Brief Communication

Alterations in Cardiac Sarcoplasmic Reticulum Calcium Transport in the Postischemic "Stunned" Myocardium

Stephen M. Krause, William E. Jacobus, and Lewis C. Becker

This study examined the possibility that the postischemic mechanical depression observed in the "stunned" myocardium is a result of an alteration in the control of intracellular calcium. Regional myocardial stunning was produced in five open-chest dogs by eight to twelve 5-minute occlusions of the left anterior descending coronary artery, alternated with 10-minute reflow periods and followed by a final 60-minute period of reperfusion. Systolic segment shortening in the postischemic zone, measured by sonomicrometry, fell from 14.9% at baseline to -1.1% at the end of reperfusion. Sarcoplasmic reticulum isolated from stunned myocardium demonstrated a 17% reduction in oxalate-supported $\text{Ca}^{2+}$ transport compared with sarcoplasmic reticulum from normal myocardium (0.93 vs. 1.12 $\mu$mol Ca$^{2+}$/mg protein/min, $p<0.005$). There was also a 20% decrease in the maximal activation by Ca$^{2+}$ of the sarcoplasmic reticulum Ca$^{2+}$-Mg$^{2+}$-ATPase (2.46 vs. 1.96 $\mu$mol P$_i$/mg protein/min, $p<0.005$), and a downward shift in the Ca$^{2+}$-activation curve of the Ca$^{2+}$-Mg$^{2+}$-ATPase. These results indicate that myocardial stunning is associated with damage to the calcium-transport system of the sarcoplasmic reticulum. Altered intracellular control may contribute to the inability of the stunned heart to maintain normal mechanical function. (Circulation Research 1989;65:526-530)

After a brief episode of ischemia, myocardial contractile function may remain compromised for several days before returning to normal, despite the absence of necrosis.1,2 This reversible ischemic damage has become well known as "stunned" myocardium3 although the subcellular mechanisms that contribute to the stunning phenomenon remain poorly understood. A loss of adenine nucleotides, which parallels the decline in function, was felt to be a major contributor to the stunning phenomenon.4,5 However, recent studies have demonstrated that depletion of the adenine nucleotide pool does not affect the recovery of function.6 In addition, catecholamine administration results in a pronounced and sustained increase in function of the stunned myocardium, which suggests that the adenine nucleotide pool should be adequate to sustain normal baseline contractile activity.7

An area that has not been adequately explored is the role of calcium fluxes in myocardial stunning. Normal contractile activity depends on proper functioning of a calcium release-uptake cycle. Intracellular free-calcium concentration rises transiently at the initiation of each contraction, principally from the release of calcium from sarcoplasmic reticulum stores.8 After the interaction of calcium with the contractile proteins for generation of contraction, relaxation is initiated by sequestration of calcium by the sarcoplasmic reticulum via an energy-requiring transport process. Since the amount of calcium sequestered by the sarcoplasmic reticulum determines the calcium available for release and, thus, activation, a defect in the function of the calcium pump would subsequently result in less releasable calcium and activation. In a nonreperfused model of global ischemia, Krause and Hess9 have demonstrated that sarcoplasmic reticulum calcium transport is remarkably sensitive to ischemia. They found a 49% depression of oxalate-supported calcium transport after only 7.5 minutes of canine normothermic global ischemia, concomitant with a reduction of Ca$^{2+}$,Mg$^{2+}$-ATPase activity required for calcium transport. However, since their study was performed without reperfusion, it is unknown whether these abnormalities of calcium transport are reversible or persistent in the reperfused, stunned

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myocardium. Our purpose in this study was determination of whether the depression in mechanical function of the myocardium after repetitive brief periods of ischemia and reperfusion was subject to an alteration in the calcium transport capabilities of the sarcoplasmic reticulum.

