We have combined time-resolved fluorescence resonance energy transfer (TR-FRET) and molecular dynamics (MD) simulations to elucidate structural changes in the phosphorylation domain (PD) of chicken gizzard smooth muscle regulatory light chain (RLC) bound to smooth muscle myosin. The PD is absent in crystal structures, leaving uncertainty about the mechanism of regulation. Donor-acceptor pairs of probes were attached to three site-directed di-Cys mutants, each having one Cys at position 129 in the C-terminal lobe and the other at position 2, 3, or 7 in the N-terminal PD. Labeled RLCs, with and without phosphorylation at S19, were reconstituted into myosin S1. Time-resolved FRET identified two structural states of the RLC, closed and open, which are present in both unphosphorylated and phosphorylated biochemical states. Phosphorylation shifts the equilibrium toward the open state by 20-30%. Molecular dynamics simulations, guided by these and previous spectroscopic studies, confirm the existence of the open and closed structural states and produce atomic resolution models of these states. In the closed state, the PD interacts with the surface of the C-terminal lobe. In the open state, the PD is more helical and straight, resides farther from the C-terminal lobe, and is more mobile. Phosphorylation stabilizes the open state by forming a specific salt bridge between R16 and phosphorylated S19. This conformational shift is consistent with a mechanism of regulation that catalyzes large structural changes within myosin at a low energetic cost. This work was supported by grants from NIH (AR32961, AR07612) and the Minnesota Supercomputing Institute. We thank Igor Negrashov for excellent technical assistance.

1838-Pos

Stimulated Actin Polymerization Induces Force Potentiation in Swine Carotid Artery

Christopher M. Rembold, Ankit Tejani.
University of Virginia, Charlottesville, VA, USA.

The phenomenon of post-tetanic potentiation, in which a single submaximal contraction or series of submaximal contractions strengthens a subsequent contraction, has been observed in both skeletal and cardiac muscle.

In this study, we describe a similar phenomenon in swine carotid arterial smooth muscle. We find that a submaximal K^+ depolarization increases the force generation of a subsequent maximal K^+ depolarization - we term this ''force potentiation.''

Force potentiation was not associated with a significant increase in crossbridge phosphorylation or shortening velocity during the maximal K^+ depolarization, suggesting that the potentiated force was not caused by higher crossbridge cycling. We found that measures of stimulated actin polymerization (higher prior Y118 paxillin phosphorylation, higher prior F actin, and transition to a more solid rheology evidenced by lower noise temperature and phase angle) present prior to the maximal K^+ depolarization predicted the degree of force potentiation.

Increased prior contraction alone did not induce force potentiation since readdition of Ca^{2+} to Ca^{2+}-depleted tissues induced a partial contraction that was not associated with changes in noise temperature or with subsequent force potentiation.

These data suggest that stimulated actin polymerization may produce a substrate for increased crossbridge mediated force, a process we observe as force potentiation.

1839-Pos

Is Slower Myosin Cross-Bridge Kinetics in Tg-D166V Preparations Due to Decreased Myosin RLC Phosphorylation?

Priya Muthu[1,2], Katarzyna Kazmierczak[1], Jingsheng Liang[1], Anna I. Rojas[1], Michelle Jones[1], Julian Borejdo[2], Danuta Szczesna-Cordary[1].
[1]University of Miami Miller School of Medicine, Miami, FL, USA,
[2]University of North Texas Health Science Center, Fort Worth, TX, USA.

In this report we have investigated a particularly malignant phenotype of Familial Hypertrophic Cardiomyopathy (FHC) associated with the 166 Aspartic Acid to Valine (D166V) mutation in the ventricular myosin regulatory light chain (RLC). We show that the rates of myosin cross-bridge attachment and dissociation are significantly different in isometrically contracting cardiac myofibrils from transgenic (Tg)-D166V compared to Tg-WT mice. A single molecule approach was taken where the fluorescence anisotropy of rhodamine phalloidin labeled actin protomers was measured in cardiac myofibrils undergoing isometric contraction. Orientation of an actin molecule oscillated between two states, corresponding to the actin-bound and actin-free states of the myosin cross-bridge. The rates of cross-bridge attachment as well as cross-bridge dissociation were significantly decreased in isometrically contracting Tg-D166V myofibrils (binding, 1.4 s^{-1}; detachment, 1.2 s^{-1}) compared to Tg-WT myofibrils (binding, 3 s^{-1}; detachment, 1.3 s^{-1}). The duty ratio of the cross-bridge cycle, equal to the fraction of the total cycle time that cross-bridge remains attached to actin, was 47% inTg-D166V myofibrils and 30% in Tg-WT. Immunoblotting of cardiac myofibrils used for kinetics studies demonstrated a large reduction in RLC phosphorylation in Tg-D166V vs. Tg-WT myofibrils. These data are in accord with our previous findings in skinned and intact papillary muscles showing slower fiber kinetics and prolonged force transients in Tg-D166V fibers compared to Tg-WT preparations (Kerrick et al., FASEB J. 23: 855, 2009). Similarly, the level of RLC phosphorylation in muscle extracts from Tg-D166V ventricles was decreased. Our cellular and single molecule data suggest that a mutation-dependent decrease in RLC phosphorylationcould initiate the slower kinetics of the D166V cross-bridgesand ultimately lead to abnormal cardiac muscle contraction. Supported by NIH-HL071778 (DSC), NIH-AR048622 (JB) and NIH-HL090786 (to JB and DSC).

1840-Pos

Biochemical Phenotypes Associated with the Myosin Essential Light Chain Mutations

Alexander Raytman, Shaurya Joshi, Katarzyna Kazmierczak,
Michelle Jones, Priya Muthu, Danuta Szczesna-Cordary.
University of Miami Miller School of Medicine, Miami, FL, USA.

To investigate the effects of familial hypertrophic cardiomyopathy (FHC) mutations in the ventricular myosin essential light chain (ELC) we have exchanged the human ventricular wild type (WT) and two FHC ELC mutants (A57G, E143K) for the endogenous porcine ELC in isolated and purified pig cardiac myosin.To elucidate the importance of the long N-terminus of cardiac myosin ELC for the actin-myosin interaction, we also exchanged the N-terminal truncation mutant ELC-Δ43 into native porcine myosin. The phenotype associated with the A57G mutation consists of a classic asymmetric hypertrophy with varying pathology and disease progression including sudden cardiac death (SCD) (Lee, *et al.* (2001) *Am Heart J***141**, 184-9). The E143K mutation is associated with a restrictive cardiomyopathy and SCD phenotype (Olson *et al.* (2002) *Circulation***105**, 2337-40). We hypothesized that FHC ELC mutants may bind to the myosin heavy chain with a lower affinity than ELC-WT, thus affecting the structural integrity of the thick filaments in muscle. SDS-PAGE demonstrated that indeed the A57G mutation yielded lower percent exchange in cardiac porcine myosin compared to WT. We further hypothesized that as a consequence the interaction of ELC-mutant myosin with actin will be affected. All ELC-mutant myosins were tested for their ability to bind actin using fluorescence spectroscopy and pyrene-labeled F-actin. We observed a significantly decreased binding affinity for both FHC mutants while the binding of the ELC-Δ43 mutant myosin to F-actin was stronger than WT. The latter supports the hypothesis that the N-terminus of ELC acts as a molecular constrain inhibiting the actin-myosin interaction. Lower binding affinity of myosin containing the A57G and E143K mutations might be responsible for the development of the pathologic cardiac phenotype observed in patients carrying these FHC mutations. Supported by NIH HL071778 and NIH HL090786.