Increased cross-bridge cycling rate in stunned myocardium

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Gao, Wei Dong, Tieying Dai, and Daniel Nyhan. Increased cross-bridge cycling rate in stunned myocardium. Am J Physiol Heart Circ Physiol 290: H886–H893, 2006. First published September 19, 2005; doi:10.1152/ajpheart.00493.2005.—Decreased Ca$^{2+}$ responsiveness of the myofilaments underlies myocardial stunning. Given that cross-bridge cycling is a major determinant of myofilament behavior, we quantified cross-bridge cycling rate in stunned myocardium. After stabilization, rat hearts were subjected to 20 min of no-flow global ischemia and 30 min of reperfusion at 37°C. Control hearts were perfused continuously at 37°C for 60 min. Trabeculae were dissected and chemically skinned with 1% Triton X-100. The muscles were then activated with solutions of varied Ca$^{2+}$ concentration ([Ca$^{2+}$]). Force-[Ca$^{2+}$] relations, rate of force redevelopment after release ($k_m$), muscle stiffness ($k_a$), and myofilament ATP consumption were determined. Maximal Ca$^{2+}$-activated force ($F_{\text{max}}$) was depressed in stunned myocardium (49 ± 5 vs. 82 ± 5 mN/mm$^2$, P < 0.01). Western immunoblotting showed degradation of troponin I in stunned myocardium. The $k_m$ at $F_{\text{max}}$ was significantly increased in stunned muscles (19.82 ± 2.74 vs. 13.19 ± 0.96 s$^{-1}$, 22°C, $P < 0.01$; 7.49 ± 0.52 vs. 5.81 ± 0.54 s$^{-1}$, 10°C, $P < 0.05$). The ratio of $k_m$ measured at 100 Hz over that at 1 Hz, during $F_{\text{max}}$, is lower in stunned muscles (8.22 ± 1.56 vs. 12.94 ± 0.71, $P < 0.05$). In comparison with $k_m$ at rigor, $k_m$ at $F_{\text{max}}$ is significantly lower in the stunned group (78.82 ± 6.11 vs. 93.27 ± 3.03%, $P < 0.05$). Myofilament ATP consumption at $F_{\text{max}}$ did not change in stunned muscles (5.901 ± 952 vs. 5.596 ± 972 pmol·μl$^{-1}$·min$^{-1}$, $P = 0.49$). These results show that cross-bridge cycling is increased in stunned myocardium. Such increases are likely the result of increased transition rate from force-generating states to non-force-generating states. Thus stunned myocardium still maintains ATP consumption in spite of lower force development, rationalizing the long-standing paradox of decreased force but unchanged oxygen consumption in the postischemic heart. Increased cross-bridge cycling; contraction; myocardial stunning
redevelopment \((k_m)\) to determine the rate of cross-bridge cycling. We found that \(k_m\) increased significantly in stunned muscles. We also performed muscle stiffness \((k_m)\) analysis by imposing sinusoidal length perturbations at 1 and 100 Hz and found that the ratio of \(k_m\) at 100 Hz over 1 Hz was reduced in stunned muscles compared with controls. Also in stunned muscles, \(k_m\) during maximal activation was lower than the stiffness during rigor, in which all cross bridges are expected to remain attached because of the absence of ATP. Myofilament ATP consumption was not different in stunned muscles compared with controls. These results show that there is an increased cross-bridge cycling rate in stunned myocardium. Stunned myocardium maintains ATP consumption in spite of decreased force development, likely because of an increased rate of cross-bridge turnover from force-generating states to non-force-generating states.

**MATERIALS AND METHODS**

**Rats.** Male LBN-F1 (~250–300 g body wt, \(n = 30\)) rats were used in these experiments. The care of the animals and the experiment protocol were approved by the Animal Care and Use Committee of The Johns Hopkins University.

