Staged Reperfusion Attenuates Myocardial Stunning in Dogs
Role of Transient Acidosis During Early Reperfusion

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Background. Acidosis during early reperfusion is reported to be beneficial for myocardial stunning. We tested in 31 dogs the hypothesis that staged reperfusion is beneficial to myocardial stunning.

Methods and Results. Contractile dysfunction was observed 3 hours after the onset of reperfusion after 15 minutes of occlusion of the coronary artery. In the staged reperfusion, pH of the coronary venous blood was lower for 20 minutes and fractional shortening was significantly improved compared with the control reperfusion group. When we increased pH of the reperfused myocardium by an intracoronary infusion of sodium bicarbonate, beneficial effects of the staged reperfusion were abolished. Furthermore, an intracoronary infusion of hydrogen chloride, which mimicked the changes in pH in coronary venous blood of the staged reperfusion, attenuated myocardial stunning.

Conclusions. These results indicate that acidosis during staged reperfusion primarily attenuates myocardial stunning. This procedure is clinically applicable for attenuation of reperfusion injury. (Circulation 1991;84:2135–2145)

Even if ischemic heart muscle is reperfused before irreversible injury occurs, contractile function remains impaired for a long period, a phenomenon known as myocardial stunning.1,2 Several lines of evidence support the hypothesis that cellular Ca overload during ischemia and/or reperfusion constitutes a primary cause of myocardial stunning.3–12 Furthermore, activation of leukocytes is reported to cause myocardial stunning13,14 by free radical generation15,16 and microcirculatory disturbances.17 Released catecholamine during ischemia18 is thought to cause myocardial hypercontraction during early reperfusion. The enhanced myocardial contraction may increase myocardial oxygen consumption, which may prolong the anaerobic myocardial metabolism during reperfusion. If these deleterious factors are attenuated, myocardial stunning may be markedly attenuated.

Acidosis during early reperfusion is one promising intervention to attenuate these deleterious factors.19–24 H+ inhibits Na-Ca exchange,25,26 slow inward Ca channels,27–29 Ca2+ release from the sarcoplasmic reticulum,30,31 and binding of Ca2+ to troponin C.32,33 The inhibition of an increase in cytosolic Ca2+ caused by acidosis may attenuate Ca overload during reperfusion and consequently myocardial stunning. Furthermore, acidosis is reported to attenuate the neutrophil activation and generation of free radicals,34 and acidosis attenuates myocardial contractility,35 both of which may also contribute to the attenuation of myocardial stunning. If transient acidosis is produced during reperfusion, contractile dysfunction is predominantly improved without any aftereffect of acidosis. Acidosis also augments the effects of adenosine,36 which may attenuate the severity of myocardial stunning.37,38 Indeed, acidosis during early reperfusion is reported to attenuate myocardial stunning in the ferret Langendorff preparations.34 In the in situ heart, one strategy to produce transient acidosis during reperfusion is staged reperfusion.39–42

We examined staged reperfusion in dogs to test the beneficial effects on myocardial stunning. Because a
long period of residual critical stenosis of the coronary artery after reperfusion is reported to be deleterious,43-45 we reperfused the heart with a limited low coronary perfusion pressure for 10 minutes after an occlusion of the left anterior descending (LAD) coronary artery.46

Methods

Instrumentation

Thirty-one mongrel dogs weighing 14–22 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.). The trachea was intubated and the animal was ventilated with room air mixed with oxygen (100% O\textsubscript{2}, 3–5 l/min). The chest was opened through the left fifth intercostal space, and the heart was suspended in a pericardial cradle. We cannulated and perfused the LAD coronary artery with blood via the left carotid artery through an extracorporeal bypass tube. Coronary blood flow (CBF) of the perfused area was measured with an electromagnetic flow probe attached at the bypass tube, and coronary perfusion pressure (CPP) was monitored at the tip of the coronary arterial cannula. A small, short collecting tube (1 mm in diameter and 7 cm in length) was cannulated into a small coronary vein near the center of the perfused area to sample coronary venous blood. The drained venous blood was collected in the reservoir placed at the level of the left atrium and was returned to the jugular vein. High fidelity left ventricular (LV) pressure was measured by a micromanometer (Konigsberg P-7, Pasadena, Calif.) placed in the LV cavity through the apex. A pair of ultrasonic crystals were placed at the anterior one third of the myocardium about 1 cm apart to measure myocardial segment length with an ultrasonic dimension gauge (Schuessler, 5 MHz, Cardiff by the Sea, Calif.). Ultrasonic crystals were inserted until endocardial resistance was felt. After the study, we confirmed by postmortem verification that the dimension gauges were placed at the inner one third of the myocardium.

