Excitation-Contraction Coupling in Postischemic Myocardium

Does Failure of Activator Ca\(^{2+}\) Transients Underlie Stunning?

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To elucidate the mechanism of contractile dysfunction in postischemic ("stunned") myocardium, time-resolved measurements of intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) were made using gated \(^{19}\)F nuclear magnetic resonance in seven perfused ferret hearts loaded with the fluorinated Ca\(^{2+}\) indicator 5F-BAPTA. Left ventricular developed pressure decreased to 65±3\% (mean±SEM) of control after 15 minutes of global ischemia at 37\°C. In stunned myocardium, diastolic [Ca\(^{2+}\)]\(_i\) (0.24±0.03 \(\mu\)M) was not changed from control (0.18±0.03 \(\mu\)M, \(p>0.10\)), but peak [Ca\(^{2+}\)]\(_i\) (1.03±0.13 \(\mu\)M) was paradoxically higher than that in control (0.61±0.06 \(\mu\)M, \(p<0.02\)). The slope of the relation between developed pressure and Ca\(^{2+}\) transient amplitude in stunned myocardium was significantly lower than that in control (\(p<0.05\), even after normalization by maximal Ca\(^{2+}\)-activated pressure. These results indicate that contractile failure in stunned myocardium is due to a decrease in the myofilament sensitivity to Ca\(^{2+}\) as well as to the previously identified decrease in maximal Ca\(^{2+}\)-activated force; failure of activator Ca\(^{2+}\) delivery cannot be implicated. The increase in the amplitude of Ca\(^{2+}\) transients would require that more ATP be spent in Ca\(^{2+}\) sequestration; thus, decreased efficiency of energy utilization in stunned myocardium would result. (Circulation Research 1990;66:1268–1276)

Stunned myocardium is defined as contractile dysfunction in heart muscle reperfused after a period of ischemia brief enough to avoid necrosis.\(^1\)\(^2\) Although the phenomenon has important implications for clinical reperfusion therapy, the cellular mechanism of the decrease in force after ischemia is not yet clear. Stunned myocardium remains responsive to catecholamines\(^3\)\(^4\)\(^5\) and to an increase in the extracellular calcium concentration ([Ca\(^{2+}\)]\(_o\))\(^6\)\(^7\) although the maximal response is attenuated.\(^6\) The nature of the impairment in force generation in stunned myocardium can best be characterized by breaking down the final common pathway of the excitation-contraction process into three elementary parameters. The first of these is the pulse of intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) that occurs during each cardiac cycle (the Ca\(^{2+}\) transient). The Ca\(^{2+}\) transient triggers the sequence of biochemical events, beginning with Ca\(^{2+}\) binding to troponin C, that lead to force generation by the contractile proteins. The two remaining parameters characterize the responsiveness of the contractile machinery to Ca\(^{2+}\): myofilament Ca\(^{2+}\) sensitivity describes the range of [Ca\(^{2+}\)] that activates contraction, and maximal Ca\(^{2+}\)-activated force represents the amplitude of the contractile response.

Most theories regarding the pathogenesis of stunned myocardium imply that force falls as a consequence of a decrease in Ca\(^{2+}\) transients. Abnormalities of the action potential that have been identified in excised, regionally stunned myocardium\(^8\) would lead to contractile dysfunction by decreasing the amount of Ca\(^{2+}\) entry during each beat. Impairment of Ca\(^{2+}\) release from the sarcoplasmic reticulum\(^9\) (e.g., by free radicals) would also lead to a decrease in cytoplasmic activator Ca\(^{2+}\) concentration.
No-reflow theories, including postischemic capillary plugging by white cells,10,11 postulate microscopic regions of persistent ischemia in which tissue is inexcitable and, therefore, lacks Ca\(^{2+}\) transients. Nevertheless, each of these specific theories is controversial, and the issue of whether or not Ca\(^{2+}\) transients are reduced has not been addressed directly. Indeed, recent work from our laboratory hints otherwise. Maximal Ca\(^{2+}\)-activated pressure is decreased in postischemic ferret hearts, indicating that at least one parameter of the myofilament responsiveness to Ca\(^{2+}\) is decreased in stunned myocardium.6 We also found an abnormally low sensitivity of contractile pressure to extracellular Ca (Ca, sensitivity), which could reflect either a decrease in Ca\(^{2+}\) transients or a shift in myofilament Ca\(^{2+}\) sensitivity to higher [Ca\(^{2+}\)]. Direct quantitation of Ca\(^{2+}\) transients is required to distinguish between these two possibilities.

