In Vitro and in Situ Structure and Function of the Cardiac Troponin C Familial Hypertrophic Cardiomyopathy-Linked Mutation, L29Q

ARTICLE *in* BIOPHYSICAL JOURNAL · JANUARY 2014

Impact Factor: 3.97 · DOI: 10.1016/j.bpj.2013.11.3996

6 AUTHORS, INCLUDING:

Ian Robertson

Government of Alberta

25 PUBLICATIONS **173** CITATIONS

SEE PROFILE

Monica X Li

University of Alberta

51 PUBLICATIONS **2,142** CITATIONS

SEE PROFILE

Yin-Biao Sun

King's College London

46 PUBLICATIONS **804** CITATIONS

SEE PROFILE

the blocked state of thin filament regulation. Because it has also been shown to stabilize the closed-state position of tropomyosin, we hypothesized that cTnI-Md is involved in the cooperativity of thin filament activation. To test this hypothesis, we generated the truncation mutant cTnI(1-167) from *R.norvegicus* wherein the entire cTnI-Md had been removed. We used passive exchange to incorporate cTnI(1-167) and FRET labeled cTnC(T13C/N51C)$_{AEDENS-DDPM}$ into left ventricular detergent skinned myocardial fibers. SDS-PAGE and Western blotting demonstrated that cTnC(T13C/N51C)$_{AEDENS-DDPM}$ and cTnI(1-167) were efficiently exchanged into skinned fibers. Intriguingly, incorporation of cTnI(1-167) resulted in biphasic Ca^{2+} dependent thin filament activation as indicated by the tightly coupled force−Ca^{2+} and N-cTnC-opening-Ca^{2+} relationships. Simultaneous force and FRET measurements showed that treatment with 1 mM orthovanadate inhibited force, reduced ensemble-averaged N-cTnC opening, and decreased the Ca^{2+}-sensitivity of activation, but did not affect the cooperativity underlying the biphasic response of N-cTnC opening to increasing Ca^{2+}. Akaike information criteria indicated that a weighted sum of two Hill equations was $>10^9$-fold superior in describing biphasic activation than a single Hill equation. Interestingly, a steady-state cooperativity model based on the concept of tropomyosin being "pinned" by cTnI (J. Mol. Biol., vol. 340, pp. 295-305) was 41-fold superior to the weighted sum of two Hill equations and suggested that blocked-state allosteric communication is severely disrupted by removal of cTnI-Md. We concluded that cTnI-Md helps facilitate allosteric communication between thin filament regulatory units in the blocked state and is therefore essential to achieving a proper contractile response to sarcomeric Ca^{2+} signals during early systole.

3661-Pos Board B389

Phosphorylation of Cardiac Troponin I at Tyrosine 26 Decreases Thin Filament Calcium Sensitivity

Hussam Salhi.
The Ohio State University, Columbus, OH, USA.

The troponin complex is a critical molecular switch involved in transducing the calcium activating signal into contraction. Troponin I (TnI), the inhibitory subunit of the complex, is phosphorylated as a key regulatory mechanism to alter the calcium regulation of contraction. Altered cardiac contraction is a hallmark of heart failure with several studies demonstrating increased myofibrillar calcium sensitivity. Recent work has identified a novel phosphorylation of TnI at Tyr-26 that is decreased in heart failure with unknown functional effects. Similar to the location of the desensitizing TnI Ser-23/24 phosphorylation, TnI Tyr-26 is located in the unique cardiac TnI N-terminal extension. These data lead us to hypothesize that the N-terminal Tyr-26 phosphorylation of TnI decreases calcium sensitivity of the thin filament, the loss of which may contribute to the altered calcium sensitivity observed in heart failure. To assess the regulatory effects of Tyr-26 phosphorylation, we employed recombinant human cardiac TnI containing phosphate at Tyr-26 induced by treatment with a tyrosine kinase and TnI Tyr-26 phosphomimetic substitutions (Glu or Asp). The effect of TnI Tyr-26 phosphorylation on myofilament calcium sensitivity was assessed by measuring calcium binding to troponin C (TnC) in reconstituted thin filaments. Results demonstrate both Tyr-26 phosphorylation and phosphomimetics decrease calcium binding to TnC compared to filaments reconstituted with non-phosphorylated TnI. To further investigate the effects of TnI Tyr-26 phosphorylation on myofilament deactivation we measured the rate of calcium disassociation from TnC. Results demonstrate filaments containing either Tyr-26 phosphorylated TnI or phosphomimetics increase the rate of calcium dissociation from TnC. Our findings suggest that TnI Tyr-26 phosphorylation functions similarly to Ser-23/24 N-terminal phosphorylation to decrease myofilament calcium sensitivity and increase myofilament relaxation. The loss of TnI Tyr-26 phosphorylation may therefore contribute to altered cardiac contraction in heart failure.