Heart rate averaged 134±3 beats per minute at control conditions and did not change significantly during each study.

Experimental Protocols

Protocol 1: Effects of staged reperfusion on myocardial stunning. Twenty-five dogs were used in this protocol. After hemodynamic stabilization, coronary arterial and venous blood were sampled for blood gas analysis and determination of lactate concentrations. Hemodynamic parameters (i.e., LV pressure [LVP], dP/dt, and segment length of the perfused area) were measured. End-diastolic length (EDL) was determined at the R wave of the electrocardiogram, and end-systolic length (ESL) was determined at the minimal dP/dt.37 Fractional shortening (FS) was calculated by (EDL–ESL)/EDL as an index of myocardial contractility of the perfused area. In 11 dogs (the control reperfusion group), the bypass tube to the LAD coronary artery was occluded for 15 minutes; four dogs from this group were excluded from the data analysis because ventricular fibrillation occurred during reperfusion. Hemodynamic parameters were observed 1, 3, 5, 7, 10, and 15 minutes during myocardial ischemia, and the clamped bypass tube was abruptly released. After the onset of reperfusion, hemodynamic parameters were measured at 15, 30, 45, and 60 seconds and 3, 5, 7, 10, 30, 60, 90, 120, 150, and 180 minutes. Coronary arterial and venous blood were sampled 1, 3, 5, 7, 10, 30, 60, 120, and 180 minutes after ischemia. Three hours after reperfusion, the myocardium perfused by LAD and the left circumflex (LCx) coronary arteries was quickly sampled in liquid nitrogen to measure myocardial tissue ATP content as described below.

In five dogs (the staged reperfusion group), CPP was kept at 40% of control CPP for 0–3 minutes after the onset of reperfusion, 60% of control CPP for 4–6 minutes, and 80% of control CPP for 7–10 minutes. Ten minutes after the onset of reperfusion, the occluded bypass was completely released. No ventricular fibrillation occurred in these five dogs. In another nine dogs, to attenuate transient acidosis during staged reperfusion, intracoronary infusion of NaHCO\textsubscript{3} was performed for 10 minutes after the onset of reperfusion: 120 μmol/kg/min of NaHCO\textsubscript{3} for the first 6 minutes, and 60 μmol/kg/min of NaHCO\textsubscript{3} for the second 4 minutes. In four of nine dogs, ventricular fibrillation occurred during 10 minutes of reperfusion, and these dogs were not used for the data analysis. The procedure for staged reperfusion was the same as in the staged reperfusion group.

During 13–14 minutes of ischemia, distal coronary back pressure and back flow were measured in the three groups.

In the preliminary study, we observed that intracoronary administration of 120 μmol/kg/min of NaHCO\textsubscript{3} increased FS from 24±1% to 34±2% (n=5) in the nonischemic control conditions.

Protocol 2: Roles of transient acidosis in myocardial stunning. To examine the cause and effect relation between acidosis during reperfusion and functional recovery, direct evidence that transient acidosis attenuates myocardial stunning is necessary. Immediately after the onset of an abrupt and complete reperfusion, intracoronary infusion of HCl was performed for 10 minutes (82 μmol/kg/min for the first 6 minutes and 27 μmol/kg/min for the following 4 minutes). Hemodynamic parameters were measured 15, 30, 45, and 60 seconds and 3, 5, 7, 10, 30, 60, 90, 120, 150, and 180 minutes after the onset of reperfusion (n=6). No ventricular fibrillation occurred in these six dogs. Coronary arterial and venous blood were sampled 1, 3, 5, 7, 10, 30, 60, 120, and 180 minutes after ischemia. During 13–14 minutes of ischemia, distal coronary back pressure and back flow were measured. Three hours after reperfusion, the myocardium perfused by LAD and LCx coronary arteries was quickly sampled in liquid nitrogen to measure myocardial tissue ATP content.48
In the preliminary study, we observed that an intracoronary administration of HCl (82 mmol/kg/min) decreased FS from 23±1% to 11±2% (n=5) in the nonischemic control conditions.