It has previously been impossible to determine Ca\(^{2+}\) transients in stunned myocardium, because the methods available to measure [Ca\(^{2+}\)] were not suitable for perfused hearts. Recently, a new approach has been developed that enables the determination of [Ca\(^{2+}\)] at various times during the cardiac cycle in isolated perfused hearts using gated nuclear magnetic resonance (NMR) spectroscopy12 and the NMR-detectable Ca\(^{2+}\) indicator 5F-BAPTA, the 5,5′-difluoro derivative of 1,2-bis-(2-aminophenoxy)-ethane-N,N,N′,N′′-tetraacetic acid.13,14 We have used this approach to determine whether Ca\(^{2+}\) transients are decreased in stunned myocardium induced by reperfusion after 15 minutes of global ischemia.

**Materials and Methods**

The experimental conditions were designed to reproduce as closely as possible those in our previous study of the pathophysiology of globally stunned myocardium,6 with various modifications for the measurement of [Ca\(^{2+}\)]. Briefly, hearts of male ferrets (10–14 weeks of age) were isolated and perfused at 30°C with a solution of the following composition (mM): NaCl 108, KCl 5, MgCl\(_2\) 1, HEPES 5, CaCl\(_2\) 2, glucose 10, and sodium acetate 20. The pH was adjusted to 7.4, and the perfusate was bubbled continuously with 100% O\(_2\). After 10–20 minutes of stabilization, coronary flow rate (controlled by a peristaltic pump) was adjusted such that perfusion pressure equaled 80–90 mm Hg. Once adjusted, the flow rate was kept constant throughout the experiment (except during global ischemia, when the pump was shut off and the perfusion line was clamped). A latex balloon tied to the end of a polyethylene tube was passed into the left ventricle through the mitral valve and connected to a Statham P23DB pressure transducer (Gould Instruments, Indianapolis, Indiana). Left ventricular pressure and perfusion pressure were recorded with a chart recorder. The balloon was filled with an aqueous solution of 1 mM 6-fluorotryptophan to an initial end-diastolic pressure of 8–12 mm Hg and then kept isovolumic throughout the experiment. Unless otherwise noted, heart rate was maintained at 0.8–1.4 Hz by right ventricular pacing.

As previously described,12,15 5F-BAPTA was loaded into cells by perfusion with its tetraacetyl-methyl ester derivative, 5F-BAPTA-AM. In this study we added 12.5 μM 5F-BAPTA-AM (Lot 8A, Molecular Probes, Eugene, Oregon) to the perfusate for 15–17 minutes to achieve a favorable signal-to-noise ratio in a 5-minute 19F NMR spectrum. The perfusate was then switched to control solution supplemented by 1 mM probenecid (Sigma Chemical, St. Louis, Missouri) to minimize the time-dependent extrusion of 5F-BAPTA from myocardial cells.12,16

**Fluorine and Phosphorus NMR Methods**

The methods of measuring Ca\(^{2+}\) transients in whole hearts using gated 19F NMR are described in detail in the accompanying article in this journal17 and elsewhere.12 All chemical shifts were referenced with respect to the 6-fluorotryptophan signal, assigned to 0 ppm, [Ca\(^{2+}\)], is calculated according to the equation [Ca\(^{2+}\)] = K\(_{d}\)·([B]/[F]), where [B] is the area under the calcium-bound 5F-BAPTA peak near 8 ppm and [F] is the area under the calcium-free 5F-BAPTA peak near 2 ppm (see Figures 5A and 5B).13,15 We have used a dissociation constant for calcium-bound 5F-BAPTA (K\(_{d}\)) of 285 nM to calibrate our signals (see Marban et al17 for details).

