Muscle Regulation

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A Two Phase Decrease in Force and Intracellular Ca^{2+} during the Relaxation of Mouse Lumbricle Muscle

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Intracellular Ca^{2+} and force were measured simultaneously in intact mouse lumbricle muscle (toe muscle). All motor units were stimulated simultaneously for a fixed period of time at varying frequencies. During the fixed period of stimulation average force and intracellular Ca^{2+} were computed. Results showed that there was a sigmoidal increase in both average force and intracellular Ca^{2+} as a function of log10 of the increasing frequency of the muscle stimulation. At the end of the stimulation period the isometric contracting muscle relaxed in two phases. Immediately following the end of stimulation there was an initial slow phase of relaxation associated with a very rapid fall in intracellular Ca^{2+} (Phase 1). Following the initial phase of slow relaxation (Phase 1) there was a very rapid relaxation associated with a much slower rate of decrease in intracellular Ca^{2+} (Phase 2). Analysis of Ca^{2+} records during the Phase 2 relaxation showed that the rapid decrease in force was associated with extra Ca^{2+} being released into the myoplasm. The initial slow phase of relaxation (Phase 1) was found to be dependent upon the initial isometric force (active state) at the time of cessation of the muscle stimulus. The relationship between phase 1 relaxation rate and active state was linear showing that a 50% decrease in maximum active state would result in approximately a 200% increase in phase 1 relaxation rate. The results suggest that the initial slow rate of cross-bridge dissociation (Phase 1) is dependent upon the activation state of the myofilaments and that the rapid relaxation during Phase 2 is associated with Ca^{2+} being released from the myofilaments . Supported by AHA 10GRNT4450042

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Properties of the Intermediate State of Actin-Tropomyosin-Troponin

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Troponin, tropomyosin and actin form an allosteric regulatory system. This regulated actin stimulates myosin ATPase activity only in the presence of positive effectors. Models of regulation often include 3 states of activity. Calcium causes partial activation and increases the fraction of the intermediate state (C state) to 50–70%. NEM labeled S1 stabilizes the active state (O state) and increases the rate beyond that observed without tropomyosin-troponin.

Deleting 14 amino acid residues from the C-terminus of TnT renders regulated actin incapable of entering the inactive state. Thus regulated actin filaments containing Δ14TnT differ from wild type in the following ways: 1) S1-ADP binding is not cooperative. 2) Acrylodan-tropomyosin fluorescence levels in EGTA are uncharacteristic of the inactive state. 3) The normalized ATPase rate in calcium is 37% of the maximum rate compared with 17% for wild type troponin I. That is, filaments with the Δ14TnT mutation spend twice as much time in the active state as wild type actin filaments. 4) The rate of S1 binding is slightly faster in EGTA but not in calcium. In the absence of calcium such actin filaments exist almost totally in the intermediate state (C state) allowing that state to be studied.

The lowest measured ATPase activity of S1 in the presence of regulated actin filaments was defined as zero. The fully activated rate in the presence of NEM-S1 was defined as 1. The activity in EGTA with actin filaments containing the Δ14TnT mutant was 1.4% of that of the fully active state. That is, the intermediate state had little ability to activate myosin S1 ATP hydrolysis. Furthermore, virtually all of the regulation of ATPase activity occurred in going from the active state to the intermediate state.

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Effects of Epigallocatechin-3-Gallate on the Activation of Cardiac Thin Filaments by Calcium and Strong-Binding Crossbridges

