. The slope m of the monoexponential EDPVR was steeper in both groups (Table 1), with no differences between the two groups.

Coronary blood flow was decreased to 84% (group 1) and 92% (group 2), these decreases being statistically not significant. Coronary resistance, in turn, was increased at constantly held coronary arterial pressure (Fig. 4). This increase reached statistical significance for group 1, but not for group 2.

Both the slope of the EDPVR and the coronary resistance were increased suggesting a cause-effect relation via the gardenhose effect. Since the perfusion pressure was held constant and since blood was used as perfusate, we did not further investigate this aspect, in particular as it was shown that the gardenhose effect does not play a significant role in the almost intact heart [10].

Total myocardial oxygen consumption (MVO$_2$) was also reduced during reperfusion to 73% of control levels for group 1 and to 82% for group 2 (Fig. 4). The pressure-volume area (PVA = measure of total mechanical energy) was reduced during reperfusion to 58 vs. 70%. The contractile efficiency (= inverse slope of the MVO$_2$-PVA relation) showed for the control group a decrease to 50% (group 1) and to 78% (group 2) during reperfusion compared with control.

4. Discussion

Myocardial ischemia and reperfusion injury is a complex process, and it is not totally clear which components of myocardial function are mostly affected and might need particular protection. In 17 isolated, blood-perfused rabbit
Fig. 3. Effects of 20 min low-flow ischemia on LV peak pressure ($LVP_{max}$; A), $dP/dt_{max}$ (B), aortic flow (AoF; C) and the end systolic pressure volume relation (ESPVR; D) during control (Ctrl) and reperfusion (Rep) after 20 min low-flow ischemia. (□ for untreated hearts and □ for ischemic preconditioned (IP) hearts). The bars represent mean values ± SD, $P < 0.05$ *Rep vs. Ctrl; **IP-Rep vs. IP-Ctrl.

Fig. 4. Effects of 20 min low-flow ischemia on $dP/dt_{min}$ (A), coronary resistance ($R_{cor}$; B), total myocardial oxygen consumption (MVO$_2$; C) and contractile efficiency (E; D) during control (Ctrl) and reperfusion (Rep) after 20 min low-flow ischemia. (□ for untreated hearts and □ for ischemic preconditioned (IP) hearts). The bars represent mean values ± SD, $P < 0.05$ *Rep vs. Ctrl.
hearts systolic and diastolic properties, coronary resistance, myocardial oxygen consumption, and its relation to systolic function were investigated for normal and reperfused myocardium. Furthermore the effects of ischemic preconditioning on these properties were investigated.

As expected, the results of this study showed a significant decline of ventricular function during reperfusion after myocardial ischemia. However, postischemic functional recovery of LV performance in terms of systolic variables and cardiac contractility assessed by dP/dtmax and the load-insensitive end-systolic pressure volume relationship (E\(_{\text{max}}\)) were better preserved with ischemic preconditioning. Diastolic properties however, were not markedly influenced by ischemic preconditioning.

In addition, myocardial oxygen consumption was overproportional high compared with the decreased mechanical function, indicating a decreased myocardial efficiency, which could be positively influenced by ischemic preconditioning.

It is concluded that the most important mechanisms for myocardial stunning, as identified so far, are a disruption of the calcium homeostasis, as reflected by a transient calcium overload, a decrease of SR function, and a damage to the contractile proteins via calcium activated proteases. This injury to the myofilaments most likely leads to a decreased calcium-sensitivity and a posts ischemic decreased contractile function [12,13], and as well, in a decreased mechanical efficiency.

4.1. Cardioprotective effects of ischemic preconditioning

It is widely accepted that hypothermia and/or cardioplegia protect the myocardium against ischemia/reperfusion injury by reducing ischemic stress during cardiac surgery. Despite the well-established protective effect of hypothermia and cardioplegia [11], intermittent ischemia with reperfusion is continued to be used with surprisingly good results [14]. In the past two decades it has been shown that the heart can be provoked to increase resistance to ischemia by one or more brief episodes of ischemia and reperfusion. Numerous experimental and clinical studies [2–7] have demonstrated that ischemic preconditioning reduces myocardial infarct size and ventricular arrhythmias. The phenomenon of preconditioning is reported for several species, including both isolated [15] and in situ [16] rabbit hearts. Since the original descriptions, ischemic preconditioning has also been shown to reduce myocardial dysfunction during reperfusion in several models [17]. But the effects on the degree on protection of myocardial function are conflicting, and the energetical aspects are less clear.

Our results demonstrate that ischemic preconditioning preserves posts ischemic systolic properties. These results

