**β-Myosin heavy chain myocytes are more resistant to changes in power output induced by ischemic conditions**

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Hinken, Aaron C., and Kerry S. McDonald. β-Myosin heavy chain myocytes are more resistant to changes in power output induced by ischemic conditions. Am J Physiol Heart Circ Physiol 290: H869–H877, 2006. First published September 19, 2005; doi:10.1152/ajpheart.00221.2005.—During ischemia intracellular concentrations of Pi and H+ increase. Also, changes in myosin heavy chain (MHC) isoform toward β-MHC have been reported after ischemia and infarction associated with coronary artery disease. The purpose of this study was to investigate the effects of myoplasmic changes of Pi and H+ on the loaded shortening velocity and power output of cardiac myocytes expressing either α- or β-MHC. Skinned cardiac myocyte preparations were obtained from adult male Sprague-Dawley rats (control or treated with 5-n-propyl-2-thiouracil to induce β-MHC) and mounted between a force transducer and servomotor system. Myocyte preparations were subjected to a series of isotonic force clamps to determine shortening velocity and power output during Ca2+ activations in each of the following solutions: 1) pCa 4.5 and pH 7.0; 2) pCa 4.5, pH 7.0, and 5 mM P; 3) pCa 4.5 and pH 6.6; and 4) pCa 4.5, pH 6.6, and 5 mM P. Added P and lowered pH each caused isometric force to decline to the same extent in α-MHC and β-MHC myocytes; however, β-MHC myocytes were more resistant to changes in absolute power output. For example, peak absolute power output fell 53% in α-MHC myocytes, whereas power fell only 38% in β-MHC myocytes in response to elevated P, and lowered pH (i.e., solution 4). The reduced effect on power output was the result of a greater increase in loaded shortening velocity induced by P in β-MHC myocytes and an increase in loaded shortening velocity at pH 6.6 that occurred only in β-MHC myocytes. We conclude that the functional response to elevated P, and lowered pH during ischemia is MHC isoform-dependent with β-MHC myocytes being more resistant to declines in power output.

The capability of the heart to pump blood is dependent on its ability to contract against a load and thus generate power. During times of reduced blood flow to the myocardium, this pumping capability is compromised. Multiple factors are involved in reducing contractile function of the myocardium during ischemia, many of which are dependent on the duration and severity of ischemia. However, a principal cause for the fall in force production during early ischemia is thought to be the accumulation of metabolic byproducts, specifically Pi and H+ (7, 8, 20, 30, 35). Some of the effects of alterations in myoplasmic electrolyte composition occurring during myocardial ischemia on contractile function have been examined by using skinned cardiac muscle. Experiments (7, 17, 18, 29) utilizing skinned strips of cardiac trabeculae in the presence of 0 to 30 mM Pi demonstrated a progressive decrease in force production with increasing [Pi]. However, not all effects appeared to be detrimental because [Pi] (up to 10 mM) increased loading shortening at loads >10% maximal isometric tension in skinned cardiac myocyte preparations (15). The increase in loaded shortening velocity at elevated Pi attenuated the fall in peak absolute power (the product of force and velocity); however, the compensation was not enough to completely prevent a decline in absolute power production. Increases in H+ (i.e., acidosis) that occur during ischemia also decrease cardiac myocyte function. In contrast to Pi, H+ has been reported to decrease unloaded and loaded shortening velocity in cardiac trabeculae preparations (33) in addition to decreasing isometric force production (10, 17, 24, 30). Studies examining increases of both Pi and H+ working in concert have primarily been limited to assessments of alterations in force production (17, 29, 39). In cardiac muscle these two additively decrease isometric force (17, 29), and the effects on loaded shortening and power output are unknown. Thus we tested the hypothesis that added Pi and H+ will act additively to decrease peak absolute power output in skinned cardiac myocyte preparations.

