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The rate of tension recovery in cardiac muscle correlates with the relative residual tension prevailing after restretch

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Abstract

Isolated cardiac muscles generate tension more quickly at higher levels of Ca^{2+} activation. We investigated the molecular mechanisms underlying this effect in permeabilized rat myocardial preparations by measuring the rate of tension recovery following brief shortening/restretch perturbations. Separate series of experiments used Ca^{2+} -activating solutions with different pH values (pH 6.75, 7.00, and 7.25) and different phosphate (P_i) concentrations (0, 2.5, and 5.0 mM added P_i) to modulate the recovery kinetics. Subsequent analysis showed that the rate of tension recovery correlated ($P < 0.001$) with the relative residual tension, that is, the minimum tension measured immediately after restretch normalized to the steady-state isometric tension for the experimental condition. This new finding suggests that the rate at which cardiac muscles develop force increases with the proportion of cross bridges bound to the thin filament and is strong evidence of cooperative contractile activation.

Keywords

cardiac contractility and energetics; muscle contraction; myocardial contractility

The rate at which myosin cross bridges generate tension is an important determinant of ventricular performance (9). It can be measured by subjecting an isolated myocardial preparation to a rapid shortening/restretch protocol in which the muscle is allowed to shorten briefly at near maximal velocity before being stepped back to its original length (1).

Numerous experiments employing this type of protocol have shown that the rate of tension recovery (k_{tr}) increases with the free Ca^{2+} concentration in the myofibrillar space (7). Activation dependence of k_{tr} could reflect a direct effect of Ca^{2+} on one or more state transitions in the actomyosin scheme (1), but Ca^{2+} could also mediate its kinetic effects indirectly by altering the dynamic state of the linear arrays of actin-binding sites and myosin S1 heads. For example, k_{tr} could vary with the free Ca^{2+} concentration if Ca^{2+} influenced the number of cross bridges bound between the myofilaments immediately after restretch, and k_{tr} was, as previously suggested by Kenneth B. Campbell (3) from Washington State University, modulated by cooperative mechanisms (7).

This work describes the results of tension recovery measurements performed with the use of permeabilized rat myocardial preparations. Analysis of an initial series of experiments performed under control conditions [pH 7.00, no added phosphate (P_i)] showed that k_{tr} values measured at different levels of Ca^{2+} activation correlated with the relative residual tension prevailing immediately after restretch. One of us (K. S. Campbell) recently reported similar behavior in rat soleus preparations (5). These observations are important because they imply that the rate at which striated muscles develop tension following a rapid shortening/restretch

maneuver could be determined by the mechanical state of the muscle immediately after the perturbation.

We tested this hypothesis by using Ca^{2+} -activating solutions with different pH values and different P_i concentrations to modulate tension recovery kinetics. To our surprise, k_{tr} increased with the relative residual tension in each experimental condition. Moreover, all of the conditions followed the same general trend. A subsequent calculation showed that the k_{tr} and relative residual tension values collated from the data sets for the five experimental conditions were significantly correlated ($P < 0.001$).

We interpret this new result to mean that Ca^{2+} exerts an indirect effect on tension recovery kinetics. The rate of tension recovery in a given preparation depends on the dynamic mechanical state of the contractile apparatus.

MATERIALS AND METHODS

Preparations

Multicellular rat cardiac preparations were obtained by mechanical disruption of hearts excised from female Sprague-Dawley animals. Animal use was approved by the Institutional Animal Care and Use Committee at the University of Kentucky. Additional details are provided as supplementary data (see supplementary data posted in the online version of this article).

Solutions

Separate batches of solutions were used to investigate 1) the pH dependence (pH values of 6.75, 7.00, and 7.25) and 2) the P_i dependence (0, 2.5, and 5.0 mM of added P_i) of myocardial mechanical properties. Solution compositions were determined by using Maxchelator 2.50 software (11). All solutions contained (in mM) 20 imidazole, 14.5 creatine phosphate, 7 EGTA, 4 MgATP, and 1 free Mg^{2+} and free Ca^{2+} ranging from 1 nM {pCa ($= -\log_{10}[\text{Ca}^{2+}]$) 9.0} to 32 μM (pCa 4.5) and sufficient KCl to adjust the ionic strength to 180 mM. Phosphate scavengers were not used. All solutions therefore contained a basal concentration of contaminant P_i previously estimated (10) at ~ 0.7 mM.

