1799-Pos

Phosphorylation of Myosin Binding Protein-C Alters the Proximity of Cross-Bridges to Actin and Accelerates Myocardial Twitch Kinetics

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The strength and kinetics of cardiac contraction vary on a beat-to-beat basis in efforts to match cardiac output in response to changing circulatory demands. In living myocardium, the beta-adrenoreceptor agonist dobutamine initiates protein kinase A (PKA)-mediated phosphorylations of Ca^{2+} handling proteins and contractile proteins including cardiac myosin binding protein-C (cMyBP-C) and cardiac troponin I (cTnI), which leads to potentiation of twitch force and faster kinetics of force development and relaxation. Our previous studies in skinned myocardium suggest that PKA phosphorylation of cMyBP-C disrupts its interaction with myosin subfragment 2 (S2), which relieves the tether-like constraint of myosin heads imposed by cMyBP-C, and thereby accelerates cross-bridge cycling kinetics. To examine the relative role of cMyBP-C phosphorylation in altering twitch kinetics, we recorded twitch force and low-angle x-ray diffraction patterns in between twitches and near maximum twitch force in intact trabeculae isolated from murine myocardium electrically stimulated at 0.5 Hz in the presence and absence of dobutamine. Our data suggest that phosphorylation of cMyBP-C caused a radial or azimuthal displacement of cross-bridges towards the thin filament *in vivo* prior to the twitch, which contributes to the accelerated contraction kinetics following twitch stimulation. These results suggest that interactions between cMyBP-C and the S2-domain of myosin heavy chain are dynamically regulated by phosphorylation of cMyBP-C and function to modulate the availability and cooperative binding of cross-bridges to actin during the myocardial twitch.

1800-Pos

Myosin Crosslinking and EPR Capture the Start of Force Generation in Muscle Fibers

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Crosslinking the two most reactive Cys (SH1 and SH2) of the myosin catalytic domain (CD) inhibits force production and ATP hydrolysis and locks myosin in a weak actin-binding conformation with the CD immobilized and orientationally disordered. These results suggest that crosslinking traps a state in which the myosin head is on the cusp of force generation. In the present study, we measured the structural dynamics of myosin's light chain domain (LCD) in skeletal muscle fibers during rigor, relaxation, and with SH1 and SH2 crosslinked. To measure LCD structural dynamics, we exchanged spin labeled RLC for native RLC in permeabilized muscle fibers, with retention of function, then used EPR spectroscopy to measure structural dynamics. EPR spectra indicate when SH1 and SH2 are crosslinked, the LCD is in an orientation intermediate between relaxation and rigor, indicative of a state beginning to generate force. The saturation transfer EPR (STEPR) spectrum from these fibers does not change with crosslinking, demonstrating that that the LCD undergoes very slow dynamics, as in rigor, and is less dynamic than relaxation. In order to relate LCD structural dynamics with those of the CD, we measured CD structural dynamics in fibers by directly crosslinking SH1 and SH2 with BSL. EPR spectra from these fibers reveal that the CD is highly disordered, with dynamics ten times slower than in relaxation. Thus when SH1 and SH2 are crosslinked, both domains exhibit structural dynamics intermediate between relaxation (pre-power stroke) and rigor (post-power stroke). This supports the conclusion that SH1-SH2 crosslinking traps a state analogous to an initial force-generating state. We propose that this state is the missing link needed to explain how myosin undergoes a transition from dynamic disorder to order as it converts chemical energy to mechanical work.

1801-Pos

Passive Properties of Single Skeletal Muscle Fibers are Altered in Heart Failure Patients