With an examination of the effects of ischemia on the myocardium, it is important to recognize that many factors, including contractile protein isoform expression, may contribute to the functional responses. Studies using skinned skeletal fibers have reported smaller declines of isometric force caused by additional Pi (29) or H+ (25) in slow-twitch fibers than in fast-twitch fibers. This may be especially significant in mammalian hearts because myosin heavy chain (MHC) isoform shifts from α-MHC toward β-MHC isoform (which is the same isoform as in slow-twitch fibers) after prolonged ischemia, infarction, and during heart failure (32, 41, 42). The potential differential effects of ischemic metabolites on power output of cardiac myocytes have not been previously examined, even though a switch toward a more resistant isoform would appear to be advantageous teleologically in situations of ischemic heart disease. Thus we examined the effects of increased metabolite concentrations on force, loaded shortening velocity, and power output of skinned cardiac myocyte preparations primarily expressing either α- or β-MHC. With the use of cardiac myocyte preparations from control rats and rats treated with 5-n-propyl-2-thiouracil (PTU, to induce β-MHC expression), force-velocity relationships were determined in the presence of Pi (5 mM) or H+ (pH 6.6) alone and together to determine the effect of simulated ischemic conditions on myocyte function. The results show that Pi and H+ act additively to depress force in both α- and β-MHC myocytes. Additionally, 5 mM Pi increased loaded shortening velocity in both α- and

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β-MHC myocytes. Lowered pH did not affect loaded shortening in α-MHC myocytes but increased loaded shortening in β-MHC myocytes. From these results it was concluded that α-MHC and β-MHC myocytes are equally sensitive to depression of force by P_i and H^+, but β-MHC myocytes are more tolerant to changes in metabolite concentrations as indexed by power-generating capacity.

METHODS

Cardiac myocyte preparations. Sprague-Dawley rats were obtained from Harlan (Madison, WI) and maintained according to guidelines set by the Animal Care and Use Committee of the University of Missouri, and this study was approved by the same institution. Skinned cardiac myocyte preparations were obtained from rats treated for ~3 wk with PTU (0.6 g/l in drinking water) or age-matched controls by mechanical disruption of hearts as described previously (22). PTU treatment has been successfully employed to shift MHC expression from primarily α-MHC to the β-MHC isoform (12). 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, all in the presence of ice-cold relaxing solution. The resulting suspension of cells was centrifuged for 65 s at 165 g, after which the supernatant was discarded. The myocytes were suspended by suspending the pellet of cells for 3 min in 0.5% ultrapure Triton X-100 (Fisher Chemical) in relaxing solution. The skinning cells were washed twice with cold relaxing solution, suspended 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. Relaxing solution in which the ventricles were disrupted, skinned, and resuspended contained (in mmol/l) 2 EGTA, 5 MgCl_2, 4 ATP, 10 imidazole, and 100 KCl at pH 7.0. Compositions of relaxing and activating solutions used in mechanical measurements contained (in mmol/l) 7 EGTA, 1 free Mg_2^++, 20 imidazole, 4 MgATP, and 14.5 creatine phosphate and Ca_2^+ concentrations of 10^{-4}–10^{-6} M of relaxing solution, 10^{-4.5} M of maximal activating solution, and sufficient KCl to adjust ionic strength to 180 mM. With a very acidic pH of ~4 to start, the final solution was brought to either pH 6.6 or 7.0 with the addition of KOH. Activating solutions containing P_i, were identical to those described above except for the inclusion of 5 mM potassium phosphate (KH_2PO_4) 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 (9).

Immediately before the activations, myocyte preparations were immersed for 60 s in a solution of reduced Ca_2^+-EGTA buffering capacity, identical to normal relaxing solution except that EGTA is reduced to 0.5 mM. This protocol resulted in a 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 (22). Briefly, myocyte preparations were attached between a force transducer and 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 long piece (0.5 mm) of 3.0 monofilament nylon suture (Ethicon) onto each end of the myocyte and then by 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) using finely shaped forceps.

Before mechanical measurements were taken, 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 by using 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 using a direct-current torque motor (model 308, Aurora Scientific), driven by voltage commands from a personal computer via a 12-bit digital-to-analog converter (DAC; AT-MIO-16E-1, National Instruments, Austin, TX). Force and length signals were digitized at 1 kHz using a 12-bit DAC, and each was displayed and stored. Input and output voltage and length commands were controlled by a personal computer using custom software based on LabView for Windows (National Instruments).

Images of the myocyte preparations during relaxation and activation were recorded digitally on a personal computer with the use of a Hamamatsu charge-coupled device camera (model 2400) and video-snapshot software. Videomicroscopy was completed by using a ×40 objective (Olympus UWD 40) and ×25 intermediate lenses. During and after each experiment, the images were reviewed to obtain myocyte length and width for cross-sectional-area calculations. Sarcomere length (SL) of these preparations was set to yield passive forces near zero and were monitored during experiments by a video-microscopy system utilizing fast Fourier transform (IonOptix, Milton, MA).

