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

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Submitted 5 July 2006; accepted in final form 14 December 2006

Campbell KS, Holbrook AM. The rate of tension recovery in cardiac muscle correlates with the relative residual tension prevailing after restretch. Am J Physiol Heart Circ Physiol 292: H2020–H2022, 2007. First published December 22, 2006; doi:10.1152/ajpheart.00714.2006.—Isolated cardiac muscles generate tension more quickly at higher levels of Ca2+ 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 Ca2+-activating solutions with different pH values (pH 6.75, 7.00, and 7.25) and different phosphate (Pi) concentrations (0, 2.5, and 5.0 mM added Pi) 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.

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 (ktr) increases with the free Ca2+ concentration in the myofibrillar space (7). Activation dependence of ktr could reflect a direct effect of Ca2+ on one or more state transitions in the actomyosin scheme (1), but Ca2+ 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, ktr could vary with the free Ca2+ concentration if Ca2+ influenced the number of cross bridges bound between the myofilaments immediately after restretch, and ktr 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 (Pi)] showed that ktr values measured at different levels of Ca2+ 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 Ca2+-activating solutions with different pH values and different Pi concentrations to modulate tension recovery kinetics. To our surprise, ktr 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 ktr 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 Ca2+ 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 Pi dependence (0, 2.5, and 5.0 mM of added Pi) of myocardial mechanical properties. Solution compositions were determined by using Maxim elator 2.50 software (11). All solutions contained (in mM) 20 imidazole, 14.5 creatine phosphate, 7 EGTA, 4 MgATP, and 1 free Mg2+ and free Ca2+ ranging from 1 nM [pCa (= −log10(Ca2+)) 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 Pi, 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 × 10−8 m2 (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 Pi). Once tension attained steady state, the muscle was rapidly shortened by 20%, held at the short length for 30 s, and then allowed to shorten freely, recording tension at 173 Hz. This protocol was repeated at 30 s intervals until 90% of the tension had recovered to steady state (14). We defined this new tension as T1. Additional data are presented in terms of ktr for each experimental condition and the relative residual tension prevailing immediately after restretch.

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Research reported in this publication was supported by the American Heart Association (Grant 0455786N). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Fig. 1. Effects of altered pH and Pi concentration on rate of tension recovery (k_r). 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_r 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 Pi, 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 Pi, at pH 7.0.

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 Pi; pH 7.00, no added Pi; pH 7.25, no added Pi; pH 7.00, 2.5 mM added Pi; and pH 7.00, 5.0 mM added Pi). 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 Ca²⁺ activations. Metabolic conditions were tested in a pseudorandom order. Steady-state isometric tension measured in control pCa 4.5 solution (pH 7.00, no added Pi) dropped by an average of 8% during an activation series.

Data analysis. k_r values were calculated as −ln(1/2)/t₁/₂, where t₁/₂ 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 Pi, 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 ≥ 0.75 and/or r² ≤ 0.98 for a monoeponential 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 Ca²⁺ activation for each pH value are shown in Fig. 1B.

The effects of different amounts of added Pi are shown in Fig. 1, C and D. The addition of 5 mM Pi 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_r from 10.3 (SD 1.4) to 19.2 s⁻¹ (SD 1.7).

Figure 2B shows k_r values from all of the experimental records represented in Fig. 1, B and D, combined, plotted against the measured P_resid/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 Pi 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_r values (P < 0.001). “Dummy variable” analysis (8) showed that the slopes of the k_r-P_resid/P_ss relationships for the different pH and Pi 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_r-P_resid/P_ss relationship for the subset of the data shown in Fig. 2B corresponding to control Ca²⁺ activations (15°C, pH 7.00, no added Pi). The triangles show the results of comparable measurements (pH

Fig. 2. Correlations between k_r values and relative residual tensions. A: an illustrative tension record (pH 7.00, no added Pi, pCa 4.5) plotted using a logarithmic time axis. P_resid, steady-state isometric tension; P_max, residual tension prevailing immediately after restretch; P_resid/P_ss, relative residual tension (ratio of these two parameters). B: symbols show k_r 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_r 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 Pi. Triangles show comparable measurements (pH 7.00, no added Pi) performed at 22°C. Dashed lines show the 95% confidence bands of each regression line.
7.00, no added Pi) performed at 22°C. Dummy variable analysis showed that the slopes of regression lines fitted to the k_tr-Presid/Pss 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 Pi 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 Pi 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_{resid}/P_{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_{resid}/P_{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_{resid}/P_{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 Pi 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 Pi concentration are three to four times faster than those produced by rapid decreases in Pi. This would be consistent with the idea that high Pi 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_tr-P_{resid}/P_{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_tr-P_{resid}/P_{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).

ACKNOWLEDGMENTS

We thank Roger Cooke (Univ. of California, San Francisco) and Kerry McDonald (Univ. of Missouri-Columbia) for helpful discussions and one of the referees for alerting us to dummy variable analysis. K. S. Campbell is the principal investigator on two research grants that are relevant to this manuscript.

GRANTS

This study was supported by American Heart Association Grant SDG-0630079N, National Institute on Aging Grant AG-028162, and the University of Kentucky Research Challenge Trust Fund.

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