Minimally saturated 31P NMR spectra were obtained12 at a spectral width of 6 kHz using 34° pulses delivered at 2-second intervals (145.8 MHz for 31P). The spectra were processed as described before.18 The amounts of inorganic phosphate (Pi), phosphocreatine (PCr), and ATP in the myocardium were obtained by planimetry of the areas under individual peaks using a digitizer. The tissue contents of Pi, PCR, and ATP were expressed as percent of the area under the ATP peak during the initial control period in each individual experiment.19

**Experimental Design**

Experiments were performed in a total of 11 hearts. Of these, seven were dedicated to the measurement of Ca\(^{2+}\) transients using 19F NMR; 31P NMR spectra were obtained in the remaining four hearts.

Figure 1 illustrates the experimental design used to measure Ca\(^{2+}\) transients before and after ischemia. The bottom row shows the time course of developed pressure in a typical experiment. The figure begins just after loading with 5F-BAPTA, when [Ca\(^{2+}\)] was increased from 2 to 8 mM to antagonize the calcium-buffering effect of 5F-BAPTA. The temperature was initially set at 30°C to minimize the slow extrusion of 5F-BAPTA that can occur at higher temperatures.15 After developed pressure reached steady state, gated 19F spectra were collected at sufficient temporal resolution to define clearly end-diastolic and peak systolic [Ca\(^{2+}\)]. The temperature was then increased to 37°C, and the heart was subjected to 15 minutes of total global ischemia (coronary flow was decreased to...
FIGURE 1. Contractile state before and after ischemia in an isolated, perfused ferret heart loaded with 5F-BAPTA. Upper panel: Experimental procedure. \([\text{Ca}]_o\), calcium concentration in perfusate; \(T\), temperature; \(CF\), coronary flow rate. Lower panel: Left ventricular developed pressure (DP) during the experiment. Measurement of calcium transient using gated \(^{19}\text{F}\) nuclear magnetic resonance (NMR) spectroscopy was performed before and after ischemia as indicated above the DP record.

0 ml/min). This temperature was maintained during ischemia to mimic as closely as possible the conditions during myocardial stunning in situ, as in our previous characterizations of contractile activation in hearts not containing a \(\text{Ca}^{2+}\) indicator.\(^6\,^{20}\) After 5 minutes of reperfusion at 37°C, the temperature was returned to 30°C. To define the \(\text{Ca}^{2+}\) transient in stunned myocardium, another set of gated \(^{19}\text{F}\) spectra was obtained in the reperfused heart when developed pressure had recovered to a new steady state (about 20 minutes). To mimic the conditions of our previous study,\(^6\) pacing was discontinued during ischemia and for the first 20 minutes of reperfusion and then restarted at the same rate used before ischemia.

Phosphorus NMR spectra were measured in four hearts with a similar protocol, except that these spectra were not gated: cyclical oscillations of phosphorus metabolites do not occur in ferret hearts,\(^12\) so that no additional information would have been obtained by gating.

**Statistical Analysis**

Data are presented as mean±SEM. Statistical analysis was performed using paired \(t\) test.\(^{21}\) A value of \(p<0.05\) was considered significant.

### Results

**Effect of 5F-BAPTA on Contractile Manifestations of Stunning**

In the accompanying article in this journal,\(^17\) we found that 5F-BAPTA was not protective against stunning when the perfusate contained 8 mM \([\text{Ca}]:\) after 20 minutes of ischemia at 30°C, developed pressure decreased by 50% compared with preischemic levels. The ischemic protocol was different in the present study, so that we again checked whether the degree of functional recovery in the present group of hearts containing 5F-BAPTA was comparable with that observed in the absence of the indicator. The single experiment shown in Figure 1 already hints that functional recovery is far from complete: developed pressure measured during reperfusion was about 40% lower than before ischemia.