Mechanical measurements. All mechanical measurements were made at 13 ± 1°C. Power output of skinned myocyte preparations was determined at varied loads as previously described (22). Briefly, myocyte preparations 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 the use of a servo-motor system, force was maintained constant for a designated time period (150–250 ms) while the length change was continuously monitored. After the force clamp was performed, 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 each of the activating solutions containing either no added P_i or 5 mM of additional P_i, each at pH 7.0 and 6.6. Each cell underwent a series of loaded contractions in each of the activating solutions; the order was chosen at random so that each cell could serve as its own control and so that pairwise statistical analysis could be performed. Isometric force was measured in activating solution before and after measurements of isotonic shortening velocities to detect the rundown of the preparation. Myocyte preparations were discarded if ≥20% decrease in isometric tension occurred.

The kinetics of force redevelopment were obtained by using a procedure previously described for skinned cardiac myocyte preparations (15). While in Ca_2^+ activating solution, the myocyte preparation was rapidly shortened by ~15% of the initial length (L_0) of the myocyte to produce zero force. The myocyte preparation was then allowed to shorten for ~20 ms; after 20 ms the preparation was rapidly restretched to a value slightly greater than L_0 for 2 ms and then returned to L_0. The slack-restretch maneuver caused dissociation of cross bridges, and subsequent force redevelopment was due to the reattachment of cross bridges and the transition to force-generating states. Force redevelopment measurements were carried out before the series of loaded contractions.

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_shortening). Velocity 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 by extrapolation of the fitted curve to 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 (14):

$$F = F_{\text{max}} \left[1 - \exp(-k_tr)\right] + F_{\text{res}}$$

(4)

where $F$ is tension at time $t$, $F_{\text{max}}$ is maximal tension, and $k_tr$ is the rate constant of tension redevelopment. $F_{\text{res}}$ represents any residual tension present immediately after the slack-restretch maneuver.

**SDS-PAGE and MHC quantification.** After mechanical measurements were taken, MHC isoform expression was determined for each myocyte preparation as previously described (12). Briefly, myocytes were removed from the experimental apparatus, suspended in 8 μl of SDS sample buffer, and stored at −80°C for subsequent SDS-PAGE analysis. The gels for SDS-PAGE were prepared with 3.5% acrylamide in the stacking gel and 12% acrylamide in the resolving gel. Samples were separated by SDS-PAGE at constant voltage (250 V) for 8.0 h. Gels were initially fixed in an acid-alcohol solution, followed by glutaraldehyde fixing. MHC isoforms were visualized by ultrasensitive silver staining, and gels were subsequently dried between mylar sheets. The relative expression of each MHC isoform was determined by using Quantiscan (Biosoft) software and an Epson scanner to measure the relative intensity and area of each MHC band.

**Statistics.** One-way repeated measures ANOVA was used to determine significant effects on force, absolute and normalized power output, and $k_tr$ from varied P, and H+ solutions. The Student-Newman-Keuls test was used post hoc to assess the differences among means. Student’s t-tests were used to assess differences in metabolite effect between myocytes expressing α-MHC or β-MHC. $P < 0.05$ was chosen to indicate significance. Values are means ± SD, unless otherwise indicated.

**RESULTS**

**Myocyte preparation characteristics and PTU-induced changes in MHC expression.** PTU treatment of rats has been employed frequently to induce MHC isoform switching with minimal changes in other myofilament protein expression or overall myocyte morphology (12, 16, 34). The addition of PTU to the drinking water decreased the expression of α-MHC protein while increasing β-MHC isoform expression. MHC content of myocytes used in mechanical experiments was determined by SDS-PAGE separation; an example of MHC separation gels is shown in Fig. 1B. Myocytes from non-PTU-treated animals expressed 91 ± 4% α-MHC protein, whereas those from age-matched animals after ~3 wk of PTU treatment yielded myocytes with 11 ± 5% α-MHC. Similar to a previous study (12), nontreated and PTU-treated myocytes were the same in length (α-MHC, 148 ± 39 μm; and β-MHC, 161 ± 43 μm) and width (α-MHC, 24 ± 9 μm; and β-MHC, 22 ± 4 μm). Resting SL of the preparations when set to yield passive forces near zero was 2.33 ± 0.07 μm and 2.25 ± 0.04 SL, μm in α-MHC and β-MHC myocytes, respectively, and SL was not significantly altered by maximal Ca2+-activation (i.e., pCa 4.5; α-MHC, 2.32 ± 0.08 μm; and β-MHC, 2.26 ± 0.05 μm), which is indicative of low compliance at the points of myocyte attachment.