3662-Pos Board B390

Effect of Amino Acid Changes in a Troponin I FHC Hotspot on Protein: Protein Binding and Calcium Sensitivity of Force Development

Ziyou Cui, Jennifer Gilda, Gaya Gomes, **Aldrin V. Gomes**.
University of California, Davis, Davis, CA, USA.

Mutations in human cardiac troponin I (cTnI) have been associated with restrictive, dilated and hypertrophic cardiomyopathies. The most commonly occurring residue on cTnI that is associated with familial hypertrophic cardiomyopathy (FHC) is arginine, which is also the most common residue at which multiple mutations occur. Two FHC mutations are known to occur at arginine 204, R204C and R204H, and both are associated with poor clinical prognosis. To determine the effect of these mutations (R204C and R204H), as well as other cTnI mutations, R204P, R204Q, and R204W, calcium-force measurements and cTnI:troponin C (TnC) and cTnI:troponin T (TnT) interactions using the mammalian two-hybrid luciferase assays were utilized. All five mutations showed significant increases in calcium sensitivity of force development ranging from ΔpCa50 0.23 (R204W) to 0.35 (R204P). The mutations associated with FHC, R204C and R204H, had ΔpCa50 values of 0.28 and 0.29 respectively. The cTnI containing the R204P mutation showed the weakest interaction with TnT when compared to wild-type cTnI or the other mutants. The R204H mutation also showed significant impairment in its ability to interact with TnT, while the R204C mutation showed mild impairment when compared to wild-type cTnI. The R204C and R204P mutations showed the greatest impairment in binding to TnC. These results suggest that mutations at the same site on cTnI could affect thin filament interactions differentially, and that significant impairment in the interaction of cTnI with TnT or TnC may be enough to cause significant changes in calcium sensitivity. If the large increase in calcium sensitivity of force development observed with these mutations is associated with the poor prognosis then other R204 mutations are likely to have a poor prognosis. This research was supported by a Hellman Fellowship.

3663-Pos Board B391

In Vivo Analysis of Troponin C Knock-In (A8V) Mice: Evidence that TNNC1 is a Hypertrophic Cardiomyopathy Susceptibility Gene

Adriano S. Martins[1], Michelle S. Parvatiyar[2], Radjeep Turna[1], Crystal Dawn Badger[1], Brittany Griffin[1], Diego Zorio[3], Milica Vukmirovic[1], Marcos A. Sanchez-Gonzalez[1,4], David Dweck[1], Edda L. Ruiz[1], Jingsheng Liang[5], Yincai Wang[5], J.M. Overton[1], Jose Renato Pinto[1].
[1]Biomedical Sciences - School of Medicine, Florida State University, Tallahassee, FL, USA, [2]Department of Integrative Biology and Physiology, UCLA, Los Angeles, CA, USA, [3]Department of Chemistry & Biochemistry, Florida State University, Tallahassee, FL, USA, [4]Family Institute, Florida State University, Tallahassee, FL, USA, [5]Department of Molecular and Cellular Pharmacology, University of Miami, Miller School of Medicine, Miami, FL, USA.