Figure 2 shows pooled data comparing the percent recovery of developed pressure and end-diastolic pressure in 11 hearts loaded with 5F-BAPTA (the present study, panel B) and in hearts containing no \(\text{Ca}^{2+}\) indicator and perfused with 2 mM \([\text{Ca}]_o\).
phosphorus nuclear magnetic resonance spectra (5-minute acquisition time) measured in a 5F-BAPTA–loaded heart before, during, and after ischemia. Panel A: Initial control phase at 37°C. Panel B: Global ischemia (10–15 minutes) at 37°C. Panel C: Reperfusion (15–20 minutes) at 30°C. Myocardial contents of inorganic phosphate (Pi), phosphocreatine (PCr), and ATP were evaluated from the individual peaks indicated in panel A.

Metabolic Changes During and After Ischemia

Stunned myocardium is characterized by mild to moderate ATP depletion; other substrates and products of energy metabolism change markedly during ischemia but return to normal levels soon after reflow. The hearts in the present protocol were no exception. Figure 3 illustrates the changes in 31P spectra observed during ischemia and after reperfusion in a heart loaded with 5F-BAPTA. During ischemia (Figure 3B), P, increased in parallel with the disappearance of PCr and a much less dramatic decrease of ATP. Intracellular pH (pH,) also decreased from 7.16 in Figure 3A to 6.46 in Figure 3B. After reperfusion, the changes in P, PCr, and pH, all reversed fully, but the partial depletion of ATP persisted. This pattern was quite similar to that in myocardium not loaded with 5F-BAPTA (compare with Figure 6 in Reference 6). The pooled data for [P], [PCr], [ATP], and pH, in Figure 4 emphasize the consistency of these changes.

Taken together, the incomplete functional recovery and the characteristic metabolic picture indicate that rather ordinary stunning occurs here despite the unusual conditions required to obtain stable measurements of Ca2+ transients before and after ischemia.

Calcium Transients in Stunned Myocardium

Figure 5 shows typical gated 19F NMR spectra taken at end diastole (panel A) and peak systole (panel B) before and after ischemia. Note first the spectra taken before ischemia (left column). In the diastolic spectrum (panel A), the area under the free peak (~2 ppm) is visibly larger than that under the bound peak (~8 ppm), signifying that diastolic [Ca2+] is low. During systole (panel B), the bound peak increases substantially while the free peak decreases, reflecting a sizable increase in [Ca2+]. Gated spectra from the same heart after reperfusion (right column) again show a striking difference between the two extremes of the cardiac cycle: the ratio of the areas under the two peaks ([B]/[F]) is much greater during systole than at end diastole. If we next make a side-to-side comparison of the spectra, we see that the predominance of the bound peak is, if anything, accentuated during reperfusion. Thus, inspection of the raw signals gives no reason to expect that the availability of activator Ca2+ is decreased in stunned myocardium.

This possibility can be assessed most easily by comparing the values for [Ca2+] derived from these and other spectra before and after ischemia, plotted in Figure 5C as a function of the time from the pacing stimulus. Before ischemia (Figure 5C, left), [Ca2+] in this particular heart increased from 0.08 μM before the stimulus to a peak of 0.65 μM in early systole. The plot of the Ca2+ transient during reflow (Figure 5C, right) confirms our suspicion that [Ca2+] has increased, particularly during systole, when [Ca2+] now reaches values as high as 1.57 μM. Diastolic [Ca2+] increased modestly in this heart to 0.22 μM after ischemia. The overall amplitude of the Ca2+ transient (i.e., systolic [Ca2+] minus diastolic [Ca2+]) was paradoxically larger in the stunned heart than in the control heart, although developed pressure fell in a typical manner in this experiment (from 45 mm Hg before ischemia to 31 mm Hg afterward).

