Inorganic phosphate speeds loaded shortening in rat skinned cardiac myocytes

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Hinken, Aaron C., and Kerry S. McDonald. Inorganic phosphate speeds loaded shortening in rat skinned cardiac myocytes. Am J Physiol Cell Physiol 287: C500–C507, 2004—Force generation in striated muscle is coupled with inorganic phosphate (P_i) release from myosin, because force falls with increasing P_i concentration ([P_i]). However, it is unclear which steps in the cross-bridge cycle limit loaded shortening and power output. We examined the role of P_i in determining force, unloaded and loaded shortening, power output, and rate of force development in rat skinned cardiac myocytes to discern which step in the cross-bridge cycle limits loaded shortening. Myocytes (n = 6) were attached between a force transducer and position motor, and contractile properties were measured over a range of loads during maximal Ca^{2+} activation. Addition of 5 mM P_i had no effect on maximal unloaded shortening velocity (V_{max}) (control 1.83 ± 0.75, 5 mM added P_i, 1.75 ± 0.58 muscle lengths/s; n = 6). Conversely, addition of 2.5, 5, and 10 mM P_i progressively decreased force but resulted in faster loaded shortening and greater power output (when normalized for the decrease in force) at all loads greater than ~10% isometric force. Peak normalized power output increased 16% with 2.5 mM added P_i, and further increased to a plateau of ~35% with 5 and 10 mM added P_i. Interestingly, the rate constant of force redevelopment (k_re) progressively increased from 0 to 10 mM added P_i, with k_re ~360% greater at 10 mM than at 0 mM added P_i. Overall, these results suggest that the P_i release step in the cross-bridge cycle is rate limiting for determining shortening velocity and power output at intermediate and high relative loads in cardiac myocytes.

DURING MUSCLE CONTRACTION myosin cross bridges cyclically interact with actin in a process that is driven energetically by hydrolysis of ATP. The chemomechanical states in the cross-bridge cycle have been investigated extensively in skinned muscle fiber preparations, and the transition rates between these states are qualitatively similar to rates derived from muscle protein studies in solution (15). A working model for the chemomechanical steps in the cross-bridge cycle is shown in Fig. 1 (40), which is based on extensive work in the field (for reviews see Refs. 3 and 15).

According to this model, the transition from weak-binding, non-force-generating cross bridges to strong-binding, force-generating states is associated with the release of inorganic phosphate (P_i) (steps 3–5, Fig. 1; Ref. 16). Force generation is maintained through the release of ADP (step 6, Fig. 1; Refs. 6 and 22), which is followed by binding of ATP and subsequent cross-bridge detachment. The binding of ATP and its hydrolysis are thought to be relatively fast processes in skinned fibers (9, 14, 23). Thus the rate-limiting step in the cross-bridge cycle is thought to occur after ATP hydrolysis, either during an isomerization of the actin-myosin ADP P_i state (step 3, Fig. 1), which is associated with the weak-to-strong binding transition, or an isomerization step of the actin-myosin ADP state just before ADP release (i.e., step 6, Fig. 1) and cross-bridge detachment (for reviews see Refs. 3 and 15).

Another factor in this cross-bridge schematic is the fact that transition rates vary as a function of load on the muscle, becoming faster as muscle load is reduced. For instance, the rate constant of force decline after rapid increase in solution P_i (k_p) varied linearly as a function of load (18). Thus the rate-limiting steps may be different as the load on the myofilaments changes. For instance, unloaded muscle shortening is likely limited by cross-bridge detachment rates, because cross bridges that remain bound ultimately become an internal load as filaments slide past each other. Thus the speed of shortening of an unloaded muscle is thought to reach its limit when compressive resistance forces equal positive forces (19). At the other intercept of the force-velocity curve, isometric force is ultimately determined by the number of force-generating cross bridges, which is limited by the balance between the rates of force-generating transitions and detachment of positively strained cross bridges. It is unknown which chemomechanical transitions are most important in determining force and shortening speeds and thus power output at intermediate loads where muscles perform work. Because elevations in P_i concentration ([P_i]; from 0 to ~15 mM) increase the rate of force development (2, 16, 37, 38) but have no effect on unloaded shortening velocity in skinned fast-twitch skeletal muscle fibers (4, 26, 42), experiments in the presence of increased [P_i] should indicate at which loads shortening velocities are most influenced by P_i transitional states, i.e., those that are associated with weak to strong-binding to force-producing states (steps 3–5, Fig. 1). Thus we investigated the effects of elevated [P_i] on loaded shortening and power output in permeabilized single cardiac myocytes to gain mechanistic insights into the cross-bridge steps that are most important in determining power output in cardiac myocytes, with particular interest in loads where power output is optimal because these are the loads that in vivo myocytes are thought to encounter during the ejection phase of the cardiac cycle.

