Muscle Regulatory Proteins - I

650-Pos Structural effects of cardiotonic compounds on the cardiac troponin C - troponin I interface

Monica X. Li, Marta Oleszczuk, Ian M. Robertson, Karen Freedman, Brian D. Sykes

Department of Biochemistry, University of Alberta, Edmonton, AB, Canada.

Board B494

The interaction of cTnC and cTnI plays a critical role in transmitting the Ca^{2+}-signal to the other myofilament proteins in heart muscle contraction. As such, the cTnC-cTnI interface constitutes a logical target for cardiotonic agents that can modulate the Ca^{2+}-sensitivity of the myofilaments. We have shown that W7, a calmodulin antagonist, binds specifically to the N-domain of cTnC, and this binding can occur together with the switch region of cTnI (Li et al., 2006, Biochemistry, *45*, 9833–9840). We have made two stable complexes, cNTnC·Ca^{2+}·$cTnI_{144-163}$·W7 and cTnC·3Ca^{2+}·$cTnI_{34–71}$·$cTnI_{128–163}$·W7 and are in the process of completing their NMR structures. The first complex involves the regulatory domain of cTnC and the key switch region of cTnI. The cTnI peptides in the second complex encompass the three functional region of cTnI that interact directly with intact cTnC. These two structures will provide a detailed understanding of the mechanism underlining the mode of action of W7 on the important functional units of cardiac troponin and generate structural insights into the features that are important for the design of cardiotoninc drugs. These structures will be compared with those of cNTnC·Ca^{2+}·$cTnI_{147–163}$·bepridil (PDB 1LXF) and cCTnC·2Ca^{2+}·EMD 57033 (PDB 1IH0) determined previously in our laboratory and the X-ray structure of sTnC·4Ca^{2+}·$sTnI_{1–182}$·$sTnT_{156–262}$·anapoe (PDB entry 1YTZ). Structural comparison will allow us to delineate the mechanism underlining the mode of action of different cardiotonic drugs on the cTnC-cTnI interface. This information can be exploited to develop new, specific, and potent cardiotonic drugs that can be used in the treatment of heart disease.

651-Pos Designing Troponin C Mutants with Dramatically Altered Calcium Dissociation Rates in Complex Biochemical Systems

Svetlana Tikunova[1], Bin Liu[1], James D. Potter[2], Aldrin V. Gomes[3], Darl R. Swartz[4], Jonathan P. Davis[1]

[1] *The Ohio State University, Columbus, OH, USA*
[2] *University of Miami, Miami, FL, USA*
[3] *University of California, Davis, Davis, CA, USA*
[4] *Purdue University, West Lafayette, IN, USA.*

Board B495

The influence of Ca^{2+} dissociation from TnC on the rate of cardiac muscle relaxation is in dispute. One way to test whether TnC controls relaxation is to directly modify the rate of Ca^{2+} dissociation from TnC by rationally engineered mutations. We have designed two mutations, V44Q and D73N, expected to dramatically affect the rate of Ca^{2+} dissociation from TnC in complex biochemical systems, without drastically altering TnI binding affinity. Neither V44Q nor D73N mutation had a dramatic effect on the affinity of Ca^{2+} saturated TnC for the regulatory fragment of TnI (residues 128–180). As predicted, V44Q increased the Ca^{2+} sensitivity of thin filament bound TnC ~ 4.8-fold and ~1.7-fold, in the absence and presence, respectively, of myosin S1. Consistent with its Ca^{2+} sensitizing effect, V44Q led to ~5.0-fold and ~1.4-fold slower Ca^{2+} dissociation rate from the thin filament bound TnC in the absence and presence, respectively, of myosin S1. On the other hand, mutation D73N decreased the Ca^{2+} sensitivity of thin filament bound TnC ~ 4.0-fold and ~3.8-fold in the absence and presence, respectively, of myosin S1. Consistent with its Ca^{2+} desensitizing effect, D73N led to ~ 4.6-fold and ~ 4.2-fold faster rate of Ca^{2+} dissociation from the thin filament bound TnC in the absence and presence, respectively, of myosin S1. Furthermore, V44Q resulted in a ~ 3.7-fold slower, while D73N resulted in ~ 2.7-fold faster Ca^{2+} dissociation rate from TnC in rigor rabbit ventricular myofibrils. These TnC mutants with dramatically altered Ca^{2+} dissociation rates can be used to examine whether TnC influences cardiac muscle relaxation.

(Supported by NIH K99 HL087462 (to SBT), R01 HL 042325 (to JDP), R01 HL073828 (to DRS); and AHA 0160174B (To AVG), 0735079N (JPD).

652-Pos Functional Effects of Newly Discovered Cardiac Troponin C-HCM Mutations

Michelle Parvatiyar[1], Jose R. Pinto[1], Michelle A. Jones[1], Michael J. Ackerman[2], James D. Potter[1]

[1] *Univ of Miami Miller School of Medicine, Miami, FL, USA*
[2] *Mayo Clinic College of Medicine, Rochester, MN, USA.*

Board B496

Troponin C has long remained elusive as a cardiomyopathy-susceptibility gene, however just recently Landstrom, et al. (AHA abstract, 2007), have reported four new HCM mutations in TnC and their effects on the Ca^{2+} sensitivity of force development. The mutations A8V, C84Y, E134D and D145E were found in a large cohort patient screen and occurred at the same prevalence as in other sarcomeric genes, thus distinguishing TnC as a HCM-susceptibility gene. In TnC depleted and reconstituted cardiac skinned fibers, A8V, C84Y and D145E showed increased Ca^{2+} sensitivity of force development whereas E134D did not. In addition to the functional characterization in fibers, we performed Actomyosin ATPase, Fluorescence and Circular Dichroism (CD) measurements to investigate the molecular properties of these mutations. Actomyosin ATPase data was collected using reconstituted filaments and demonstrated that these mutations had varying degrees of activation, in the presence of Ca^{2+}. No differences were observed in the Actomyosin ATPase inhibition by these mutants. Fluorescence analysis was evaluated using IAANS as extrinsic probe. Of the four mutants, only D145E showed a small increase in the Ca^{2+} affinity of the Ca^{2+}

specific site in the isolated state. In the CD experiments, there were no modifications to the secondary structure in the APO state. Our results clearly show that the Ca^{2+} binding properties of the isolated TnC mutants do not predict the changes observed in the Ca^{2+} sensitivity of force development.

Supported by NIH HL 42325 and NIH HL 67415.

