the four important S4 arginine residues on the voltage-sensing paddle is still unknown, with some models placing them in an aqueous crevice, and others a lipid environment. To learn more about the intricate role of lipid in the structure and function of potassium channels we have studied deuterium and phosphate ESEEM on spin-labeled, liposome reconstituted KcsA. By scanning the trans-membrane helices of KcsA, we show that deuterium coupling can be used to determine residue depth within a lipid bilayer. In addition, residues that interact with the phosphate head-groups of the lipid can be determined by phosphate coupling, and their precise location modeled.

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Platform AF: Cardiac Muscle II

1905-Plat

Direct Evidence In Man For Haploinsufficiency As The Mechanism Of Action Of Myosin-binding Protein C Mutations That Cause Hypertrophic Cardiomyopathy

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Sarcomeric mutations in MyBPC that cause hypertrophic cardiomyopathy (HCM) may act as dominant negative alleles by encoding 'poison polypeptides'or as null alleles resulting in haploinsufficiency. To resolve this we have studied left ventricular muscle samples from patients undergoing surgical myectomy for obstructive HCM and compared these with samples from non-failing (donor) heart muscle. Seven out of 27 myectomy samples were found to contain mutations in MyBP-C: two previously described missense alleles (Glu258Lys, Arg502Trp) and five premature terminations (truncating in domains C3, C5, C7 [x2], C10). Western blots were performed using an antibody shown to recognise specifically the N-terminal region (C0-C2) of MyBPC. MyBPC content was quantified by ELC and densitometry and normalised to staining with an anti-actin antibody.

No truncated peptides were detected in whole muscle homogenates, or the myofibrillar fraction, of HCM tissue (including in overloaded gels). However, the overall level of MyBP-C in myofibrils was reduced by 24 ± 4% in myofibrils from tissue containing a MyBP-C mutation: 0.76+/-0.04 (n=39) vs 1.00+/-0.05 in non-failing (n=19)* and 1.01+/-0.05 (n=24) in non-MyBPC mutant myectomies. *p=0.0005. Four of the myectomy samples individually showed statistically significant differences from the non-failing group; these included both truncation and missense samples.

The absence of detectable lower molecular weight protein suggests that the truncated MyBPC proteins are degraded, arguing against their incorporation in the myofibre and any dominant negative effect. In contrast, the lowered relative level of full length MyBPC in the myofibre argues strongly for haploinsufficiency as the disease mechanism (potentially for missense as well as truncation alleles). Previous work on partial extraction of MyBPC suggests that lowered MyBPC stoichiometry would be expected to alter muscle function.

1906-Plat

The Effects of Troponin T Heterogeneity on Reducing Myocardial Efficiency

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Cardiac TnT variants with abnormal splicing in the N-terminal region have been found in avian and mammalian cases of dilated cardiomyopathy. Similar abnormality also occurs in myopathic and failing human hearts. These cardiac TnT variants may play a role in the pathogenesis and pathophysiology of cardiomyopathy and heart failure. Without losses of function, the cardiac TnT variants result in only minor differences in thin filament Ca^{2+} sensitivity. Therefore, we hypothesize that the heterogeneity resulting from the presence of two or more functionally distinct cardiac TnT variants in the normally uniform adult cardiac muscle thin filaments desynchronizes myofilament activation and decreases the contractile efficiency. We studied transgenic mouse hearts expressing one or two of the myopathy-related cardiac TnT variants together with the wild type adult cardiac TnT. The function of isolated working hearts was examined for pumping efficiency in the absence of neurohumoral influence. The results showed that at heart rate of 480 beat per minute and pressure load of 90 mmHg, contractile and relaxation velocities were lower in the transgenic mouse hearts than that in the wild type hearts. Left ventricular pumping efficiency calculated by the ratio of ejection integral to total systolic integral was also lower in the transgenic mouse hearts than that in wild type controls. When stressed by pacing at 600 beats per minute and giving 10 nM isoproterenol, the transgenic mouse hearts exhibited shorter ejection time and decreased cardiac efficiency than that of wild type hearts. These results indicate a chronic pathogenic mechanism that TnT heterogeneity leads to decreased myocardial efficiency due to desynchronized responses to intracellular Ca^{2+} transient.

1907-Plat

The N-terminus of Cardiac Myosin Binding Protein-C Contains Multiple Binding Sites for F-actin