METHODS

Cardiac myocyte preparation. Normal Sprague-Dawley rats were obtained from Harlan (Madison, WI), maintained, and killed humanely according to guidelines set by the Animal Care and Use Committee of the University of Missouri. Single skinned cardiac myocytes were obtained by mechanical disruption of hearts from Sprague-Dawley rats as described previously (24). Rats were anesthetized by inhalation of isoflurane (0.05 mg) for 2–4 min in an airtight 1-liter container, and their hearts were excised and rapidly placed in ice-cold relaxing solution. The ventricles were dissected
away from the atria, cut into 2- to 3-mm pieces, and further disrupted for 5–10 s in a Waring blender. The resulting suspension of cells was centrifuged for 65 s at 165 g, after which the supernatant was discarded. The myocytes were skinned by resuspending the pellet of cells for 3 min in 0.5% ultrapure Triton X-100 (Pierce Chemical) in relaxing solution. The skinned cells were washed twice with cold relaxing solution, resuspended in 10–15 ml of relaxing solution, and kept on ice during the day of the experiment. Myocytes were used within 12 h of isolation.

**Solutions.** The relaxing solution in which the ventricles were disrupted, skinned, and resuspended contained (in mM) 2 EGTA, 5 MgCl₂, 4 ATP, imidazole 10, and 100 KCl at pH 7.0. Compositions of relaxing and activating solutions used in mechanical measurements were as follows (in mM): 7 EGTA, 1 free Mg²⁺, 20 imidazole, 4 MgATP, 14.5 creatine phosphate, pH 7.0, Ca²⁺ concentrations of 10⁻⁹ M (relaxing solution) and 10⁻⁴ M (maximal activating solution), and sufficient KCl to adjust ionic strength to 180 mM. Activating solutions used in phosphate experiments were identical to those described above except for inclusion of 2.5, 5, and 10 mM KH₂PO₄ before adjustment to ionic strength of 180 mM. The final concentrations of each metal, ligand, and metal-ligand complex at 13°C were determined with a computer program (8). Immediately before activation, muscle preparations were immersed for 60 s in a solution of reduced Ca²⁺-EGTA buffering capacity, identical to normal relaxing solution except that EGTA was reduced to 0.5 mM. This protocol resulted in more rapid steady-state force development and helped preserve the striation pattern during activation.

**Experimental apparatus.** The experimental apparatus for physiological measurements of myocyte preparations was similar to one previously described in detail (28) and modified specifically for cardiac myocyte preparations (24). Briefly, myocyte preparations were attached between a force transducer and a torque motor by gently placing the ends of the myocyte into stainless steel troughs (25 gauge). The ends of the myocyte were secured by overlaying a 0.5-mm-long piece of 3.0 monofilament nylon suture (Ethicon) onto each end of the myocyte and then tying the suture into the troughs with two loops of 10.0 monofilament suture (Ethicon). The attachment procedure was performed under a stereomicroscope (approximately × 100 magnification) with finely shaped forceps.

Before mechanical measurements the experimental apparatus was mounted on the stage of an inverted microscope (model IX-70, Olympus Instrument), which rested on a pneumatic antivibration table with a cutoff frequency of ~1 Hz. Force measurements were made with a capacitance-gauge transducer (model 403, sensitivity of 20 mV/mg plus a ×10 amplifier and resonant frequency of 600 Hz; Aurora Scientific, Aurora, ON, Canada). Length changes during mechanical measurements were introduced at one end of the preparation with a direct current (DC) torque motor (model 308; Aurora Scientific) driven by voltage commands from a personal computer via a 12-bit digital-to-analog converter (AT-MIO-16E-1; National Instruments, Austin, TX). Force and length signals were digitized at 1 kHz with a 12-bit analog-to-digital converter, and each was displayed and stored on a personal computer with custom software based on LabView for Windows (National Instruments).