Table 1

<table>
<thead>
<tr>
<th></th>
<th>c</th>
<th>m</th>
<th>CBF (ml/min per 100 g)</th>
<th>R(_{\text{cor}}) (mmHg/ml per min)</th>
<th>PVA (mmHg/ml)</th>
<th>MVO(_2) (ml/beat per 100 g)</th>
<th>E%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>0.6 ± 1.0</td>
<td>3.1 ± 1.4</td>
<td>82 ± 13</td>
<td>0.9 ± 0.4</td>
<td>520 ± 120</td>
<td>0.068 ± 0.009</td>
<td>28 ± 16</td>
</tr>
<tr>
<td>Rep</td>
<td>2.4 ± 3.2*</td>
<td>4.1 ± 1.6</td>
<td>62 ± 11*</td>
<td>1.4 ± 0.7*</td>
<td>380 ± 110*</td>
<td>0.051 ± 0.014</td>
<td>14 ± 7*</td>
</tr>
<tr>
<td>Ctrl-IP</td>
<td>0.6 ± 0.9</td>
<td>3.2 ± 1.6</td>
<td>83 ± 12</td>
<td>0.9 ± 0.5</td>
<td>550 ± 160</td>
<td>0.065 ± 0.012</td>
<td>27 ± 17</td>
</tr>
<tr>
<td>Rep-IP</td>
<td>2.2 ± 1.8*</td>
<td>3.9 ± 1.9</td>
<td>79 ± 12</td>
<td>1.0 ± 0.4</td>
<td>430 ± 130</td>
<td>0.053 ± 0.016</td>
<td>21 ± 10*</td>
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</tbody>
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* Data are shown during control (Ctrl) and at 30 min reperfusion after 20 min low-flow ischemia, for untreated (n = 9) and for preconditioned (IP, n = 8) hearts. c equals LVPed-axis intercept and m equals LV stiffness, coronary blood flow (CBF), coronary resistance (R\(_{\text{cor}}\)), the pressure-volume area (PVA), total myocardial oxygen consumption (MVO\(_2\)) and contractile efficiency (E). Mean values ± SD, P < 0.05 *Rep vs. Ctrl; **IP-Rep vs. IP-Ctrl.
are in contrast to those of Ovize and coworkers [4], and Johania and coworkers [18], their data are based on regional ischemia in dogs and swine, and therefore might not be comparable to ours, because of the interference between the ischemic and the non-ischemic myocardium in their studies. The ischemic preconditioning protocol of this study was not identical to all cited studies. We used one ischemia reperfusion cycle to precondition the hearts. The group of Hearse et al. [11] showed that increasing the number of preconditioning cycles to two or three cycles failed to enhance the protective effect of ischemic preconditioning, these findings were supported by several other studies. According to these data we assume that fundamental pathophysiological differences between the models may be the underlying reason, like hemodynamic factors, preservation of electrical activity, and the amount of collateral flow, while in the isolated heart subjected to global ischemia, the entire muscle is rendered ischemic, the heart is essentially quiescent, and is not exposed to hemodynamic factors.

Interestingly, the diastolic function was not improved by ischemic preconditioning in this study. This discrepancy between systolic and diastolic recovery has not been reported in other studies, that describe an improvement for both, systolic and diastolic function [19]. In contrast to other studies, we used the end-diastolic pressure-volume relation (= ventricular stiffness), for describing diastolic properties. Ventricular stiffness is related to the amount and distribution of the cytoskeleton within the cells and the residual interactions between the thin and thick filaments modulated by the troponin molecules. The postischemic increased stiffness could very well be related to ATP-deficiency and myofilament damage, as described by Marban and coworkers [20]. Despite immediate recovery of systolic function, ventricular stiffness remained increased and could not be prevented by ischemic preconditioning.

The decrease in inotropic state is associated with a comparable decrease in relaxation properties in the stunned myocardium. Compared with the improved recovery of systolic variables by ischemic preconditioning, the impaired early relaxation, documented by dP/dtmin, could be also influenced by the increased stiffness, thus slowing ventricular relaxation.

In this study coronary flow was significantly decreased during reperfusion. The reduced flow in stunned myocardium, in particular in the subendocardium, could either reflect injury of the coronary vessels, i.e. the capillaries, or the reduced energy demand of the myocardium. But calculation of coronary resistance showed a significant increase during reperfusion in the untreated hearts, and only a slight increase in the preconditioned hearts. The postischemic increase of coronary resistance is described as microvascular stunning [21] and is explained by morphological alterations and interstitial edema that develops during reperfusion. Therefore, ischemic preconditioning exerts also a protective effect on the coronary vasculature.

In addition, our results show a beneficial effect of ischemic preconditioning on myocardial efficiency, i.e. the ratio of ventricular function to myocardial energy consumption. The pressure-volume area (PVA, Fig. 1A) as a measure of total mechanical work has been proven to be a useful tool for investigation cardiac dynamics [9]. The relation between PVA and MVO2 has shown to be linear, and this framework allows calculation of myocardial efficiency. It has previously been shown that contractile efficiency in the stunned myocardium is clearly decreased [11,12].

So far, no other studies have investigated the effect of ischemic preconditioning on myocardial energetics. Summarizing a number of studies investigating protein kinases [23,24], ischemic preconditioning exerts a beneficial effect by activating selected PKCs and other still unknown proteins that induce protection resulting in an improved myocardial integrity. The improved myocardial energetics after ischemic preconditioning might be explained by the smaller damage of the myofilaments and the better preserved conversion of energy to mechanical activity compared to the untreated hearts.

For clinical application of ischemic preconditioning, it is desirable to maximize the extent of protection. Increasing the number of cycles of transient ischemia and reperfusion to induce ischemic preconditioning may achieve this goal [22]. However, in a separate series of this study, we have seen that every cycle of global ischemia compromised ventricular function, so that during the 5 min reperfusion after ischemia, ventricular function recovered to only 90–95% of the preceding control. Applying two or more cycles of ischemia and reperfusion, already impaired ventricular function and, much worse, the following hypoperfusion period would result in a more severe myocardial dysfunction. Therefore, we decided to apply only one preconditioning cycle.

4.2. Summary

Brief episodes of ischemia not only induce systolic but also diastolic and vascular stunning at almost maintained MVO2. The decreased mechanical efficiency clearly indicates an impaired O2-utilization of the contractile apparatus. Ischemic preconditioning did not improve diastolic function during reperfusion, but it improved systolic function and protected from vascular stunning and, in addition, improved myocardial energetics.

References