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

Steven Marston[1], O'Neal Copeland[1], Adam Jacques[1], William McKenna[2], Victor Tsang[2], Karen Livesey[3], Sebastian Carballo[3], Charles Redwood[3], Hugh Watkins[3].
[1]Imperial College London, London, United Kingdom, [2]University College London, London, United Kingdom, [3]University of Oxford, Oxford, United Kingdom.

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

Hanzhong Feng, J.-P. Jin.
Evanston northshore University Healthsystem and Northwestern University Feinberg School of Medicine, Evanston, IL, USA.

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

Justin F. Shaffer[1,2], Robert W. Kensler[3], Samantha P. Harris[2].
[1]University of Washington, Seattle, WA, USA, [2]University of California, Davis, Davis, CA, USA, [3]University of Puerto Rico, San Juan, PR, USA.

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

Sabine J. van Dijk[1], Dennis Dooijes[2], Cris dos Remedios[3], Jos M.J. Lamers[2], Folkert J. ten Cate[2], Ger J.M. Stienen[1], Jolanda van der Velden[1].
[1]VU University Medical Center, Amsterdam, Netherlands, [2]Erasmus Medical Center, Rotterdam, Netherlands, [3]Muscle Research Unit, Sydney, Australia.

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