**Effects of ischemic conditions on mechanical properties of cardiac myocytes.** Previous experiments on skinned cardiac trabeculae (17, 18, 29) and myocytes (15, 39) have shown that increased [P]i and/or [H+•] decreased maximal Ca2+-activated force. Comparable force and declines in force were observed in this study during the addition of P, and H+ alone and together, with α-MHC and β-MHC myocytes having equal decreases. Force and force per cross-sectional area in rat-skinned cardiac myocytes are summarized in Tables 1 (α-MHC myocytes) and 2 (β-MHC myocytes). In α-MHC myocytes, maximal Ca2+-activated force per cross-sectional area was 62%, 62%, and 40% of control tension in solutions containing 5 mM P, at pH 6.6 (solution 2), and 5 mM P, at pH 6.6 (solution 4), respectively. β-MHC myocytes produced 58%, 59%, and 35% of maximal Ca2+-activated tension with 5 mM P, at pH 6.6, and 5 mM P, at pH 6.6, respectively. These results indicate that P, and H+-induced decreases in force are independent of mammalian MHC isoform.

The rate constant of force redevelopment ($k_{tr}$) was assessed in α- and β-MHC myocytes with each experimental condition. The addition of P, increased $k_{tr}$ in α-MHC myocytes at both pH 7.0 and 6.6, whereas a lowered pH alone did not affect force redevelopment rates (Table 1). Similar alterations in $k_{tr}$ with P, and H+• have previously been reported for α-MHC myocytes (1, 15). The rates of force development were affected by P, and H+• qualitatively the same in β-MHC myocytes as in α-MHC myocytes, i.e., the addition of P, increased $k_{tr}$ at both pH 7.0 and 6.6, whereas a lowered pH alone did not affect force redevelopment rates (Table 2). Thus P, and H+• affected both isometric force and isometric force development rates similarly, independent of MHC isoform.

Force-velocity and power-load relationship characteristics are given in Tables 1 and 2 for α-MHC and β-MHC myocytes, respectively. Figure 1 displays a representative absolute force-velocity and power-load relationship from a non-PTU-treated myocyte (i.e., α-MHC myocyte) under all four of the experimental conditions. The addition of P, or the lowering the pH of the activator solution resulted in a leftright shift of the absolute force-velocity relationship (Fig. 1) because of lower isometric force. Also, even though the addition of 5 mM P, and the lowered pH to 6.6 reduced force to the same extent, there was a significantly greater peak absolute power output with P, than with lowered pH (Fig. 1D). The absolute power output of α-MHC myocyte preparations fell ~20% with 5 mM P, ~40% at pH 6.6, and ~50% with 5 mM P, at pH 6.6, all significantly less than power output without additional P, at pH 7.0. However, the fall in power with additional P, at either pH 7.0 or 6.6 was less than what was expected from the fall in force, whereas the fall in power at pH 6.6 without added P, was equivalent to the fall in force. The reason for the lesser decline in power output than force with P, at (pH 7.0 or 6.6) in α-MHC myocytes is more easily seen with force-velocity relationships normalized to isometric force. Normalized α-MHC myocyte force-velocity relationships (Fig. 2) reveal an increase in the...
velocity of shortening that occurred with the addition of Pi, whereas increased \( H^+ \) alone had no effect on loaded shortening velocity. The increase in loaded shortening velocity increased power at all loads of greater than \( \sim 15\% \) isometric force in all \( \alpha \)-MHC myocytes, with normalized peak power output being \( \sim 30\% \) greater with 5 mM Pi, unchanged at pH 6.6, and 16\% greater with 5 mM Pi at pH 6.6. Interestingly, the Pi-induced increase in loaded shortening velocity was attenuated in combination with elevated \( H^+ \).

In contrast to \( \alpha \)-MHC, \( \beta \)-MHC myocyte absolute power-generating capacity was not diminished by either 5 mM Pi or pH 6.6; however, power did fall when both Pi and \( H^+ \) were increased (Table 2). The normalized force-velocity relationships of \( \beta \)-MHC myocytes (Fig. 3) reveal increased velocity of
shortening with the addition of P<sub>i</sub> and H<sup>+</sup> alone and in combination. Increased loaded shortening velocity occurred at all loads greater than ~5% with P<sub>i</sub> (at both pH values) and ~15% at the lower pH. Moreover, these increases were much greater than those observed in α-MHC myocytes, producing a 73% increase with 5 mM P<sub>i</sub>, a 43% increase at pH 6.6, and an 88% increase in peak normalized power output with 5 mM P<sub>i</sub> at pH 6.6. These increases in loaded shortening velocity provided the basis for the preserved absolute power generating capacity of β-MHC myocytes. Also of interest, the P<sub>i</sub>-induced increase in loaded shortening velocity was augmented by added H<sup>+</sup> in β-MHC myocytes, which was the opposite of that seen in α-MHC myocytes. Larger increases in loaded shortening velocity with metabolites in β-MHC myocytes than in α-MHC myocytes resulted in similar power-generating capacity between the two myocyte types. Normally, as with control conditions, α-MHC myocytes generate significantly more power than do β-MHC myocytes; however, the addition of P<sub>i</sub> or H<sup>+</sup> abrogated this difference, resulting in similar power production between α-MHC and β-MHC myocytes.

