Progressive loss of perfusion-contraction matching during sustained moderate ischemia in pigs

RAINER SCHULZ,1 HEINER POST,1 TILL NEUMANN,1 PETRA GRES,1 HARTMUT LÜSS,2 AND GERD HEUSCH1
1Abteilung für Pathophysiologie, Zentrum für Innere Medizin des Universitätsklinikums Essen, 45122 Essen; and 2Institut für Pharmakologie und Toxikologie, Universität Münster, 48149 Münster, Germany

Received 25 October 2000; accepted in final form 15 December 2000

Schulz, Rainer, Heiner Post, Till Neumann, Petra Gres, Hartmut Lüss, and Gerd Heusch. Progressive loss of perfusion-contraction matching (PCM) during sustained moderate ischemia in pigs. Am J Physiol Heart Circ Physiol 280: H1945–H1953, 2001.—It is unclear whether perfusion-contraction matching (PCM) is maintained during prolonged myocardial ischemia. In 27 anesthetized pigs, left anterior descending coronary arterial inflow was reduced to decrease an anterior work index (WI) at 5 min of hypoperfusion by 40% and then maintained at this level for 12 or 24 h. With 12 h of hypoperfusion, the myocardium remained viable in 6 of 7 pigs (with triphenyltetrazolium chloride; TTC) and with 24 h of hypoperfusion in 5 of 11 pigs (TTC, histology). The reduction in WI to 62 ± 4 and 62 ± 3% of baseline in the two groups was matched to the reduction of transmural blood flow (TBF; microspheres) at 5 min of hypoperfusion, averaging 59 ± 2 and 60 ± 2% of baseline. With prolonged hypoperfusion, WI decreased to 30 ± 5% at 12 h and 18 ± 3% at 24 h; TBF remained unchanged (53 ± 4 and 54 ± 4%). The added calcium concentration required for the half-maximal increase in WI increased from 121 ± 25 μg/ml blood at baseline to 192 ± 26 μg/ml blood at 12 h of hypoperfusion. Thus, with hypoperfusion for 24 h, PCM is progressively lost, and calcium responsiveness is reduced.

The underlying mechanism of perfusion-contraction matching is unclear (13). Decreased perfusion pressure for 30 min reduced the calcium transient in isolated ferret hearts (16), and reduced calcium responsiveness was demonstrated at 90 min of hypoperfusion in pig hearts in vivo (15). One potential explanation for the loss of perfusion-contraction matching during prolonged ischemia, therefore, relates to further progressive alterations in excitation-contraction coupling.

The present study is the first to maintain truly constant coronary hypoperfusion from an extracorporeal circuit for 12 and 24 h and then study the issues of perfusion-contraction matching and the maintenance of viability. Calcium responsiveness was assessed at baseline and after 12 h of hypoperfusion.

METHODOLOGY

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.

Experimental Model

Twenty-seven Göttinger miniswine (20–40 kg) of either sex were initially sedated using ketamine hydrochloride (1 g im) and then anesthetized with thiopental (Trapanal, 500 mg iv). Through a midline cervical incision, the trachea was incised.
intubated for connection to a respirator (Dräger, Lübeck, Germany). Anesthesia was then maintained using enflurane (1–1.5%) with an oxygen-nitrous oxide mixture (40:60%). Arterial blood gases were monitored frequently in the initial stages of the preparation until stable and then periodically throughout the study (Radiometer, Copenhagen, Denmark). Rectal temperature was monitored, and body temperature was kept above 37°C by the use of a heated surgical table and drapes.

Through the cervical incision, both common carotid arteries and internal jugular veins were isolated. The arteries were cannulated with polyethylene catheters, one for the measurement of arterial pressure and the other to supply blood to the extracorporeal circuit. The jugular veins were cannulated for volume replacement using warmed 0.9% NaCl and for the return of blood to the animal from the coronary venous line.

A left lateral thoracotomy was performed in the fourth intercostal space and the pericardium opened. A micromanometer (P7; Konigsberg, Pasadena, CA) was placed in the left ventricle through the apex together with a saline-filled polyethylene catheter (used to calibrate the micromanometer in situ). Ultrasonic dimension gauges were implanted in the left ventricular myocardium to measure the thickness of the anterior and posterior (control) walls (System 6; Triton Technologies, San Diego, CA).