In these protocols, the perfusion area was determined by injection of Evans blue dye in the bypass tube. The mean tissue weights of the perfused area in the control reperfusion, staged reperfusion, staged reperfusion with NaHCO₃, and HCl reperfusion groups were 38±5, 33±4, 39±4, and 35±4 mg, respectively.

Chemical Analysis

Coronary arterial and venous blood oxygen difference (AVo₂D) was assessed by the difference between coronary arterial and venous oxygen contents. Myocardial oxygen consumption (MVo₂) (ml/100 g/min) was calculated by CBF (ml/100 g/min)×AVo₂D (ml/dl). Lactate was assessed by enzymatic assay, and lactate extraction ratio (LER) was obtained by coronary arteriovenous difference in lactate concentration multiplied by 100 and divided by arterial lactate concentration.

ATP Measurement

The method of ATP measurement has been previously described. After the last hemodynamic and metabolic measurements, small myocardial samples of LAD and LCx coronary arterial areas were removed (40–100 mg) and frozen with precooled stainless steel tongs and tongs in liquid nitrogen, and immediately stored at −80°C in liquid nitrogen. The frozen myocardial tissue was powdered and homogenized at 4°C in 1 ml of ice-cold 6% trichloroacetic acid, and then centrifuged at 2,500g for 20 minutes. The supernatant fluid was removed and extracted three times with 3 ml of diethyl ether saturated with water and stored in the freezer (−80°C). A luciferine-luciferase solution was prepared by dissolving FLE-50 (Sigma Co.) in 10 ml of distilled water, followed by centrifugation at 8,000g in ice-cold temperature for 1 hour. Supernatant (0.2 ml) was added to 5 ml of the ATP standard solution or ATP extract of myocardium, and the intensity of bioluminescence generated from the ATP luciferine-luciferase mixture was measured with a bioluminescence reader. The ATP standard solution was prepared by diluting ATP-2 Na salts with distilled water. The ATP concentration was computed from the intensity of chemiluminescence.

Statistical Analysis

Statistical analysis was performed with paired and unpaired t tests. The repeated-measures analysis of variance was also used to test the difference of each parameter versus time between the two groups. All values were expressed as mean±SEM, and a value of p<0.05 was considered significant.

Results

Before myocardial ischemia, there were no significant changes in regional FS and metabolic parameters between the control reperfusion condition, the staged reperfusion conditions with and without infusion of NaHCO₃, and the acidic reperfusion condition (Tables 1 and 2, Figures 1, 2, 5, and 7).

During ischemia, FS in each group decreased to identical extents and no significant differences were observed in both FS at 15 minutes of ischemia and time courses in the FS decline (Figures 1, 2, 5, and 7). Heart rate in each group did not significantly change during ischemia and reperfusion (control, 134±4 beats per minute; 15 minutes of ischemia, 138±4 beats per minute; 180 minutes after reperfusion, 130±4 beats per minute).

Table 2 shows that there were no significant changes in systemic hemodynamic parameters (LVP and dP/dtmax) between each group throughout the study. The extent of increases in end-diastolic pressure during ischemia was comparable in the four groups, and there were no significant changes in end-diastolic pressure in the control and at 3 hours of reperfusion.

Effects of Staged Reperfusion on Contractile Dysfunction During Reperfusion

Figure 1 shows the representative tracing of the systemic and regional hemodynamic parameters before, during, and after 15 minutes of coronary occlusion followed by abrupt reperfusion (control). Figure 2 shows the representative tracing of the hemodynamic parameters in the staged reperfusion group. In the groups in the staged reperfusion, the functional recovery (FS at 3 hours of reperfusion, 27.3%) was better than that in the control reperfusion group (FS at 3 hours of reperfusion, 10.7%; Figure 1). Figure 3 shows the changes in CPP (panel B) and CBF (panel C) after the onset of reperfusion. Abrupt coronary reperfusion returned CPP to the control level imme-