653-Pos Effects of Single Residue Mutations in Site II of Cardiac Troponin C on Calcium Binding Affinilty and Myofilament Calcium Sensitivity

Karen L. Reece, Richard L. Moss

University of Wisconsin School of Medicine and Public Health, Madison, WI, USA.

Board B497

Myocardial contraction is initiated when calcium binds to site II of cardiac troponin C (cTnC). This 12-residue EF-hand loop (NH_2-**D***E***D**G*S*G*T*V**D***F**D***E**-COOH) contains six residues (bold) that coordinate calcium binding and six residues that do not appear to influence calcium binding directly. We have made six single cysteine substitutions (italics) within site II of cTnC to investigate whether these residues are essential for calcium binding affinity in isolation and calcium sensitivity of force development in single muscle fibers. Calcium binding properties of mutant proteins were examined in solution and after substitution into rat skinned soleus fibers. While mutation of the serine eliminated a coordinating oxygen thereby disrupting calcium binding, cysteine substitution had no effect on calcium binding on cTnC in solution. However, as part of the myofilament, the threonine mutation (T71C) reduced calcium sensitivity while the phenylalanine mutation (F74C) increased calcium sensitivity. Structural analysis using the available crystal structures revealed that T71C introduced an additional hydrogen bond with S35 in site I while F74C eliminated a hydrogen bond with F20 in the A helix. There were no changes in hydrogen bonding with the remaining three mutations of non-coordinating residues. These results suggest that subtle changes in cTnC structure alter myofilament calcium sensitivity. This study also reveals that function of modified cTnC in solution does not necessarily correlate to in situ function.

This work was supported by R01-HL61635.

654-Pos Troponin C as a Therapeutic Target to Increase Cardiomyocyte Contraction Without Affecting Relaxation

F. Steven Korte, Anthony G. Rodriguez, Jin Dai, Charles E. Murry, Michael Regnier

University of Washington, Seattle, WA, USA.

Board B498

Current pharmaceutical therapies for heart failure often target or alter intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$), which can have significant side-effects such as arrhythmogenesis or adverse affects on diastolic function. These drugs can also have unwanted actions in non-target areas. In this study, we directly targeted cardiac thin filament activation to enhance intact cardiomyocyte contraction independent of altered $[Ca^{2+}]_i$. Specifically, cardiac thin filament activation was enhanced through adenovirally-mediated over-expression of a cardiac troponin C (cTnC) mutant designed to have increased Ca^{2+} binding affinity conferred by single amino acid subsititution (L48Q). In skinned cardiac trabeculae and myofibrils we have shown that substitution of L48Q cTnC for WT cTnC increases Ca^{2+} sensitivity of force and the maximal rate of force development. Over-expression of L48Q cTnC (identified by co-expression with GFP) significantly increased the rate (L48Q $= 1.32 \pm 0.32$ ML/s; control $= 0.45 \pm 0.14$ ML/s) and extent (L48Q $= 9.1 \pm 2.2\%$; control $= 4.6 \pm 1.4\%$) of shortening as compared to control (GFP-expression only) in spontaneously contracting neonatal rat cardiomyocytes. Importantly, the rate of relaxation was unaffected (L48Q $= 0.43 \pm 0.11$ ML/s; control $= 0.27 \pm 0.09$ ML/s) by the presence of L48Q cTnC. Expression of L48Q cTnC was confirmed by western blot analysis of skinned myofibrils from transduced cardiomyocytes, which indicated replacement of ~40% of WT cTnC with L48Q cTnC. Future experiments will target cultured adult rat cardiomyocytes. These experiments demonstrate the feasibility of directly targeting cardiac thin filament proteins to enhance cardiac contractility without altering diastolic function.

Supported by NIH HL07828 (FSK).

655-Pos Regulation of Asynchronous Insect Flight Muscle by Troponin

Belinda Bullard[1], Uros Krzic[2], Gian Felice De Nicola[3], Kevin R. Leonard[4], Annalisa Pastore[3], Wolfgang A. Linke[5]

[1] *University of York, York, United Kingdom*
[2] *EMBL, Heidelberg, Germany*
[3] *NIMR, London, United Kingdom*
[4] *EMBL/EBI, Cambridge, United Kingdom*
[5] *University of Munster, Munster, Germany.*

Board B499

Asynchronous indirect flight muscle (IFM) is activated by sinusoidal length changes at low $[Ca^{2+}]$. Work is produced as a result of delayed activation by stretch and deactivation on release. The area of loops on tension vs. length plots gives the work done by the fibre. During oscillatory contraction, work loops are superimposed on calcium-dependent isometric tension. In *Lethocerus* fibres, maximal oscillatory work was obtained at pCa 6, when isometric tension was low. IFM has two isoforms of TnC: F1 with a single calcium bound to the C-terminal lobe is needed for stretch-activation, and F2 with calcium bound to C- and N-lobes is needed for isometric contraction; the ratio of F1:F2 is 7:1. We replaced endogenous TnC with F1 or F2, or with varying proportions of the isoforms. Work was maximal at F1:F2 = 100:1, and often exceeded that for the native fibre; with more F2, work was reduced as isometric tension increased. At pCa 6, work produced by fibres with the *in vivo* F1:F2 ratio was close to that of the native fibre. The structure of F1 and the interaction with TnI were studied by NMR and fluorescence spectroscopy. The N-lobe is in the closed conformation and, unexpectedly, the C-lobe is in the open conformation in both apo and

calcium-loaded forms. The C-lobe interacts with the N-terminus of TnI and, at lower affinity, with the switch and inhibitory peptide region; both interactions are independent of calcium. The N-lobe does not interact with TnI. Thus stretch activation needs a TnC that binds calcium in the C-lobe without producing a structural change.

656-Pos A Novel Method of Measuring Troponin Phosphorylation Levels

Andrew E. Messer, Clare E. Gallon, Steven B. Marston

Imperial College - NHLI, London, United Kingdom.

Board B500

We recently demonstrated that levels of troponin I (TnI) and troponin T (TnT) phosphorylation could be measured in isolated troponin from human tissue samples using SDS-PAGE and the Pro-Q Diamond phosphoprotein specific gel stain. We have now developed a new method that enables us to determine the level of troponin subunit phosphorylation directly without the need for calibrated standards.

