digestion studies indicate that cTnI Delbp529AA is degraded at a faster rate than wild-type cTnI. These results suggest that the poor prognosis of patients carrying these RCM-linked mutants is due to protein dysfunction at multiple levels and suggest possible mechanisms of RCM pathology.

1851-Pos

Identification of Unknown Protein Kinase Cα Phosphorylation Sites on Both Human Cardiac Troponin I and T

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Protein kinase C (PKC) isoforms have been shown to play an important role in the development of heart failure. Most research performed on PKCα has been done in rodents and direct evidence in human heart failure is limited. Our previous study showed a decrease in Ca^{2+}-sensitivity in failing tissue upon PKCα treatment of cardiomyocytes via phosphorylation of cardiac troponin I (cTnI), cardiac troponin T (cTnT) and myosin binding protein C (cMyBP-C). This study aims to determine the targets of PKCα on cTnI and cTnT. Western immunoblotting revealed that PKCα is less abundant but more active in failing compared to donor tissue. PKCα treatment of donor and failing tissue was able to phosphorylate Thr-143, which is a known PKCα site on cTnI, but endogenous phosphorylation levels were very low. LC-MS analysis of purified human recombinant cTn complex incubated with PKCα identified two novel phosphorylation sites, Ser-199 located on cTnI and Ser-189 on cTnT. Both sites are located in conserved regions on cTnI and cTnT. The PKA sites Ser23/24 on cTnI are phosphorylated by PKCα in purified human recombinant cTn complex, but there is no cross phosphorylation in donor and failing tissue. In conclusion, endogenous Thr-143 phosphorylation is low, which makes its involvement in heart failure unlikely. Exogenous PKCα phosphorylation of Thr-143 and Ser-199 on cTnI and Ser-189 on cTnT could possibly explain the decrease in Ca^{2+}-sensitivity observed and further research on the site-specific effects is warranted.

1852-Pos

Rescuing Myopathic Phenotype of Abnormal Cardiac Troponin T with a Single Amino Acid Substitution in Cardiac Troponin I

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Troponin T (TnT) and troponin I (TnI) are two subunits of the troponin complex. We previously found a single amino acid substitution in the TnT-binding helix of cardiac TnI (cTnI) in wild turkey hearts that expressed abnormally spliced myopathic cardiac TnT (cTnT) (Biesiadecki *et al., JBC* 279:13825-32, 2004). To test the potential role of this cTnI modification in rescuing a cTnT abnormality, we developed transgenic mice expressing the cTnI variant (K118C) with or without a deletion of endogenous cTnI gene to mimic homozygote and heterozygote wild turkeys. Double and triple transgenic mice were created to combine the cTnI-K118C allele with an allele encoding the abnormally spliced cTnT (exon 7 deletion). Functional analysis in *ex vivo* working hearts found that cTnI-K118C had no destructive effect on cardiac muscle and baseline heart function but was able to rescue the decreases in cardiac function caused by cTnT exon 7 deletion. Further characterizations showed that cTnI-K118C significantly blunted the inotropic response of cardiac muscle to β-adrenergic stimulation whereas the PKA-dependent phosphorylation of cTnI was unchanged. These data indicate that TnI-TnT interaction is a critical link in Ca^{2+} signaling and β-adrenergic regulation of myocardial contraction, providing a novel target for the treatment of heart failure.

1853-Pos

H2-Helical Region of Cardiac Troponin T Contributes to Length-Dependent Regulation of Cardiac Contractile Activation

