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

flight muscle fibers from *Drosophila melanogaster* indicate faster cross-bridge cycling kinetics during lengthening and slower cycling during shortening, compared to isometric contraction. During isometric contraction we estimate a myosin attachment time of 5.0 ms, and 4.6 versus 5.5 ms during the lengthening and shortening transients, respectively. These initial applications of the white-noise system analysis technique show promise for future studies probing molecular processes that underlie complicated length transients associated with normal muscle contraction.

1804-Pos

The Isotonic Velocity Transient Following a Sudden Rise in Force Imposed on the Muscle Sarcomere During Unloaded Shortening Reveals a Rate Limiting Step in Detached Myosin Motors

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Energy balance studies indicate that ATP splitting by myosin motors during rapid shortening of skeletal muscle is not sufficient to account for the energy (mostly heat) output (Rall et al., *J Gen Physiol* 68(1), 13, 1976; Homsher et al., *J Physiol* 321, 423, 1981). We investigated the kinetic step of the myosin ATP-ase cycle related to this phenomenon in single muscle fibers from *Rana esculenta* (~2.15 μm sarcomere length, 4°C), by recording the isotonic velocity transient following a force step from zero to the isometric tetanic value (T_0). Once the isometric tetanus had developed, the force was first clamped to zero for a range of times from 10 ms to 18 ms (during which the fiber shortened at the maximum velocity by 30 nm hs^{-1} to 50 nm hs^{-1}) and then raised again to T_0 in a stepwise manner (~120 μs). The elastic lengthening induced by the force step was followed by a transient isotonic lengthening, the size of which ranged from 40 to 60 nm hs^{-1} depending on the size of the preceding shortening. The lengthening velocity was larger for larger shortening size and progressively decreased to approach the isometric condition with a half-time of 2-3 ms. Similarly, the half-sarcomere stiffness recovered the isometric value e_0 from the unloaded shortening value of 0.4 e_0 with an exponential time course with τ ~3 ms. We conclude that during rapid shortening a ~3 ms-transition between detached states of the myosin motor, likely related to the ATP hydrolysis, becomes rate limiting. Accumulation of motors in the state preceding the hydrolysis step can account for the unexplained energy during rapid shortening. Supported by MIUR (Italy).

1805-Pos

Effect of Inorganic Phosphate on the Rate of ADP Release During Ramp Shortening in Activated Permeabilized Fibers from Rabbit Psoas Muscle

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A coumarin-labeled recombinant phosphorylated nucleoside diphosphate kinase (P~NDPK-IDCC; West et al., 2009; Biophys.J. 96:3281-3294), was used as a fluorescence probe for time-resolved measurement of changes in [MgADP] during steady shortening of single permeabilized rabbit psoas fibers at 12°C (pCa 4.5, pH 7.1, ATP 5.7mM). A fiber contracted from the relaxed state by immersion into a Ca^{2+} activation solution at 0°C. Temperature activation was then initiated by immersion of the fiber into silicone oil at 12°C. The activation solutions were prepared with either zero added P_i or with 10 mM added P_i at constant ionic strength (0.15 M). The decline in fluorescence intensity emission (470nm) associated with MgADP-dependent dephosphorylation of P~NDPK-IDCC (60 μM) was monitored during activation and during a period of isovelocity shortening. Fluorescence emission (580 nm) of a rhodamine dye was measured simultaneously to correct the P~NDPK-IDCC signal for the effects of fiber movements and volume changes. The rate of MgADP release in the absence of added P_i increased from 0.7 ± 0.07 mM·s^{-1} at 0.2 fiber-lengths·s^{-1} to approximately 3.4 ± 0.25 mM·s^{-1} for shortening velocities between 1 and 2 fiber-lengths·s^{-1}. When 10 mM P_i was added, the rate of ADP release at 0.2 fiber-lengths·s^{-1} was 0.48 ± 0.05 mM·s^{-1} and 2.6 ± 0.4 mM·s^{-1} at 1-2 fiber-lengths·s^{-1}. In the absence of added Pi, the rate of ATP hydrolysis calculated from the appearance of ADP is similar to that calculated previously from the appearance of P_i using MDCC-labeled phosphate binding protein, over the same range of fiber shortening speeds (He et al., 1999, J. Physiol. 517: 839-854). Thus P_i slows the ATPase rate by 25-30%, both in the isometric and isotonic state. The energetic consequences will be discussed.