### Table 1. Coronary Hemodynamic and Metabolic Parameters Before and After Pharmacological Interventions Before Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>CPP (mm Hg)</th>
<th>CBF (ml/100 g/min)</th>
<th>LER (%)</th>
<th>MVo₂ (ml/100 g/min)</th>
<th>pH(a)</th>
<th>pH(v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>102±5</td>
<td>91±2</td>
<td>27.1±3.8</td>
<td>7.20±0.27</td>
<td>7.41±0.01</td>
<td>7.39±0.01</td>
</tr>
<tr>
<td>Staged reperfusion</td>
<td>108±13</td>
<td>92±3</td>
<td>21.4±2.5</td>
<td>7.16±0.61</td>
<td>7.42±0.01</td>
<td>7.41±0.01</td>
</tr>
<tr>
<td>Staged reperfusion with NaHCO₃</td>
<td>105±12</td>
<td>90±2</td>
<td>26.5±5.2</td>
<td>7.08±0.70</td>
<td>7.42±0.03</td>
<td>7.42±0.02</td>
</tr>
<tr>
<td>Acidotic reperfusion</td>
<td>104±4</td>
<td>90±2</td>
<td>26.6±3.6</td>
<td>7.07±0.66</td>
<td>7.42±0.02</td>
<td>7.42±0.01</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

CPP, coronary perfusion pressure; CBF, coronary blood flow; LER, lactate extraction ratio; MVo₂, myocardial oxygen consumption; pH(a) and pH(v), pH in coronary arterial and venous blood, respectively.
TABLE 2. Serial Changes Before, During, and After Myocardial Ischemia

<table>
<thead>
<tr>
<th>Hemodynamic parameter by group</th>
<th>Time after ischemia</th>
<th>Time after reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>3 Min</td>
</tr>
<tr>
<td>LVP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>117±7</td>
<td>115±7</td>
</tr>
<tr>
<td>Staged</td>
<td>117±11</td>
<td>118±11</td>
</tr>
<tr>
<td>Staged with NaHCO₃</td>
<td>120±11</td>
<td>117±11</td>
</tr>
<tr>
<td>Acidotic</td>
<td>114±7</td>
<td>112±7</td>
</tr>
<tr>
<td>dP/dtmax (mm Hg/sec)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3,130±130</td>
<td>3,030±210</td>
</tr>
<tr>
<td>Staged</td>
<td>3,120±140</td>
<td>3,080±150</td>
</tr>
<tr>
<td>Staged with NaHCO₃</td>
<td>3,280±210</td>
<td>3,280±260</td>
</tr>
<tr>
<td>Acidotic</td>
<td>3,270±220</td>
<td>3,150±190</td>
</tr>
<tr>
<td>EDP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.1±0.4</td>
<td>5.4±0.4</td>
</tr>
<tr>
<td>Staged</td>
<td>4.5±0.7</td>
<td>5.0±1.0</td>
</tr>
<tr>
<td>Staged with NaHCO₃</td>
<td>5.5±0.4</td>
<td>5.4±0.4</td>
</tr>
<tr>
<td>Acidotic</td>
<td>5.3±0.5</td>
<td>5.5±0.5</td>
</tr>
<tr>
<td>EDL (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.8±0.5</td>
<td>12.1±0.5</td>
</tr>
<tr>
<td>Staged</td>
<td>10.8±0.7</td>
<td>11.8±0.9</td>
</tr>
<tr>
<td>Staged with NaHCO₃</td>
<td>10.7±0.8</td>
<td>12.2±1.0</td>
</tr>
<tr>
<td>Acidotic</td>
<td>10.9±0.7</td>
<td>11.9±0.7</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LVP, left ventricular pressure; EDP, end-diastolic pressure; EDL, end-diastolic length. There are no significant differences between LVP, dP/dtmax, EDP, and EDL at each time between each group.

diately and caused the marked coronary reactive hyperemic flow (peak flow, 325±27 ml/100 g/min at 15 seconds of reperfusion). When the coronary artery was gradually reperfused with and without NaHCO₃ (panel A, 40% of control CPP for 0–3 minutes after the onset of reperfusion, 60% of control CPP for 4–6 minutes, and 80% of control CPP for 7–10 minutes), the measured values of the CPP were consistent with the set values (panels A and B). Coronary reactive hyperemic flow in both of the staged reperfusion groups was significantly (p<0.001) attenuated for 6 minutes of reperfusion (panel C).