Images of the myocyte preparations were recorded digitally on a personal computer while myocytes were relaxed and during activation with a Hamamatsu charge-coupled device camera (model 2400) and video snapshot software (Fig. 2). Videomicroscopy was completed with a ×40 objective (Olympus UWD 40) and ×25 intermediate lenses. During and after each experiment, the images were reviewed to obtain sarcomere length measurements from the myocyte while relaxed and activated; myocyte length and width for cross-sectional area calculations were also obtained from these images. The sarcomere length of these preparations was set to yield passive forces near zero. The dimensions of the skinned cardiac myocyte preparations are reported in Table 1.

**Mechanical measurements.** All mechanical measurements were made at 13 ± 1°C. Power output of single skinned myocyte preparations was determined at varied loads as previously described (24). Briefly, myocytes were placed in activating solution and, once steady-state force developed, a series of force clamps (less than steady-state force) were performed to determine isotonic shortening velocities. With a servo system, force was maintained constant for a designated period of time (150–250 ms) while the length change was continuously monitored. After the force clamp, the myocyte preparation was slackened to reduce force to near zero to allow estimation of the relative load sustained during isotonic shortening; the myocyte was subsequently reextended to its initial length.

Isotonic shortening velocities were measured in activating solutions containing varied amounts of additional Pi (0, 2.5, 5, and 10 mM). Each cell underwent a series of loaded contractions in each of the Pi solutions, whose order was chosen at random. In this way, each cell served as its own control and pairwise statistical analysis could be performed. Isometric force was measured in activating solution with no additional Pi before and after measurements of isotonic shortening velocities to detect rundown of the preparation. Myocytes were discarded if a 20% or greater decrease in isometric tension occurred.

The kinetics of force redevelopment were obtained by following a procedure previously described for skinned cardiac myocyte preparations (21). While in Ca²⁺-activating solution, the myocyte preparation was rapidly shortened by ~15% of the myocyte’s initial length (L₀) to produce zero force. The myocyte preparation was then allowed to shorten for ~20 ms; after 20 ms the preparation was rapidly stretched to ~105% of L₀ for 2 ms and then returned to L₀. The slack-restretch maneuver caused dissociation of cross bridges, and subsequent force redevelopment was due to reattachment of cross bridges and transition to force-generating states. Force redevelopment measurements were carried out before the series of loaded contractions at each [Pi].

V₀ was measured during maximal Ca²⁺ activations by the slack test method (7, 33). Once steady-state force was reached, the myocyte preparation was rapidly (~<2 ms) slackened to a predetermined value between 5% and 20% of its initial length. The time between the imposition of the slack step and the onset of force redevelopment was measured from the intersection of two lines fitted by eye through the zero-force baseline and the initial phase of force redevelopment (Fig. 3). The length of release was plotted against the duration of unloaded

<table>
<thead>
<tr>
<th>Weak binding</th>
<th>Strong binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM → AM ATP</td>
<td>4 → AM ADP P₁</td>
</tr>
<tr>
<td>AM ADP P₁</td>
<td>5 → AM ADP P₁</td>
</tr>
<tr>
<td>M ATP</td>
<td>10 → AM ATP</td>
</tr>
</tbody>
</table>

Fig. 1. Working model for the chemomechanical steps in the cross-bridge cycle. A, actin; M, myosin; Pi, inorganic phosphate.
shortening, and $V_o$ was determined from the slope of a line fitted to the data by linear regression analysis.

**Data analysis.** Myocyte preparation length traces were fit to a single decaying exponential equation:

$$L = Ae^{-kt} + C$$  

where $L$ is cell length at time $t$, $A$ and $C$ are constants with dimensions of length, and $k$ is the rate constant of shortening ($k_{\text{shortening}}$). Velocity

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Fig. 2. Effect of added $P_i$ on myocyte force-velocity and power-load relationships. A: photomicrograph of a skinned cardiac myocyte preparation while relaxed (pCa 9.0) and during maximal Ca$^{2+}$ activation (pCa 4.5). B: representative length traces during force clamps of a rat skinned cardiac myocyte preparation during maximal Ca$^{2+}$ activations in the presence of 0, 2.5, 5, and 10 mM added $P_i$. C: force-velocity and normalized power-load curves shifted upward in response to increased concentrations of added $P_i$. ML, muscle lengths. D: absolute force-velocity and power-load curves in presence of 0 and 2.5 mM added $P_i$. Data points are means ± SE.