**Whole hearts.** The hearts were exposed via midsternotomy after the animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (100 mg/kg). 0.3–0.5 ml (300–500 U) heparin was injected in the left atrium, and the aorta was clamped. The heart was then rapidly excised, and the aorta was cannulated. The hearts were perfused retrogradely (~15 ml/min) with Krebs-Henseleit (K-H) solution equilibrated with 95% O2–5% CO2. The K-H solution is composed of (in mmol/l): 120 NaCl, 20 NaHCO3, 5 KCl, 1.2 MgCl, 10 glucose, and 1.0 CaCl2, pH 7.35–7.45. The hearts were beating at 270–300 beats/min. Isovolumic left ventricular pressure was measured with an intracavity balloon filled with water and connected to a pressure transducer. The volume of the balloon was adjusted to a diastolic pressure of ~10 mmHg, which was kept constant throughout the experiments. The hearts were placed in a water-jacketed container to maintain a constant temperature of 37°C. All hearts were perfused at constant flow (15 ml/min). An implantable temperature probe was put inside the ventricle, and the temperature was kept at 37°C. After stabilization, the hearts were divided into the following groups. In control group, the hearts were perfused continuously for 60 min. In stabilization, the hearts were divided into the following groups. In put inside the ventricle, and the temperature was kept at 37°C. After ischemia, the hearts were reperfused for 30 min. After control group, the hearts were perfused continuously for 60 min. In

**Measurement of myofilament ATP consumption.** Isometric ATPase activity was measured using the following coupled enzyme system (15), which was later adopted by Wannenburg et al. (37) in cardiac trabeculae (37):

1. pyruvate kinase converts: ADP + phosphoenolpyruvate → ATP + pyruvate;
2. lactate dehydrogenase converts: pyruvate + NADH + H+ → NAD+ + lactate.

In this coupled system, ATP is added to fuel contraction. ADP thus produced is converted back to ATP by the oxidation of NADH to NAD. Also in this system, one mole of NADH is converted to NAD for one mole of ATP to ADP. Because NADH fluoresces at 470 nm (emission) under ultraviolet radiation at 340 nm (excitation), decreases in fluorescence at 340 nm excitation in the solution reflects a reduction in NADH amount in the solution and thus the rate of ATP hydrolysis by the myofilaments (i.e., ATPase activity). Pyruvate kinase and lactate dehydrogenase, and their substrates (NADH and phosphoenolpyruvate), were added to both relaxing and activating solutions. To allow better diffusion into myofibrils, muscles were bathed in relaxing solution containing these substrates for a few minutes before the next activation. The concentrations of these substrates in both relaxing and activating solutions were as follows: 0.6 mmol/l NADH, 10 mmol/l phosphoenolpyruvate, 100 U/ml pyruvate. The steady state force-[Ca2+] relations were fit with a function of the Hill equation

\[
F = F_{\text{max}}[\text{Ca}^{2+}]^{t} / (C_{\text{Ca}^{2+}} + [\text{Ca}^{2+}]^{t})
\]

(1)

where \(F\) is the steady-state force at varied \([\text{Ca}^{2+}]\) values, \(F_{\text{max}}\) is the maximal \([\text{Ca}^{2+}]\)-activated force, \(C_{\text{Ca}^{2+}}\) is the \([\text{Ca}^{2+}]\) required for 50% of maximal activation, and \(n\) is the Hill coefficient.

**Western immunoblotting of troponin I.** In separate experiments, frozen (~80°C) tissue samples (100–200 mg) from control and stunned hearts were ground in liquid nitrogen and decanted into preweighed ice-cold Eppendorf tubes with lysozyme buffer (6.0 mmol/l urea, 2.0 mol/l thiourea, 4% CHAPS, and 40 mmol/l Tris, pH 8.8). The mixture (10% wt/vol) was homogenized by sonication and centrifuged at 12,000 rpm for 40 min at 4°C. The supernatant was collected. Protein contents were determined with the Bio-Rad Protein Assay Kit. Myofilament proteins separated by SDS-PAGE were transferred to a nitrocellulose membrane. After being blotted, the membrane was incubated with anti-Tnl (31-35; epitope 175–189 fragment, dilution 1:30,000; Spectral Diagnostic) for 1 h at room temperature with gentle agitation. After being washed, the membrane was probed with anti-mouse IgG (1:30,000) conjugated to horseradish peroxidase (HRP). HRP activity was visualized with Super Signal Chemiluminescent Substrate (Pierce) for 5 min, and the membranes were exposed to X-ray film (KODAK BioMax XAR Film) for 1–120 s to detect the blot.