Figure 4 shows the changes in pH in the coronary venous blood after the onset of reperfusion. In the control abrupt reperfusion condition; pH quickly returned to the normal condition; however, staged reperfusion significantly (p<0.001) prolonged acidosis up to 20 minutes. NaHCO₃ administration returned low pH during the staged reperfusion to the levels of the control reperfusion condition. Indeed, NaHCO₃ administration slightly increased pH in blood of the coronary vein 6 minutes after the onset of reperfusion. There were no significant changes in pH at 30 minutes of reperfusion with and without 10-minute administration of NaHCO₃, indicating that the aftereffects of NaHCO₃ administration disappear at 30 minutes of reperfusion. Figure 5 shows the time courses of FS after 15 minutes of ischemia in the control reperfusion group and the staged reperfusion groups with and without NaHCO₃ infusion. Although FS was significantly (p<0.05) decreased in the staged reperfusion without NaHCO₃ group compared with the control reperfusion groups up to 10 minutes of reperfusion, it was significantly (p<0.001) improved for 10–180 minutes of reperfusion. Staged reperfusion without NaHCO₃ significantly (p<0.005) improved the contractile dysfunction at 3 hours of reperfusion compared with the control reperfusion group; however, this beneficial effect of staged reperfusion was abolished by the intracoronary infusion of NaHCO₃ during reperfusion.

The changes in LER and MVo2 in these three groups are depicted in Tables 3 and 4. There were no significant changes in LER during reperfusion with and without staged reperfusion. However, in the staged reperfusion group with NaHCO₃, MVo2 during 30 minutes of reperfusion was significantly (p<0.05) attenuated compared with the control reperfusion and the staged reperfusion with NaHCO₃ groups.

The coronary retrograde pressure and retrograde flow during ischemia were not different in the abrupt reperfusion (21.9±1.5 mm Hg and 3.6±0.5 ml/100 g/min), staged reperfusion (22.0±2.4 mm Hg and 3.4±0.8 ml/100 g/min), and staged reperfusion with NaHCO₃ administration (23.0±2.3 mm Hg and 3.4±0.6 ml/100 g/min, respectively).

These results indicate that staged reperfusion significantly attenuates myocardial stunning and suggest that this beneficial effect of staged reperfusion is due to transient acidosis during reperfusion.

**Role of Transient Acidosis in Improvement of Myocardial Stunning**

Although we showed that staged reperfusion is beneficial for myocardial stunning possibly because
of transient acidosis during early reperfusion, the direct evidence that transient acidosis per se improves contractile dysfunction is necessary to prove the cause and effect relation between extents of acidosis during early reperfusion and improvements of myocardial stunning in the staged reperfusion condition. To determine this unique cause and effect relation, we tested whether transient acidosis produced by intracoronary infusion of HCl attenuates myocardial stunning in the abrupt reperfusion condition.

Figure 6 shows the changes in pH of coronary venous blood. Intracoronary infusion of HCl up to 20 minutes of reperfusion significantly (p < 0.001) decreased pH compared with the control reperfusion group, which is comparable with the staged reperfusion group. In accordance with this transient low pH, Figure 7 demonstrates that functional recovery 3 hours after reperfusion is significantly improved compared with the control reperfusion group, and the extent of improvement of FS in this group was comparable with that of the staged reperfusion group (Figure 5). The reactive hyperemic flow was comparable with the abrupt reperfusion condition (peak flow, 346 ± 29 ml/100 g/min at 15 seconds of reperfusion).

The coronary retrograde pressure and retrograde flow during ischemia were not different in the abrupt reperfusion (21.9 ± 1.5 mm Hg and 3.6 ± 0.5 ml/100 g/min) and acidosis reperfusion (22.2 ± 2.3 mm Hg and 3.0 ± 1.4 ml/100 g/min, respectively) groups.

These results strongly indicate that transient acidosis during early reperfusion attenuates myocardial stunning.

Role of ATP in Improvement of Myocardial Stunning

Table 5 shows the ATP contents in the control reperfused, staged reperfused, and acidic reperfusion myocardium. In both the epicardial and endocardial myocardium, ATP content of the reperfused myocardium was significantly (p < 0.001) lower than that in the nonischemic myocardium; however, the extents of decreases in ATP in these four groups were not significantly different.