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of shortening at any given time \( t \) was determined as the slope of the tangent to the fitted curve at that time point. In this study, velocities of shortening were calculated at the onset of the force clamp (i.e., \( t = 0 \)). Hyperbolic force-velocity curves were fit to the relative force-velocity data using the Hill equation (17):

\[
P = a \cdot V + b \cdot V^2
\]

where \( P \) is force during shortening at velocity \( V \), \( P_o \) is the peak isometric force, and \( a \) and \( b \) are constants with dimensions of force and velocity, respectively. Power-load curves were obtained by multiplying force times velocity at each load on the force-velocity curve. The optimum force for mechanical power output (\( F_{opt} \)) was calculated with the equation (43):

\[
F_{opt} = (a^2 + a \cdot P_o)^{1/2} - a
\]

Curve fitting was performed with a customized program written in Qbasic as well as commercial software (SigmaPlot).

### RESULTS

The addition of \( P_i \) resulted in progressive decrease in maximal \( Ca^{2+} \)-activated force in skinned cardiac myocyte preparations. Force declined from a control value of 21 \( \pm 7 \) kN/m\(^2\) to 14 \( \pm 5 \), 12 \( \pm 5 \), and 7 \( \pm 3 \) kN/m\(^2\) in the presence of 2.5, 5, and 10 mM added \( P_i \), respectively. These effects of added \( P_i \) on skinned myocyte preparations were quantitatively similar to those on permeabilized multicellular cardiac muscle preparations at pH 7.0 (29).

<table>
<thead>
<tr>
<th>Length, ( \mu )m</th>
<th>Width, ( \mu )m</th>
<th>Sarcomere Length, ( \mu )m</th>
</tr>
</thead>
<tbody>
<tr>
<td>131 ( \pm ) 28</td>
<td>22.8 ( \pm ) 2.6</td>
<td>2.36 ( \pm ) 0.05</td>
</tr>
<tr>
<td>2.29 ( \pm ) 0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SD; \( n = 6 \).

Fig. 3. Effect of 5 mM added \( P_i \) on unloaded shortening velocity (\( V_o \)) in a skinned myocyte preparation. A: length traces and force traces are shown during a slack test before and after addition of 5 mM \( P_i \). Unloaded shortening times after length changes were similar before and after the addition of \( P_i \). B: slack plot before and after addition of \( P_i \). \( V_o \) for this skinned myocyte preparation was 1.29 ML/s before and 1.18 ML/s after the addition of 5 mM \( P_i \). Additionally, in this same myocyte preparation, loaded shortening was measured and peak normalized power output was ~35% greater after addition of 5 mM \( P_i \) (data not shown), which is consistent with the results from the 6 myocyte preparations reported in Table 2.
Table 2. Effects of Pi addition on mechanical properties of skinned cardiac myocyte preparations

<table>
<thead>
<tr>
<th>Added Pi</th>
<th>Maximum Force, $\mu$N</th>
<th>Maximum Force, kN/m$^2$</th>
<th>$F_{\text{opt}}$, PiP$^*$</th>
<th>$a$, PiP$^*$</th>
<th>$V_{\text{opt}}$, ML/s</th>
<th>Peak Absolute Power Output, pW</th>
<th>Peak Absolute Power Output, mW/mg</th>
<th>Peak Normalized Power Output PiP$^*$, ML/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM</td>
<td>7.0±1.4</td>
<td>21±7</td>
<td>0.28±0.03</td>
<td>0.18±0.05</td>
<td>0.35±0.07</td>
<td>85±12</td>
<td>1.95±0.82</td>
<td>0.102±0.009</td>
</tr>
<tr>
<td>2.5 mM</td>
<td>4.8±1.0$^*\dag\dag$</td>
<td>14±5$^*$</td>
<td>0.32±0.03</td>
<td>0.32±0.11</td>
<td>0.36±0.06</td>
<td>71±14$^*$</td>
<td>1.61±0.61$^*$</td>
<td>0.118±0.019$^*$</td>
</tr>
<tr>
<td>5 mM</td>
<td>4.1±1.3$^\dag\dag$</td>
<td>12±5$^*$</td>
<td>0.33±0.04</td>
<td>0.36±0.19</td>
<td>0.43±0.07$^*$†</td>
<td>71±12$^*$</td>
<td>1.65±0.77$^*$</td>
<td>0.140±0.025$^*$†</td>
</tr>
<tr>
<td>10 mM</td>
<td>2.4±0.9$^*\dag\dag$</td>
<td>7±3$^*$</td>
<td>0.33±0.03</td>
<td>0.35±0.11</td>
<td>0.41±0.04$^*$†</td>
<td>41±13$^*\dag\dag$</td>
<td>0.99±0.58$^*\dag\dag$</td>
<td>0.136±0.022$^*\dag\dag$</td>
</tr>
</tbody>
</table>