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An alpha-helical region of cardiac troponin T, cH2(T), is centrally positioned in the core domain of the troponin complex (Takeda et al., Nature 2003 vol.424(6944):p35-41). cH2(T)-troponin I and cH2(T)-troponin C interactions have been illustrated through biochemical studies and visualized in the crystal structure of the core domain of human cardiac troponin, suggesting an important regulatory role for cH2(T). However, little is known about how the cardiac-specific structure of cH2(T) relates to cardiac-specific contractile function. To better understand the functional significance of cH2(T), we created a chimeric rat cardiac troponin T (TnT) in which cH2(T) was replaced by the corresponding helical region of rat slow skeletal TnT and studied how replacement of native TnT with chimeric TnT affected contractile function of rat cardiac muscle. We measured isometric force, ATPase activity, and length-dependent contractile dynamics in detergent skinned papillary muscle bundles reconstituted with either wild-type or chimeric TnT, held at either sarcomere length (SL) 2.0 μm or 2.2 μm. Preliminary studies suggest that the SL dependence of Ca^{2+}-activated maximal force production and tension cost was depressed in bundles containing chimeric TnT. For example, bundles containing the chimeric TnT showed an 8.4% decrease in force production when SL was decreased from 2.2 μm to 2.0 μm, whereas bundles containing the wild-type TnT showed a 43.9% decrease in force production. In addition, length-dependent contractile dynamics were significantly altered in bundles containing the chimeric TnT. For example, the length-dependent rate constant of crossbridge recruitment was slower, and the rate constant of crossbridge detachment was faster in bundles containing the chimeric TnT. Thus, our data suggest that cH2(T) plays a role in cardiac-specific length-mediated myofilament activation, an important mechanism underlying the Frank-Starling relationship.

1854-Pos

Structural and Functional Characterization of the TNT1 Domain of Cardiac Troponin T

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Familial Hypertrophic Cardiomyopathy (FHC) is a primary cardiac muscle disorder and a common cause of sudden cardiac death among young people in the field. The majority of disease-causing mutations in the thin filament protein hcTnT are found within the TNT1 domain. This domain has not been crystallized and its structural details are poorly defined, limiting our ability to understand the mechanism of disease for these mutations. A highly charged region is found at the C-terminal end of TNT1 (158-RREEEENRR-166) in which this highly alpha helical domain may unwind to create a flexible hinge that is necessary for normal function. We aim to determine the structural details and function of this region using SDSL-EPR and regulated in vitro motility (R-IVM) assays. The purpose of our R-IVM experiments is two-fold: to functionally analyze our spin labeled proteins and to gain insight into the function of TNT1 in the presence of cysteine substitutions. R-IVM data shows a progressive increase in the severity of the functional effects of cysteine substitution and spin labeling across the putative hinge region, suggesting that this region is dynamically important and may be making critical interactions with other components of the sarcomere. CW-EPR spectra of spin labeled hcTnT in Troponin ternary complexes show an increase in spin label mobility from residue 153 to 157 and 177, consistent with a decrease in alpha helical character across the putative hinge region. Preliminary doubly labeled CW-EPR experiments show that interspin distance between hcTnT residues 157 and 177 exceed 25A. Interspin distance measurements using doubly labeled hcTnT will further elucidate the secondary and tertiary structure of this region. Additional spin label pairs are currently being investigated using both CW-EPR and DEER techniques to determine the structural details of this important region.

1855-Pos

Tropomyosin-Kappa Alters Cardiac Dynamics in a Mouse Heart Model

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Tropomyosin-kappa (TPM1κ), a novel TM isoform, is exclusively expressed in the human heart. Alternative splicing of the α-TM gene generates TPM1κ, in which the skeletal muscle exon 2b is replaced by the smooth muscle exon 2a. We previously reported that TPM1κ expression was increased in the hearts of patients with chronic dilated cardiomyopathy (DCM). To understand how the TPM1κ isoform affects cardiac dynamics, we generated transgenic (TG) mice expressing TPM1κ in the myocardium. Most of the native TM (90%) is replaced by TPM1κ. In situ cardiac dynamics were determined by echocardiographic analysis. Results demonstrated that the TG hearts exhibited a diastolic dysfunction associated with a dilation of the left ventricle compared with the non transgenic (NTG) controls. We also compared force-pCa relations in detergent extracted (skinned) fiber bundles isolated from NTG and TG-TPM1κ hearts at sarcomere lengths (SL) 1.9 μm and 2.3μm. Our data demonstrated a significant decrease in the Ca2+ sensitivity of the

myofilaments from TG hearts at both SL compared to NTG ones. There was an equal leftward shift of the force-pCa curves from both NTG and TG hearts when SL was increased to 2.3 μm. In addition, NEM-S1, a mimic of strongly bound, rigor cross-bridges, was not able to induce activation of TG myofilaments to the same extent as in the NTG controls. To determine whether isoform switching affects sarcomeric protein phosphorylation, we performed two-dimensional difference in gel electrophoresis (2D-DIGE) and Western blots using TM, TPM1κ, and Serine-283P specific antibodies. We observed an increase in the total phosphorylation of TPM1κ compared with that of α-TM. MLC2, TnI, and TnT phosphorylation was not significantly affected. Our results demonstrate that the increased cardiac expression of TPM1κ alters cardiac dynamics in a similar way to DCM-linked point mutations of TM.