The proximal left anterior descending coronary artery (LAD) was dissected over a distance of 1.5 cm, ligated, and cannulated, and the distal LAD was perfused from an extracorporeal circuit. Before coronary cannulation, the pigs were anticoagulated with 20,000 IU heparin sodium; additional doses of 10,000 IU were given at hourly intervals. The system included a roller pump, windkessel, and two side ports, one for calcium infusion (15). Coronary arterial pressure was measured from the side arm of a polyethylene "T"-connector (Cole-Parmer, Chicago, IL) used as catheter tip with an external transducer (Bell and Howell type 4-327I, Pasadena, CA) used as catheter tip with an external transducer (Bell and Howell type 4-327I, Pasadena, CA) used as catheter tip with an external transducer (Bell and Howell type 4-327I, Pasadena, CA). The large epicardial vein parallel to the LAD was dissected and cannulated. Coronary venous blood was drained to an unpressurized reservoir and then returned to a jugular vein by use of a second roller pump. Heart rate was controlled throughout the study by left atrial pacing (Hugo Sachs Elektronik type 215/T, Hugstetten, Germany). The completed preparation was allowed to stabilize for at least 30 min before control measurements were made. The flow-constant perfusion pump was adjusted so that the minimum coronary arterial pressure was not less than 70 mmHg under control conditions to avoid initial hypoperfusion. Therefore, mean coronary arterial pressure exceeded peak left ventricular pressure.

**Regional Myocardial Function**

End diastole was defined as the point when left ventricular dP/dt (first derivative of pressure development over time) started its rapid upstroke after crossing the zero-line. Regional end systole was defined as the point of maximal wall thickness within 20 ms before peak negative left ventricular dP/dt (37). Systolic wall thickening was calculated as end-systolic wall thickness minus end-diastolic wall thickness divided by the end-diastolic wall thickness.

Because the regional calcium infusion changed the contraction pattern of the stimulated area, such that an augmented early systolic thickening was followed by late systolic thinning, the regional myocardial work performed by the LAD-perfused myocardium was estimated in addition to systolic wall thickening. A regional myocardial work index was calculated as the sum of the instantaneous left ventricular pressure-wall thickness product over the time of the cardiac cycle, using the equation

\[ WI_n = \sum_{ed}^{m} (LVP_{n,m} - LVP_{n,\text{min}})(WTh_{n,m} - WTh_{n,m-1}) \]

where ed is end diastole, n is actual cardiac cycle, m is sampling point within cardiac cycle n at a sampling frequency of 5 ms, LVP_{n,m} is instantaneous left ventricular pressure within cardiac cycle n and at sampling point m, LVP_{\text{min}} is minimum left ventricular pressure, and WTh is wall thickness. The maximal work index value during systole is reported as WI (15).

Ischemia decreased the baseline value of WI. Therefore, to analyze responses of WI to intracoronary calcium infusion (see below) during control conditions and during hypoperfusion, increases in WI were expressed as a fraction of the maximal increase during the respective intervention, as reported before (15).

**Regional Myocardial Blood Flow**

Radiolabeled microspheres (15-µm diameter, 141Ce, 114In, 51Cr, 113Sn, 103Ru, 95Nb or 46Sc; NEN, DuPont, Boston, MA) were injected into the coronary perfusion circuit (1–2 × 10^5 suspended in 1 ml saline) to determine the regional myocardial blood flow and its distribution throughout the LAD perfusion bed. This procedure for the determination of blood flow has been validated extensively (29). Blood flow to the tissue at the site of the ultrasonic crystals is reported, and this piece of tissue was divided into transmural one-thirds of approximately equal thickness. The average tissue sample size was 1.02 ± 0.02 g.

**Regional Myocardial Metabolism**

Oxygen content was measured using anaerobically sampled blood drawn simultaneously from the cannulated coronary vein and an artery (Cavitron/LexO2-Con-k, Waltham, MA). Oxygen consumption of the anterior myocardial wall (MV_{O2}) was calculated by multiplying the arterial-coronary venous oxygen difference by the transmural blood flow at the crystal site.

Transmural myocardial biopsies (~10 mg each) were taken with a modified dental drill from the LAD perfusion bed for analysis of creatine phosphate (CP) content. Care was taken to ensure that the biopsies originated from within the LAD perfusion territory (using epicardial arteries as landmarks) but distal to the site of the ultrasonic dimension gauges and blood flow measurements. Samples requiring >1–2 s for acquisition were not used for this analysis. Holes in the myocardium were closed using a shallow purse-string suture. CP content was measured using HPLC on an anion-exchanger column (Protein Pak DEAE 5 PW; Millipore-Waters, Eschborn, Germany), as described previously (22). The retention time for CP was 8 min.