Discussion

Prompt reperfusion of the acute ischemic myocardium is a primary and rational way of limiting the extent of eventual contractile dysfunction and myocardial necrosis. Recent clinical advances in emergency coronary bypass surgery and percutaneous transluminal coronary recanalization have provided potentially feasible methods of reperfusion of the ischemic myocardium. However, despite these clinical efforts of early revascularization, the contractile function remains impaired for long periods, a
FIGURE 2. Representative recording of systemic hemodynamic parameters (LVP, left ventricular pressure; and dP/dt), regional myocardial function (SL, segment length), and coronary perfusion parameters (CPP, coronary perfusion pressure; CBF, coronary blood flow) before ischemia (control), at 15 minutes of ischemia, and at 10 and 180 minutes of reperfusion in the staged reperfusion group. Fractional shortening was restored at 3 hours of reperfusion (27.3% vs. 28.0% at control), showing attenuation of contractile dysfunction during reperfusion.

Phenomenon known as myocardial stunning.\textsuperscript{1,2} Reperfusion is reported to generate free radicals\textsuperscript{16,55} and cause Ca overload and overcontraction,\textsuperscript{7-12} either of which may be responsible for myocardial stunning.

In the present study, we showed evidence that staged reperfusion attenuates myocardial stunning and that the beneficial effect of staged reperfusion is attributed primarily to transient acidosis during early reperfusion. However, several lines of evidence suggest that graded reperfusion is deleterious to the reperfused myocardium.\textsuperscript{43-45} In these studies, because the duration of limited coronary flow is rather long (>4 hours) compared with that of the present study, anaerobic myocardial metabolism caused by low-flow ischemia may be prolonged, which masks the beneficial effects of staged reperfusion. The staged reperfusion procedure in the present study was performed within 10 minutes, suggesting that anaerobic myocardial metabolism did not persist for a long period. Indeed, LER during early reperfusion in the staged reperfusion condition was not significantly different from that in the abrupt reperfusion group (Table 3).

Mechanisms Wherein Staged Reperfusion Attenuates Myocardial Stunning

Although we concluded that transient acidosis during staged reperfusion primarily attenuates myocardial stunning, we need to consider other possibilities.

Low perfusion pressure during staged reperfusion procedures may affect myocardial contractility,\textsuperscript{56,57} which attenuates myocardial stunning. Increases in H\textsuperscript{+} during staged reperfusion also attenuate myocardial contractility through competition of troponin C.\textsuperscript{32,33} This depressed cardiac contractility during staged reperfusion may preserve ATP contents of the reperfused myocardium, leading to an improvement of cardiac dysfunction. However, ATP contents of the reperfused myocardium were not significantly different between the control and staged reperfusion groups. Furthermore, the extent of recovery from ATP depletion does not necessarily determine the contractile dysfunction of stunned myocardium\textsuperscript{5,6,58,59} because repletion of ATP does not restore normal contractile function.\textsuperscript{90} Although ATP depletion of stunned myocardium was between 40% and 60%...
A

B

C

FIGURE 3. Changes in the set (panel A) and real (panel B) coronary perfusion pressure (CPP) and coronary blood flow (CBF, panel C) during early reperfusion with and without the staged reperfusion procedure. The changes in real values of CPP (panel B) followed the changes in set CPP (panel A) by the adjustment of the occluder attached at the coronary bypass tube. In the staged reperfusion group with and without NaHCO<sub>3</sub> infusion, coronary reactive hyperemia up to 6 minutes of reperfusion was significantly (p<0.001) attenuated compared with the control reperfusion group.

Staged reperfusion in the present study also reduced coronary hyperemic flow during early reperfusion, which may affect contractile dysfunction during reperfusion. Free radicals are reported to be predominantly generated in the oxygenated hyperemic myocardium, and this may cause myocardial stunning. Although hyperemic flow was also limited...

Table 5), as in the previous studies, such decreases in ATP content of stunned myocardium are not likely to cause contractile dysfunction observed in the present study because these extents of ATP depletion are not sufficient to compromise the ionic pump activities of the cellular membrane or the sarcoplasmic reticulum.
in the staged reperfusion with NaHCO₃, this procedure could not attenuate myocardial stunning (Figure 3). Furthermore, during the acidic reperfusion with HCl infusion, the peak hyperemic flow was comparable with that during abrupt control reperfusion; nevertheless, myocardial stunning was improved. These results indicate that limited hyperemic coronary flow during staged reperfusion is not the primary factor in attenuating myocardial stunning. However, this limited hyperemic flow may further increase adenosine release during early reperfusion, and this released adenosine may attenuate myocardial stunning because adenosine is reported to attenuate myocardial stunning and reperfusion injury. Furthermore, acidosis is reported to enhance the effect of adenosine, which may contribute to attenuation of myocardial stunning. We cannot exclude this possibility.