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We previously demonstrated that myosin heavy chain (MHC) content is decreased in skeletal muscle fibers from heart failure patients, thereby altering Ca^{2+}-activated (pCa 4.5) contractile properties. In this study, we examined chemically-skinned, single human skeletal muscle fibers under relaxed conditions (low $[Ca^{2+}]$, pCa 8) using small amplitude sinusoidal analysis to determine whether the loss of MHC content affects their viscoelastic properties. We obtained *vastus lateralis* (quadriceps) muscle from needle biopsies of 9 patients and 5 sedentary controls. Surprisingly, Type I (slow twitch) and Type IIA (fast twitch) fibers produced slight but significant positive oscillatory work under relaxed conditions at 25°C, indicating the presence of cross-bridge cycling. This positive oscillatory work persisted when $[Ca^{2+}]$levels were lowered even further (to pCa 9). Addition of 40 mM 2,3-butanedione monoxime (BDM) or 100 μM blebbistatin, inhibitors of skeletal muscle myosin ATPase, to a subset of Type I and IIA fibers eliminated their positive work output and reduced their high frequency elastic and viscous moduli. Type I fibers from heart failure patients had lower isometric tensions as well as lower elastic and viscous moduli compared to controls; whereas, Type IIA fibers from patients had tension values similar to controls, while the frequency of maximum work output (an indicator of myosin kinetics) was greater. Taken together, these results demonstrate that cross-bridges contribute to the viscoelastic properties of human skeletal muscle in the 'relaxed' state (low intracellular $[Ca^{2+}]$) and that cycling myosin heads may contribute to resting muscle tone. As under Ca^{2+}-activated conditions, heart failure affects relaxed muscle properties differentially depending upon fiber type. Decreased resting muscle tone in heart failure patients may decrease postural stability and contribute to a reduced ability to perform activities of daily living.

1802-Pos

Correction of Error in Fiber Length Due to Lever Arm Rotation During Mechanical Tests of Single Muscle Fibers

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The single muscle fiber preparation is a popular technique to assess mechanical properties at the cellular level and how these properties change with aging, disease, exercise, etc. Fibers are usually attached to wires extending perpendicularly from the lever arm of a high speed motor and force transducer that are then mounted to the stage of a microscope. Because of lever arm rotation, the fiber does not move along the optical plane, introducing a length measurement error if length is measured along the optical plane. The purpose of this study was to 1) calculate the error of lever arm movement and 2) provide a correction equation. The error was calculated assuming 2 mm fiber length at up to 50% displacement, because this is a commonly used stretch or slack magnitude (~15% slacking with unloaded shortening velocity, ~30% lengthening response to eccentric contraction, ~50% stretch with passive tension). The range of error was hyperbolically enlarged as either lever arm length or optical plane stretch magnitude increased, and this error was exacerbated with the increase of both factors. For example, with a 5 mm connector attached to the lever arm, the relative error [100×(actual displacement-target displacement)/target displacement] was 0.59%, 1.36%, and 1.78% with 10, 30, and 50% of stretch or slacking, respectively. However, with a 10 mm connector, the relative error was 2.38%, 5.66%, and 7.64%, and in the case of a 15 mm connector, the relative error increased dramatically up to 5.36%, 12.69%, and 17.10%. We suggest the following correction equation to eliminate potential errors; $q=b+f-r/mT+1//(m^2T^2+m^2+T^2+1)^{1/2}$, where q = observed fiber length, b=connector length, f=fiber length, h=lever arm length, $r=(b^2+h^2)^{1/2}$, $m=-h/(b+f)$, $T=[p^2-(d-r)^{1/2}/(d^2+r^2-p^2)]^{1/2}$, p=target fiber length, and $d=[(b+f)^2+h^2]^{1/2}$.

1803-Pos

Using White Noise to Probe Actomyosin Cycling Kinetics During Shortening and Lengthening in Drosophila Flight Muscle Fibers

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Our laboratory routinely measures demembranated muscle fiber mechanics using small amplitude sinusoidal length perturbation analysis and estimates kinetics of actomyosin cycling and myosin attachment time. We have developed a complementary measurement technique using small, random changes in muscle length (white-noise length stimuli) that simultaneously cover a broad frequency spectrum, comparable with the sinusoidal length perturbations. We find the white-noise technique provides a description of the viscoelastic properties that is consistent with sinusoidal analysis measurements, rapidly capturing much of the physiological behavior associated with contracting muscle fibers. The white-noise technique samples a vast range of system behavior in a fraction of the time required to complete sinusoidal analysis, and does not require the linear response underlying sinusoidal analysis methods. Thus, we combined the white-noise stimuli with a linear shortening and lengthening transients to probe cross-bridge cycling behavior during these periods of varied load. Preliminary measurements using demembranated dorsal longitudinal