1856-Pos

Distribution of Voltage Gated Sodium Channels in Rabbit Cardiomyocytes During Development

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We and others have postulated that in neonatal cardiomyocytes sodium currents may influence Ca^{2+} influx through reverse-mode NCX activity by inducing strong membrane depolarization and/or local sodium accumulation profoundly modify contractility. To study the role of voltage-gated sodium channels ($Na_V1.X$) and reverse mode NCX activity through development, the expression and distribution of $Na_V1.X$ was determined using Western blot analysis, immunocytochemistry, confocal microscopy and image analysis in cardiomyocytes isolated from 3 and 56 days old rabbits. Immunoblot analysis revealed a robust expression of the skeletal muscle isoform ($Na_v1.4$) in neonatal cardiomyocytes which decreased 6-fold by 56 days ($p<0.01$). The neuronal isotype $Na_v1.1$ was found to have comparatively low levels of expression throughout development and followed a similar pattern as that of Nav1.4. $Na_v1.1$ levels decreased by 9 fold in the 56 day cardiomyocyte ($p<0.01$). The cardiac isoform ($Na_v1.5$) expression was also robust in the neonatal and adult cardiomyocytes but the protein levels did not vary significantly throughout development by western blot analysis. The distribution of $Na_v1.4$ and $Na_v1.5$ was punctate in nature on the cell periphery in both 3 and 56 day cardiomyocytes. Both $Na_v1.4$ and $Na_v1.5$ co-localized with NCX in 3 and 56 day cardiomyocytes. In neonatal cardiomyocytes, $Na_v1.4$-NCX and $Na_v1.5$-NCX colocalization relationship remained the same, while in the adult, $Na_v1.5$-NCX colocalization decreased by 50% due to the increase in the separation distances between $Na_v1.5$ and NCX in the adult. Taken together, our results suggest that in the neonate heart, $Na_v1.4$ may dictate the role of NCX in regulating Ca^{2+} influx during contraction.

1857-Pos

Characterization of L-Type Ca Channel Currents ($I_{Ca,L}$) in Zebrafish Cardiomyocytes

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The zebrafish is an important model for the study of vertebrate cardiac development with a rich array of genetic mutations for functional interrogation. The similarity of the zebrafish cardiac action potential duration with that of humans further enhances the role of this model to study cardiac arrhythmias. Despite this, little is known about basic excitation-contraction coupling in the zebrafish heart. Single cardiomyocytes were isolated from the adult zebrafish heart by enzymatic perfusion of the cannulated ventricle. Using an amphotericin-perforated patch clamp in whole cell configuration, $I_{Ca,L}$ was characterized in the zebrafish cardiomyocytes at RT. Simultaneous recordings of the voltage dependence of $I_{Ca,L}$ amplitude and cell shortening showed a typical bell-shaped I-V relationship for $I_{Ca,L}$ with a maximum at 10 mV whereas cell shortening showed a monophasic increase with membrane depolarization, and reached a plateau at membrane potentials above 20 mV. $I_{Ca,L}$ was 53, 100, and 17% of maximum at −20, +10 and +40 mV while cell shortening was 62, 95, and 96% of maximum respectively, suggesting that $I_{Ca,L}$ is the major contributor to the activation of contraction at voltages below 10 mV whereas the contribution of reverse-mode NCX becomes increasingly more important at membrane potentials above 10 mV. The $T_{1/2}$ for the recovery of $I_{Ca,L}$ from inactivation was 96 ms and the $V_{1/2}$ for voltage-dependent inactivation was −27.6 mV. In conclusion, we demonstrate that: 1) healthy and viable myocytes can be obtained by enzymatic perfusion of the heart; 2) ventricular myocytes exhibited large $I_{Ca,L}$ density (>12 pA/pF) over a range of stimulation frequencies (0.5 to 3 Hz) and 3) a monophasically increasing contraction - voltage relationship which is in contrast to the bell-shaped relationship observed in human and other mammalian cardiomyocytes.