**Morphology**

At the end of each study, the heart was removed and sectioned from base to apex into five transverse slices in a plane parallel to the atrioventricular groove. The tissue slices were immersed in a 0.09 M sodium phosphate buffer (pH 7.4) containing 1.0% triphenyltetrazolium chloride (TTC; Sigma, Deisenhofen, Germany) and 8% dextran (mol wt 77,800) for 20 min at 37°C to detect infarcted tissue. In pigs undergoing
Table 1. Systemic hemodynamics, regional myocardial function, flow, and metabolism at baseline and during 12-h hypoperfusion

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>5 min</th>
<th>90 min</th>
<th>6 h</th>
<th>12 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>94 ± 2</td>
<td>95 ± 2</td>
<td>94 ± 2</td>
<td>96 ± 2</td>
<td>97 ± 2</td>
</tr>
<tr>
<td>LVEDP</td>
<td>3 ± 2</td>
<td>5 ± 1</td>
<td>3 ± 1</td>
<td>7 ± 6</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>LVFP</td>
<td>95 ± 2</td>
<td>92 ± 3</td>
<td>88 ± 3</td>
<td>79 ± 3*</td>
<td>79 ± 4*</td>
</tr>
<tr>
<td>dP/dt\text{max}</td>
<td>1.095 ± 39</td>
<td>1.053 ± 52</td>
<td>1.007 ± 77</td>
<td>805 ± 39*</td>
<td>803 ± 43*</td>
</tr>
<tr>
<td>AoP</td>
<td>78 ± 3</td>
<td>76 ± 4</td>
<td>71 ± 3</td>
<td>63 ± 3*</td>
<td>60 ± 3*</td>
</tr>
<tr>
<td>CAP</td>
<td>112 ± 3</td>
<td>42 ± 1*</td>
<td>41 ± 2*</td>
<td>14.1 ± 0.7*</td>
<td>14.1 ± 0.7*</td>
</tr>
<tr>
<td>CBF</td>
<td>24.8 ± 1.7</td>
<td>14.0 ± 0.8*</td>
<td>14.1 ± 0.7*</td>
<td>17.6 ± 1.1*</td>
<td>11.8 ± 2.1†</td>
</tr>
<tr>
<td>AWThd</td>
<td>10.89 ± 0.54</td>
<td>10.15 ± 0.39</td>
<td>9.91 ± 0.44</td>
<td>9.30 ± 0.20</td>
<td>9.92 ± 0.37</td>
</tr>
<tr>
<td>AWTh</td>
<td>32.4 ± 9.8</td>
<td>21.8 ± 2.0*</td>
<td>27.1 ± 2.5</td>
<td>17.6 ± 1.1*</td>
<td>11.8 ± 2.1†</td>
</tr>
<tr>
<td>Endo</td>
<td>0.94 ± 0.06</td>
<td>0.47 ± 0.04*</td>
<td>0.51 ± 0.05*</td>
<td>0.34 ± 0.06*</td>
<td>0.35 ± 0.05*</td>
</tr>
<tr>
<td>O₂\text{ven}</td>
<td>5.4 ± 0.7</td>
<td>2.7 ± 0.4*</td>
<td>3.9 ± 0.7*</td>
<td>3.4 ± 0.6*</td>
<td>5.9 ± 0.8†</td>
</tr>
<tr>
<td>MVO₂</td>
<td>66.6 ± 3.8</td>
<td>48.7 ± 6*</td>
<td>47.5 ± 4.3*</td>
<td>36.6 ± 2.9*</td>
<td>32.3 ± 2.6†</td>
</tr>
<tr>
<td>CP</td>
<td>8.66 ± 1.48</td>
<td>3.86 ± 0.56*</td>
<td></td>
<td></td>
<td>7.02 ± 1.54</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. HR, heart rate (in beats/min); LVEDP, left ventricular end-diastolic pressure (in mmHg); LVFP, left ventricular peak pressure (in mmHg); dP/dt\text{max}, maximum of the first derivative of left ventricular pressure (in mmHg/s); AoP, mean aortic pressure (in mmHg); CAP, coronary arterial pressure (in mmHg); CBF, coronary blood flow (in ml/min); AWTh, anterior systolic wall thickening (%); Endo, subendocardial blood flow (in ml/min); MVO₂, myocardial oxygen consumption (in ml/100 g/min); CP, creatine phosphate content (in μmol/g wet wt). *P < 0.05 vs. baseline. †P < 0.05 vs. 5- and 90-min hypoperfusion.