SDS-PAGE gels were made incorporating a phosphate-chelating molecule linked to acrylamide. When these were run, the mobility of phosphoproteins was retarded in relation to the unphosphorylated proteins and therefore formed separate bands according to their phosphorylation level. We tested this system using recombinant human cardiac TnI (supplied by C. Redwood, Oxford) phosphorylated by PKA. On phosphate-chelating gels, stained with SYPRO Ruby, retarded bands corresponding to 1P, 2P, 3P and 4P appeared whilst the original unphosphorylated TnI band disappeared. The sum of the individual band volumes was constant.

The phosphorylated bands were identified by staining with Pro-Q Diamond which also showed that the staining intensity (Pro-Q/SYPRO Ruby) of the 1P band was half that of the 2P band. The 1P species appeared with a half-life of 3–4minutes, peaked at 35minutes and slowly declined to half its maximal level at 120minutes. The 2P species appeared with a half-life of 19minutes and levelled off after 60minutes. The 3P and 4P species were first detected at 120minutes. At 240minutes the TnI species were 0P, 3.9%, 1P, 39.6%, 2P, 41.3%, 3P, 11.9% and 4P, 3.4% indicating that PKA can phosphorylate sites in addition to serines 23 and 24. The total phosphorylation time-course could be reconstructed from the band volumes without the need for external calibration and this showed that the TnI phosphorylation level reached 1.8molsPi/mol after 120minutes and was very similar to the curve determined using radioactive ATP.

657-Pos Cardiac Troponin T: a Novel Scaffold Protein, Anchors PKA to the Myofilaments

C. Amelia Sumandea, Catherine H. Bozio, Levi Lampe, Mary L. García-Cazarín, R. John Solaro, C. William Balke, Marius P. Sumandea

University of Kentucky, Lexington, KY, USA.

Board B501

Cardiac troponin is a heterotrimeric protein complex that plays an important role in regulating cardiac muscle contraction and relaxation. Increasing evidence suggests that cardiac myofilament dynamics are modulated by cTn alterations such as phosphorylation, proteolysis etc. Using a yeast two-hybrid screen of a human heart cDNA library, we identified previously nine novel proteins that interact with cTnT, including cyclic AMP-dependent protein kinase A (PKA). We repeated the screen using a different human heart library and identified 159 clones that grew on high-stringency plates and turned blue in the presence of α-gal, indicating positive interactions with hcTnT. DNA sequencing of the clones is underway.

In the present study, we focused on elucidating the mechanism by which cTnT anchors PKA to the myofilaments and leads to cTnI phosphorylation. HA-tagged PKA regulatory subunits Iα (RIα) and IIα (RIIα), as well as myc-tagged cTnT, were individually expressed using a rabbit reticulocyte lysate system. Association between myc-TnT and HA-PKAs was determined by immunoprecipitation (IP) with agarose conjugated anti-myc and anti-HA antibodies. Our results demonstrate that both HA-PKA-RI and -RII associate with myc-cTnT, *in vitro*. Next, a GST-cTnT fusion protein was used in a pull-down assay, followed by immuno-blotting, to verify whether endogenous PKAs from adult rat ventricular myocytes bind to cTnT. In order to determine the affinity of each PKA subunit for cTnT and cTnI, a solid-phase ELISA assay was used. To produce enough recombinant proteins for binding and subsequent phosphorylation studies, PKA -RIα, -RIIα, and -catalytic domains were expressed in *E. Coli*, and purified either by affinity (8-AEA-cAMP-Sepharose) or by ion-exchange chromatography. Our study suggests that cTnT is a novel scaffold protein, anchoring PKA to the myofilament in close proximity to cTnI, thereby facilitating rapid phosphorylation of cTnI-Ser^{23}/Ser^{24} following β-adrenergic stimulation.

658-Pos Slow Skeletal Troponin T Isoforms Decrease the Ca^{2+} Sensitivity of Force Development and Increase the Maximal Force in Skinned Cardiac Muscle Fibers

Jose Renato Pinto, Michelle A. Jones, Jingsheng Liang, James D. Potter

Univ of Miami Miller School of Medicine, Miami, FL, USA.

Board B502

Slow skeletal troponin T (SSTnT) mRNA expression has been shown to be upregulated in the hearts of patients with end-stage heart failure (Barton, et al, *Mol Cell Biochem*, 2004. *263*:1 p. 91–97). Nevertheless, the role of SSTnT in diseased hearts remains unknown. At least three SSTnT isoforms have been found to exist in slow skeletal muscle. To evaluate the function of each human SSTnT isoform in cardiac muscle regulation, we have cloned, expressed, purified, and performed skinned fiber experiments using SSTnT1 (+ Exons 5 and 12), SSTnT2 (+5, −12), SSTnT3 (−5, −12) and $SSTnT_{HYP}$ (−5, +12 theoretical). All of the SSTnTs were less soluble than adult cardiac troponin T (HCTnT3) at three

different pHs. Skinned fiber experiments were performed using a well established method of sequential TnT displacement and reconstitution with a HCTnI.TnC complex. The extent of TnT displacement was analyzed by the unregulated tension at pCa 8.0 after TnT treatment and by western blot analyses. HSSTnT1, 2 and 3 showed a decrease in the Ca^{2+} sensitivity of force development compared to HCTnT3. The maximal recovered force was significantly increased in fibers displaced with HSSTnT1 and 3. In contrast, $HSSTnT_{HYP}$, showed an increase in the Ca^{2+} sensitivity of force development and no difference in maximal force recovered compared to HCTnT3. The HSSTnT2 and $HSSTnT_{HYP}$ isoforms showed improved relaxation of contraction. We conclude that HSSTnT1, 2 and 3 decrease the Ca^{2+} sensitivity of force development and increase maximal force. The $HSSTnT_{HYP}$ isoform showed the opposite. These results suggest that the SSTnT isoforms play distinct functional roles in muscle regulation and function in both normal adult and diseased hearts.

Supported by NIH - AR050199, HL 42325 and HL 67415.

659-Pos Calcium Overloading Induces Restricted N-terminal Truncation of Cardiac Troponin T

Zhiling Zhang, Hanzhong Feng, J.-P. Jin

Evanston Northwestern Healthcare, Evanston, IL, USA.