**DISCUSSION**

This study directly examined the effect of increases in P<sub>i</sub> and H<sup>+</sup> on cardiac myocyte force production, rates of force development, loaded shortening velocity, and power output. Metabolite concentrations employed here are similar to those reported during the first 20–30 min of low-flow ischemia in which [P<sub>i</sub>] increases from ~1 mM (19) toward ~5 mM and pH declines ~0.4 units (5). In addition to a simple alteration of metabolite concentrations, experiments also utilized myocyte preparations expressing predominantly either α-MHC or β-MHC to ascertain whether there is a cardiac MHC-dependent response to ischemic metabolites. The main findings of this study were as follows: 1) maximal Ca<sup>2+</sup>-activated force and rates of force development changed comparably in α-MHC and β-MHC myocytes with P<sub>i</sub> and lowered pH both alone and together; 2) the addition of P<sub>i</sub> increased loaded shortening velocity in both α-MHC and β-MHC myocytes; 3) lowered pH had no effect on velocity of shortening in α-MHC myocytes but increased loaded shortening velocity in β-MHC myocytes; and 4) power output of α-MHC myocytes fell to a greater extent than did β-MHC myocytes with increased P<sub>i</sub> and H<sup>+</sup>, resulting in comparable power-generating capacity of α-MHC and β-MHC myocytes. From these results it can be concluded that the type of MHC does not affect the force or rate of force development response to metabolite accumulation in cardiac myocytes; however, MHC is a determinant of the effect on loaded shortening and power output. The differential effects of these conditions on shortening velocity between α- and β-MHC myocytes may represent a mechanism whereby the heart compensates to maintain function during pathological conditions (repeated ischemia or congestive heart failure) by expressing the more tolerant MHC isoform β-MHC.

**MHC as a determinant of mechanical properties of cardiac myocytes during simulated ischemic conditions.** The inhibitory effects of ischemic metabolites on force production in muscle have been investigated extensively in skinned skeletal and cardiac preparations. Some of these studies have provided the possibility of an inverse relationship between preparation diameter and force decline (18, 37), which is thought to exist because of lower P<sub>i</sub> accumulation from myofibrillar ATPases in thinner preparations during Ca<sup>2+</sup> activation. Importantly, though, this would indicate that the rate of ATPase activity also may be a determinant of force sensitivity to metabolites,

**Table 1. Effects of P<sub>i</sub> addition and lowered pH on force-velocity, power-load, and k<sub>tr</sub> properties of α-MHC cardiac myocytes**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Maximum Force, kN/m²</th>
<th>Peak Absolute Power Output, μW/mg</th>
<th>a&lt;sub&gt;P&lt;sub&gt;i&lt;/sub&gt;&lt;/sub&gt;</th>
<th>F&lt;sub&gt;opt&lt;/sub&gt;, P/P&lt;sub&gt;o&lt;/sub&gt;</th>
<th>V&lt;sub&gt;opt&lt;/sub&gt;, ML/s</th>
<th>Peak Normalized Power Output, P/P&lt;sub&gt;o&lt;/sub&gt;/ML·s&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>k&lt;sub&gt;tr&lt;/sub&gt;, s&lt;sup&gt;-1&lt;/sup&gt;</th>
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<tr>
<td>pH 7.0</td>
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<td></td>
</tr>
<tr>
<td>0 mM P&lt;sub&gt;i&lt;/sub&gt;</td>
<td>25.3±12.5</td>
<td>2.68±1.3</td>
<td>0.31±0.12</td>
<td>0.32±0.03</td>
<td>0.35±0.06</td>
<td>0.114±0.02</td>
<td>6.1±1.5</td>
</tr>
<tr>
<td>5 mM P&lt;sub&gt;i&lt;/sub&gt;</td>
<td>15.6±6.6*</td>
<td>2.14±1.0*</td>
<td>0.46±0.16*</td>
<td>0.35±0.02</td>
<td>0.41±0.08*</td>
<td>0.145±0.02*</td>
<td>10.5±4.8*</td>
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<td>pH 6.6</td>
<td></td>
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<tr>
<td>0 mM P&lt;sub&gt;i&lt;/sub&gt;</td>
<td>15.7±8.1*</td>
<td>1.65±0.7*†</td>
<td>0.36±0.13</td>
<td>0.33±0.03</td>
<td>0.35±0.06†</td>
<td>0.115±0.02†</td>
<td>7.2±1.9†</td>
</tr>
<tr>
<td>5 mM P&lt;sub&gt;i&lt;/sub&gt;</td>
<td>10.0±4.6†‡</td>
<td>1.26±0.6†‡</td>
<td>0.28±0.09†</td>
<td>0.32±0.03†</td>
<td>0.42±0.07†‡</td>
<td>0.132±0.02†‡</td>
<td>13.4±7.3†‡</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 9 myocytes. *P < 0.05, significantly different from pH 7.0 and 0 mM P<sub>i</sub>; †P < 0.05, significantly different from pH 6.6 and 0 mM P<sub>i</sub>; ‡P < 0.05, significantly different from pH 7.0 and 5 mM P<sub>i</sub>.