In addition, H⁺ has several cardiac effects that may account for the attenuation of myocardial stunning. First, acidosis is reported to attenuate myocardial oxygen consumption. During the early reperfusion period, overshoot of myocardial oxygen consumption was observed (Table 4 and Figure 5) despite the existence of anaerobic myocardial metabolic condition. This overshoot phenomenon, possibly caused by released catecholamine or Ca overload, may be deleterious to the reperfused myocardium. Acidosis during staged reperfusion significantly attenuated the increase in oxygen consumption during early reperfusion. The reduction of mechanical work load caused by acidosis may be beneficial for recovery from contractile dysfunction. H⁺ also attenuates the activation of neutrophils and attenuates free radical generation. Activation of neutrophils is reported to be one of the causes of myocardial stunning.

### Table 3. Serial Changes in Lactate Extraction Ratio After Onset of Reperfusion

<table>
<thead>
<tr>
<th>Time after reperfusion</th>
<th>1 Min</th>
<th>3 Min</th>
<th>5 Min</th>
<th>7 Min</th>
<th>10 Min</th>
<th>30 Min</th>
<th>60 Min</th>
<th>120 Min</th>
<th>180 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control reperfusion</td>
<td>-37.9±7.3</td>
<td>-22.5±5.1</td>
<td>8.4±2.6</td>
<td>10.7±4.3</td>
<td>13.1±6.4</td>
<td>24.1±6.0</td>
<td>16.8±3.8</td>
<td>16.6±2.6</td>
<td>22.1±2.1</td>
</tr>
<tr>
<td>Staged reperfusion</td>
<td>-36.8±13.0</td>
<td>-15.7±10.2</td>
<td>10.8±7.1</td>
<td>21.4±6.6</td>
<td>15.8±5.2</td>
<td>20.2±4.2</td>
<td>23.9±4.9</td>
<td>23.8±4.3</td>
<td>24.0±3.8</td>
</tr>
<tr>
<td>Staged reperfusion with NaHCO₃</td>
<td>-56.9±15.7</td>
<td>-25.2±15.0</td>
<td>2.3±4.0</td>
<td>8.6±5.5</td>
<td>15.0±5.2</td>
<td>15.0±5.2</td>
<td>24.5±7.3</td>
<td>24.3±3.0</td>
<td>21.4±3.2</td>
</tr>
<tr>
<td>Acidotic reperfusion</td>
<td>-32.2±12.2</td>
<td>-5.1±6.7</td>
<td>12.2±6.0</td>
<td>11.8±3.8</td>
<td>23.9±3.0</td>
<td>26.7±3.7</td>
<td>24.8±5.5</td>
<td>23.5±2.5</td>
<td>23.5±3.1</td>
</tr>
</tbody>
</table>

Values (%) are mean±SEM. There are no significant differences in lactate extraction ratio at each time between each group.

### Table 4. Serial Changes in Myocardial Oxygen Consumption After Onset of Reperfusion

<table>
<thead>
<tr>
<th>Time after reperfusion</th>
<th>1 Min</th>
<th>3 Min</th>
<th>5 Min</th>
<th>7 Min</th>
<th>10 Min</th>
<th>30 Min</th>
<th>60 Min</th>
<th>120 Min</th>
<th>180 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control reperfusion</td>
<td>6.00±0.24</td>
<td>6.39±0.19</td>
<td>7.14±0.22</td>
<td>6.83±0.41</td>
<td>6.95±0.37</td>
<td>7.25±0.44</td>
<td>6.96±0.39</td>
<td>7.05±0.57</td>
<td>7.16±0.37</td>
</tr>
<tr>
<td>Staged reperfusion</td>
<td>3.67±0.37*</td>
<td>4.82±0.41†</td>
<td>5.08±0.60‡</td>
<td>5.26±0.47‡</td>
<td>5.81±0.28‡</td>
<td>7.38±0.55</td>
<td>7.04±0.42</td>
<td>7.15±0.20</td>
<td>7.15±0.53</td>
</tr>
<tr>
<td>Staged reperfusion with NaHCO₃</td>
<td>6.15±0.28</td>
<td>5.82±0.23</td>
<td>6.53±0.25</td>
<td>6.72±0.37</td>
<td>6.79±0.37</td>
<td>7.10±0.68</td>
<td>7.08±0.30</td>
<td>7.42±0.46</td>
<td>7.26±0.45</td>
</tr>
<tr>
<td>Acidotic reperfusion</td>
<td>3.73±0.24*</td>
<td>4.35±0.35*</td>
<td>6.09±0.34‡</td>
<td>6.14±0.59</td>
<td>6.32±0.31</td>
<td>7.44±0.34</td>
<td>7.46±0.39</td>
<td>7.07±0.31</td>
<td>7.43±0.34</td>
</tr>
</tbody>
</table>