24 h of hypoperfusion, transmural tissue specimens from the ischemic and the control area were taken and analyzed by histology (25). DNA strand breaks were detected in situ by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) (19).

Calcium Regulatory Proteins

In pigs undergoing 24 h of hypoperfusion, calcium regulatory proteins were quantified in transmural tissue specimens from the hypoperfused and control area (21).

Experimental Protocols

Group 1. The protocol of group 1 (n = 10) is as follows. After measurements of systemic hemodynamics, regional myocardial blood flow, function, and metabolism at baseline, LAD inflow was decreased to achieve a 40% reduction in WI at 5 min of hypoperfusion and then maintained at this level. Measurements were repeated at 10 and 85 min and 6 and 12 h of hypoperfusion. The myocardium was reperfused for 2 h.

To determine myocardial calcium responsiveness, calcium chloride was infused into the perfusion system at increasing doses at baseline and after 12 h of hypoperfusion (14, 15). At baseline, the calcium-containing solution was infused into the perfusion system with a syringe pump, starting at an infusion rate of 10 ml/h and with stepwise increases up to 240 ml/h. These calcium infusion rates were normalized for the actual LAD inflow and finally expressed as added calcium (in μg/ml blood). The recruitment of maximal calcium-activated work was verified by the lack of further increases of WI despite further increased calcium infusion rates. Because intracoronary calcium infusion increased contractile function, it was also expected to deteriorate the metabolic status of the myocardium during ischemia, as previously shown for dobutamine (29). Therefore, after 12 h of hypoperfusion, only the three dosages of intracoronary calcium that resulted in an ~10% increase, half-maximal increase, and maximal increase in WI at baseline were repeated. The infusion rates were corrected for the reduced coronary inflow during hypoperfusion; these dosages are referred to as Ca1, Ca2, and Ca3.

Group 2. The protocol of group 2 (n = 17) was identical to that of group 1 except that hypoperfusion was maintained for 24 h. Retrospectively, group 2 was further subdivided into group 2A, including pigs surviving 24 h of hypoperfusion without the development of any infarction, and group 2B, including pigs surviving 24 h of hypoperfusion with the development of patchy infarction.

Data Analysis and Statistics

Data are reported as mean values ± SE. Statistical analysis was composed of one-way ANOVA for repeated measures and Fisher’s least-significance difference tests when significant overall effects were detected. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. Overall effects were detected. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests. The percentage of tissue samples in blood flow intervals of 0.15 ml/min was compared by paired -tests.
Table 2. Systemic hemodynamics, regional myocardial function, flow, and metabolism at baseline and during 24-h hypoperfusion in pigs with viable myocardium