**Table 2. Effects of P<sub>i</sub> addition and lowered pH on force-velocity, power-load, and k<sub>tr</sub> properties of β-MHC cardiac myocytes**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Maximum Force, kN/m²</th>
<th>Peak Absolute Power Output, μW/mg</th>
<th>a&lt;sub&gt;P&lt;sub&gt;i&lt;/sub&gt;&lt;/sub&gt;</th>
<th>F&lt;sub&gt;opt&lt;/sub&gt;, P/P&lt;sub&gt;o&lt;/sub&gt;</th>
<th>V&lt;sub&gt;opt&lt;/sub&gt;, ML/s</th>
<th>Peak Normalized Power Output, P/P&lt;sub&gt;o&lt;/sub&gt;/ML·s&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>k&lt;sub&gt;tr&lt;/sub&gt;, s&lt;sup&gt;-1&lt;/sup&gt;</th>
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<td>pH 7.0</td>
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<tr>
<td>0 mM P&lt;sub&gt;i&lt;/sub&gt;</td>
<td>30.1±9.2</td>
<td>1.48±0.7</td>
<td>0.10±0.06</td>
<td>0.22±0.05</td>
<td>0.21±0.04</td>
<td>0.049±0.01</td>
<td>2.3±0.7</td>
</tr>
<tr>
<td>5 mM P&lt;sub&gt;i&lt;/sub&gt;</td>
<td>17.3±5.0*</td>
<td>1.46±0.5</td>
<td>0.18±0.07*</td>
<td>0.28±0.03*</td>
<td>0.30±0.04*</td>
<td>0.085±0.02*</td>
<td>4.0±2.0*</td>
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<tr>
<td>pH 6.6</td>
<td></td>
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<td></td>
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<tr>
<td>0 mM P&lt;sub&gt;i&lt;/sub&gt;</td>
<td>17.8±5.1*</td>
<td>1.28±0.4</td>
<td>0.11±0.04†</td>
<td>0.24±0.02</td>
<td>0.30±0.05*</td>
<td>0.070±0.01†</td>
<td>2.7±0.5</td>
</tr>
<tr>
<td>5 mM P&lt;sub&gt;i&lt;/sub&gt;</td>
<td>10.6±3.8†‡</td>
<td>0.92±0.3†‡</td>
<td>0.13±0.03†</td>
<td>0.25±0.02</td>
<td>0.35±0.06†‡</td>
<td>0.092±0.02†‡</td>
<td>4.7±1.2†‡</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 8 myocytes. *P < 0.05, significantly different from pH 7.0 and 0 mM P<sub>i</sub>; †P < 0.05, significantly different from pH 6.6 and 0 mM P<sub>i</sub>; ‡P < 0.05, significantly different from pH 7.0 and 5 mM P<sub>i</sub>.