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Cardiac muscle is activated via the single Ca^{2+}-binding site (site II) in the N-domain of cardiac troponin C (cTnC). However, the discovery of cardiomyopathy-associated mutations in the C-domain of cTnC has called attention to the possible role of this domain in contractile regulation. Our previous work suggested that the C-domain Ca^{2+}/Mg^{2+} binding sites (III and IV) can modulate myosin-mediated activation of the thin filament (Biochem Biophys Res Comm 408:697, 2011). Additional evidence for a regulatory role for the C-domain has come from the discovery of pharmacological agents which specifically bind to the C-domain and modulate Ca^{2+} activation. The green tea polyphenol epigallocatechin-3-gallate (EGCg) binds to the C-domain and reduces myofilament Ca^{2+} sensitivity. This study compared the effects of EGCg on Ca^{2+} activation and myosin-mediated activation of rat cardiac myofibrils. To assay for effects on Ca^{2+} activation the myofibrillar ATPase activity was measured at pCa 5.0 in the presence of 0-100μM EGCg. Myosin-mediated activation was assayed by measuring the change in ATPase activity at pCa 8.0 following the addition of strong-binding S-1 heads generated by treatment with N-ethylmaleimide (NEMS-1). The Ca^{2+}-activated ATPase activity was inhibited by ~30% in the presence of 50μM EGCg and ~60% with 75–100 μM EGCg. However, in the absence of Ca^{2+} (pCa 8.0) 50μM EGCg had no significant effect on the increase in ATPase activity induced by the addition of 1 μM NEMS-1. Thus EGCg inhibits the Ca^{2+}-dependent component of thin filament activation, with no apparent effect on the strong-binding myosin S-1 component of activation. EGCg may be a useful reagent for dissecting the roles of Ca^{2+} and S-1 in cardiac thin filament activation.

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Cardiac Thin Filament Activation Modulation by Sarcomere Length

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Myofilament Length Dependent Activation (LDA) forms the cellular basis of the Frank-Starling law observed on the heart. It has been previously shown in isolated myocardium that the responsiveness of cardiac myofilaments to activating calcium ions is increased with stretch. However, the molecular mechanism underlying LDA is still unknown. The aim of our study was to investigate whether LDA is modulated by increased calcium binding to the thin filaments, and whether or not thick filaments modulate this mechanism. Accordingly, experiments were performed under strict sarcomere length control on skinned guinea pig multicellular preparations isolated from the left ventricle. Myofilament calcium sensitivity was measured at both short (1.9 μm) and long (2.3 μm) sarcomere length. Simultaneously, calcium binding to cTnC was monitored using fluorescently labeled cTnC (cTnCT53C*) with an environmentally sensitive probe (2-(4′-iodoadcetamidoanilo)-napthalene-6-sulfonic acid (IAANS)). The involvement of thick filaments in the regulation of calcium binding to cTnC was evaluated either in the presence of Blebbistatin to inhibit cross bridges formation or in rigor solution to inhibit cross-bridges detachment. Calcium dependent modulation of IAANS fluorescence showed calcium dependent decrease in IAANS fluorescence, indicating that the label was correctly incorporated into a functional TnC. The labeled TnC was then incorporated into recombinant Tn complex (Tn*) and endogenous Tn was exchanged for Tn*. Preliminary data show that Tn exchange did not alter the myofilament properties as demonstrated by preserved Tension-pCa curves at both short and long SL. Our data also show alteration of IAANS emitted fluorescence upon calcium activation in exchanged skinned muscles. Moreover, fluorescence was also altered by stretch in relaxed muscles. These data suggest that LDA may be due to altered thin filament activation in response to stretch secondary to a direct influence of sarcomere length on troponin structure.

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Dynamic Mapping of Cardiac Thin Filament Regulatory Proteins

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In cardiac and other striated muscles, actions of the thin filament proteins troponin and tropomyosin comprise the primary on-off switch controlling muscle contraction. This regulation is dynamic in several respects, so the dynamic properties of these two proteins are of particular interest. To elucidate these properties in depth, dynamic maps of both troponin and tropomyosin are undergoing detailed delineation by hydrogen-deuterium exchange mass spectrometry (HDX-MS). In this method, specific, induced perturbations in protein structure can be examined globally within a protein, i.e., examined for both proximate and remote effects on dynamics that are determined quantitatively as HDX rates. For cardiac troponin, we are examining both the single and combined effects of (1) regulatory site Ca^{2+} binding abrogation via TnC D65A mutation, and (2) other troponin mutations at sites believed critical for structure and/or function. A principle finding is that Ca^{2+} binding to the TnC regulatory site stabilizes the TnC N-lobe and the TnI switch helix as might be expected, but also has the unexpected effect of allosterically destabilizing a remote portion of the troponin core domain near the base of the TnT-TnI coiled-coil. In contrast to troponin, tropomyosin resists HDX study by usual HDX-MS methods, because pepsin digests tropomyosin poorly under post-exchange, low pH, HDX-quench conditions. Successful digestion and peptide fragment identification have been achieved by use of Rhizopuspepsin rather than pepsin, and by including denaturant during post-exchange, digestion conditions.