<table>
<thead>
<tr>
<th>Baseline</th>
<th>5 min</th>
<th>90 min</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>108 ± 1</td>
<td>108 ± 1</td>
<td>111 ± 2</td>
<td>109 ± 3</td>
</tr>
<tr>
<td>LVEDP</td>
<td>4 ± 1</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>LVPP</td>
<td>109 ± 4</td>
<td>105 ± 3</td>
<td>98 ± 5</td>
<td>89 ± 2*</td>
</tr>
<tr>
<td>dP/dt_max</td>
<td>1,346 ± 51</td>
<td>1,211 ± 37</td>
<td>1,067 ± 77</td>
<td>1,042 ± 96*</td>
</tr>
<tr>
<td>AoP</td>
<td>93 ± 5</td>
<td>89 ± 3</td>
<td>80 ± 4</td>
<td>68 ± 1*</td>
</tr>
<tr>
<td>CAP</td>
<td>117 ± 3</td>
<td>48 ± 3*</td>
<td>43 ± 4*</td>
<td>46 ± 3*</td>
</tr>
<tr>
<td>CBF</td>
<td>37.4 ± 2.3</td>
<td>19.7 ± 1.1*</td>
<td>19.6 ± 1.2*</td>
<td>19.8 ± 1.2*</td>
</tr>
<tr>
<td>AWThrd</td>
<td>11.72 ± 1.49</td>
<td>10.88 ± 1.27</td>
<td>10.83 ± 1.46</td>
<td>10.61 ± 1.14</td>
</tr>
<tr>
<td>AWT</td>
<td>33.9 ± 1.5</td>
<td>23.2 ± 1.6*</td>
<td>27.3 ± 2.5</td>
<td>12.9 ± 1.1††</td>
</tr>
<tr>
<td>Endo</td>
<td>0.85 ± 0.10</td>
<td>0.41 ± 0.07*</td>
<td>0.42 ± 0.05*</td>
<td>0.35 ± 0.07*</td>
</tr>
<tr>
<td>O₂ven</td>
<td>4.5 ± 0.7</td>
<td>2.6 ± 1.2*</td>
<td>2.3 ± 0.4*</td>
<td>2.4 ± 0.3*</td>
</tr>
<tr>
<td>MVO₂</td>
<td>71.7 ± 7.9</td>
<td>53.4 ± 8.7</td>
<td>51.4 ± 3.7</td>
<td>39.1 ± 4.3††</td>
</tr>
<tr>
<td>CP</td>
<td>9.02 ± 0.48</td>
<td>3.71 ± 1.77*</td>
<td>8.03 ± 2.41</td>
<td>8.03 ± 2.41</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5. See legend to Table 1 for abbreviations. *P < 0.05 vs. baseline. †P < 0.05 vs. 5- and 90-min hypoperfusion.

Regional Myocardial Blood Flow Distribution

The distribution of regional myocardial blood flow was shifted leftward at 5 min of hypoperfusion in all groups (Fig. 1, A–C). Whereas the blood flow distribution did not further change significantly in groups 1 and 2A (Fig. 1, A and B), a significant further leftward redistribution of flow occurred in group 2B (Fig. 1C) when hypoperfusion was prolonged to 24 h; i.e., the percentage of myocardial samples with blood flow below 0.15 ml·min⁻¹·g⁻¹ increased. In group 2B, 84% of necrotic tissue samples, but only 14% of viable tissue samples, had blood flow below 0.15 ml·min⁻¹·g⁻¹. Blood flow in infarcted myocardium averaged 0.095 ± 0.011 ml·min⁻¹·g⁻¹ at 24 h of hypoperfusion and was thus significantly lower than that of viable myocardium (0.387 ± 0.012 ml·min⁻¹·g⁻¹).

Relation Between Flow and Function and Between Oxygen Consumption and Function

The reduction in WI to 62 ± 4, 62 ± 3, and 58 ± 3% of baseline was closely matched to the reduction of transmural myocardial blood flow at 5 min of hypoperfusion, which averaged 59 ± 4, 60 ± 2, and 54 ± 5% of baseline in groups 1, 2A, and 2B, respectively. However, when hypoperfusion was prolonged to 6 h or longer, WI progressively decreased in viable myocardium to 40 ± 7% at 6 h (group 1), 30 ± 5% at 12 h (group 1), and 18 ± 3% at 24 h (group 2A), although transmural myocardial blood flow did not change (53 ± 4, 53 ± 4, and 54 ± 4%; i.e., perfusion-contraction mismatch developed (Fig. 2). Similarly, WI decreased from 59 ± 4% at 5 min of hypoperfusion to 15 ± 6% at 24 h of hypoperfusion in hearts with patchy necrosis (group 2B), although transmural myocardial blood flow slightly increased (54 ± 5% at 5 min of hypoperfusion vs. 66 ± 7% at 24 h of hypoperfusion). The close relationship between regional MVO₂ and WI, however, was maintained (Fig. 3), because regional MVO₂ decreased along with WI. This decrease in regional MVO₂ was not caused by insufficient oxygen supply, because coronary venous oxygen content rather tended to increase during prolonged hypoperfusion (Tables 1–3).