Materials and Methods

Five mongrel dogs weighing 20–25 kg were anesthetized with sodium pentobarbital (30 mg/kg), intubated, and placed on a mechanical ventilator. Through a left thoracotomy, an inflatable occluder was placed around the left anterior descending coronary artery (LAD) between the first and second main diagonal branches, approximately 2 cm from the origin of the LAD. A Silastic tube was sutured to the superior portion of the left atrium for the insertion of a Millar pressure transducer-tipped catheter (Millar Instruments, Houston, Texas). A pair of ultrasonic crystals was implanted approximately 1 cm apart in the midwall of the LAD perfusion territory for measurement of segment length changes. A second pair of ultrasonic crystals was placed in the nonischemic region of the free left ventricular (LV) wall. LV pressure, the differentiated LV pressure signal (dP/dt), segment length, and lead II of the electrocardiogram were monitored continuously. For measurement of segment length changes, the ultrasonic signals were digitized at 150 Hz and stored on a floppy disk. End-systolic length was measured 20 msec before peak negative LV dP/dt, and end-diastolic length was measured just before the onset of positive LV dP/dt. Percent shortening was calculated as (end-diastolic minus end-systolic length) times 100 divided by end-diastolic length.

Protocol

Regionally stunned myocardium was produced by occlusion of the LAD for 5 minutes, followed by 10 minutes of reperfusion. This sequence was repeated eight to 12 times, followed by a reperfusion period of 60 minutes. This repetitive occlusion procedure resulted in reproducible stunning characterized by akinesia or dyskinesia in the postischemic bed. Previous studies that used this model have shown an absence of necrosis.10

After the final 60-minute reperfusion period, 10-g transmural tissue samples were taken from the center of the ischemic and nonischemic zones (judged by the epicardial vascular pattern) for the isolation of subcellular fractions enriched with sarcoplasmic reticulum according to the methods of Krause and Hess.9 Briefly, the tissue samples were minced in 10 mM imidazole (pH 7.1, 3 ml/g wet weight) and homogenized at 4° C for 1 minute. The homogenate was then subjected to differential centrifugation (4,000×g max for 20 minutes; 10,000×g max for 15 minutes; 31,000×g max for 60 minutes; 198,000×g max for 60 minutes) for isolation of the sarcoplasmic reticulum-enriched fraction, which was suspended in 30% sucrose and 20 mM imidazole (pH 7.1) and stored at -20° C. When isolated in this fashion, the activity of the sarcoplasmic reticulum fraction is representative of sarcoplasmic reticulum activity in the whole heart homogenate11 or of sarcoplasmic reticulum released during the homogenization process.9

The integrity of the calcium transport system was determined by measurement of steady-state oxalate-supported calcium uptake and Ca2+,Mg2+-dependent ATPase activity at 37° C as previously described.9 For the Ca2+-transport studies, the reaction medium contained 100 mM KCl, 20 mM imidazole (pH 7.1), 10 mM NaN3, 10 mM potassium oxalate, 5 mM MgCl2, 5 mM Na2ATP, 200 μM CaCl2, and 0.05 μCi 45CaCl2/ml at a protein concentration of 0.15 mg/ml. The reaction was started by the addition of MgATP and CaCl2 to an otherwise complete incubation medium. Aliquots of the incubation medium were removed and filtered through 0.45-μm filters (Millipore, Bedford, Massachusetts) to stop the reaction. A 100-μl aliquot of the filtrate was counted in a liquid scintillation counter, and the amount of Ca2+ sequestered by the sarcoplasmic reticulum was determined from the decrease in 45Ca in the filtrate. Total Ca2+ and Mg2+-ATPase activity was determined from an aliquot of the same filtrate by monitoring the amount of inorganic phosphate by use of the method of Penny.12 The Ca2+ dependency of the ATPase activity was also determined over a range of free calcium concentrations from 0.1 to 32 μM as previously described.13 The incubation medium contained 100 mM KCl, 3.2 mM MgATP, 3.2 mM MgCl2, 10 mM EGTA, 20 mM imidazole (pH 7.1), and 2 μM A23187 in addition to the calcium.

The statistical significance of differences between stunned and nonischemic myocardium was determined by use of the t test for paired comparisons. Results are expressed as mean±SEM.

Results

Mechanical and Hemodynamic Effects of Stunning

Postischemic stunning was characterized by an increase in end-systolic segment length, a decrease in percent systolic shortening, and a delay in the onset of shortening. This mechanical dysfunction was seen after the first ischemia/reperfusion episode, became cumulative with repeated episodes of ischemia/reperfusion, and persisted to the end of the final 1-hour reperfusion period. By the end of recovery, percent systolic shortening had decreased from 14.9±1.5% to 11.1±1.9% (p<0.005) without significant change in end-diastolic segment length. There were no significant changes in heart rate or LV systolic or diastolic pressure (Table 1).