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meaning a more active ATPase will result in greater accumulation (at a given diameter). This is in agreement with data showing more responsiveness in fast-twitch compared with slow-twitch skeletal muscle fibers (27, 29, 38). However, this idea remains controversial because other studies have reported greater metabolite sensitivity in slow-twitch fibers (6) or no difference (31) between fiber-type force responses. The disparity of these results may lie in experimental solutions or conditions, such as temperature, because force sensitivity to metabolites is temperature dependent (6). Nevertheless, the potential difference between skeletal fiber-type sensitivities to metabolites suggests the potential for a MHC dependence of force response to Pi and H\(^+\) in cardiac muscle. In vertebrates, two MHC isoforms are expressed in the myocardium, α-MHC and β-MHC, with considerable homology containing 93% identical amino acids (23) that are functionally distinct. For example, α-MHC exhibits approximately three times the actin-activated ATPase activity (21) and generates two to three times more power than β-MHC (12). However, analyses of differential force responses to P\(_i\) and H\(^+\) with cardiac MHC content has not been addressed to our knowledge. Comparing the results from some studies may offer potential support for a MHC dependence of force response in cardiac muscle. For example, van der Velden et al. (39) reported a ~35% decline in force per decade of P\(_i\) in human donor and heart failure ventricular myocytes, both of which likely express β-MHC primarily, whereas others have reported larger (45–65%) declines per decade in rat cardiac preparations expressing primarily α-MHC (7, 15, 17). Yet a direct comparison of these results may be errant because they were collected under different experimental conditions with preparations of varied size, which, as discussed above, may be an important variable in the responses to metabolites. Although, in regard to differential P\(_i\) effect due to size, our previous work (15) and the van der Velden study (39) both utilized single ventricular cardiac myocytes and yet produced very different force responses to 10 mM P\(_i\), namely, 35% and 65% declines in α-MHC and β-MHC myocytes, respectively. The reason for this difference is unclear at this time but may be the result of differential myofibrillar protein phosphorylation status as proposed (39), solu-
tions used, myocyte preparation, or myofilament protein expression. However, with regard to the former mechanism, we observed no difference in basal phosphorylation status of cardiac myosin binding protein C or cardiac troponin I between PTU- and non-PTU-treated rats in this study (data not shown). As for the response of cardiac myocytes to acidosis, Solaro et al. (36) showed that neonatal myocardium is less sensitive to acidosis than adult myocardium. However, the authors concluded that the change in sensitivity of force production observed was the result of a shift in troponin I isoform from the slow skeletal isoform in neonates to the cardiac isoform in adults, although heavy chain expression was not completely characterized. In our study, equivalent decreases in force were observed between α-MHC and β-MHC myocytes with Ca²⁺-activating solutions at lower pH or elevated Pi levels, implying cardiac MHC independence to these conditions (Fig. 4). This supports that ATPase rate, and consequent metabolite production, is not a major determinant of metabolite sensitivity of force, at least in myocyte preparations where the diameter is small and equivalent. Finally, the combined effects of Pi and H⁺ on force production were comparable in α-MHC and β-MHC myocytes, and although additive, there was no synergistic effect (whole greater than the sum of the parts) in combination, in agreement with previous studies (17, 29, 39) in cardiac muscle.

Previous reports have found that the rates of force development increased with Pi in both cardiac myocytes (1, 15) and skeletal muscle fibers (26), whereas no changes occurred with lower pH in skeletal muscle fibers during maximal Ca²⁺ activation (25). Importantly, it is the rate of force development that likely determines how quickly the ventricles complete isovolumic contraction (phase 2 of the cardiac cycle), allowing the remaining time of systole for ejection (phase 3). α-MHC myocytes develop force two to three times more quickly than do β-MHC myocytes at maximal Ca²⁺ activation (the current study and Ref. 11), which likely allows for the ejection phase to commence earlier and to last longer in working ventricles. Our results indicate that α-MHC and β-MHC myocytes appear to benefit from this Pi-induced increase in force development in a similar manner. This may ultimately mean that even though α-MHC and β-MHC myocytes exhibit reduced power output-generating capacity in response to ischemic metabolites, global ventricular function may be maintained due to faster isovolumic contraction allowing for more ejection time.

In contrast to the effects on force, we found that Pi and H⁺ affected loaded shortening differentially in myocytes expressing α-MHC and β-MHC. The greater effect of Pi and H⁺ on loaded shortening velocity of β-MHC myocytes tended to maintain the absolute power-generating capacity of β-MHC myocytes even with the fall in force. The maintenance of power-generating capacity may be functionally important during the ejection phase of the cardiac cycle when the ventricle is shortening against a load and generating power. If the velocity of shortening is sustained at a given load, then the extent to which the myocytes shorten and, consequently, the ventricles contract during a heartbeat will remain similar, thereby helping to alleviate a fall in ejection volume during ischemia. Under similar Ca²⁺-activated conditions, α-MHC myocytes can generate more power and thus shorten faster at a given load than β-MHC myocytes. However, the difference in power capacity between α-MHC and β-MHC myocytes is diminished with increased Pi and/or H⁺ to the point where there is essentially no difference in power generation between α-MHC and β-MHC myocytes. Furthermore, increases in metabolites to concentrations greater than those employed in this study may actually result in β-MHC myocytes maintaining their functional capacity, whereas α-MHC myocytes continue to decline. We were unable to test this idea because force falls to levels that become experimentally limiting in our myocyte preparation.