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Cardiac myosin binding protein-C (cMyBP-C), long known to interact with thick filaments, also interacts with thin filaments (actin) through its N-terminus. However, a single actin binding site has not been identified and it is unclear whether one or more N-terminal domains of cMyBP-C interact with actin. In this study we aimed to characterize the interaction of the N-terminus of cMyBP-C with actin using recombinant proteins consisting of various cMyBP-C N-terminal domains. Results from high speed cosedimentation binding assays showed that recombinant proteins containing the C1 domain and the MyBP-C motif bound to F-actin at a 1:1 molar ratio with a dissociation constant (K_d) ~ 10 uM. In contrast, proteins containing either C1 or the motif showed reduced binding at a 1:2 molar ratio. Proteins containing both C1 and the motif also bundled actin filaments, suggesting multiple actin interaction sites. Binding of recombinant proteins to Ca^{2+} regulated thin filaments was similar to binding to F-actin alone. Strongly bound myosin cross-bridges (myosin S1, no ATP) abolished cMyBP-C binding to actin, while weakly bound cross-bridges (myosin S1 plus ATP) diminished, but did not abolish, binding. Recombinant myosin ΔS2, which binds to the MyBP-C motif *in vitro* (~6 uM), did not affect cMyBP-C binding to actin. However, phosphorylation of the motif or alkaline pH both reduced binding. Together, these results suggest that the N-terminus of cMyBP-C contains at least two binding sites for actin and that binding is modulated through electrostatic interactions. Supported by NIH HL080367 to SPH and a NSF Graduate Research Fellowship to JFS.

1908-Plat

Myosin Binding Protein C Mutations and Hypertrophic Cardiomyopathy: Haploinsufficiency, Deranged Phosphorylation and Cardiomyocyte Dysfunction

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Background Mutations in the *MYBPC3* gene, encoding for cardiac myosin binding protein C (cMyBP-C), are a frequent cause of familial hypertrophic cardiomyopathy (FHCM). In the present study we investigated if protein composition and function of the sarcomere are altered in a homogenous FHCM patient group with truncating mutations in *MYBPC3* ($MYBPC3_{mut}$).

Methods and Results Comparisons were made between cardiac samples from *MYBPC3* mutant carriers (c.2373dupG, n=7; c.2864_2865delCT, n=4) and non-failing donors (n=8). Western Immunoblotting using antibodies directed against different parts of cMyBP-C did not reveal truncated cMyBP-C in $MYBPC3_{mut}$. Protein expression of cMyBP-C was significantly reduced in $MYBPC3_{mut}$ by 33 ± 5%. Cardiac MyBP-C phosphorylation in $MYBPC3_{mut}$ samples was similar to the values in donor samples, whereas the phosphorylation status of troponin I (cTnI) was reduced by 84 ± 5%, indicating divergent phosphorylation of the two main contractile target proteins of the beta-adrenergic pathway. Force measurements in mechanically isolated Triton-permeabilized cardiomyocytes demonstrated a decrease in maximal force per cross-sectional area of the myocytes in $MYBPC3_{mut}$ (21.4 ± 3.9 kN/m^2) compared to donor (34.5 ± 1.7 kN/m^2). Moreover, Ca^{2+}-sensititivy was higher in $MYBPC3_{mut}$ (pCa_{50}=5.60 ± 0.04) than in donor (pCa_{50}=5.52 ± 0.03), consistent with reduced cTnI phosphorylation. Treatment with exogenous protein kinase A, to mimic beta-adrenergic stimulation, did not correct reduced

maximal force, but abolished the initial difference in Ca^{2+}-sensititivy between $MYBPC3_{mut}$ (pCa_{50}=5.46 ± 0.03) and donor (pCa_{50}=5.48 ± 0.02).
Conclusions Truncating *MYBPC3* mutations cause haploinsufficiency, deranged phosphorylation of contractile proteins and reduced maximal force generating capacity of cardiomyocytes. The enhanced Ca^{2+}-sensititivy in $MYBPC3_{mut}$ is due to hypophosphorylation of troponin I secondary to mutation-induced dysfunction.

1909-Plat
Truncation Of Titin'S Elastic PEVK Region Leads To Cardiomyopathy With Diastolic Dysfunction
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The giant protein titin is important for proper myofilament assembly and structure, as well as the passive mechanical properties of the sarcomere. Within the sarcomeric I-band region of titin reside two elastic elements - the cardiac specific N2B element and the PEVK element. Unlike the N2B element that has been linked to metabolism and signal transduction, the PEVK region has so far solely been regarded as a mechanical spring. Here we have used a loss of function approach to delete exons 219-225 from the mouse titin gene, which encode all residues of the PEVK element present in N2B titin, the main titin isoform expressed in the heart. Homozygous PEVK knockout (KO) mice survived to adulthood and were fertile. Titin-based passive tension was highly increased, accompanied by diastolic dysfunction, as determined by echocardiography, isolated heart experiments, and muscle mechanics. Surprisingly, PEVK-KO mice had dilated and hypertrophied hearts and had increased FHL2 expression, contrasting the cardiac atrophy and decreased FHL2 levels that result from the deletion of the N2B element. This work indicates a concerted action of titin's elastic elements in providing proper diastolic function, but a distinct effect on the trophic properties of the heart.