Mechanical measurements

Preparations [length, 621 μm (SD 173)] were attached between a force transducer (resonant frequency, 600 Hz; model 403, Aurora Scientific, Aurora, Ontario, Canada) and a motor (step time, 0.6 ms; model 312B, Aurora Scientific) and stretched to a sarcomere length of 2.27 μm (SD 0.01) in a pCa 9.0 solution. Cross-sectional area [$1.42 \times 10^{-8} \text{ m}^2$ (SD 0.96)] was estimated assuming a circular profile. With the exception of some data shown in Fig. 2C, all measurements were performed at 15°C. Experiments were performed using SLControl software (6).

Each preparation was initially activated in a control pCa 4.5 solution (pH 7.00, no added P_i). Once tension attained steady state, the muscle was rapidly shortened by 20%, held at the short length for 20 ms, and then stepped back to its original length. Steady-state isometric tension for this condition equaled 24.2 kN m^{-2} (SD 12.1). The muscle was then immersed in the pCa 9.0 solution corresponding to one of the five metabolite conditions (pH 6.75, no added P_i ; pH 7.00, no added P_i ; pH 7.25, no added P_i ; pH 7.00, 2.5 mM added P_i ; and pH 7.00, 5.0 mM added P_i). Tension recovery measurements were subsequently performed for that condition in solutions with pCa values ranging from 6.5 to 4.5. Some stable preparations were subjected to a second series of Ca^{2+} activations. Metabolite conditions were tested in a pseudorandom order. Steady-state isometric tension measured in control pCa 4.5 solution (pH 7.00, no added P_i) dropped by an average of 8% during an activation series.

Data analysis

k_{tr} values were calculated as $-\ln(1/2)/(t_{1/2})$, where $t_{1/2}$ is the time required for tension to rise from P_{resid} to $1/2(P_{max} + P_{resid})$ and where P_{max} is the maximum tension attained after restretch (see Fig. 2 of Ref. 5). P_{resid} was defined as the minimum tension prevailing after restretch (Fig. 2A). Control resting tensions (measured in pH 7.00, no added P_i , pCa 9.0 solution) were subtracted from all measured force values to correct for passive elastic properties (Fig. 2A). Records were excluded from further analysis if they had $P_{resid}/P_{ss} \geq 0.75$ and/or $r^2 \leq 0.98$ for a monoexponential fit to the recovery time course, where P_{ss} is steady-state isometric tension. This ensures that the statistical analysis is restricted to unambiguous tension recovery records that are reasonably characterized by a single time constant. Summary results are presented as means (SD).

RESULTS

Figure 1A shows superposed recordings for a single myocardial preparation subjected to a brief shortening/restretch perturbation in each of three different pCa 4.5 solutions. Steady-state isometric tension was greatest in the solution with a pH of 7.25, but the recovery to steady state in this condition took longer than during either the pH 7.00 or the pH 6.75 activations. Averaged data collected from multiple preparations at different levels of Ca^{2+} activation for each pH value are shown in Fig. 1B.

The effects of different amounts of added P_i are shown in Fig. 1, C and D. The addition of 5 mM P_i reduced pCa 4.5 steady-state isometric force to 0.68 (SD 0.07) of the control value and increased the measured value of k_{tr} from 10.3 (SD 1.4) to 19.2 s⁻¹ (SD 1.7).

Figure 2B shows k_{tr} values from all of the experimental records represented in Fig. 1, B and D, combined, plotted against the measured P_{resid} -to- P_{ss} ratio (P_{resid}/P_{ss}) for each trial. (Example P_{resid} and P_{ss} values are shown in Fig. 2A. Superposed plots for the different pH and P_i conditions are presented as supplementary data in the online version of this article.) The P_{resid}/P_{ss} ratios define the relative residual tension prevailing immediately after restretch in each record and correlate with the measured k_{tr} values ($P < 0.001$). “Dummy variable” analysis (8) showed that the slopes of the k_{tr} - P_{resid}/P_{ss} relationships for the different pH and P_i conditions were not significantly different ($P > 0.05$, see supplementary data in the online version of this article).

The squares in Fig. 2C show the k_{tr} - P_{resid}/P_{ss} relationship for the subset of the data shown in Fig. 2B corresponding to control Ca^{2+} activations (15°C, pH 7.00, no added P_i). The triangles show the results of comparable measurements (pH 7.00, no added P_i) performed at 22°C. Dummy variable analysis showed that the slopes of regression lines fitted to the k_{tr} - P_{resid}/P_{ss} relationships for the two temperature conditions were significantly different ($P < 0.001$).

DISCUSSION

Figure 1, B and D, shows k_{tr} values measured at different levels of Ca^{2+} activation plotted against relative isometric tension. Each panel shows three distinct relationships. This suggests that adjusting either the pH or the P_i concentration in the myofibrillar space results in a contractile apparatus that exhibits distinctly different actomyosin kinetics.