Values are means ± SD. Pi, inorganic phosphate; $F_{\text{opt}}$, optimal force for mechanical power output; PiP, force during shortening; $a$, constant with dimension of force; ML, muscle lengths. $^*P < 0.05$ vs. 0 mM Pi, $^\dag P < 0.05$ vs. 2.5 mM Pi, $^\dag\dag P < 0.05$ vs. 5 mM Pi.

Previous studies reported that maximal velocity of shortening (i.e., unloaded) is minimally affected by addition of up to 15 mM added Pi, in fast-twitch skeletal muscle fibers (4, 26, 42). We addressed whether Pi alters $V_o$ in cardiac myocyte preparations with slack tests before and after the addition of 5 mM Pi. Figure 3 shows force traces after rapid slack steps in a cardiac myocyte preparation during maximal Ca$^{2+}$ activation in the presence and absence of 5 mM added Pi. Consistent with skeletal muscle reports, $V_o$ was unchanged in response to added Pi in cardiac myocyte preparations (control 1.83 ± 0.75, 5 mM added Pi 1.75 ± 0.58 muscle lengths/s). This finding suggests that cross-bridge interaction transitions (2, 27) but has no effect on detachment rates (of compressively strained cross bridges) determine power output over most load ranges in cardiac myocytes. Interestingly, though, the increase in loaded shortening with added Pi, was not great enough to offset the Pi-induced fall in force to maintain absolute power output (Fig. 2D). Peak absolute power output (85 ± 12 pW with no additional Pi) decreased 16% (71 ± 14 pW) with 2.5 mM Pi, added, 16% (71 ± 12 pW) with 5 mM Pi, and 52% (41 ± 13 pW) with 10 mM Pi. Thus added Pi increases power output at a given relative load, but not at a specific absolute load, because of its marked effect of depressing force generation capacity.

We also addressed the effects of added Pi on the rate of myocyte force development after a mechanical perturbation to assess the potential role of this process in determining loaded shortening and power output. The rate constant of force redevelopment ($k_r$) increased in the presence of raising [Pi] (Fig. 4): $k_r$ increased from 6.1 ± 1.4 s$^{-1}$ in controls to 8.5 ± 1.3 s$^{-1}$ in the presence of 2.5 mM Pi, representing a 40% increase from control. Addition of 5 mM and 10 mM Pi further increased $k_r$ 94% (11.8 ± 3.1 s$^{-1}$) and 379% (28.1 ± 7.8 s$^{-1}$) above control, respectively. Interestingly, the residual force (i.e., the

Fig. 4. Effect of added Pi on force development rates. A: length trace and force development traces during maximal Ca$^{2+}$ activation in the presence of 0, 5, and 10 mM added Pi in the same cardiac myocyte preparation. B: the rate constant of force redevelopment ($k_r$) progressively increased as a function of added Pi. Data points are means ± SD.
force just before force redevelopment) was consistently higher as added Pi increased. Residual forces were 20 ± 3%, 30 ± 3%, 37 ± 4%, and 47 ± 11% of isometric force at 0, 2.5, 5, and 10 mM added Pi, respectively. The exact reason for this is unclear, but it may involve faster transition(s) to force-generating cross bridges as a function of [Pi]. Because of this potentially confounding influence of residual force, we also examined the rate of force redevelopment after a ~10% slack step. The rates of force development were 4.77 ± 1.10 s⁻¹ with no added Pi and 8.57 ± 2.30 s⁻¹ with 5 mM Pi (P = 0.003, n = 6); this result is quantitatively similar to that observed with the slack-restretch maneuver. Overall, these results are quantitatively different from the Pi-induced increase in normalized power output, suggesting that cross-bridge steps that determine power output differ from the transitions that limit force redevelopment in cardiac muscle.