Board B503

We previously reported that the N-terminal region of cardiac troponin T (cTnT) could be selectively cleaved by mu-calpain during myocardial ischemia reperfusion (Zhang et al., *Biochemistry 45*:11681–94, 2006). To investigate the regulatory mechanisms, we found that *ex vivo* myocardial infarction from ligation of the left anterior descending coronary artery induced heart failure and produced the N-terminal truncated cTnT (cTnT-ND) not only in the infarct but also in remote zones. Acute increases in the afterload of left OR right ventricle induced the production of cTnT-ND in BOTH ventricles. This whole organ proteolytic response in isolated working heart preparations indicates an independence of systemic neurohumural mechanisms. The activating signal is likely transduced from the stressed area to the whole ventricles via a syncytium-based mechanism. Ca(2+)-overloading conditions could directly induce myocardial production of cTnT-ND. Therefore, we propose that increased calcium influx into cardiomyocytes in response to ischemia reperfusion or pressure overload may play a role to activate the restricted proteolytic modification of cTnT. We are testing this hypothesis by correlating the level of cytosolic Ca(2+) and the production of cTnT-ND in primary cardiomyocyte cultures. Ca(2+) overloading conditions are investigated for the induction of cTnT-ND production. Together with our finding that the phosphorylation states of cTnT may determine the production of cTnT-ND. It is possible that intracellular calcium overload activates a protein kinase pathway that phosphorylates cTnT to confer a higher sensitivity to calpain cleavage. This study not only provides information for the mechanism of cTnT-ND production in myocardial ischemia reperfusion but also explores a potential new target for manipulating myocardial contractility.

660-Pos Expression of α- and β-Isoforms of Fast Troponin T During Skeletal Muscle Differentiation and Adaptation

Stephen M. Chong, J.-P. Jin

ENH Research Institute, Evanston, IL, USA.

Board B504

The COOH-terminal region of troponin T (TnT) is largely conserved among the three muscle fiber type isoforms. However, a segment in the COOH-terminal region of fast skeletal muscle TnT is regulated by alternative RNA splicing that produces the α- and β-isoforms corresponding to the mutually exclusive utilization of exon 16 or 17, respectively. This segment of TnT directly binds troponin I (TnI) in the I-T arm of troponin and, therefore, its structural regulation may have physiological importance. Splicing error can cause a skipping of both exon 16 and exon 17, resulting in an mRNA encoding truncated TnT protein. Using site-specific monoclonal antibodies, we found that the truncated fast TnT protein may exist in normal skeletal muscles. Tropomyosin and TnI binding experiments are carried out to investigate the potential negative impact of the aberrant splicing product. We have previously reported that the α-isoform of fast TnT is developmentally up-regulated and specific to the mature fast skeletal muscle. We have further investigated the distribution of the α- and β-isoforms in different types of adult skeletal muscle tissues and fibers. Specific antibodies and quantitative PCR were employed to analyze representative mouse skeletal muscles (extensor digitorum longus, soleus and diaphragm muscles) as well as in myoblasts during in vitro differentiation for the α- and β-fast TnT contents. The regulation of exon 16/17 expression during muscle adaptation is investigated in unloaded rat leg muscles for correlations with the reduction of slow fiber contents and the increase in high molecular fast TnT from N-terminal alternative splicing.

661-Pos In Silico and In Vitro Characterization of Cardiac Troponin T Mutations Known to Cause Familial Hypertrophic Cardiomyopathy

Pia J. Guinto, Edward P. Manning, Rachel K. Moore, Steven D. Schwartz, Jil C. Tardiff

Albert Einstein College of Medicine, Bronx, NY, USA.

Board B505

FHC is a primary cardiac muscle disorder that is one of the most common causes of sudden death in young people. The FHC "hotspot" mutations at residue 92 in cardiac troponin T (cTnT), a central modulator of thin filament regulation of myofilament activation, flank the proposed α-helical TNT1 tail domain. The flexibility of TNT1 has been suggested to be important in normal protein-protein interactions within the thin filament. Through Molecular Dynamics (MD) simulations, we have shown that FHC mutations Arg92Leu and Arg92Trp result in increased flexibility at a critical hinge region 18 Angstroms distant from the mutation. How do

primary biophysical changes induced by these mutations cause complex cardiomyopathies? MD shows replacement of residue Arg92 with hydrophobic Leu and Trp results in local helical compaction about the mutation site and significant expansion within the hinge region. MD simulations of Arg92Gln, a polar, uncharged substitution, also show increased flexibility about the hinge region with differences in α-helical characteristics compared to the hydrophobic substitutions. Arg92Lys simulations (a mutation with a residue of similar polarity and charge) show no alteration in flexibility or compaction-expansion regions in α-helical structure. These observations imply a significant cause and effect relationship due to the removal of an important polar, charged residue. We hypothesize the flexibility alterations and compaction-expansion regions in mutational segments would lead to electrostatic perturbations, possibly interfering with cTnT-TM complex formation and thin filament function. *In vitro* motility assays with wild type cTnT and hotspot FHC-cTnT mutants are in progress to directly correlate predicted alterations with resultant functional changes. Additional simulations and motility assays comparing wild type cTnT with FHC mutants will address the relative importance of electrostatic, structural, and functional contributions to FHC pathogenesis.

662-Pos Alterations in myofibrillar mechanics by psuedo-phosphorylation of cardiac troponin I

Aaron C. Hinken[1], Laurin M. Hanft[2], Sadayappan Sakthivel[3], Jeffery Robbins[3], Pieter P. de Tombe[1], Kerry S. McDonald[2], R John Solaro[1]

[1] *University of Illinois-Chicago, Chicago, IL, USA*
[2] *University of Missouri, Columbia, MO, USA*
[3] *The Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA.*

Board B506

The phosphorylation of specific serine and threonine residues on the inhibitory subunit of cardiac troponin (cTnI) by various kinases represents a major physiological mechanism for alteration of myofilament properties. Furthermore, altered thin filament function plays an important role in the contractile dysfunction associated with pathological outcomes such as heart failure. In this study we investigated the effect(s) of chronic cTnI phosphorylation on myofilament function using skinned preparations from transgenic animals in which either only PKA phosphorylation sites (Ser-23/Ser-24) (PP) or all of the possible PKA and protein kinase C (PKC) phosphorylation sites (Ser-23/Ser-24/Ser-43/Ser-45/T-144) (All-P) were replaced with aspartic acid to mimic a state of phosphorylation, as compared to wild-type cTnI replacement (WT). Left ventricular cardiac myocytes from All-P transgenic mice exhibited less Ca^{2+} sensitivity of force and produced lower levels of maximal force (29 ± 15 kN/m^2) than both WT (52 ± 10 kN/m^2) and PP transgenic myocytes (55 ± 13 kN/m^2). In addition, the economy of force production was decreased in All-P left ventricular papillary strips as compared to WT and PP. Moreover, while PKA treatment yielded faster loaded shortening at high loads in WT and PP transgenic myocytes, this effect was often blunted in myocyte preparations from All-P transgenic mice. These results implicate pseudo-phosphorylation at PKC sites on cTnI are important modulators of maximal force, Ca^{2+} sensitivity of force, and tension cost of cardiac myofibrils. In addition, PKC phosphorylation of cTnI attenuates the PKA-induced acceleration of myocyte loaded shortening, a process that appears to be modulated, at least in part, by phosphorylation of MyBP-C. The data show PKC phosphorylation of cTnI plays a primary role in depressing contractility, and therefore may contribute to the maladaptive behavior exhibited during the progression to heart failure with increased PKC isozyme activity.

