Less afterload sensitivity in short-term hibernating than in acutely ischemic and stunned myocardium

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Schulz, Rainer, Jochen Rose, Heiner Post, Andreas Skyschally, and Gerd Heusch. Less afterload sensitivity in short-term hibernating than in acutely ischemic and stunned myocardium. Am J Physiol Heart Circ Physiol 279: H1106–H1110, 2000.—Short-term hibernating myocardium is characterized by reduced contractile function during persistent moderate ischemia, the recovery of metabolic parameters, and the absence of necrosis. To study the afterload dependence of regional wall excursion in short-term hibernating myocardium, in 11 enflurane-anesthetized swine the left anterior descending coronary artery was cannulated and hypoperfused for 90 min to reduce anterior systolic wall thickening (WT, sonomicrometry) by 60%. Under control conditions, at 5 and 90 min ischemia the descending thoracic aorta was acutely constricted to increase left ventricular (LV) pressure by 30 mmHg. Under control conditions, increased LV pressure resulted in decreased WT [i.e., a negative slope of the relationship between WT and LV end-systolic pressure: \(-11.2 \pm 4.2\) (SD) \(\mu m/mmHg\)]. This slope was further significantly decreased at 5 min ischemia \((-26.5 \pm 8.8 \mu m/mmHg\)) but returned toward control values in short-term hibernating myocardium at 90 min ischemia \((-17.2 \pm 6.6 \mu m/mmHg\)). At 30 min reperfusion, the slope was once more significantly decreased \((-27.8 \pm 8.1 \mu m/mmHg\)). In conclusion, WT in short-term hibernating myocardium is less afterload dependent than in acutely ischemic and reperfused myocardium.

Acutely ischemic myocardium has increased afterload sensitivity, i.e., in anesthetized dogs, the inflation of an aortic balloon during a single cardiac cycle (20), aortic constriction (5, 10, 21), or infusion of phenylephrine (15) resulted in a larger decrease in mean circumferential fiber shortening (20), systolic wall thickening (5), systolic segment shortening (10, 15), or left ventricular (LV) pressure-segment length loop area (21) than in nonischemic control myocardium. In reperfused myocardium of anesthetized pigs, the reduction in systolic segment shortening and LV pressure-segment length loop area in response to increased afterload was also more pronounced than under control conditions (4), and stunned myocardium is "hypersensitive" for loading conditions in general (2).

The effects of increased afterload on regional myocardial function in short-term hibernating myocardium have not been studied so far. Short-term hibernating myocardium is characterized by reduced contractile function during persistent moderate ischemia, the recovery of metabolic parameters despite ongoing ischemia, and the absence of necrosis (for review see Ref. 7). In the present study, the effect of an acute aortic constriction on acutely ischemic, short-term hibernating, and reperfused myocardium was compared.

MATERIALS AND METHODS

The experimental protocols employed in this study were approved by the bioethical committee of the district of Düsseldorf, and they adhere to the guiding principles of the American Physiological Society.

The experimental model has been described previously (8); in brief, in 11 enflurane-anesthetized Göttinger miniswine, a micromanometer was placed in the left ventricle. The descending thoracic aorta was dissected, and a plastic tube was wrapped around it for later aortic constriction. Heart rate was controlled by left atrial pacing. Ultrasonic dimension gauges were implanted in the LV myocardium to measure the thickness of the anterior and posterior (control) walls. The proximal left anterior descending coronary artery (LAD) was cannulated and perfused from an extracorporeal circuit. Radiolabeled microspheres were injected to assess regional myocardial ischemia; hibernating myocardium; ventricular function.

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myocardial blood flow, and flow is reported for the myocardium with the dimension gauges. The large epicardial vein parallel to the LAD was cannulated for the withdrawal of blood samples to determine myocardial lactate consumption. Transmural myocardial biopsies were taken from the LAD perfusion bed for analysis of myocardial creatine phosphate content.

**Regional Myocardial Function**

End diastole was defined as the point when the first derivative of LV pressure (LV dP/dt) started its rapid upstroke after crossing the zero line. Global LV end systole was defined as the time point of peak negative LV dP/dt, and regional end systole was defined as the point of maximal wall thickness within 20 ms before peak negative LV dP/dt (22). End-diastolic wall thickness was normalized to an end-diastolic wall thickness of 10 mm under control conditions to account for interindividual differences (16). Systolic wall thickening was calculated as end-systolic wall thickness minus end-diastolic wall thickness.