Table 3. Systemic hemodynamics, regional myocardial function, flow, and metabolism at baseline and during 24-h hypoperfusion in pigs with patchy necrosis

<table>
<thead>
<tr>
<th>Baseline</th>
<th>5 min</th>
<th>90 min</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>101 ± 2</td>
<td>101 ± 2</td>
<td>102 ± 2</td>
<td>101 ± 1</td>
</tr>
<tr>
<td>LVEDP</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>LVPP</td>
<td>93 ± 1</td>
<td>88 ± 2</td>
<td>88 ± 2</td>
<td>80 ± 3*</td>
</tr>
<tr>
<td>dP/dt_max</td>
<td>1,222 ± 48</td>
<td>1,073 ± 53</td>
<td>1,048 ± 36</td>
<td>807 ± 51*</td>
</tr>
<tr>
<td>AoP</td>
<td>84 ± 1</td>
<td>78 ± 1</td>
<td>78 ± 2</td>
<td>65 ± 4*</td>
</tr>
<tr>
<td>CAP</td>
<td>123 ± 7</td>
<td>49 ± 2*</td>
<td>50 ± 4*</td>
<td>45 ± 7*</td>
</tr>
<tr>
<td>CBF</td>
<td>32.8 ± 2.2</td>
<td>17.3 ± 1.5*</td>
<td>17.4 ± 1.3*</td>
<td>16.8 ± 2.3*</td>
</tr>
<tr>
<td>AWThrd</td>
<td>10.96 ± 0.61</td>
<td>10.36 ± 0.67</td>
<td>9.94 ± 0.61</td>
<td>10.40 ± 0.60</td>
</tr>
<tr>
<td>AWT</td>
<td>37.6 ± 2.7</td>
<td>26.3 ± 2.5*</td>
<td>24.2 ± 2.0*</td>
<td>10.7 ± 3.5††</td>
</tr>
<tr>
<td>Endo</td>
<td>0.72 ± 0.06</td>
<td>0.32 ± 0.02*</td>
<td>0.36 ± 0.04*</td>
<td>0.39 ± 0.04*</td>
</tr>
<tr>
<td>O₂ven</td>
<td>5.7 ± 0.7</td>
<td>2.3 ± 0.4*</td>
<td>2.4 ± 0.4*</td>
<td>2.8 ± 0.5*</td>
</tr>
<tr>
<td>MVO₂</td>
<td>69.2 ± 8.2</td>
<td>52.5 ± 3.3*</td>
<td>47.6 ± 4.5*</td>
<td>44.2 ± 2.0*</td>
</tr>
<tr>
<td>CP</td>
<td>8.55 ± 0.80</td>
<td>3.06 ± 1.02*</td>
<td>3.06 ± 1.02*</td>
<td>3.06 ± 1.02*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. See legend to Table 1 for abbreviations. *P < 0.05 vs. baseline. †P < 0.05 vs. 5- and 90-min hypoperfusion.
Calcium Responsiveness

Plasma calcium concentration averaged 51.8 ± 1.2 μg/ml at baseline and remained unchanged when hypoperfusion was prolonged to 12 h (50.5 ± 1.2 μg/ml). Increasing concentrations of added calcium increased WI at baseline and after 12 h of hypoperfusion (Table 4 and Fig. 4). The added calcium concentration for the half-maximal increase in WI averaged 121 ± 25 μg/ml blood at baseline and increased to 192 ± 26 μg/ml blood after 12 h of hypoperfusion.

Calcium Regulatory Proteins

The protein expression of sarcoplasmic reticulum calcium ATPase, phospholamban, calsequestrin, tropomin inhibitor, and heat shock protein (HSP)-72 in the posterior (control) and the viable anterior wall was not significantly different after 24 h of hypoperfusion (group 2A, Table 5).

Survival and Morphology

Seven out of 10 and 11 out of 17 pigs survived the 12- and 24-h hypoperfusion period, respectively. After 12 h
of hypoperfusion and 2 h of reperfusion, the myocardium remained completely viable in six pigs (TTC); in the seventh pig, 4% of the myocardium at risk was necrotic (blood flow of 0.053 ± 0.011 ml·min⁻¹·g⁻¹). After 24 h of hypoperfusion and 2 h of reperfusion, only five pigs had completely viable myocardium by TTC; also, in these pigs, no microinfarction was detected in either the anterior or posterior wall using light microscopy (Fig. 5A). Only 0.02 ± 0.01% of myocytes stained TUNEL positive in the anterior wall, whereas 0.11 ± 0.06% of myocytes stained TUNEL positive in the posterior (control) wall. In the remaining six pigs surviving 24 h of hypoperfusion and 2 h of reperfusion, infarct size (TTC) averaged 14.3 ± 2.2%, and leukocyte infiltration was detected with the use of light microscopy (Fig. 5B). In the anterior wall, 1.32 ± 0.80% of myocytes stained TUNEL positive, whereas 0.01 ± 0.01% of myocytes stained TUNEL positive in the posterior (control) wall (P < 0.05).