The paradoxical increases in systolic [Ca2+] and in the amplitude of the Ca2+ transient after stunning
were observed quite consistently. Figure 6 summarizes the data: the slight increase in diastolic [Ca\textsuperscript{2+}], from 0.18±0.03 to 0.24±0.03 μM was not significant (p>0.10), but [Ca\textsuperscript{2+}], at peak systole did increase (0.61±0.06 μM in control vs. 1.03±0.13 μM in stunned hearts; p<0.02). Concomitantly, the amplitude of Ca\textsuperscript{2+} transients in stunned myocardium was significantly higher than that before ischemia. We also ascertained whether the time course of the Ca\textsuperscript{2+} transients was altered. Neither the average time-to-peak [Ca\textsuperscript{2+}], (93±7 msec in control vs. 104±9 msec after ischemia; p>0.40) nor the time constant of the declining phase of the Ca\textsuperscript{2+} transient (40±5 msec in control vs. 36±9 msec after ischemia; p>0.70) was changed, thus simplifying the interpretation of the results.\textsuperscript{22} These findings demonstrate that contractile failure during reflow is not due to a critical failure of any of the mechanisms that regulate cytoplasmic activator Ca\textsuperscript{2+}.

**Implications of the Disparity Between Ca\textsuperscript{2+} Transient Amplitude and Developed Pressure**

The increase in the amplitude of Ca\textsuperscript{2+} transients is in the direction opposite to which would help explain the decrease of developed pressure in stunned myocardium. Despite a larger chemical trigger, the myofilaments generate less force after reflow. This dissociation between [Ca\textsuperscript{2+}], and contractile force indicates there must be a decrease in the responsiveness of the myofilaments to activator Ca\textsuperscript{2+}. The new information regarding the amplitude of Ca\textsuperscript{2+} transients now allows us to dissect out the individual components of this decrease in myofilament Ca\textsuperscript{2+} responsiveness. As summarized in the introduction, this could either represent a shift in the range of activation (myofilament Ca\textsuperscript{2+} sensitivity) or a decrease in the amplitude of the maximal response (maximal Ca\textsuperscript{2+}-activated pressure), or both.
Table 1 summarizes the values for developed pressure and Ca\(^{2+}\) transient amplitude in control and after stunning. Assuming there is no active force generation in diastole, we calculated the slopes for the apparent relation between developed pressure and \([\text{Ca}^{2+}]\). If a decrease in Ca\(^{2+}\) transients had been the sole cause of stunning, the slopes should have been comparable: a given increment in \([\text{Ca}^{2+}]\), should have produced a similar rise in twitch pressure.\(^{22}\) Instead, we find that the slope of the relation in stunned myocardium is much lower than that in control \((p<0.05)\). Nevertheless, we know from previous work in our laboratory⁶ that maximal Ca\(^{2+}\)-activated pressure is decreased in stunned myocardium. Can the decrease in maximal Ca\(^{2+}\)-activated pressure account entirely for the decrease in myofilament Ca\(^{2+}\) responsiveness in stunned myocardium? When the two slopes were normalized by the corresponding maximal Ca\(^{2+}\)-activated pressures (MCAPs) measured previously (270 mm Hg in control, 216 mm Hg in stunned hearts), the difference was still significant \((21\pm6 \% \text{MCAP}/\mu\text{M in control, } 9\pm3 \% \text{MCAP}/\mu\text{M in stunned hearts}; p<0.05)\). These results indicate that contractile failure in stunned myocardium is due to a decrease in the myofilament sensitivity to Ca\(^{2+}\) as well as to the previously identified decrease in maximal Ca\(^{2+}\)-activated force.

Discussion
What Goes Wrong in Excitation-Contraction Coupling After Ischemia?

Most hypotheses regarding the pathogenesis of contractile dysfunction focus on a specific initial mechanism while ignoring the convergent downstream links in contractile activation. Having determined three fundamental end points of excitation-contraction coupling, we now cast doubt on the entire category of hypotheses that imply that stunning is due to a decrease in cytoplasmic activator Ca\(^{2+}\). Instead, our findings suggest that the focus in stunned myocardium should be shifted to determination of the proximate cause of the abnormally low Ca\(^{2+}\) responsiveness of the contractile proteins. Both components of myofilament Ca\(^{2+}\) responsiveness, maximal Ca\(^{2+}\)-activated force⁶ and myofilament Ca\(^{2+}\) sensitivity (present study), are abnormally low in the stunned heart. The combined effect of these two
Factors are severe enough to more than neutralize the observed increase in \( \text{Ca}^{2+} \) transients.