Figure 2B shows that all of the k_{tr} values from Fig. 1, B and D, follow a single linear relationship if they are plotted against their respective relative residual tensions (P_{resid}/P_{ss}). This graph suggests an alternate view of contractile regulation in which pH and P_i exert their kinetic effects by altering the P_{resid}/P_{ss} ratio.

These interpretations are dramatically different and probably represent two extreme viewpoints. On reflection, we favor an intermediate view of contractile regulation in which the rate at which muscles develop tension following rapid shortening/restretch maneuvers is determined by the mechanical state of the muscle, the metabolic environment, and many other factors.

The effects of altered metabolite concentrations on the actomyosin cycle are already well documented (7,12). How might the $P_{\text{resid}}/P_{\text{ss}}$ ratio exert its kinetic effects? One answer is through cooperative activation of the contractile apparatus (3).

Burton et al. (2) suggest that P_{resid} in skeletal fibers (termed T_{min} in their work) reflects attached cross bridges that “slip” along the thin filament during rapid restretch movements. [Stiffness measurements reported by K. S. Campbell (5) also support this conclusion.] If P_{resid} reflects attached cross bridges in myocardial preparations as well as in skeletal fibers, the $P_{\text{resid}}/P_{\text{ss}}$ ratio calculated in this work could be indicative of the proportion of cross bridges (expressed relative to the number attached at steady state) linking the myofilaments immediately after restretch. The linear relationship between the $P_{\text{resid}}/P_{\text{ss}}$ ratio and k_{tr} shown in Fig. 2B could then be explained if k_{tr} increases with the probability of actin-binding sites being available for cross-bridge attachment (4), and this probability is, in turn, related via a cooperative mechanism to the proportion of cross bridges bound to the thin filament (7). pH and P_{i} could then modulate tension development kinetics by influencing the number of cross bridges that “slipped” along the thin filament during the restretch movement as well as by influencing the rate at which attached cross bridges progressed to strongly bound force-generating states.

The mechanism outlined above could also help to explain the intriguing finding of Tesi et al. (12) that tension transients induced in isometrically contracting psoas myofibrils by rapid increases in P_{i} concentration are three to four times faster than those produced by rapid decreases in P_{i} . This would be consistent with the idea that high P_{i} concentrations speed actomyosin kinetics indirectly by cooperatively activating the thin filament.

Figure 2C shows that raising the experimental temperature significantly increased the slope of the $k_{\text{tr}}-P_{\text{resid}}/P_{\text{ss}}$ relationship. This result reinforces the point that tension recovery kinetics are not determined solely by the proportion of cross bridges bound between the filaments immediately after restretch and must reflect other factors including sarcomere length, regulatory protein isoforms, and myosin heavy chain expression (7). The increased slope of the $k_{\text{tr}}-P_{\text{resid}}/P_{\text{ss}}$ relationship at 22°C could be explained by faster molecular state transitions at the higher temperature or by potential temperature dependence of the cooperative unit size (7).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1.

Effects of altered pH and P_i concentration on rate of tension recovery (k_{tr}). *A*: tension records for a myocardial preparation activated in pCa 4.5 solutions with pH values of 7.25, 7.00, and 6.75. A logarithmic time axis has been used to emphasize the behavior of the muscle immediately after restretch. *B*: symbols show means (SD) of k_{tr} values measured with the use of multiple preparations ($n = 6$ to 21 for the different conditions) in different pCa solutions plotted against relative tension (normalized to steady-state isometric tension measured in the pH 7.00, no added P_i , pCa 4.5 solution) for the pH 6.75, 7.00, and 7.25 conditions. *C* and *D*: as in *A* and *B* but showing the effects of 0, 2.5, and 5.0 mM added P_i at pH 7.0.

Fig 2.

Correlations between k_{tr} values and relative residual tensions. **A**: an illustrative tension record (pH 7.00, no added P_i , pCa 4.5) plotted using a logarithmic time axis. P_{ss} , steady-state isometric tension; P_{resid} , residual tension prevailing immediately after restretch; P_{resid}/P_{ss} , relative residual tension (ratio of these two parameters). **B**: symbols show k_{tr} and P_{resid}/P_{ss} values for all of the experimental data summarized in Fig. 1, **B** and **D**. The solid line indicates the least-squares linear fit. k_{tr} correlates with P_{resid}/P_{ss} ($P < 0.001$). **C**: squares show the subset of the data from **B** corresponding to pCa solutions of pH 7.00 with no added P_i . Triangles show comparable measurements (pH 7.00, no added P_i) performed at 22°C . Dashed lines show the 95% confidence bands of each regression line.