**Mechanical measurement of cross-bridge kinetics.** The quick release/restretch protocol was used to measure \(k_m\). The skinned muscle was mounted between a force transducer and a motor arm via aluminum foil T-clips, the arms of which were folded and cramped directly on both ends of the muscle. The muscle length was decreased by a rapid release step (20% of muscle length) to drop force to zero in 1 ms, held for 25 ms, and then stretched back to the original length. The redeveloped force was recorded. These experiments were performed at both 10 and 22°C. The redeveloped force was fitted with the following single exponential equation

\[
F_t = F_{\text{max}}(1 - e^{-kt})
\]

(2)

where \(F_t\) is the level of force redevelopment at any time \(t\) after release and \(k\) is \(k_m\).
CROSS-BRIDGE CYCLING IN STUNNED MYOCARDIUM

kinase, 12 U/ml lactate dehydrogenase, 0.2 mmol/l A2P5, and 100 
mmol/l leupeptin. To ensure adequate mixing of all substrates, the 
volume of the bath was limited to 100 µl, and the solution was stirred 
by air bubbles from a microneedle placed at one end of the bath. The 
small disturbance in the solution allows adequate mixing but does not 
interfere with fluorescent measurement. Calibration was performed 
separately, using ADP (10 nmol/l) that was manually injected in the 
bath containing the coupled enzyme system. The step drop in fluo-
rescence (in mV) after ADP injections was measured and was later 
used to calculate ATPase conversion (decrease in mV was used as the 
denominator). In most of our experiments, ATPase measurements 
were performed only during maximal activations. We therefore in-
jected ADP in the same maximal activating solution after removing 
the muscle and calculated ATPase activity based on the drop for that 
muscle. We also injected ADP in the bath containing the coupled 
enzyme system during rest. We pooled the data of the injections and
determined that the step drop of fluorescence after 10 nmol/l ADP was 6.5 ± 0.3 (SD) mV in <20 s (n = 7). Thus, in our system, there were 
only small variations in the step drops after ADP injection across 
experiments. The decreases of fluorescence at 340 nm were monitored 
during activations, and the rate of NADH decrease (and thus the rate 
of NADH concentrations of 0 – 400 nmol/l, a range over which our 
results were linearized. We also injected ADP in the same maximal activating solution after removing 
the muscle. We also injected ADP in the bath containing the coupled 
enzyme system during rest. We pooled the data of the injections and
determined that the step drop of fluorescence after 10 nmol/l ADP was 6.5 ± 0.3 (SD) mV in <20 s (n = 7). Thus, in our system, there were 
only small variations in the step drops after ADP injection across 
experiments. The decreases of fluorescence at 340 nm were monitored 
during activations, and the rate of NADH decrease (and thus the rate 
of NADH concentration and fluorescence intensity was nonlin-
erar. However, a linear relationship was determined in the range of 
NADH concentrations of 0–400 nmol/l, a range over which our 
experiments were performed.

Statistics. Student’s t-test and one-way ANOVA was used for 
statistical analysis of the data (32, 38). A value of P < 0.05 was 
considered to indicate significant differences between groups. Unless 
otherwise indicated, pooled data are expressed as means ± SE.

RESULTS

Force-[Ca2+] relations in skinned stunned muscles. Figure 
1 shows the pooled data from all muscles studied. In Fig. 1A 
the data are shown in absolute values, and in Fig. 1B the data 
are normalized to their maximal values. These results are 
consistent with our previous findings in stunned muscles (11). 
Fmax decreased significantly in stunned muscles (49 ± 5 vs. 
82 ± 5 mN/mm², P < 0.01). Ca₅₀ increased in stunned 
muscles (1.68 ± 0.14 vs. 1.22 ± 0.14 μmol/l in control 
muscles, P = 0.04), with no changes in the Hill coefficient n 
(2.5 ± 0.41 in stunned muscles vs. 2.1 ± 0.19 in control 
muscles, P = 0.5). Thus stunned muscles showed decreased 
Ca²⁺ responsiveness.