**DISCUSSION**

The major findings of the present study are that 1) in some but not all pigs the myocardium remained completely viable after 24 h of controlled hypoperfusion, and 2) the close matching between perfusion and contraction was lost when hypoperfusion was prolonged to 6 h or longer.

The present model of moderate myocardial hypoperfusion with its strengths and limitations has been discussed in detail before (14, 15, 28). The degree of flow reduction, the decrease in regional MVO₂, and the contractile dysfunction measured early during hypoperfusion in the present study were comparable with those in former studies (14, 28).

**Perfusion-Contraction Matching**

In prior studies, 2–5 h of moderate coronary stenosis in chronically instrumented conscious dogs induced a proportionate reduction in coronary blood flow and contractile function, and this situation was associated with the lack of infarction in the previously dysfunctional myocardium (23, 34, 35). In anesthetized, closed-chest pigs subjected to 60% of baseline inflow by a hydraulic occluder for 24 h, the relation of reduced blood flow to reduced wall thickening appeared to be maintained; however, coronary inflow varied substantially during the protocol, some animals developed patchy necrosis, and regional myocardial blood flow data (microspheres) were not reported such that the existence of perfusion-contraction matching on a regional level was not verified (5, 6). In contrast, in sedated chronically instrumented pigs subjected to 60% of inflow by manipulation of a hydraulic occluder, loss of perfusion-contraction matching resulted and patchy necrosis developed, affecting 40% of the endocardial surface area (17). Again, in this preparation, fluctuations in coronary inflow and episodes of excitement with superimposed stress-induced ischemia cannot be excluded.

The present study is the first to avoid variations in coronary inflow and used controlled hypoperfusion for
12 or 24 h from an extracorporeal circuit. Between 90 min and 6 h of hypoperfusion, WI decreased at almost unchanged transmural myocardial blood flow, leading to loss of perfusion-contraction matching. The progressive loss of contractile function was, however, not related to the development of microinfarction (Fig. 5A), which occurred in some but not all pigs. Also, in contrast to prior studies (7, 18), apoptosis was absent in pigs with viable myocardium.

**Calcium Responsiveness**

Partial proteolysis of troponin I occurs during ischemia/reperfusion in rats (12) and pigs (24), and the incorporation of altered cardiac troponin I regulatory complex into rabbit skeletal muscle reduces its calcium sensitivity (24). However, in the present study, troponin I protein levels were not reduced, and the expression of the other calcium regulatory proteins was also unaltered, although an unaltered protein expression does not necessarily reflect unaltered protein function. Also, the reduced calcium responsiveness was probably not related to enflurane anesthesia, because in guinea pig papillary muscles, the intracellular calcium concentration-isometric tension curve was not affected by 2.2% enflurane, indicating unaltered myofibrillar calcium responsiveness (2).

In a previous study from our laboratory using the same model, calcium responsiveness was reduced after 90 min of hypoperfusion at unchanged calcium sensitivity (15), however, and the expression of calcium regulatory proteins was also unchanged (21). In the present study, as in the previous one, calcium responsiveness was reduced at 12 h of hypoperfusion, and the expression of calcium regulatory proteins again remained unchanged even when hypoperfusion was prolonged to 24 h. However, in contrast to the previous finding (15), calcium sensitivity was reduced at 12 h of hypoperfusion. This decrease in calcium sensitivity in the presence of an unaltered blood calcium concentration could indeed explain the observed progressive decrease in baseline regional myocardial function. Such decreased calcium sensitivity was recently demonstrated in anesthetized pigs during only 90 min of hypoperfusion when nitric oxide synthase was blocked (14). Thus loss of endogenous nitric oxide (due to loss of substrate and/or active protein, scavenging of nitric oxide by an increase in oxygen free radicals or free hemoglobin concentration secondary to some inevitable degree of hemolysis) during prolonged hypoperfusion could underlie the observed decrease in calcium sensitivity and, consequently, baseline contractile function. Unfortunately, regional myocardial nitric oxide production was not measured in the present study to substantiate this speculative explanation.