**DISCUSSION**

This study examined the effects of added Pi on force, unloaded shortening, loaded shortening, power output, and rates of force development in rat skinned cardiac myocyte preparations. Addition of Pi lowered isometric force, had no effect on V_o, but increased loaded shortening, power output, and rates of force development. The decrease in force exceeded the decline in peak absolute power output because of the Pi-induced increase in loaded shortening velocity. The increase in loaded shortening velocity yielded greater peak myocyte power output after normalizing for changes in isometric force. These results together suggest that peak power output is determined more by force-generating steps in the cross-bridge cycle than by cross-bridge detachment rates because Pi is known to speed force-generating transitions but appeared to have no effect on detachment of compressively strained cross bridges.

The effects of Pi on contractile properties have been examined extensively in both skinned skeletal and cardiac muscle preparations. Interestingly, the contractile properties of cardiac muscle preparations appear to be more sensitive to added Pi than those of skeletal muscle preparations. For instance, Nosek et al. (29) reported that 10 mM added Pi decreased cardiac muscle force ~60% compared with a 40% force decline in skeletal muscle preparations in the same study. We also observed that force declined ~65% in the cardiac myocyte preparation in response to 10 mM added Pi. Although data from skeletal muscle fiber preparations demonstrate a wide range of force decline in response to added Pi (~30–55%), more recent data from myofibrils from fast-twitch skeletal muscle have demonstrated greater effects of Pi (e.g., 70% decline in force) with 10 mM Pi (36). The exact reasons for the differences in Pi responsiveness between fibers and myofilament preparations are unclear but may involve the procedures used to reduce contaminating Pi, as described by Pate et al. (30) and used in the myofibril experiments (36, 37). Also, there is likely an inverse relationship between preparation diameter and force decline in response to added [Pi] because of greater accumulation of Pi from myofibrillar ATPases (and thus higher baseline [Pi]) in thicker preparations (20, 32). Regardless of contractile properties, added Pi has been reported to increase k_P (the rate constant of force decline after rapid increases in solution Pi) to a greater extent in cardiac myocyte preparations than in fast-twitch skeletal muscle fibers (2, 41). Additionally, the increase in k_P with added [Pi] (from 0 to 10 mM) was upward of threefold in our skinned cardiac myocyte preparations vs. a 50% increase in fast-twitch skinned skeletal muscle fibers (38). Together these results suggest that contractile properties appear to be more sensitive to elevations in [Pi] in cardiac muscle than skeletal muscle at least over a range of 0–10 mM added Pi. This may have implications in the response of these two striated types of myofilaments to metabolite (i.e., Pi) buildup associated with ischemia. For cardiac muscle, [Pi] is thought to rise to as high as 30 mM during ischemia (1); such a rise in [Pi] would likely result in a marked fall in force-generating cross bridges, but the faster rates of force development and loaded shortening would tend to compensate to possibly help sustain adequate stroke volume and cardiac output.

A primary purpose of this study was to assess which step(s) in the cross-bridge cycle may be most important in determining myocyte shortening and power output at various loads. In looking at a force-velocity curve (Fig. 5), shortening velocity at low loads is thought to be limited by detachment of compressively strained cross bridges, which appears to be limited by ADP release from actomyosin (31, 44) and/or by mechanical detachment of highly compressed cross bridges (5). Conversely, loaded shortening and power at high loads are thought to be determined by force-generating steps in the cross-bridge cycle, which are thought to be coupled to Pi release (16) and/or an isomerization that is in rapid equilibrium with Pi release from the actomyosin complex (27, 41). The question then arises as to what load the cross-bridge step(s) that limits power shifts from force-generating transitions to detachment rates.

**Fig. 5.** Schematic addressing which steps in the cross-bridge limit power output. Shortening velocity and power at low loads are thought to be limited by detachment of compressively strained cross bridges, whereas loaded shortening and power at high loads are thought to be limited by force-generating transitions. The question arises as to what load the cross-bridge step(s) that limits power shifts from force-generating transitions to detachment rates. 