Western immunoblots of TnI. The role of TnI degradation in the 
stunned myocardium is controversial. To determine if TnI 
degradation occurred in our experiments, we performed West-
ern immunoblot analysis of troponin I in tissues from hearts 
that underwent 20 min global ischemia followed by 30 min 
reperfusion. Figure 2 reveals a degradation band of TnI in 
stunned myocardium. Brief ischemia (20 min) alone did not 
cause TnI degradation. Similar results were obtained in three 
stunned hearts. These results are consistent with our previous 
studies (12), confirming that TnI is indeed partially and selec-
tively degraded in stunned myocardium.

Changes in ktr after stunning in skinned muscles. One of the 
major changes in stunned myocardium is the depression of 
Fmax (see Fig. 1A). We thus investigated whether altered 
kinetic behavior of cross bridges affected force development 
during maximal activation. Brenner (3, 4) showed that the 
turnover kinetics of the cross bridges is important in regulating 
force development. When the turnover rate of the attached 
cross-bridges changes, the force-Ca relationship changes. We 
performed quick-step releases during Fmax to assess changes in 
ktr, to probe the behavior of the cross bridges in stunned 
myocardium. Figure 3A shows the typical traces of force 
development after a quick-release step of 25 ms duration. 
After normalization, it is clear that the rate of force recovery 
is faster in stunned trabecula than control (Fig. 3, A and B, insets). 
These force redevelopment traces were fitted with a single 
exponential equation, and ktr values were determined. Figure 
3C shows the pooled data of ktr from both control and stunned 
muscles. The ktr increased significantly in stunned muscles 
compared with control muscles (19.82 ± 2.74 vs. 13.19 ± 0.96 
μmol/l, P < 0.02, temperature 22°C). We repeated these experi-
ments at 10°C to verify these changes (Fig. 3, B and D). 
Despite slowing of ktr as temperature drops, it remains 
accelerated in stunned muscles (7.49 ± 0.52 vs. 5.81 ± 0.54 s⁻¹, 
P < 0.05). We also performed quick-release experiments at

![Fig. 1. Force-Ca²⁺ concentration ([Ca²⁺]) relationships in control and stunned trabeculae. A: pooled data of force-[Ca²⁺] relations in control and stunned muscles. These muscle were skinned chemically with Triton (1%), and varied levels of forces were achieved with different [Ca²⁺] values. Steady-state forces were plotted against [Ca²⁺] values in both groups. Note that maximal Ca²⁺- 
activated force is decreased and [Ca²⁺] required for 50% of maximal activa-
tion is increased in stunned muscles (see text for details). B: normalized force-[Ca²⁺] relations in the two groups of muscles. Data were normalized to 
the maximal force in each group. A rightward shift of the force-[Ca²⁺] relations in stunned muscles is demonstrated (see text for details). Data are 
means ± SE; n = 8–10 experiments in each group.](http://ajpheart.physiology.org/)

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two submaximal \([Ca^{2+}]\) (Table 1). The \(k_t\) was not statistically different between control and stunned groups at these submaximal activations.

Because \(k_t\) is the sum of \(f_{app}\) (the rate constant for cross bridges from a non-force-generating state to a force-generating state) and \(g_{app}\) (the rate constant from a force-generating state to a non-force-generating state), we derived \(f_{app}\) and \(g_{app}\) based on \(k_t\) measured at maximal \([Ca^{2+}]\) and \(k_m\) at rigor and during \(F_{max}\) (see below). Table 2 shows the derived \(f_{app}\) and \(g_{app}\) in control and stunned muscles. Thus the rate for cross bridges from a force-generating state to a non-force-generating state is increased in stunned muscles.