1806-Pos

Cross-Bridges and Sarcomere Stiffness in Single Intact Frog Muscle Fibers

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The number of cross-bridges formed in activated skeletal muscles is a key information for both energetics and mechanics of muscle contraction. In this study we determined the cross-bridge number in single fibers by measuring the tension $P_c$which forcibly detached the cross-bridge by fast stretches (Bagni et al. 2005, *J. Physiol* 565). Fibers, isolated from the tibialis anterior muscle of *Rana esculenta*, were mounted between an electromagnetic motor and a fast force transducer. Sarcomere length was measured by means of a striation follower device. Measurements were made during tetanus rise in normal Ringer and in sub-maximal tetanic contractions in Ringer-BTS (*N*-benzyl-p-toluene sulphonamide, 1 μM) at 5°C at sarcomere length of 2.1 μm.
The results were compared with fiber stiffness, another indicator of cross-bridge number, measured with 4 kHz sinusoidal length oscillations (1 $nmhs^{-1}$ p-p amplitude). The stiffness-tension relation was the same both during the tetanus rise and Ringer-BTS and showed the non-linearity expected from the myofilament compliance. However, the data could not be fitted satisfactorily with a simple model made of cross-bridge and linear filament compliances in series. A good fit was obtained by assuming that a fraction (~14%) of attached bridges at tetanus plateau was generating no-force. Relative myofilament and cross-bridge compliance resulted 0.37 and 0.63 respectively. The stretch data showed a linear relation between $P_c$and tension with a slope consistent with the presence of the non-force generating bridges suggested by stiffness data. These results suggests the existence of a possible non-linearity between cross-bridge force and stiffness and show that the relation between fiber stiffness and cross-bridge number is not as simple as usually assumed.

1807-Pos

Ultrafine Striations in Skeletal Muscle Revealed by 3D Super-Resolution Fluorescence Microscopy

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Super-resolution three-dimensional imaging was achieved using newly synthesized photoactivatable quantum dot probes. Semiconductor quantum dots are nanoparticles with high photostability and brightness. They were modified with a novel quencher system to make them photoactivatable. The unique properties of these photoactivatable quantum dots enable single-fluorophore localization in three dimensions using a confocal microscopy optical sectioning method with a piezo scanner. To image skeletal muscle at resolutions exceeding that of the standard confocal microscope, the photoactivatable quantum dots were conjugated to secondary antibodies. Myofibrilar bundles were dual- labeled using both a primary antibody to myosin rod with the secondary antibody-conjugated photoactivatable 655 nm quantum dot and a primary antibody against tropomyosin with the secondary antibody-conjugated photoactivatable 525 nm quantum dot. During the 3D acquisition on a spinning disk confocal with piezo scanner, different individual quantum dots were photoactivated during each of hundreds of cycles. A sufficient number of single quantum dots were localized, reduced to their center of mass and then reconstructed to a super-resolution image. The resulting super-resolution image shows a sub-diffraction resolution in both lateral and axial directions. The broad absorbance band of quantum dots enables the excitation of both quantum dots with the same laser type. This technique enables the relative localization of two different myofibril proteins at nanometer scale resolutions in solution demonstrates ultrafine striations in the staining pattern with widths less than 70 nm in axial and lateral dimensions that are not evident by conventional confocal microscopy due to its resolution constraints. The bands appear to be related to the presentation of epitopes at the surface of thin and thick filaments and may be related to thick and thin filament binding proteins and/or structural variations in the actin and myosin filaments.

1808-Pos

Structural and Functional Gradients with Temperature in the Flight Muscle of Manduca Sexta

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The force/extension curve of the flight muscle of the Hawkmoth, *Manduca sexta* is remarkably similar to that of mammalian cardiac muscle suggesting that it may serve as a useful model system for certain aspects of cardiac muscle structure and function (J Exp Biol. 2004;207:2455). More recently, it was discovered that these animals maintain an astonishing thermal gradient of 8.8 C in the 5 mm distance dorsal to ventral in their dorso-longitudinal flight muscles (DLMs). Does the existence of this thermal gradient necessarily imply a functional gradient? Do these changes in function have, as their basis, changes in structure? Twitch dynamics of individual fibers within the DLM in intact animals are temperature dependent so that mechanical power output (and it's phase dependence) varies with depth in the tissue. A surprising observation was that all five sub-units in the DLM were simultaneously activated. Cooler