663-Pos AMP Kinase Phosphorylates Cardiac Troponin I on Multiple Sites

Raquel M. Sancho Solis[1], Lisa Xu[1], Huan He[2], Carole L. Nilsson[2], Alan G. Marshall[2], Ying Ge[1], Jeffery W. Walker[1]

[1] *University of Wisconsin - Madison, Madison, WI, USA*
[2] *Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory, Tallahassee, FL, USA.*

Board B507

AMP kinase is a serine/threonine protein kinase that becomes activated when cellular AMP:ATP ratios rise, thereby serving as a key regulator of cellular energetics. Among the known target proteins phosphorylated by AMP kinase are catabolic and anabolic enzymes (e.g. cardiac phosphofructokinase 2, acetyl CoA carboxylase), but little is known about its ability to regulate the cardiac contractile apparatus. Upon treatment of Triton X-100 skinned mouse myocytes with recombinant catalytic subunit of AMP kinase activated by the LKB1/STRAD/MO25 complex, we observed incorporation of ^{32}P from ^{32}P-γ-ATP into cardiac troponin I (cTnI) and an unidentified protein at ~50 kDa. Purified human cTnI was also readily phosphorylated by AMP kinase, in contrast to human cardiac troponin T which was a poor substrate. Using a combination of recombinant mouse cTnI with candidate sites mutated to alanine, phosphospecific antibodies and high resolution MS/MS analysis of synthetic peptide substrates, we identified Ser^{22} as one of at least two preferred sites phosphorylated by AMP kinase. Ser^{23} was not phosphorylated by AMP kinase under the conditions employed. By screening synthetic peptides mimicking selected regions of cTnI, a second potential AMP kinase site was identified at Ser^{149}. AMP kinase was essentially refractory toward other sites including Thr^{30} and three major PKC sites at Ser$^{41/43}$ and Thr^{142}. The results indicate that AMP kinase phosphorylates cardiac troponin I with a unique pattern characterized by selective targeting of one PKA site and one p21-activated kinase (PAK) site, but no PKC sites. Functional consequences on the contractile apparatus and its regulation are under investigation.

664-Pos Altered Phosphorylation Of cTnT And cTnI In SHR Myocardium

Mary L. Garcia-Cazarin, Prasanna K. Challa, Krishna S. Vyas, Gail A. Sievert, Ye Chen-Izu, C. William Balke, Marius P. Sumandea

University of Kentucky, Lexington, KY, USA.

Board B508

Spontaneously hypertensive rats (SHR) are the most extensively studied animal models for human hypertensive heart disease. The animals are pre-hypertensive during the first 4–6 weeks of life, rapidly develop high systolic pressure, become fully hypertensive at 8–12 weeks of age and suffer sustained hypertension there after. Cardiac hypertrophy develops gradually under hypertension between 3–18 months of age. Most animals suffer heart failure at 18–24 months of age. The consistent pattern of a long period of stable hypertrophy followed by a transition to failure provides a useful model to study mechanisms of heart failure and to test different phases of the disease process. The transition from compensated hypertrophy to failure is accompanied by marked changes in cardiac function, which are associated with altered active and passive mechanical properties of the myocardial tissue resulting in a decrease force of contraction. However, the mechanisms responsible for this depression in function are poorly understood.

In this study, we test the hypothesis that changes in phosphorylation of cardiac troponin (cTnT and cTnI) in the aging SHR myocardium leads to the progression of contractile dysfunction and ultimately heart failure. Myofibrils isolated from left ventricular myocardium of male SHR and age-matched WKY rats were probed for total (ProQ Diamond) as well as site-specific (phospho-antibodies) phosphorylation patterns. Our preliminary results indicate that cTnI (Ser23/24; Thr144) and cTnT (Thr206; Ser278) are hyper-phosphorylated in the pre-hypertensive and hypertensive animals compared to WKY controls. Interestingly, failing SHR myocardium exhibits a dramatic de-phosphorylation of cTnI and cTnT. These data implicate that regulation of cardiac myofilament function through troponin phosphorylation is altered in SHR animals.

665-Pos Kinetic Study of the Interaction between cTnI and Actin: Effects of Ca^{2+}, Strongly Bound S1 and PKA Phosphorylation of cTnI

Jun Xing[1], Herbert C. Cheung[1], Wen-Ji Dong[2]

[1] *University of Alabama at Birmingham, Birmingham, AL, USA*
[2] *Washington State University, Pullman, WA, USA.*

Board B509

In cardiac muscle the interaction between the regulatory/inhibitory (Rr/Ir) region of cTnI and actin is regulated by Ca^{2+} and strong crossbridge and fine tuned by cTnI phosphorylation. The kinetics associated with the interaction was investigated using FRET-based stopped-flow to monitor the changes in the distance from actin (Cys374) labeled with DABM as acceptor to the single cysteine residues 151 or 167 of cTnI mutant labeled with AEDANS as donor. Studies with the thin filament (TF) reconstituted with labeled actin, labeled cTnI, wild-type cTnC, cTnT and tropomyosin showed that Ca^{2+}-binding induced the Rr/Ir region to move away from actin by 11~12 Å. Ca^{2+} sensitivity and Ca^{2+} dissociation-induced kinetics of this structural transition were 5.70 ~ 5.74 (pCa_{50}) and 85 ~ 120 s^{-1}, respectively. The strongly bound S1 caused the Rr/Ir region to move further away by 3~7 Å and significantly increased Ca^{2+} sensitivity and slowed down the kinetics. Phosphorylation of cTnI by PKA decreased the Ca^{2+} sensitivity and increased the kinetics regardless of the presence or absence of the strongly bound S1. Kinetics of the structural transition between the Rr/Ir region of cTnI and actin (Cys374) induced by the strongly bound S1 was measured by mixing the Ca^{2+}-saturated thin filament with S1 in the presence of ADP. On the TF reconstituted with wild-type cTnC, the kinetics of the structural transition was 3 s^{-1} and independent of cTnI phosphorylation. However, when the Ca^{2+}-binding site II of cTnC was deactivated in the TF reconstituted with cTnC(D65V/D67A), the kinetic rate decreased to 0.6 s^{-1}. These results suggest that the presence of Ca^{2+} promotes kinetics of strong crossbridge formation, but the PKA phosphorylation of cTnI has no direct effects on the kinetics of the crossbridge formation.