\(k_m\) in stunned myocardium. In this study, we also determined \(k_m\) by use of sinusoidal muscle length perturbations at frequencies of 1 and 100 Hz. In an active muscle, \(k_m\), when measured at 100 Hz of length perturbations, increases as more cross bridges are attached, whereas \(k_m\) at 1 Hz remains nearly the same compared with a nonactivated muscle (19, 36). We have also shown previously that measurement of the \(k_m\) ratio (100 over 1 Hz) is a sensitive method to establish whether active cross-bridge cycling is present. In each individual muscle during \(F_{max}\), \(k_m\) was first measured at 1 Hz and then at 100 Hz, and the ratio of \(k_m\) at 100 Hz over 1 Hz was determined (Fig. 4). The ratio of \(k_m\) at 100 over 1 Hz is greater in control muscles compared with stunned muscles, indicating a higher number of cross bridges during \(F_{max}\) in control muscles.

Rigor defines a state when cross bridges are unable to detach when ATP is absent. During rigor, all cross bridges are “locked” and \(k_m\) is expected to be maximal. To determine

**Table 1.** \(k_t\) and myofilament ATPase activity in control and stunned muscles at submaximal activations

<table>
<thead>
<tr>
<th>[(Ca^{2+})] (\mu)mol/l</th>
<th>(k_t), s(^{-1})</th>
<th>ATPase, pmol·s(^{-1})·l(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Stunned</td>
<td>Control</td>
</tr>
<tr>
<td>3.67</td>
<td>4.47±0.46</td>
<td>6.34±1.05</td>
</tr>
<tr>
<td>8.68</td>
<td>10.45±0.76</td>
<td>15.05±2.05</td>
</tr>
</tbody>
</table>

Data are means ± SE; \(n = 4–6\) rat hearts in each group. [\(Ca^{2+}\)], \(Ca^{2+}\) concentration; \(k_t\), rate of force redevelopment after release. Temperature = 22 °C.
whether a decreased capability of forming cross bridges is responsible for the decreased \( F_{\text{max}} \), we induced rigor in stunned muscles by exposing the muscles to ATP-free activating solutions containing maximal \( Ca^{2+} \), and \( k_m \) was measured at 100 Hz. There is no change in \( k_m \) at rigor in stunned muscles (4,799.81 ± 544.83 vs. 5,385.58 ± 887.58 mN·mm⁻²·μm⁻¹, \( P = 0.49 \)). This indicates that the total number of cross bridges available for attachment is not different in stunned myocardium compared with control. However, the maximal active \( k_m \) during \( F_{\text{max}} \) decreased over 20% when comparing with \( k_m \) at rigor in stunned muscles (Fig. 5). In control muscles, \( k_m \) during \( F_{\text{max}} \) decreased ~7% in comparison with the \( k_m \) at rigor.

Myofilament ATP consumption in stunned myocardium. To complement the above findings, we assessed myofilament actomyosin-ATPase activity in separate groups of skinned muscles (Fig. 6). These measurements would add the analysis of cross bridge in stunned myocardium. Because the major change of \( Ca^{2+} \) responsiveness in stunned myocardium is depression of force development during maximal activations, we focused our measurement of myofilament ATP consumption at \( F_{\text{max}} \). Figure 7 shows the summary of the data. In spite of significant decreases in \( F_{\text{max}} \), myofilament ATP consumption was not reduced in stunned muscles. We also measured ATP consumption at two different submaximal activations, and there was no difference between control and stunned groups.
DISCUSSIONS

This study in stunned myocardium reveals increased $k_{tr}$, decreased $k_m$ during maximal activations with no changes in $k_m$ at rigor, and no changes in myofilament ATP consumption despite reduced maximal force development. These findings suggest that cross-bridge cycling rate is increased in stunned myocardium. The increased cross-bridge cycling results in decreased $F_{max}$ and maintained myofilament ATP consumption.