Values (ml/100 g/min) are mean±SEM. *p<0.001, †p<0.005, ‡p<0.05 vs. control reperfusion group at each time between each group.
because of free radical generation and microcirculatory disturbances. Furthermore, transient acidosis may reduce Ca overload and attenuate myocardial stunning. An increase in intracellular H+ concentration can inhibit several pathways of Ca movement to intracellular sites. An increase in intracellular H+ leads to a decrease in Ca influx through voltage-dependent slow Ca channels. Acidosis is also known to inhibit the release of Ca2+ from sarcoplasmic reticulum. The most likely source of Ca overload is through the reverse Na-Ca exchange.

**FIGURE 6.** Serial changes in pH in coronary venous blood during control reperfusion and acidotic reperfusion. The acidotic reperfusion procedure significantly (p<0.001) decreases in pH up to 20 minutes of reperfusion compared with the control reperfusion group.

**FIGURE 7.** Serial changes in fractional shortening during control reperfusion and acidotic reperfusion. There are no significant differences in fractional shortening at baseline and extents of decreases in fractional shortening during ischemia in the control reperfusion and acidotic reperfusion groups. Although FS in the acidotic reperfusion group is significantly (p<0.05) reduced up to 10 minutes of reperfusion, it conversely increases (p<0.001) during 10–180 minutes of reperfusion compared with the control group.

**TABLE 5.** ATP Contents of Stunned and Nonstunned Myocardium

<table>
<thead>
<tr>
<th>Group</th>
<th>LAD area</th>
<th>LCx area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>End</td>
<td>Epi</td>
</tr>
<tr>
<td>Control reperfusion</td>
<td>2.9±0.1</td>
<td>3.1±0.2</td>
</tr>
<tr>
<td>Staged reperfusion</td>
<td>3.0±0.2</td>
<td>3.3±0.3</td>
</tr>
<tr>
<td>Staged reperfusion with NaHCO3</td>
<td>2.8±0.2</td>
<td>3.0±0.3</td>
</tr>
<tr>
<td>Acidotic reperfusion</td>
<td>3.1±0.1</td>
<td>3.2±0.1</td>
</tr>
</tbody>
</table>

Values (μmol/wet wt/g) are mean±SEM. LAD, left anterior descending coronary artery; LCx, left circumflex coronary artery; End, endocardial layer; Epi, epicardial layer.
fusion group. Since the damages caused by ischemia are inevitable even in the acidosis reperfusion groups, the late, more functional recovery after acidosis reperfusion suggests that acidosis may inhibit triggering mechanisms during early reperfusion.

Clinical Relevance and Limitations

Recent advances in coronary revascularization technique lead us to face a new aspect of myocardial injury. Although the beneficial effects of early reperfusion are unquestionable, myocardial stunning can persist for days to weeks. This myocardial stunning may be one of the causes of acute heart failure in the ischemic, reperfused heart.2,1,2 Our results suggest that reperfusion injury can be attenuated because the maneuver in the present study attenuated myocardial stunning.

Our results merit further evaluation in realistic clinical settings. Percutaneous transluminal coronary revascularization and thrombolysis are often applied in acute myocardial infarction. When the balloon is deflated after coronary revascularization, gradual deflation of the balloon instead of abrupt deflation may cause transient acidosis. This transient acidosis during early reperfusion may attenuate the contractile dysfunction and infarct size. Indeed, there are several reports that staged reperfusion accelerates the recovery of the contractile dysfunction after a long period of ischemia.13-42 If coronary stenosis is prolonged for 2 or 3 hours, anaerobic metabolism may sustain and attenuate the beneficial effect of acidosis.43-45 Thus, in clinical settings, we should evaluate what duration and extent of residual stenosis and balloon inflation are beneficial.

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**Key Words**: reperfusion injury · calcium overload · contractile dysfunction · acidosis · sodium-calcium exchange
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