666-Pos Shift In Myosin Heavy Chain Isoform Influences The Effect Of Slow Skeletal Troponin I On Mouse Cardiac Contractile And Myofiber Dynamic Functions

Steven J. Ford[1], R. John Solaro[2], Murali Chandra[1]

[1] *Washington State University, Pullman, WA, USA*
[2] *University of Illinois at Chicago, Chicago, IL, USA.*

Board B510

In addition to changes in Myosin Heavy Chain (MHC) isoform expression, developing murine embryos also undergo a transition in Troponin I (TnI) isoform expression. The functional significance of slow skeletal TnI (ssTnI) in the developing heart is not well understood because ssTnI is expressed in the developing heart only when β-MHC is the predominant isoform. Most studies on the effects of ssTnI on cardiac function have been performed using normal adult mice expressing a native α-MHC background. We studied muscle fibers from native transgenic (TG) and non-transgenic (NTG) mice expressing cardiac specific ssTnI cardiac TnI, respectively, as well as TG and NTG mice treated with 6-propyl-2-thiouracil (PTU, a hypothyroidic chemical resulting in β-MHC expression). Contractile function and myofiber dynamic measurements were made in detergent-skinned fibers harvested from left-ventricular papillary bundles of these mice. Myofilament calcium sensitivity, as measured by pCa_{50}, was significantly higher in ssTnI TG mice only in the presence of α-MHC, (pCa50 = 5.78±0.01 in NTG mice vs 6.11±0.01 in TG mice). β-MHC expression blunted this effect of ssTnI dependent increase in Ca2+ sensitivity (pCa50 = 5.85±0.01 NTG vs 5.90±0.01 TG). ssTnI increased the muscle-length-dependent increase in the rate of XB-recruitment by 15% (b = 16.72±1.23 NTG vs 19.16±0.66 TG) in the presence of α-MHC and by 57% (b = 5.50±0.24 NTG vs 8.61±0.57 TG) in the presence of β-MHC. Thus, ssTnI affected myofiber contractile dynamics more significantly in the presence of β-MHC. These and other contractile function and myofiber dynamic measurement data will be discussed in more detail at the meeting.

667-Pos Structure And Fly-casting Mechanism Of The C-terminal Region Of Troponin I By SDSL-EPR Spectroscopy

James A. Cooke[1], Paul M.G. Curmi[2], Peter G. Fajer[3], Louise J. Brown[1]

[1] *Macquarie University, Sydney, NSW, Australia*
[2] *The University of New South Wales, Sydney, NSW, Australia*
[3] *Florida State University and The National High Magnetic Field Laboratory, Tallahassee, FL, USA.*

Board B511

Several regions of the Troponin complex are highly flexible causing complications in structural elucidation by traditional high-resolution techniques. One such important region is the C-terminal region of the Tn inhibitory subunit (C-TnI), an alleged second actin-binding domain. Futhermore, numerous mutations in C-TnI are implicated in hypertrophic cardiomyopathy (HCM) thus inferring significant functional importance for this region. Several contradicting structural models of C-TnI have been suggested with most describing a highly dynamic domain with at most a nascent secondary structure (Biophys. J *90*:2436; J. Mol. Biol. *352*:178). These structures were proposed from data collected in the absence of the actin thin filament - the proposed C-TnI binding partner.

We have utilised Site-Directed Spin Labeling Electron Paramagnetic Resonance (SDSL-EPR) to elucidate the structure of the C-TnI region of cardiac troponin in the reconstituted thin filament. The MTSSL spin label was introduced at 9 consecutive cysteine residues (cardiac 175–183) as engineered by mutagenesis of cardiac TnI. The mobility of this region was highly dynamic but several regions exhibited EPR trends consistent with a helical region. The C-TnI was found to weakly interact with actin and we propose stronger binding with actin further towards the TnI C-terminus. Inter-residue spin distance measurements between labels within this C-TnI domain are also consistent with this conclusion. Conformational changes in the C-TnI region upon actin and Ca^{2+} binding from ongoing EPR scanning experiments in this region will be discussed. Thin filament residue interactions within this region are further compared to HCM mutations for structural/functional relevance.

668-Pos The Switch Peptide And N-terminus cTnI Conformational Changes As Measured By Deer And Dipolar Epr

jean chamoun, Likai Song, Brenda Schoffstall, Janush Pyka, Peter G. Fajer

FSU, Tallahassee, FL, USA.

Board B512

The regulatory protein troponin complex (TnI, TnT and TnC) triggers muscle contraction upon calcium binding. In the ON-state ($+Ca^{2+}$), the position of the cTnI switch peptide (150–159) is in close proximity to the N-lobe of TnC (Takeda et al., Nature, 2003) whereas in the OFF-state ($-Ca^{2+}$) its location is unknown. One of many possibilities is that the peptide is close to the coiled-coil region of cTnT (226–275) and cTnI (90–136). We designed mutants with labels on the N-lobe of TnC (TnC55) and adjacent to the switch peptide (cTnI160) to probe the ON-state and double mutants of cTnI with labels in the coiled-coil region (cTnI160/129, cTnI160/115, and cTnI160/138) to probe the OFF state. Conventional dipolar EPR and Double Electron-Electron Resonance (DEER) were used for distance measurements between the different mutants in the reconstituted troponin complex. For the ON-state the measured distance (TnC55/TnI160, 30 Å) is in agreement with the crystal structure. In the OFF-state the switch peptide is closer to the coiled-coil (cTnI160/129, 17 Å). These results support the proposed hypothesis where the switch peptide moves towards the coiled-coil in the absence of calcium. Cardiac muscle is regulated by the phosphorylation of serines (23/24) in the N-terminal extension which was truncated in the construct used for crystal structure (Takeda et al., Nature, 2003). We suspect that phosphorylation induces the movement of the N-terminal extension toward either the inhibitory region (137–148) or the C-terminus of cTnI (200–210). We measured the relative distance in both un-&-bisphosphorylated states between a) N-terminus extension and the inhibitory region (TnI9/142) b) the N-terminus extension and the C-terminus of cTnI (TnI9/209) in the presence of calcium. Our preliminary results showed no distance variation between un-&-bisphosphorylated states of TnI9/209 (48 Å ± 3 Å).