Cross-bridge cycling in stunned myocardium. We have previously modeled the cross-bridge behavior of stunned myocardium using a cross-bridge model with experimentally constrained rate constants (10). Using measured intracellular $[Ca^{2+}]$ transients as inputs, we predicted that decreased force production could be the result of decreased cross-bridge attachment rates and increased cross-bridge detachment rates. In this study, we assessed cross-bridge cycling directly using a quick-release method to determine $k_{tr}$.

Based on the current cross-bridge model (2), $k_{tr}$ obtained from the quick-release experiments (under conditions of low $P_i$ and maximal $Ca^{2+}$ activation) is the sum of $f_{app}$ and $g_{app}$. Also, force development depends on $f_{app}$

$$F = N \times F_o \times f_{app}/(f_{app} + g_{app})$$

(3)

where $N$ indicates the number of cross bridges available, $F_o$ indicates the mean force produced by each strongly attached cross bridge, and $f_{app}/(f_{app} + g_{app})$ represents the occupancy of force-generating states (2, 5). We have shown in this study that $k_{tr}$ increased significantly in stunned muscles (Fig. 3). Either increased $f_{app}$ or $g_{app}$, or both, would cause the increase in $k_{tr}$. However, an increase in $f_{app}$ would result in higher force development. On the other hand, increases in $g_{app}$ would predict a reduced occupancy of force-generating states of cross bridges and thus lower force development (Table 2).

The $k_{tr}$ did not change significantly at the submaximal activations in stunned muscles tested in this study. However, this does not necessarily mean cross-bridge cycling was not affected. Recent investigations have suggested that cooperative activation of the thin filament because of strong-bound cross bridges plays an important role in force redevelopment (6, 7), especially at submaximal activations. Thus $k_{tr}$ at submaximal $Ca^{2+}$ is not only dependent on $Ca^{2+}$ (4, 5) but also on cooperativity (6). More detailed experiments at submaximal $Ca^{2+}$ will be needed to define cross-bridge behavior at submaximal activations in stunned myocardium.

Changes in $k_m$ found in this study also support that the occupancy of cross bridges in force-generating states is lower (Table 1). ATPase activity at rest was not different between control and stunned groups (results not shown).
at maximal activation in stunned myocardium. The $k_m$ at rigor reflects all available cross bridges that are formed and locked in the attached states. Thus $k_m$ at rigor represents maximal stiffness under the same experimental conditions for the muscle. We have found that $k_m$ during $F_{\text{max}}$ is not different from the $k_m$ at rigor in control muscles. This suggests that the total number of cross bridges available for attachment and contraction is not different in stunned and control myocardium. However, $k_m$ during $F_{\text{max}}$ is 23% less than the $k_m$ at rigor in stunned muscles (Fig. 5), suggesting $>20\%$ less attached cross bridges during $F_{\text{max}}$. This could be caused by either reduced availability of the cross bridges (i.e., $N$ in Eq. 3) or reduced occupancy of the cross bridges in force-generating states (i.e., the total number of cross bridges engaged in cycling is not reduced but, as a result of increased $g_{\text{app}}$, the number of cross bridges at any given time is reduced). The fact that the $k_m$ at rigor is not different between control and stunned muscles indicates that stunned myocardium is capable of recruiting the same total number of cross bridges (i.e., the same $N$) as control, negating that a reduction in the number of cross bridges available for force generation is responsible for lower force development at maximal activation. Therefore, increased $g_{\text{app}}$ results in less $F_{\text{max}}$ in stunned myocardium. Our results show that $k_m$ during $F_{\text{max}}$ is $\sim93\%$ of rigor stiffness in control muscles. A similar high ratio of $k_m$ during $F_{\text{max}}$ at rigor has also been reported from other laboratories (30). One potential explanation for the high ratio is the formation of rigor-like cross bridges during $F_{\text{max}}$ (36). If this is true, we could have overestimated the $k_m$ during $F_{\text{max}}$ in stunned myocardium. This high ratio also limits our ability to conclude how much $g_{\text{app}}$ and $g_{\text{app}}$ have changed in stunned muscles based on Table 2.