Recently, the *TNNC1* gene that encodes cardiac troponin C (cTnC) was found as a target for many hypertrophic cardiomyopathy (HCM) mutations in humans, eliciting alterations in the Ca^{2+} binding properties of the N-domain of cTnC. We genetically engineered knock-in mice containing the HCM-associated A8V mutation in cTnC (heterozygote = KI-TnC-A8V$^{+/-}$; homozygote = KI-TnC-A8V$^{+/+}$) in order to characterize its *in vivo*, molecular and cellular effects. ECHO revealed that at 3 months old (mo) KI-TnC-A8V$^{+/+}$ mice display increased IVRT and E/A ~1 compared to WT, suggesting diastolic dysfunction; whereas KI-TnC-A8V$^{+/-}$ showed signs of cardiac restriction at 14 mo. Histopathology of both genotyped hearts revealed papillary muscle hypertrophy, interstitial fibrosis, and myofibrilar disarray. Real-time PCR analysis at 3 mo demonstrated increases in BNP, α-MHC and β-MHC mRNA levels in the right ventricles of the KI-TnC-A8V mice (only ANP increased in the left ventricle). We identified in intact KI-TnC-A8V$^{+/-}$ and KI-TnC-A8V$^{+/+}$ cardiomyocytes: a significant decrease in the sarcomere length at several stimulation frequencies; prolonged Ca^{2+} and contractile transient kinetics at 4Hz; uncoupling between Ca^{2+} decay (delayed) and contractile (no change) transients at 6Hz; suggesting a mechanical frequency-dependent uncoupling from the Ca^{2+} transient. Furthermore, a decrease in the baseline Ca^{2+} fluorescence and in Ca^{2+} peak percentage was also detected in KI-TnC-A8V$^{+/-}$ and KI-TnC-A8V$^{+/+}$, indicating increased myofilament Ca^{2+} buffering. The calcium sensitivity of contraction in skinned fibers increased in a gene dose fashion: KI-TnC-A8V$^{+/+}$ > KI-TnC-A8V$^{+/-}$ > WT. The rate of relaxation in KI-TnC-A8V$^{+/+}$ cardiac skinned fibers investigated by flash photolysis was found increased, compared to WT. These results suggest that the A8V mutation in cTnC increases Ca^{2+} binding affinity to its N-domain eliciting changes in intracellular Ca^{2+} homeostasis and cellular mechanical function, ultimately leading to diastolic dysfunction.

3664-Pos Board B392

In Vitro and in Situ Structure and Function of the Cardiac Troponin C Familial Hypertrophic Cardiomyopathy-Linked Mutation, L29Q

Ian M. Robertson[1,2], Ivanka Sevrieva[1], Monica X. Li[2], Malcolm Irving[1], Yin-Biao Sun[1], Brian D. Sykes[2].
[1]Randall Divison of Cell & Molecular Biophysics, King's College London, London, United Kingdom, [2]Biochemistry, University of Alberta, Edmonton, AB, Canada.

Familial Hypertrophic Cardiomyopathy (FHC) is characterized by severe abnormal cardiac muscle growth. The traditional view is that mutations associated with FHC result in an increase in Ca^{2+}-sensitivity of cardiac muscle contraction; however, recent studies indicate that their pathogenesis may stem from a diminished response to troponin I phosphorylation. The mutation L29Q, found in the Ca^{2+}-sensitive muscle regulatory protein, troponin C, has been tenuously linked to cardiac hypertrophy. L29Q is in the regulatory domain

of cardiac troponin C's defunct Ca^{2+}-binding site (site 1). In this study, we combined in vitro and in situ structural and functional techniques to elucidate the role this mutation may play in the modulation of troponin's function. We used Nuclear Magnetic Resonance to solve the structure and characterize the backbone dynamics of the regulatory lobe of troponin C with this mutation. The overall structure and dynamics of troponin C was not significantly altered by L29Q; however there was a slight rearrangement of site 1 making it more similar to trout cardiac troponin C, which also has a glutamine at position at residue 29 and displays increased Ca^{2+} sensitivity. Backbone dynamics measurements indicated that Q29 was more flexible than L29. The structure and function of L29Q was also assessed in demembranated ventricular trabeculae using Fluorescence for In Situ Structure. The structure and/or orientation of the regulatory lobe of troponin C was slightly perturbed by L29Q in relaxing conditions and was unaffected at activating Ca^{2+} concentrations. The Ca^{2+} sensitivity of the structural change and contractility were both unaltered by the L29Q mutation, suggesting that while this may cause a small change in the structure of troponin C, this does not translate to a large functional effect in cardiac muscle.

3665-Pos Board B393

Troponin I Ser-150 Phosphorylation Sustains Troponin Ca^{2+} Sensitivity in an Acidic Environment

Benjamin R. Nixon, Shane D. Walton, Jonathan P. Davis, Brandon J. Biesiadecki.
Physiology and Cell Biology and Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA.