Our earlier work (15) demonstrated an increase in loaded shortening velocity of cardiac myocytes with increased [Pi]; here we extend that work by reporting the effects of the addition of Pi and lowered pH individually and in combination.
which commonly occurs during ischemia in vivo in α-MHC and β-MHC myocytes. The P_i-induced increase in loaded shortening velocity occurred in both α- and β-MHC myocytes, resulting in greater peak power output when normalized for the fall in isometric force. The addition of P_i is thought to decrease force production by shifting populations of cross bridges from a force-generating state in which the actomyosin (AM) complex has ADP alone bound (AM-ADP) to a weakly or strongly bound lower force-generating state in which both ADP and P_i are bound (AM-ADP-P_i) (13). P_i also speeds the kinetics of transition through the force-generating steps leading to P_i release (1). We (15) previously concluded that loaded shortening and power output at intermediate and high loads are determined by force-generating transitions coupled to P_i release and/or an isomerization that is in rapid equilibrium with P_i release from the AM complex. On the other hand, H^+ alone had no effect in α-MHC myocytes, but it attenuated the P_i-induced increase in loaded shortening velocity when used in combination with P_i. Our result of lowered pH having no effect on loaded shortening velocity is in contrast to a previous report (33) where velocity decreased. The reason for the discrepancy between the results is not clear, but it may be due to preparation sizes, because the trabecular preparation used in that study had >20 times the cross-sectional area of our myocytes or differences in solution compositions. Attenuation of the P_i effect by lower pH that occurred may be the result of H^+ reducing the pool of strongly bound cross bridges by driving them to a weakly bound state, which has been postulated previously (17). Alternatively, it may be that H^+ directly slows P_i release from the ATP-binding cleft, which may also slow loaded velocity. In contrast to α-MHC myocytes, lowered pH did not attenuate the P_i-induced increase in loaded shortening velocity in β-MHC myocytes; in fact, loaded shortening was augmented toward faster velocities at the same load. Just how H^+ and P_i in combination tend to increase loaded shortening in β-MHC myocytes is unclear, but each metabolite may act independently on the steps that limit power output, because H^+ and P_i alone both increased loaded shortening velocity. Nevertheless, there is a MHC-dependent difference in responses to P_i at a lower pH in cardiac myocytes, and the submolecular mechanisms are unknown.

Possible implications for impaired function in heart disease. Myocardial tissue exposed to ischemic conditions has been shown to have greater relative expression of β-MHC in adult rat cardiac myocytes than in control tissues (41, 42). The expression of β-MHC in myocytes reduces their power-generating capacity (12), but in doing so these myocytes appear to become both more economical (34) and more resistant to the effects of ischemic metabolites (as shown in this study). Thus β-MHC appears to provide more resistance to falls in energy charge because it produces force at a much smaller energy cost (34) and faster loaded shortening velocity with increased metabolites. In agreement with this, postischemic function was greater in hearts expressing some (∼25%) β-MHC compared with control rat hearts (3). Theoretically, myocardium exposed to repeated bouts of ischemia may attempt to offset reductions in myocyte function that occurs as a result of lowered [ATP] or free energy of ATP hydrolysis by expressing the more economical isoform of MHC, thus conserving ATP as less is consumed in the myofilaments. Decreased AM activity and ATP conservation may also be important, because a decrease in [ATP], leading to a decrease in ATPase activity, specifically ones associated with Ca^2+ -extrusion (sarcoplasmic reticulum and sarcocemellar Ca^2+ ATPases), is thought to be causal for apoptosis and more chronic dysfunction (as reviewed in Ref. 2 and 4). In addition to β-MHC expression conceivably being protective to myocardium that is frequently made ischemic, increased β-MHC expression also commonly occurs during the progression of heart failure, experimentally and clinically (32, 41, 42). Thus β-MHC expression also appears to be important in the transition to a compensated heart in response to pathophysiological stimuli, perhaps by preserving myocardial function during energetic challenge.

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