Biochemical Alterations in Cardiac Sarcoplasmic Reticulum

The effect of myocardial stunning on the rate of oxalate-supported Ca2+ uptake and the Ca2+,Mg2+-
ATPase activity of the calcium pump during the time that calcium was being actively sequestered are shown in Figure 1. Calcium uptake in sarcoplasmic reticulum isolated from the stunned zone was reduced 17%, from 1.12±0.06 to 0.93±0.08 μmol Ca\(^{2+}\)/mg protein/min (p<0.005). Ca\(^{2+}\),Mg\(^{2+}\)-ATPase activity was reduced concomitantly by 20% from 2.46±0.06 to 1.96±0.06 μmol P\(_i\)/mg protein/min (p<0.005). The coupling ratio, defined as moles of calcium transported per mole of ATP hydrolyzed, was not significantly different, although there was a trend toward a lower value in the stunned region (0.49±0.03 vs. 0.47±0.03, p=0.23).

For further definition of the effects of stunning on Ca\(^{2+}\),Mg\(^{2+}\)-ATPase activity, the calcium sensitivity was determined over a range of free-calcium concentrations from 0.1 to 10 μM (Figure 2). There is a downward shift in the ATPase activity of the stunned sarcoplasmic reticulum as compared with control. This decrease reached statistical significance (p<0.05) from pCa 5.75 to 5.0 (1.8-10 μM), which is in the range of the 1-3 μM value reported for the cytosolic calcium concentration during contractile protein activation.\(^{14}\)

**Discussion**

This study has shown that sarcoplasmic reticulum isolated from stunned myocardium demonstrates a decrease in the ability to transport calcium, concomitant with a reduction in the activity of the associated Ca\(^{2+}\),Mg\(^{2+}\)-ATPase activity. Although sarcoplasmic reticulum function has been shown to be affected by long periods of ischemia and reperfusion\(^ {15}\) that result in cellular damage and necrosis, alterations have not been documented in the stunned myocardium. Previous electron microscopic studies in this canine model of stunning have demonstrated an absence of necrosis, despite the very pronounced abnormality of regional function. Therefore, it is possible that damage to the sarcoplasmic reticulum and its calcium-transport function may contribute to the contractile dysfunction of the stunned myocardium through a decrease in the activation level of the contractile proteins.

The calcium that activates the contractile proteins is apparently derived solely from the sarcoplasmic reticulum and does not directly involve transsarcolemmal calcium influx.\(^{8}\) Therefore, any

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**TABLE 1. Effect of Reperfusion on Myocardial Contractile Function**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Occlusion 1</th>
<th>Reflow 1</th>
<th>Postischemic recovery (60 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>126±4</td>
<td>125±10</td>
<td>137±13</td>
<td>128±10</td>
</tr>
<tr>
<td>Left ventricular systolic pressure (mm Hg)</td>
<td>113±6</td>
<td>106±6</td>
<td>110±2</td>
<td>130±3</td>
</tr>
<tr>
<td>Left ventricular diastolic pressure (mm Hg)</td>
<td>4±1</td>
<td>6±1</td>
<td>5±1</td>
<td>4±1</td>
</tr>
<tr>
<td>End diastolic length (mm)</td>
<td>10.7±1.2</td>
<td>11.5±1.4</td>
<td>10.3±2.0</td>
<td>11.0±1.1</td>
</tr>
<tr>
<td>Systolic shortening (%)</td>
<td>14.9±1.5</td>
<td>-13.4±2.1*</td>
<td>4.9±8.6</td>
<td>-1.1±1.9*</td>
</tr>
</tbody>
</table>