A hallmark of cardiac ischemia is decreased intracellular pH which can affect a number of cellular processes. Such an acidic environment alters cardiac troponin (Tn) myofilament regulation to decrease Ca^{2+} sensitive force production. Tn also undergoes cardiac ischemia-induced AMPK troponin I (TnI) Ser-150 phosphorylation. We recently characterized the effects of TnI Ser-150 phosphorylation demonstrating that it blunted the functional effects of canonical TnI Ser-23/24 phosphorylation; however, the role of Ser-150 phosphorylation in ischemia remains unknown. As an initial step, we sought to investigate the effect of acidic pH on myofilament regulation in the presence of TnI Ser-150 phosphorylation alone and in combination with Ser-23/24 phosphorylation. We first investigated the effect of in vivo cardiac ischemia on levels of TnI Ser-150 and Ser-23/24 phosphorylation. Exposure to 30 minutes of regional ischemia resulted in elevation of both TnI Ser-150 and Ser-23/24 phosphorylation. Next we determined the effects of TnI Ser-150 pseudo-phosphorylation (S150D) on the myofilament by measuring troponin C (TnC) Ca^{2+} binding properties at normal and acidic pH. Results demonstrate acidic pH decreases steady-state Ca^{2+} binding to TnC in reconstituted thin filaments across all Tn (WT, S150D, S23/24D, and S23/24/150D) such that TnI S150D Ca^{2+} sensitivity at pH 6.5 is similar to WT at pH 7. Decreasing the pH had no effect on Ca^{2+} dissociation such that compared to WT, S23/24/150D remained fast while S150D was slowed. We conclude that TnI Ser-150 phosphorylation imparts resistance to acidic pH-induced myofilament Ca^{2+} desensitization while retaining increased Tn Ca^{2+} dissociation when in combination with Ser-23/24 phosphorylation suggesting the potential for an increase in force while maintaining accelerated Ca^{2+} dissociation. Future investigations are aimed at examining the effect of TnI Ser-150 and Ser-23/24 phosphorylation on protease cleavage of TnI.

3666-Pos Board B394

Deficiency of Slow Skeletal Muscle Troponin T Causes Atrophy of Type I Slow Fibers and Decreases Tolerance to Fatigue

Bin Wei, Yingru Lu, J.-P. Jin.
Physiology, Wayne State University, Detroit, MI, USA.

Loss of slow skeletal muscle troponin T (ssTnT) due to a nonsense mutation at codon Glu180 in exon 11 of the TNNT1 gene causes a severe form of recessive nemaline myopathy (Amish nemaline myopathy, ANM). To investigate the pathogenesis and muscle pathophysiology of ANM, we studied the phenotypes of partial and total loss of ssTnT in Tnnt1 gene targeted mice. An insertion of neomycinR cassette in intron 10 of Tnnt1 caused approximately 60% decrease in ssTnT protein expression whereas deletion of exons 11-13 using cre-loxP approach resulted in total loss of ssTnT as that seen in the muscle of ANM patients. In diaphragm and soleus muscles of the knockdown and knockout mouse models, we demonstrated that ssTnT deficiency resulted in significantly decreased levels of other slow fiber-specific myofilament proteins while fast fiber-specific myofilament proteins were increased. Histology studies revealed that ssTnT deficiency caused significant atrophy of type I slow fibers and a hypertrophic growth of type II fast fibers. Along with the slow fiber atrophy and the changes in myofilament protein isoform contents, ssTnT deficiency in soleus muscle shifted the force-frequency relationship toward the fast muscle type and significantly reduced the tolerance to fatigue. ssTnT deficient soleus muscle also exhibited a significant number of smaller size central nuclei type I fibers, indicating an adaptive regeneration. ssTnT deficient mouse soleus muscle contained apparently normal number of spindles, in which intrafusal fibers were positive for type I myosin with a trend of atrophic morphology. The results demonstrate the essential function of ssTnT in skeletal muscle and the causal effect of its loss on the pathology of ANM.

3667-Pos Board B395

Attentuating the Depressive Effect of Acidosis with Mutations in Troponin and with 2-Deoxy-Atp

Thomas J. Longyear[1], Matthew A. Turner[1], Brandon J. Biesiadecki[2], Joseph Lopez[2], Jonathan P. Davis[2], Edward P. Debold[1].
[1]University of Massachusetts, Amherst, MA, USA, [2]The Ohio State University, Columbus, OH, USA.