The slope of the relationship between systolic wall thickening and LV end-systolic pressure during aortic constriction was calculated using the linear equation

$$WT = m \text{LVESP} + b$$

where WT is systolic wall thickening, LVESP is left ventricular end-systolic pressure, $m$ is slope of the regression line, and $b$ is y-axis intercept.

**Protocol**

Following control measurements including a gradual aortic constriction over 6–10 cardiac cycles to increase LVESP by 30 mmHg, blood flow to the LAD was reduced to a level sufficient to reduce the anterior systolic wall thickening by ~60%. This level of flow reduction has been previously demonstrated to allow the development of short-term hibernating myocardium. Measurements were performed at 5 and 90 min ischemia. Thereafter, the myocardium was reperfused, and final measurements were obtained at 30 min reperfusion. After 2 h of reperfusion, absence of necrosis was verified by triphenyl tetrazolium chloride staining.

**Statistics**

Single cardiac cycles during aortic constriction were analyzed separately, and data of the first and the last cardiac cycle during aortic constriction are presented. Steady-state systemic hemodynamics and regional myocardial dimension data as well as regional myocardial blood flow and metabolism were compared using a one-way analysis of variance for repeated measures. The first and the last cardiac cycle of the aortic constrictions during control conditions, at 5 and 90 min ischemia and at 30 min reperfusion, were compared by a two-way analysis of variance. When significant differences were detected, individual mean values were compared using post hoc tests. Linear regression analyses were calculated between anterior and posterior systolic wall thickening and LV end-systolic pressure. The slopes (4) and intercepts of the calculated regression lines were compared using a two-way analysis of variance for repeated measures. All data are reported as mean values ± SD, and a $P$ value less than 0.05 was accepted as significant.

**RESULTS**

In none of the animals was myocardial necrosis detected after 90 min of ischemia and 120 min of reperfusion.

**Steady-State Data**

In two animals, the data from the ultrasonic crystals in the posterior myocardium were excluded from further analysis due to their position in the border zone of the ischemic area.

### Table 1. Steady-state systemic hemodynamics, regional myocardial dimensions, blood flow, and metabolism during control, at 5 and 90 min of moderate ischemia, and at 30 min of reperfusion

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Control</th>
<th>Ischemia, 5 min</th>
<th>Ischemia, 90 min</th>
<th>Reperfusion, 30 min</th>
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<tr>
<td><strong>Systemic Hemodynamics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HR</td>
<td>11</td>
<td>98 ± 10</td>
<td>98 ± 11</td>
<td>98 ± 10</td>
<td>99 ± 12</td>
</tr>
<tr>
<td>LVEDP</td>
<td>11</td>
<td>6.6 ± 2.8</td>
<td>11.0 ± 4.2</td>
<td>10.2 ± 4.8</td>
<td>9.7 ± 3.6</td>
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<tr>
<td>LVPP</td>
<td>11</td>
<td>99 ± 14</td>
<td>94 ± 13</td>
<td>89 ± 12</td>
<td>87 ± 10</td>
</tr>
<tr>
<td>LV dP/dt$_{max}$</td>
<td>11</td>
<td>1,400 ± 300</td>
<td>1,200 ± 240</td>
<td>1,110 ± 200*</td>
<td>1,060 ± 200*</td>
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<tr>
<td>CAP</td>
<td>11</td>
<td>117 ± 12</td>
<td>46 ± 8*</td>
<td>45 ± 8*</td>
<td>106 ± 7†‡</td>
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<td><strong>Regional Myocardial Dimensions</strong></td>
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<tr>
<td>AW$_{ed}$</td>
<td>11</td>
<td>10</td>
<td>8.92 ± 0.33*</td>
<td>9.15 ± 0.68</td>
<td>10.71 ± 1.87†‡</td>
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<tr>
<td>AWT</td>
<td>11</td>
<td>4.55 ± 1.23</td>
<td>1.87 ± 0.61*</td>
<td>1.63 ± 0.59*</td>
<td>2.02 ± 0.71*</td>
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<td>PW$_{ed}$</td>
<td>9</td>
<td>10</td>
<td>9.60 ± 0.26</td>
<td>9.52 ± 0.55</td>
<td>9.75 ± 0.53</td>
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<td>PWT</td>
<td>9</td>
<td>2.84 ± 0.72</td>
<td>2.82 ± 0.75</td>
<td>2.65 ± 0.91</td>
<td>2.59 ± 0.74</td>
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<td><strong>Regional Myocardial Blood Flow and Metabolism</strong></td>
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<tr>
<td>TMF</td>
<td>11</td>
<td>0.70 ± 0.20</td>
<td>0.33 ± 0.11*</td>
<td>0.32 ± 0.08*</td>
<td>0.82 ± 0.30†‡</td>
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<td>ENDO</td>
<td>11</td>
<td>0.77 ± 0.23</td>
<td>0.19 ± 0.06*</td>
<td>0.20 ± 0.07*</td>
<td>0.89 ± 0.48‡</td>
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<tr>
<td>CP</td>
<td>11</td>
<td>9.5 ± 1.4</td>
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<td>6.9 ± 2.6</td>
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<td>MV$_{LAC}$</td>
<td>11</td>
<td>1.8 ± 1.2</td>
<td>-2.2 ± 1.3*</td>
<td>-0.9 ± 1.0*</td>
<td>-0.2 ± 0.9*‡</td>
</tr>
</tbody>
</table>