669-Pos The Restrictive Cardiomyopathy Mutation In Troponin I, R192H, Slows The Rate Of Ca2+ Dissociation From Troponin C In Reconstituted Thin Filaments

Bin Liu[1], Svetlana B. Tikunova[1], Jack A. Rall[1], Darl R. Swartz[2], Jonathan P. Davis[1]

[1] *The Ohio State University, Columbus, OH, USA*
[2] *Purdue University, West Lafayette, IN, USA.*

Board B513

The human restrictive cardiomyopathy mutation, troponin I R192H, is associated with severe heart disease. This mutation in TnI is located in the putative second tropomyosin-actin binding site. In transgenic mice, the homologous human mutation causes diastolic dysfunction in-vivo and gene transfer in rats leads to impaired myocyte relaxation in-vitro. Previous studies have also demonstrated that the R192H mutation drastically increases the Ca^{2+} sensitivity of acto-myosin ATPase activity in reconstituted thin filaments and force development in skinned cardiac preparations. We hypothesized that the impaired relaxation and increased Ca^{2+} sensitivity of the myofilaments was due to a slower rate of Ca^{2+} dissociation from troponin C. At 15°C, the rate of Ca^{2+} dissociation from isolated Tn containing TnI R192H was not different from that containing the wild type TnI. This result is consistent with a previous study that

showed the Ca^{2+} sensitivity of isolated Tn was not affected by TnI R192H. However, incorporation of TnI R192H into reconstituted thin filaments (7 actin : 1 tropomyosin : 0.88 Tn) in the absence and presence of myosin S1 (2 S1 per 7 actin) slowed the rate of Ca^{2+} dissociation from TnC ~2-fold compared to the wild type TnI. Thus, on the thin filament, the rate of Ca^{2+} dissociation from Tn containing TnI R192H was always slower than the control and mechanistically explains the higher Ca^{2+} sensitivity of the myofilaments caused by the TnI mutation. Furthermore, the data are consistent with the hypothesis that slowing the rate of Ca^{2+} dissociation from TnC slows cardiac muscle relaxation.

(Supported by NIH K99 HL087462 (to SBT); RO1 HL 073828 (to DRS); RO1 AR020792 (to JAR); and AHA SDG 0735079 (to JPD)).

670-Pos Basal contractile function in myocytes expressing cardiac troponin I Ser43/45Ala

Dustin Robinson, Julie Rogers, Sarah Jimines, Margaret V. Westfall

University of Michigan, Ann Arbor, MI, USA.

Board B514

In earlier work, protein kinase C-mediated phosphorylation of cardiac troponin I Ser43/45 (cTnISer43/45) decreased myofilament Ca^{2+} sensitivity and maximum force in permeabilized cells. However, it remains unclear whether the absence of phosphorylation at this cluster directly influences basal contractile function in intact myocytes. In addition, replacement of endogenous cTnI with mutants containing substitutions of this site has not been examined in myocytes from larger mammals. The goal of the present study was to determine whether troponin I with an Ala substitution at Ser43/45 is incorporated equally well in rabbit and rat myocytes, and whether this substitution significantly influences basal myocyte contractile function. Expression and sarcomere shortening were measured in adult rat and rabbit myocytes after gene transfer of cTnISer43/45Ala with and without a FLAG epitope. Western analysis showed >85% replacement of endogenous cTnI with cTnIFLAG or cTnISer43/45AlaFLAG in both rabbit and rat myocytes 4 days after gene transfer. Expressed cTnISer43/45AlaFLAG was incorporated into the sarcomere based on the comparable levels of replacement measured in intact and permeabilized myocytes. Sarcomere shortening was measured in electrically paced, adult rat myocytes and results in myocytes expressing cTnISer43/45Ala were compared to myocytes expressing cTnIFLAG 4 days after gene transfer. In preliminary functional studies, peak shortening and the rates of contraction and relaxation were comparable in myocytes expressing cTnISer43/45Ala and cTnIFLAG. These early results provide evidence to indicate that basal Ser43/45 phosphorylation does not significantly influence basal sarcomere shortening and re-lengthening in intact myocytes. Ongoing studies are now focused on determining whether absence of this Ser cluster significantly influences the contractile response to PKC activation by endothelin.

671-Pos S23D/S24D, S23A/S24A Mutations In Rat Cardiac Troponin I Depress Myofilament Ca^{2+} Sensitivity, Maximum Tension And ATPase Activity In Reconstituted Rat Cardiac Muscle Fibers

RANGANATH MAMIDI, JASON DOBRY, STEVEN J. FORD, MURALI CHANDRA

Washington State University, PULLMAN, WA, USA.

Board B515

Phosphorylation of cardiac troponin I (cTnI) at S23/24 is known to decrease myofilament Ca^{2+} sensitivity. In this study, we used a substitution mutant of cTnI (S23D/S24D) to determine if these mutations altered contractile function other than myofilament Ca^{2+} sensitivity.

Preliminary studies in our lab showed that reconstitution of both S23D/S24D, S23A/S24A mutants resulted in a significant change in contractile function parameters. Maximum Tension (S23D/S24D: 33.91 ± 1.79, S23A/S24A: 31.77 ± 2.09), maximum ATPase activity (S23D/S24D: 146.68 ± 7.86, S23A/S24A: 150.19 ± 8.77) and myofilament Ca^{2+} sensitivity, as measured by pCa_{50} (S23D/S24D: 5.38 ± 0.02, S23A/S24A: 5.42 ± 0.01) of the fibers reconstituted with mutant Tn were significantly lower than fibers reconstituted with wild type recombinant RcTnI. Contractile parameters of RcTnI were as follows: maximum tension (50.81 ± 2.97), maximum ATPase activity (210 ± 6.0) and pCa_{50} (5.67 ± 0.02).

Further experiments will be done to determine the underlying source of the effects of cTnI mutants. Our preliminary data suggest these mutations affect the structure and function of cTnI as observed by changes in the aforementioned contractile parameters.

672-Pos Troponin I interacts near residue 146 of tropomyosin in a Ca^{2+}-dependent manner

Wasana A. Mudalige, Terence Tao, Sherwin S. Lehrer

Boston Biomedical Research Institute, Watertown, MA, USA.