Repeated, intense contractile activity compromises the ability of skeletal muscle to generate force and velocity, which defines fatigue. The decrease in velocity is thought to be due, in part, to the intracellular build-up of acidosis inhibiting the function of the contractile proteins myosin and troponin; however, the underlying molecular basis of this process remains unclear. We sought to gain novel insight into the decrease in velocity by determining if the depressive effect of acidosis could be altered by 1) introducing Ca^{++}-sensitizing mutations into troponin (Tn) or 2) by agents that directly affect myosin function, including inorganic phosphate (P_i) and 2-deoxy-ATP (dATP) in an *in vitro* motility assay. Acidosis reduced regulated thin filament velocity (V_{RTF}) at both maximal and sub-maximal Ca^{++} levels in a pH-dependent manner. A truncated construct of the inhibitory subunit of Tn, R156, and a Ca^{++}-sensitizing mutation in the Ca^{++}-binding subunit of Tn, V43Q, increased V_{RTF} at sub-maximal Ca^{++} under acidic conditions, but had no effect on V_{RTF} at maximal Ca^{++} levels. In contrast, both 15mM P_i and replacement of ATP with dATP reversed much of the acidosis-induced depression of V_{RTF} at saturating Ca^{++} (0.7 ± 0.1 control, 2.0 ± 0.3 with P_i, 1.8 ± 0.3 with dATP, 3.8 ± 0.1 with both P_i and dATP), with the combined effect fully restoring the V_{RTF} to the value under control conditions. Interestingly, despite producing similar magnitude increases in V_{RTF}, the combined effects of P_i and dATP were additive, suggesting different underlying mechanisms of action. These results suggest that the major mechanism by which acidosis slows V_{RTF} is through directly slowing myosin's rate of detachment from actin.

3668-Pos Board B396

The Effect of Truncated Troponin Components on Activation of Lethocerus Flight Muscle

Belinda Bullard[1], Bogos Agianian[2], Gian-Felice de Nicola[3], Annalisa Pastore[3], Kevin Leonard[4].
[1]Department of Biology, University of York, York, United Kingdom, [2]Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece, [3]National Institute of Medical Research, London, United Kingdom, [4]European Bioinformatics Institute, Cambridge, United Kingdom.

Indirect flight muscle (IFM) of *Lethocerus* is activated by periodic stretches at a constant priming concentration of calcium. The muscle is unusually stiff and stress is transmitted to the thick and thin filaments by kettin, which reinforces links between both filaments and the Z-disc. The activating effect of stress on thin filaments is likely to affect troponin. The isoforms of troponin in IFM differ from those in other muscles. TnT has a C-terminal extension not present in vertebrate TnT; TnH is an isoform of TnI with a C-terminal extension rich in Pro and Ala; TnC is present in two isoforms: F1 binds a single calcium in the C-lobe and is needed for stretch-activation; F2 binds one calcium in both N- and C-lobes and is needed for isometric force. Under conditions of low ionic strength, native fibres have a force-pCa curve that shows high calcium-sensitivity and low cooperativity ($pCa_{50} = 6.2$, $n_H = 1.3$). Fibres with F2 alone have a pCa curve similar to that of cardiac muscle, ($pCa_{50} = 5.8$, $n_H = 3.2$). A fragment of F1 without the N-lobe (F1-Ct) inhibits stretch-activation; therefore the N-lobe of F1 is necessary, although it does not bind calcium or TnH. F1-Ct is displaced by F2 and isometric force is restored, but not stretch-activation. We hope to show the effect of replacing endogenous troponin in fibres with a complex containing TnT truncated at the C-terminus, TnH with TnI sequence but without the Pro-Ala extension, and either F1 or F2. This will show how important the IFM isoforms of troponin are to the stretch-activation response.

3669-Pos Board B397

Changes in the Orientation of the Myosin Light Chain Domain (LCD) Associated with Thick Filament-Based Regulation of Skeletal Muscle

Luca Fusi, Zhe Huang, Malcolm Irving.
Randall Division of Cell and Molecular Biophysics, King's College London, London, United Kingdom.

The dependence of myosin LCD orientation on temperature, myofilament lattice spacing and sarcomere length was determined using fluorescence