* p<0.005 vs. control.
A decrease in the amount of calcium stored in the sarcoplasmic reticulum as a result of a reduction in the calcium pump activity could diminish contractile protein activation via attenuated calcium release. The observed downward shift in the Ca\(^{2+}\),Mg\(^{2+}\)-ATPase curve is consistent with a decrease in the number of ATPase sites in the sarcoplasmic reticulum. This is similar to a loss in calcium "pumps," and would be expected to result in a lowered ability to remove calcium from the cytoplasm. The consequence of such an impairment in intracellular calcium sequestration could be far-reaching. For example, slower removal of calcium from the myofibrils would be expected to result in delayed relaxation and abnormal diastolic properties of stunned myocardium. Compensatory mechanisms for decreasing cytosolic calcium would have to be called into play, including transport across the sarcolemma or into the mitochondria, both of which could be detrimental to the cell. The inappropriately high oxygen consumption found in the stunned myocardium could be related to a need for increased calcium transport activity requiring concomitant energy expenditure.\(^{16}\)

A decrease in the level of activation of the contractile proteins could also occur by a change in the sensitivity of troponin C for calcium at the level of the myofibrils. This can probably be ruled out since even 30 minutes of normothermic global ischemia, producing cellular necrosis, has been shown to have no appreciable effect on the calcium activation of cardiac myofibrils.\(^{13}\) In addition, a recent study in the globally stunned, isolated rabbit heart has indicated that there is no change in the calcium activation of the myofibrillar ATPase.\(^{17}\)

The sarcoplasmic reticulum is an organelle that is apparently highly susceptible to ischemic damage.\(^{9,11}\) It has been shown previously that after prolonged irreversible ischemia, the loss in mechanical function is correlated with decreased sarcoplasmic reticulum activity.\(^{15}\) In the current study, we have demonstrated for the first time that myocardial stunning resulting from repetitive short-term ischemic episodes may lead to a similar alteration in sarcoplasmic reticulum function. However, in contrast with studies in nonreperfused hearts,\(^{9,11}\) the damage to sarcoplasmic reticulum function is not as severe in the reperfused, stunned myocardium. This observation indicates that reperfusion after brief periods of ischemia can limit subcellular damage. In addition, previous studies have demonstrated that the administration of catecholamines\(^{7}\) or calcium\(^{18}\) can return function of the stunned myocardium essentially to normal. This suggests that the depressed sarcoplasmic reticulum calcium transport present in stunned myocardium might also be modified by inotropic interventions. Catecholamines are known to stimulate calcium transport into the sarcoplasmic reticulum by means of increased phosphorylation of phospholamban, leading to increased activity of the Ca\(^{2+}\)-ATPase.\(^{19}\) In contrast, restoration of function related to an increased extracellular calcium is probably mediated by increased transsarcolemmal calcium influx into the cell.

It should be pointed out that in contrast with studies of zero-flow global ischemia, the ischemic zone in this model probably contained a mixture of heterogeneously ischemic tissue. For this reason, the extent of altered calcium transport and Ca\(^{2+}\),Mg\(^{2+}\)-ATPase activity probably represent conservative estimates of sarcoplasmic reticulum damage. If sarcoplasmic reticulum could have been isolated from only the most ischemic cells, the calcium titration curve of the ATPase activity probably would have been shifted even further down and to the right, reflecting even greater abnormalities of calcium transport and Ca\(^{2+}\),Mg\(^{2+}\)-ATPase.

Based on the abnormalities we have described in sarcoplasmic reticulum function, one might anticipate abnormalities in intracellular calcium concentration in stunned myocardium. Total cell calcium might or might not be increased, depending on the ability of transsarcolemmal extrusion to compensate for impaired sarcoplasmic reticulum activity. More likely, however, the distribution of cellular free calcium might be altered with a relative excess in the cytoplasm and mitochondria and a relative deficiency in the sarcoplasmic reticulum. Marban et al\(^{20}\) and Steenbergen et al\(^{21}\) have recently shown, by use of \(^{19}\)F nuclear magnetic resonance, that the total cytosolic calcium rises during ischemia, but Marban et al\(^{20}\) demonstrated a rapid return during reperfusion to baseline levels within the first 5–10 minutes. Although informative, these studies do not address the subcellular distribution of calcium or provide information on the kinetics of the calcium fluxes. Thus, even though the cell may be able to return mean cytosolic calcium to normal levels, intracellular calcium regulation by the sarcoplasmic reticulum may still be abnormal and contribute to the depressed function of stunned myocardium.
References


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Key Words · sarcoplasmic reticulum · calcium transport · ischemia · reperfusion injury · calcium
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