The present finding that systolic \([\text{Ca}^{2+}]_i\), is increased in stunned myocardium has escaped detection in previous estimates of time-averaged \([\text{Ca}^{2+}]_i\) in reperfused hearts.\(^{15,17}\) This insensitivity of the time-averaged measurements is not surprising, given our finding that the time during which \([\text{Ca}^{2+}]_i\) is much higher than the diastolic level comprises only a fraction of the duration of an entire cardiac cycle (Figure 5C). Thus, a doubling of peak \([\text{Ca}^{2+}]_i\), with an unchanged diastolic \([\text{Ca}^{2+}]_i\), would produce at most a 20% increase in the true time-averaged \([\text{Ca}^{2+}]_i\), when heart rate is constant, a difference that could easily be missed in the estimates of time-averaged \([\text{Ca}^{2+}]_i\). The insensitivity of the time-averaged measurements is compounded by the properties of the indicator: SF-BAPTA can only give a lower-limit estimate for \([\text{Ca}^{2+}]_i\), when there is underlying spatiotemporal \( \text{Ca}^{2+} \) inhomogeneity.\(^{17}\) Thus, the observed changes in \( \text{Ca}^{2+} \) transients in this study are not discrepant with previous observations; indeed, they serve to emphasize the importance of time-resolved measurements of \([\text{Ca}^{2+}]_i\), in the investigation of mechanisms of contractile dysfunction.

Our observation that a reduction of \( \text{Ca}^{2+} \) transients cannot explain stunned myocardium agrees well with recent results from aequorin-injected papillary muscles subjected to simulated ischemia.\(^{23}\) Contractile recovery after such an intervention is substantially impaired despite the fact that \( \text{Ca}^{2+} \) transients are not decreased in amplitude or in absolute magnitude. These results are technically complementary to ours: aequorin does not buffer \( \text{Ca}^{2+} \), and its potential errors in the estimation of \([\text{Ca}^{2+}]_i\) are opposite those of SF-BAPTA.\(^{24,25}\) The agreement between the two very different models provides reassurance for the validity of the central observation in the present study.

### Table 1. Relation Between Developed Pressure and \( \text{Ca}^{2+} \) Transient Amplitude Before and After Ischemia

<table>
<thead>
<tr>
<th>Condition</th>
<th>( \Delta[\text{Ca}^{2+}]_i ) (( \mu \text{M} ))</th>
<th>Slope (mm Hg/( \mu \text{M} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.43±0.06</td>
<td>57±17</td>
</tr>
<tr>
<td>Stunned</td>
<td>0.78±0.12</td>
<td>20±7</td>
</tr>
</tbody>
</table>

Values are mean±SEM. DP, developed pressure; \( \Delta[\text{Ca}^{2+}]_i \), \( \text{Ca}^{2+} \) transient amplitude. The slopes are an index of the \( \text{Ca}^{2+} \) responsiveness of the myofilaments.

### Mechanism of the Decrease in Myofilament \( \text{Ca}^{2+} \) Responsiveness

The precise mechanism of the decrease in myofilament \( \text{Ca}^{2+} \) responsiveness remains to be determined. Unlike the situation during ischemia or hypoxia, \( \text{pH} \), and \( P \), are normal in stunned hearts; the diminished \( \text{Ca}^{2+} \) responsiveness cannot be due to the well-known effects of either of these metabolites on the myofilaments.\(^{18,26}\) The observed ATP depletion is not nearly severe enough to affect the myofilaments directly.\(^{27}\) nor is the free energy that can be derived from ATP hydrolysis compromised.\(^{28}\) Recent preliminary characterization of the myofibrillar ATPase from stunned hearts suggests that the \( \text{Ca}^{2+} \) dependence of ATP hydrolysis is unaffected.\(^{29}\) If confirmed, this latter observation would point to a downstream effect on the \( \text{Ca}^{2+} \) sensitivity of the force-generating elements.