1858-Pos

Electrophysiological Determinants for Arrhythmogenesis Following Premature Stimulation In Murine Hearts

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Background: Circus type re-entry is classically associated with reduced action potential (AP) conduction velocity through partially refractory tissue resulting in unidirectional conduction block. We assessed the extent to which premature extrasystolic APs under such conditions resulted in ventricular arrhythmogenesis in isolated Langendorff-perfused murine hearts.

Methods and Results: A novel programmed electrical stimulation (PES) protocol applied trains of 8 S1 stimuli at 100 ms intervals followed by extrasystolic S2 stimuli at successively decreasing S1S2 intervals. S2 stimulus strengths required to overcome refractoriness, reduce ventricular effective refractory period (VERP) and thereby elicit extrasystolic APs, increased with shortened S1S2 intervals, despite constant durations at 90% recovery (APD_{90}) of the preceding APs. Critical interval, CI, the difference APD_{90}-VERP, consequently increased with stimulus strength. The corresponding latencies and peak amplitudes of the extrasystolic APs consequently sharply increased and decreased respectively with CI thereby potentially replicating necessary conditions for re-entrant, circus-type, arrhythmia. The dependence of CI upon stimulus strength tended to consistent limiting values expected from approaches to absolute refractory periods. These values were greater in arrhythmogenic (mean CI 18.9 ± 0.55 ms, n=4) than in non-arrhythmogenic hearts (mean CI 15.1 ± 0.37, n=4; P=0.001, ANOVA), despite their statistically indistinguishable APD_{90} (arrhythmogenic hearts: 40.9 ± 2.23 ms, n=4 vs non-arrhythmogenic hearts: 36.5 ± 2.61ms, n=4; p>0.05, ANOVA) or VERP values (arrhythmogenic hearts: 22.5 ± 2.66 ms, n=4 vs non-arrhythmogenic hearts: 21.8 ± 2.53 ms, n=4; p>0.05, ANOVA).

Conclusions: These findings suggest existence of a specific CI (CI*) in turn corresponding to specific conditions of latency and action potential amplitude that would be sufficient to result in arrhythmogenesis.

1859-Pos

Effect of Sodium Homeostasis on Action Potential Duration Alternans in Cardiac Ventricular Cells

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Beat-to-beat alternation of action potential (AP) duration (alternans) is a precursor of fatal cardiac arrhythmias. The effect of the time course of intracellular Ca2+ transient on AP duration (APD) alternans was studied extensively, and the Na+/Ca2+ exchanger (INCX) was identified as a major coupling link between Ca2+ alternans and APD alternans. However, the role of Ca2+ -independent factors such as the Na+/K+ pump (INaK) in the coupling between the Ca2+ and AP subsystems has been overlooked.

We used computational models of AP and Ca2+ cycling in guinea-pig and canine myocytes to study effects of rate-dependent Na+ homeostasis on APD and the Ca2+ transient. We found that rate-dependent Na+ accumulation increases both the amplitude and frequency range of APD alternans in the guinea-pig, but decreases the amplitude of APD alternans in canine cells. The mechanism is as follows: in canine, Ca2+ and APD alternans are concordant (large Ca2+ is accompanied by long APD) and APD prolongation is due to inward INCX enhancement at a late phase of the AP. INaK enhancement by Na+ accumulation blunts the effect of INCX and decreases APD alternans amplitude. In the guinea pig, alternans are discordant (large Ca2+ transient with short APD) due to enhanced Ca2+ -dependent inactivation of L-type Ca2+ current and increased Ca2+-dependent slow delayed rectifier IKs at high Ca2+. Additional APD shortening by INaK increases the amplitude of the discordant alternans.

In conclusion, INaK enhancement due to Na+ accumulation decreases the amplitude of concordant APD-Ca2+ alternans and increases the amplitude of discordant APD-Ca2+ alternans. This mechanistic insight is relevant to arrhythmogenesis in heart failure where INCX is upregulated and INaK downregulated, amplifying APD alternans in larger mammals (canine) and possibly humans.