This study, however, cannot rule out the possibility that the force generated by each individual cross bridge is reduced in stunned myocardium (i.e., lower $F_0$ in Eq. 3), thus causing decreased $F_{\text{max}}$. Some recent studies have focused on the structural alterations within the cross-bridge complex in stunned myocardium and have suggested decreased strains in the cross bridges formed in the presence of truncated troponin I (8). Direct evidence of decreased force production by each individual cross bridge is still lacking. Moreover, $k_u$ would not necessarily be affected when $F_0$ is decreased.

The results from this study contrast those of McDonald et al. (22), who found decreased unloaded shortening velocity in a porcine model of regional stunning. The discrepancy can be the result of a couple of factors: 1) different models of stunning can cause different changes in myofilaments and thus differences in cross-bridge behavior. For example, in the regional stunning in pigs, no proteolysis of troponin I is seen (17); 2) differences in experimental protocol (e.g., loaded vs. unloaded shortening, slack-length vs. rapid-release stretch back, cell segments vs. intact fibers) could account for the different observations.

**Myofilament ATP consumption in stunned myocardium.** We have measured myofibrillar ATP consumption during maximal activations and found that the myofibrillar ATP consumption remained unchanged compared with controls, whereas maximal force development decreased significantly (Fig. 7). Our findings are consistent with a number of other studies. It has long been recognized that stunned myocardium consumes more oxygen in spite of decreased contraction (18, 26). The increased $MV_\text{O}_2$ is independent of coronary flow and tissue oxygen extraction (31). A recent study has shown that oxygen consumption by processes involved in excitation-contraction coupling and by myofibrils increased significantly in stunned myocardium (33). Foster et al. (8) have provided more direct evidence that the myofibrillar ATPase activity was increased in stunned myocardium. Using COOH-terminal truncated cardiac troponin I (cTnI1–192) to reconstruct the troponin complex, they found that there is a $>50\%$ increase in Ca$^{2+}$-activated S1 ATPase activity, whereas force development was $\sim76\%$ compared with myofilaments composed of reconstructed full-length cTnI. The mechanism of maintained ATP utilization in spite of decreased force in stunned myocardium is not entirely clear. Increases in the rate of cross-bridge detachment, $g_{\text{app}}$, predict increased ATP usage since cross-bridge detachment occurs when ATP is bound to the actomyosin complex. However, at a molecular level, the nature of the increased cross-bridge detachment remains elusive. We did not calibrate ATPase activity during activations, and this is a limitation to our study. Given that accurate ATPase calibration may be altered by solution volume, light intensity, NADH signal, and basal ATPase activity, we could not completely rule out the contribution of these confounding variables contributing to quantitative changes in ATPase activity.

When viewed in the context of the three-states model of thin filament regulation of contraction (13, 24, 35), the increased ATP consumption in spite of decreased force is likely the result of destabilization of the strongly bound actomyosin intermediates of the cross-bridge cycle (i.e., during the open state). In the absence of Ca$^{2+}$, troponin stabilizes tropomyosin in a position that blocks myosin binding to actin (the blocked state). In the presence of Ca$^{2+}$, troponin shifts tropomyosin and allows myosin to bind actin (the closed state). In the presence of myosin binding, tropomyosin adopts a state that permits myosin to bind all exposed sites on actin and hydrolyzes ATP (the open state). It is conceivable that the states of tropomyosin are affected by the troponins. In stunned myocardium, the position of tropomyosin in the open state is likely altered such that the transition to other states is accelerated and the strongly bound cross bridges are not fully supported as a result of degradation of troponin I (12, 23, 34). Thus ATP consumption by the cross bridges would be maintained in spite of decreased force generation.

We conclude that cross-bridge cycling rate is increased in stunned myocardium. The increased cycling rate is caused by an increased rate of transition from force-generating states to non-force-generating states ($g_{\text{app}}$), resulting in lower force development. Myofilament ATP consumption remained unchanged in spite of decreased force development. These findings have important implications for our understanding and management of stunned myocardium clinically.

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REFERENCES