Values are means ± SD; $n$, number of animals; HR (beats/min), heart rate; LVEDP (mmHg), left ventricular end-diastolic pressure; LVPP (mmHg), mean coronary arterial pressure; AW$_{ed}$ (mm), anterior end-diastolic wall thickness; AWT (mm), anterior systolic wall thickening; PW$_{ed}$ (mm), posterior end-diastolic wall thickness; PWT (mm), posterior systolic wall thickening; TMF (ml/min·1·g$^{-1}$), transmural myocardial blood flow; ENDO (ml/min·1·g$^{-1}$), subendocardial blood flow; CP (µmol/g wet wt), myocardial creatine phosphate concentration; and MV$_{LAC}$ (µmol/min·1·g$^{-1}$), myocardial lactate consumption. *$P < 0.05$ vs. control. †$P < 0.05$ vs. preceding value. ‡$P < 0.05$ vs. 5 min ischemia.
The data demonstrate the successful development of short-term hibernating myocardium followed by myocardial stunning after reperfusion (Table 1). In brief, reducing coronary inflow decreased anterior transmural and subendocardial blood flows as well as anterior systolic wall thickening. At 5 min ischemia, creatine phosphate concentration was decreased, and the myocardium produced lactate. Prolonging ischemia to 90 min did not change transmural and subendocardial blood flows or anterior systolic wall thickening while metabolic parameters recovered toward control values. At 30 min reperfusion, anterior transmural and subendocardial blood flows had recovered, whereas anterior systolic wall thickening was still depressed.

**Aortic Constriction Data**

**Hemodynamics.** With aortic constriction at constant heart rate, LV peak pressure increased by ∼30 mmHg during control conditions, at 5 and 90 min ischemia, as well as at 30 min reperfusion (Table 2). Maximum of LV dP/dt max did not change, whereas LV end-diastolic pressure increased during aortic constrictor.

**Regional myocardial dimensions.** Anterior and posterior end-diastolic wall thickness tended to be decreased in the last cardiac cycle of the aortic constriction [not significant (NS)]. The relationships between anterior systolic wall thickening and LV end-systolic pressure had r values of 0.94 ± 0.04 under control conditions and 0.96 ± 0.03, 0.97 ± 0.06, and 0.96 ± 0.05 at 5 min and 90 min ischemia and 30 min reperfusion, respectively. The intercept decreased from 5.09 ± 1.33 mm under control conditions to 3.29 ± 1.04, 2.46 ± 0.98, and 3.22 ± 1.00 mm at 5 min and 90 min ischemia and 30 min reperfusion, respectively (all three P < 0.05 vs. control, NS vs. each other). Under control conditions, aortic constriction resulted in a slightly decreased anterior systolic wall thickening.
Acutely ischemic myocardium had greater afterload sensitivity of regional wall excursion than control myocardium. After successful adaptation to ischemia, i.e., after short-term myocardial hibernation had developed, the afterload sensitivity was normalized again, indicating better inotropic competence of short-term hibernating than acutely ischemic myocardium. Such inotropic competence deteriorated again after reperfusion, i.e., in stunned myocardium.