In chronically instrumented pigs, 3 mo of coronary stenosis induced a decrease in the mRNA and protein expression of calcium regulatory proteins in the dysfunctional myocardium (9). The decrease in the content of calcium regulatory proteins was inversely related to blood flow reserve, recruited by infusion of adenosine. The authors concluded that the decrease in the expression of calcium regulatory proteins most likely represented the consequence of repetitive stunning rather than that of prolonged myocardial ischemia (9).

Therefore, the progressive loss of regional myocardial function during the initial hours of hypoperfusion may indeed result from decreased calcium sensitivity, possibly as a consequence of lack of nitric oxide. With more prolonged periods of moderate ischemia or repetitive ischemia/reperfusion over weeks to months, however, additional decreases in the expression of calcium regulatory proteins may further deteriorate excitation-contraction coupling, thereby inducing more pronounced contractile dysfunction.
Sustained Ischemia and Hibernation

The present study clearly indicates also that during longer periods of hypoperfusion, the myocardium can remain viable, and energetic parameters, such as creatine phosphate content, can recover (13). However, the incidence of necrosis increases with prolongation of hypoperfusion (1 out of 7 pigs after 12 h and 6 out of 11 pigs after 24 h developed patchy necrosis). In sedated chronically instrumented pigs, reduction of myocardial blood flow to 60% of baseline for 24 h resulted in multifocal patchy necrosis in all animals, affecting 40% of the endocardial surface area (17). Whereas in that prior study in sedated pigs episodes of excitement with secondary alterations in coronary blood flow and superimposed stress-induced ischemia cannot be excluded such that the extent of necrosis is possibly overestimated, the lower inotropic state and the use of volatile anesthetics (8, 39) and heparin (1), both of which may be cardioprotective per se, may have delayed and reduced the development of infarction in our study.

The distribution of blood flow during hypoperfusion appears to determine the maintenance of viability. In hearts that remained completely viable during hypoperfusion, blood flow distribution did not change between 5 min and 12 or 24 h. However, in partially infarcted myocardium, the number of samples with blood flow below 0.15 ml·min⁻¹·g⁻¹, flow values that are associated with infarction already after 90 min in the same animal model (31), was increased (Fig. 1C). This finding confirms prior data in chronically instrumented pigs with normal blood flow in viable myocardium and severely reduced blood flow in necrotic areas after 24 h of coronary stenosis (17).

The increase in end-diastolic pressure over time was probably not responsible for the redistribution of blood flow in the present study, as the increase in end-diastolic pressure was similar in groups 2A and 2B. The mechanism(s) responsible for such blood flow redistribution is unclear at present.

Reduction of myocardial blood flow by >40–50% for 60 min increased HSP expression in anesthetized dogs (20). Similarly, repetitive episodes of ischemia/reperfusion increased HSP72 expression in chronically instrumented pigs (9). Overexpression of HSP72 protected isolated myocytes or mice hearts against ischemic injury, and the increased expression of HSP has been suggested to be involved in cardioprotection achieved by late ischemic preconditioning (36). In the present study, however, no increase in HSP72 protein expression was found during prolonged moderate ischemia with reduction of transmural myocardial blood flow by 40–45%, suggesting that the successful adaptation to ischemia did not depend on an upregulation of stress proteins. The present data therefore once more support the notion that ischemic preconditioning and short-term hibernation, although both are cardioprotective phenomena initiated during ischemia, are mechanistically different, as previously demonstrated for the involvement of adenosine (32, 33), opioids (27), and activation of ATP-dependent potassium channels (30, 33).

Our conclusions are as follows. Apparently, perfusion-contraction matching is progressively lost with increasing duration of ischemia, and the incidence of necrosis is progressively increased. The loss of perfusion-contraction matching is not related to the development of necrosis but reflects a progressive disturbance of excitation-contraction coupling. Maintained myocardial viability, in turn, does not require perfusion-contraction matching, because viability was maintained in most hearts after 12 h and in some hearts after 24 h of hypoperfusion when perfusion-contraction matching was always lost. Loss of myocardial viability is related to a progressive leftward shift of blood flow distribution during hypoperfusion. Therefore, truly chronic hibernation cannot result from sustained perfusion-contraction matching. However, perfusion-contraction matching in chronic hibernation may develop as a result of repetitive stunning (4).

We thank Dr. Claus Martin for the chemical analyses and Dr. Andreas Skyschally for technical support. This study was supported by the German Research Foundation (Schu 9/3-1).

REFERENCES