1910-Plat
Protein Kinase G Modulates Human Myocardial Passive Stiffness By Phosphorylation Of The Titin Springs
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The sarcomeric titin springs influence myocardial distensibility and passive stiffness. Titin-isoform composition and protein kinase-A-dependent titin phosphorylation are variables contributing to diastolic heart function. However, diastolic tone is lowered, left ventricular extensibility is increased, and relaxation is accelerated also by activating protein kinase-G (PKG). Here we studied using back-phosphorylation assays whether PKG can phosphorylate titin and affect titin-based stiffness in skinned myofibers and isolated myofibrils. PKG in the presence of 8-pCPT-cGMP (cGMP) phosphorylated the two main cardiac titin isoforms, N2BA and N2B, in human donor and dog left ventricle. In human myofibers/myofibrils dephosphorylated prior to mechanical analysis, passive stiffness dropped 10-20% upon application of cGMP-PKG. Autoradiography and anti-phosphoserine blotting of recombinant human I-band-titin domains established that PKG phosphorylates titin's N2-B and N2-A domains. Using site-directed mutagenesis, a serine residue near the COOH-terminus of the cardiac N2-B-unique sequence (N2-Bus) was identified as a PKG-phosphorylation site. To address the mechanism of the PKG-effect on titin stiffness, single-molecule AFM force-extension experiments were performed on engineered N2-Bus-containing constructs. The presence of cGMP-PKG increased the bending rigidity of the N2-Bus to a degree that explained the overall PKG-mediated decrease in cardiomyofibrillar stiffness. Thus, the mechanically relevant site of PKG-induced titin phosphorylation is most likely in the N2-Bus, whereas phosphorylation of other titin sites could affect protein-protein interactions. Results suggest that reducing titin stiffness through PKG-dependent phosphorylation of the N2-Bus can benefit diastolic function. Since failing human hearts revealed lower PKG-mediated basal titin phosphorylation than donor hearts, titin-phosphorylation deficits may contribute to diastolic dysfunction in heart failure.

1911-Plat
Proteasome Dysfunction in Troponin Related Cardiomyopathies
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Hypertrophic cardiomyopathy (HCM) is a disease characterized by small to significant increases in calcium sensitivity of force development. Our results suggest that patients with mutations which are associated with large increases in calcium sensitivity of force development show poor prognosis. Investigation of two cardiac troponin T (cTnT) transgenic mice, I79N and F110I, which are associated with HCM using a functional proteomics approach showed that several key proteins which are known to be degraded by the proteasome are altered when compared to wild-type cTnT transgenic mice. Skinned fiber studies from I79N and F110I transgenic mice showed that these mutations both caused similar increases in calcium sensitivity of force development (change in pCa50 = approx. 0.22) when compared to wild-type transgenic mice. The 179N and F110I mice both showed significant decreases in 20S proteasome activities related to wild-type cTnT transgenic mice suggesting that proteasomal dysfunction may be a contributing factor to the pathogenesis of HCM. The levels of polyubiquitinated proteins present in the I79N and F110I were also increased relative to wild-type cTnT transgenic mice. Transgenic mice expressing cTnT I79N or cTnT F110I do not develop significant hypertrophy or ventricular fibrosis even after chronic exercise challenge. This suggests that increased heart size may not the critical factor for causing proteasome dysfunction in some cardiomyopathies. These results suggest that the UPS is an important system involved in the pathogenesis of troponin related HCM which is initially caused by significant increases in calcium sensitivity of force development.

1912-Plat
Engineering Cardiac Contractility from the Sarcomere to Tissue-Scale
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Engineering myocardium with specific contractile properties is a major goal of cardiac regenerative medicine, both for in vivo therapy and in vitro disease models. Yet maintaining the multiscale coupling and uniaxial orientation from nanometer-scale actin-myosin motors to centimeter-scale muscle tissue remains a major obstacle. Inspired by the collagen fibrillar network and capillary beds in myocardium, we hypothesized that these microscale heterogeneities act as boundary conditions that direct cardiomyocyte alignment and functional coupling. Specifically, our interest is the role these boundary conditions play in the sub-cellular alignment of the sarcomeres. To tackle this problem we developed a cardiac tissue engineering methodology that allows us to control and quantify sub-micron sarcomere orientation and measure macroscale contractile force and electrical conduction. We evaluate the deformation of 2-dimensional (2D) myocardial sheets that mimic the lamellar layers of the ventricular wall. Cardiomyocytes are grown onto a free-standing film of polydimethylsiloxane elastomer, referred to as a muscular thin film (MTF). Microscale heterogeneities are created using microcontact printing to direct 2D myogenesis, either (i) isotropic with no cell or sarcomere alignment, (ii) anisotropic with uniaxial cell and sarcomere alignment or (iii) an array of 20 micrometer wide myocardial strands with enhanced uniaxial cell and sarcomere alignment. For contractility experiments, MTFs are fashioned into cantilevers, mounted in an organ bath and electrically stimulated at 0.5 Hz. Results demonstrate that isotropic myocardium generates ~1 kPa peak systolic stress. In stark contrast the anisotropic myocardium generates an order-of-magnitude greater peak systolic stress of ~10 kPa. The 1D myocardial strands generate the greatest peak systolic stress of ~17 kPa, or ~35 kPa when normalized for muscle mass. This demonstrates that microscale heterogeneities can have a profound effect on sarcomere alignment and muscle contractility, defining new strategies for optimizing electro-mechanical function in engineered muscle.