**Critique of Methods**

The present model of regional short-term myocardial hibernation with its strengths and limitations has been discussed in detail before (8). In the present study, the descending thoracic aorta was only briefly constricted to avoid baroreflex changes in autonomic nerve activity (1). Indeed, the lack of an altered autonomic nerve activity is reflected by the unchanged LV dP/dt max (Table 2). Absolute rather than percent systolic wall thickening was used to measure regional wall excursion (4), since changes in percent systolic wall thickening may overestimate afterload sensitivity due to the tendency for decreased end-diastolic wall thickness associated with aortic constriction and myocardial ischemia (Table 2). Use of percent systolic wall thickening is further complicated when comparing anterior and posterior walls, since the decrease in end-diastolic wall thickness is more pronounced in the anterior wall; this effect is partially related to the reduced amount of blood in the ischemic wall (14). LV pressure-wall thickness or LV pressure-segment length loop area (4) were not used, since, in this case, LV pressure would be used as both dependent and independent variable in the relation of wall function vs. LV end-systolic pressure.

**Afterload Sensitivity in Ischemic and Reperfused Myocardium**

The present study confirms previous findings in anesthetized dogs and swine demonstrating a substantial reduction in regional wall excursion during an acute increase in aortic pressure in acutely ischemic and stunned myocardium (4, 5, 10, 15, 20, 21). However, the present study for the first time demonstrates an attenuation of such decrease in systolic wall thickening during acute aortic constriction in short-term hibernating myocardium. This attenuation becomes particularly obvious when the slope of the relationship between systolic wall thickening and LV end-systolic pressure of the anterior wall is normalized to that of the posterior wall. The ratio of 0.94 under control conditions is increased to 1.61 at 5 min ischemia and returns to 0.95 at 90 min ischemia to increase again to 1.46 at 30 min reperfusion. In some cases, such as in Fig. 1, the relationship between systolic WT and LVesP at 85 min ischemia was rotated counterclockwise vs. that at 5 min ischemia, such that at lower end-systolic pressures, systolic wall thickening was actually greater in acutely ischemic than in short-term hibernating myocardium. However, this was never the case for short-term hibernating vs. stunned myocardium, i.e., our main point of comparison.

Preload recruitment was not different in acutely ischemic, short-term hibernating, and stunned myocardium, as reflected by equivalent decreases in anterior end-diastolic wall thickness (Table 2), suggesting, in the concept of preload reserve and afterload mismatch (17, 19), that short-term hibernating myocardium had greater afterload sensitivity of regional wall excursion than control myocardium. After successful adaptation to ischemia, i.e., after short-term myocardial hibernation had developed, the afterload sensitivity was normalized again, indicating better inotropic competence of short-term hibernating than acutely ischemic myocardium. Such inotropic competence deteriorated again after reperfusion, i.e., in stunned myocardium.
dium is characterized by a better inotropic competence than acutely ischemic and stunned myocardium. Such better inotropic competence of short-term hibernating myocardium is primarily a descriptive finding, and we can only speculate about the underlying mechanism(s).

Such better inotropic competence of short-term hibernating myocardium is not related to an altered calcium responsiveness, as calcium responsiveness is comparable in acutely ischemic, short-term hibernating and stunned myocardium in this in situ heart model (8). Maximal calcium-activated work is decreased in ischemic and reperfused myocardium compared with control conditions; the reduction in maximal calcium-activated work, however, is not different among short-term hibernating myocardium, acutely ischemic and stunned myocardium (8).

The energetic situation of acutely ischemic, short-term hibernating and stunned myocardium differs significantly. Although the free energy change of ATP hydrolysis is reduced early during ischemia, it recovers to almost normal values in short-term hibernating myocardium (13). Therefore, part of the observed load dependence of acutely ischemic myocardium could have been related to an altered energetic situation. During reperfusion, however, there is no difference in the creatine phosphate content and ATP content compared with 85 min ischemia (11), and the free energy change of ATP hydrolysis is probably normalized at this time as well (6, 9), although the myocardium once again becomes load dependent to the same extent as seen during early ischemia. Therefore, it appears unlikely that alterations in the energy metabolism are the main determinant of the load dependence of acutely ischemic and reperfused myocardium.

Clearly, the functional characteristics of acutely ischemic, hibernating, and stunned myocardium differ, even at equivalent baseline dysfunction.

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REFERENCES