This question was addressed in this study by constructing force-velocity and power-load curves after the addition of Pi, which is known to speed force-generating transitions but have little or no effect on detachment of compressively strained cross bridges. Because added Pi increased normalized power over most relative loads, this implies that force-generating transitions are most important in determining myocyte power.
Our experiments were designed to alter one of these processes (i.e., force-generating transitions) but not the other (i.e., detachment rates). In this regard, elevations in $P_i$ (from 0 to ~15 mM) have been reported to speed the rates of force generation in skeletal (37, 38) and cardiac (2) muscle preparations but had minimal effects on unloaded shortening velocity in fast-twitch skeletal muscle fibers (4, 26, 42). We also found no effect of 5 mM $P_i$ on $V_o$ in skinned cardiac myocyte preparations (Fig. 3). Thus we examined how added $P_i$ affects power-load curves; if force-generating steps were the primary determinants over a large range of loads, added $P_i$ would increase power over much of the curve. If, on the other hand, force-generating steps only determine power at high loads, accelerating these steps with added $P_i$ should only increase power at high loads. Interestingly, loaded shortening and power output increased with 2.5 mM added $P_i$ over all relative loads greater than ~10% isometric force (Fig. 2C). In fact, both loaded shortening and power output continued to increase and reached a plateau at all loads >10% isometric force when added $P_i$ was increased to 5 mM. These results suggest that force-generating steps associated with $P_i$ release are most important in limiting power output over most loads in cardiac myofibrils, at least under our experimental conditions.

Our results are also consistent with similar studies on skeletal muscle fiber preparations that sought insight into which chemomechanical transitions might determine power output at various loads. For example, a study by Ford et al. (13) examined power output over a range of loads in rabbit skinned fast-twitch skeletal muscle fibers and discovered that osmotic compression of fibers resulted in reduced velocity and power at low loads but had little effect at intermediate and high loads. Because osmotic compression reduced $V_o$, this implies that cross-bridge detachment limits power output at low loads. Consistent with this idea, lowering ATP concentrations or substituting ATP with CTP also reduced $V_o$ and decreased power output only at low loads in rat skinned slow-twitch skeletal muscle fibers (39). A recent study also found that increased $[P_i]$ sped loaded shortening in fast-twitch skeletal muscle fibers at loads greater than ~15% isometric force (11). Together these studies suggest that power output in striated myofibrils is rate limited at low loads by the same chemomechanical transitions that limit unloaded shortening speeds and at intermediate and high loads by chemomechanical transitions that are associated with force generation.

Interestingly, the addition of $P_i$ also increased the rates of force development but to a much greater extent than increases in peak normalized power. The exact reasons for the differing $P_i$ dependencies of force development and power are unclear. One possibility is that force development is limited by the cooperative activation of near-neighbor regulatory units on the thin filament by strongly binding cross bridges (10, 12, 34) and addition of $P_i$ increases the number of strongly binding non-force-generating cross bridges (actin-myosin ADP $P_i$). This is consistent with the finding that $P_i$ addition, like addition of strongly binding N-ethylmaleimide (NEM)-modified cross bridges (35), eliminated the slow phase of unloaded shortening after slack steps in skinned fast-twitch skeletal muscle fibers during submaximal $Ca^{2+}$ activations (26). Loaded shortening, on the other hand, is not likely limited by the number of strongly bound non-force-generating cross-bridges (actin-myosin ADP $P_i$), at least during maximal $Ca^{2+}$ activations (25).

Rather, shortening velocity and power output (over most loads) appear to be determined by $P_i$ release steps in the cross-bridge cycle, which are modulated to a different extent than thin filament activation rates by addition of $P_i$.

In summary, this study implies that myocyte power output is determined by $P_i$-sensitive force-generating transitions in the cross-bridge cycle as opposed to detachment of compressively strained cross bridges. However, even though $P_i$ speeds loaded shortening at given relative loads, the increase in velocity does not appear to be great enough to compensate for the $P_i$-induced decrease in force; this yields an overall decrease in absolute power-generating capacity, providing a myofibrillar mechanism for depressed myocardial work capacity and function during ischemia.

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