In principle, the decrease in myofilament \( \text{Ca}^{2+} \) sensitivity might be functional rather than static. If triggered \( \text{Ca}^{2+} \) oscillations were to occur in the reperfused heart, in analogy with calcium-overload states such as digitalis toxicity, such oscillations would lead to incoordinate contraction and a decrease in overall pressure development.\(^{30}\) Our finding that diastolic \([\text{Ca}^{2+}]_i\), is not significantly elevated argues against this possibility, although our estimates of \([\text{Ca}^{2+}]_i\), would be biased downward if there were underlying spatial \([\text{Ca}^{2+}]_i\), inhomogeneity.\(^{17}\) Real-time, spatially localized \( \text{Ca}^{2+} \) measurements will be required to assess the role of diastolic \([\text{Ca}^{2+}]_i\), oscillations in posts ischemic myocardium.

A number of lines of evidence suggest that the decrease in myofilament \( \text{Ca}^{2+} \) responsiveness is a lingering aftereffect of the transient increase in \([\text{Ca}^{2+}]_i\), during ischemia and early reflow. First of all, our previous measurements of time-averaged \([\text{Ca}^{2+}]_i\), have shown that such an increase occurs as early as 10–15 minutes of total ischemia and persists during the early moments of reflow.\(^{17}\) Reperfusion with solutions of low \( [\text{Ca}] \) improves functional recovery,\(^{6,31}\) as does the induction of intracellular acidosis during the initial stage of reflow.\(^{20}\) Both maneuvers would tend to decrease the amount of calcium that binds to intracellular sites during reperfusion. Perhaps the most telling evidence implicating calcium comes from our previous observation that transient calcium overload even in the absence of ischemia mimics stunning physiologically, metabolically, and histologically.\(^{32}\) Calcium overload can activate protein kinases in myocardium.\(^{6,33,34}\)
and possibly induce changes in Ca\(^{2+}\) sensitivity and/or maximal Ca\(^{2+}\)-activated force through phosphorylation of one or more of the contractile proteins. Although this is a very plausible mechanism for stunning, no evidence for or against such changes in the myofilaments has yet been reported in response to Ca\(^{2+}\)-induced phosphorylation. Covalent modification of contractile proteins by Ca\(^{2+}\)-activated proteases also merits future consideration. Such modification would help explain why stunning reverses over several days, a time course consistent with the expected rate of de novo protein synthesis.\(^3\)

**Functional and Energetic Implications**

It is worth considering whether the observed changes in Ca\(^{2+}\) transients are likely to be adaptive or maladaptive. Conventional wisdom might dictate that stunning represents a state of decreased energy demand that “rests” the myocardium after a bout of ischemia. Although the observed increase in Ca\(^{2+}\) transients tends to preserve function by offsetting partially the decreased Ca\(^{2+}\) responsiveness of the myofilaments, the cycling of Ca\(^{2+}\) by the cell exacts its own cost. Excitation-contraction coupling consumes a large fraction of the energy used by the heart\(^36\); this fraction is even greater during maneuvers, such as exposure to catecholamines, that are known to increase Ca\(^{2+}\) transients.\(^36,37\) Thus, the increase in Ca\(^{2+}\) transients is adaptive in that it minimizes the degree of contractile impairment, but it is possibly maladaptive in that additional energy must be spent in the cycling of intracellular Ca\(^{2+}\). Maneuvers such as \(\beta\)-adrenergic stimulation\(^3-5\) that offset the decrease in force in stunned myocardium probably do so by increasing Ca\(^{2+}\) transients\(^5\) and would thus be expected to aggravate the potential imbalance in energy metabolism.

The observation that Ca\(^{2+}\) transients are increased certainly helps explain the reported inefficiency of energy utilization in stunned myocardium.\(^38,39\) Oxygen consumption in stunned myocardium is much higher than in control when normalized for the degree of force generation.\(^40,41\) Preliminary calculations reveal that the free energy change available from ATP hydrolysis is actually increased in stunned myocardium despite the decrease in force generation.\(^28\) These results suggest that either energy transfer to contraction or energy consumption in noncontractile work is inappropriately high in stunned myocardium. The energy demands imposed by the cycling of increased intracellular Ca\(^{2+}\) imply that at least the latter mechanism contributes to the decreased efficiency of energy utilization in stunned myocardium.

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