Board B516

The Ca^{2+}-dependent interaction of troponin I (TnI) with actin-tropomyosin in the muscle thin filament is an important step in the regulation of muscle contraction. Previous studies have suggested that TnI interacts specifically with tropomyosin (Tm) in the reconstituted muscle thin filament and is involved in the Ca^{2+}-induced movement of Tm (Potter & Gergely, Biochemistry, 1974; Zhou et al. and Geeves et al., Biochemistry, 2000; Luo et al., JMB, 2002). To obtain direct evidence for this interaction, and to determine the sites of interaction we performed photochemical crosslinking studies with 4-maleimidyl benzophenone (BPmal) labeled Tm at a single Cys at position 146 or 174 (Tm146* or Tm174*), reconstituted with

troponin (TnI +TnT +TnC) and actin. After uv-irradiation, SDS-gels of the Tm146* system showed 3 new high MW bands confirmed to be Tm146*-TnI, Tm146*-TnC and Tm146*-TnT using both fluorescently labeled TnI and mass spectrometry. In contrast, for Tm174* one main crosslinked band predominated attributed to Tm174*-TnT. Interestingly, the Tm146*-TnI band was much more intense when crosslinked in the absence of Ca^{2+}, in contrast to the other crosslinked species which did not show a Ca^{2+} dependence. These data show that in the thin filament:

5. a region of TnI interacts with Tm near position 146 in the absence but not in the presence of Ca^{2+};
6. a region of TnC is near Tm 146;
7. regions of TnT are near both Tm positions 146 and 174.

These data support a model in which the TnI-Tm interaction near Tm146 is involved in the Ca^{2+}-dependent Tm movement and will also aid in the location of the Tn complex along Tm in the muscle thin filament.

Muscle Regulatory Proteins - II

673-Pos Tropomyosin Pseudo-phosphorylation Alters its Interaction Within the Thin Filament

Brandon J. Biesiadecki, Janel Jin, R. John Solaro

University of Illinois at Chicago, Chicago, IL, USA.

Board B517

The head-to-tail overlap region of the tropomyosin dimer is critical to striated muscle contractile function. Tropomyosin contains a single phosphorylation site at Ser-283 that is located directly within the head-to-tail overlap region. During cardiac muscle development tropomyosin phosphorylation is elevated while phosphorylation is decreased in the adult suggesting a significant role for tropomyosin phosphorylation in either sarcomeric assembly or in the regulation of cardiac muscle contraction. To retain physiological function the tropomyosin molecule must be acetylated at its N-terminal amino acid residue. To date, all but a single study have investigated the biochemical properties of phosphorylated tropomyosin using either tropomyosin purified from muscle, that is likely to contain other post-translational modifications, or recombinant E. coli expressed tropomyosin containing an AS- tag. To investigate the effects of native N-terminal acetylated tropomyosin phosphorylation in the absence of other potentially confounding post-translational modifications we purified wild type and recombinant alpha tropomyosin containing either phosphorylation-null (S283A) or pseudo-phosphorylation (S283D) mutations expressed in insect cells. ELISA epitope analysis demonstrates tropomyosin pseudo-phosphorylation alters the binding of the monoclonal antibody CH1 to a central epitope in the tropomyosin molecule suggesting a long-range conformational effect of Ser-283 phosphorylation on the central tropomyosin molecule. ELISA solid phase protein binding assays demonstrate a significant increase in maximal binding of the pseudo-phosphorylated tropomyosin to both troponin T and the troponin complex compared to wild type and phosphorylation-null tropomyosin in the absence of a significant effect on binding affinity. These results demonstrate for the first time that recombinant pseudo-phosphorylated tropomyosin exhibits altered interaction with the troponin complex, further suggesting a role for tropomyosin phosphorylation in cardiac muscle function.

674-Pos Effects of the Novel Tropomyosin-Kappa on Modulating the Mechanical Properties of the Heart

Chehade N. Karam[1], Sudarsan Rajan[2], Chao Yuan[1], David F. Wieczorek[2], R John Solaro[1]

[1] *University of Illinois at Chicago, Chicago, IL, USA*
[2] *University of Cincinnati, Cincinnati, OH, USA.*

Board B518

Tropomyosin-kappa (TPM1κ) is a newly discovered TM isoform that is exclusively expressed in the human heart. Preliminary studies indicate an increased expression of TPM1κ in hearts of patients with dilated cardiomyopathy (DCM). TPM1κ results from the alternative splicing of the α-TM gene where the skeletal muscle exon 2b is replaced with the smooth muscle exon 2a. In this study we sought to determine the effect of the presence of TPM1κ isoform on sarcomeric response to Ca^{2+}. We generated transgenic (TG) mice expressing TPM1κ in the cardiac compartment. About 60% of the native TM was replaced with TPM1κ. We compared force-pCa relations in detergent extracted fiber (skinned) bundles isolated from hearts of non transgenic (NTG) controls (n=13) and TG-TPM1κ (n=11) at a sarcomere length of 1.9 μm. Our data (mean ± SEM) demonstrated a significant decrease in the Ca^{2+} sensitivity of the myofilaments (NTG: $pCa_{50}= 5.89 \pm 0.01$; TG: $pCa_{50}= 5.72 \pm 0.01$; $\Delta pCa_{50}= 0.18 \pm 0.01$; $P<0.0001$) with no change in the maximum developed tension (NTG: $F_{max}= 32.12 \pm 0.66$ mN/mm^2; TG: $F_{max}= 29.92 \pm 0.43$ mN/mm^2). To determine the effect of isoform switching on TM phosphorylation, we performed two-dimensional difference gel electrophoresis (2D-DIGE) followed by Pro-Q staining. There was a decrease in the total phosphorylation of TPM1κ compared with that of α-TM. Our results fit with reports showing a linkage of DCM to point mutations that decrease Ca^{2+} sensitivity, and suggest a potential role of altered TM phosphorylation.

675-Pos Oxidation Of Tropomyosin By Reactive Oxygen Species In The Myocardium

Benjamin S. Avner, Lei Xiao, R. John Solaro

University of Illinois at Chicago, Chicago, IL, USA.

Board B519

Reactive oxygen species [ROS] generation is linked to acute and chronic cardiac pathophysiological change and injury, and is known to be increased in aging tissue. Sarcomeric components such as tropomyosin [Tm] are critical in cardiac function and may be a target of modification by ROS. The data presented test the hypothesis that ROS in ventricular tissue act through oxidation of sarcomeric