

manner and inhibits the ability of endothelin to produce phosphorylation of MyBP-C. These results suggest that phosphorylation of cMyBP-C may be a molecular component of the vascular endothelial cell - cardiac myocyte cross-talk. Coupled with already published work, the results also suggest that cMyBP-C phosphorylation may contribute to the regulation of the turnover of myofibrillar proteins.

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Comparative Effects of the Proline-Alanine Rich Regions of Human and Murine Cardiac Myosin Binding Protein-C

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The N-terminus of cMyBP-C can activate actomyosin interactions in the absence of Ca^{2+} , but it is unclear which sequences mediate the activating effects. Herron et al. (Circ Res, 98:1290-8, 2006) found that the Pro-Ala rich region (P-A) of human cMyBP-C could activate tension in the absence of Ca^{2+} , whereas Razumova et al. (J Gen Physiol, 132:575-85, 2008) found that murine C1 and M domains activated tension. The different results might be explained by isoform differences, especially in P-A which is only 46% identical between mouse and human cMyBP-C. The goal of this study was to determine if species-specific differences in P-A account for the different activating effects of murine and human cMyBP-C. Recombinant chimeric proteins containing the C0, P-A, and C1 domains (C0C1) from either human or murine cMyBP-C were engineered and their activating effects assessed using *in vitro* motility and ATPase assays. Consistent with previous observations, human C0C1 activated actomyosin interactions in the absence of Ca^{2+} , whereas murine C0C1 did not. However, substituting human P-A for murine P-A conferred activating properties to murine C0C1, whereas substituting murine P-A for human P-A depressed the activating effects of human C0C1. Activating effects of the chimera proteins were intermediate between those of murine and human C0C1, suggesting that C0 or C1 also contribute to activation properties. Further chimeric substitutions of C0 and C1 demonstrated that the human C1 domain also contributed to activation, whereas the C0 domain did not. These results suggest that the human P-A and C1 domains are sufficient to activate actomyosin interactions in the absence of Ca^{2+} , and that species-specific differences are likely to contribute to functional differences of cMyBP-C. Supported by NIH HL080367 to SPH and a NSF Graduate Research Fellowship to JFS.

2860-Pos

Single Sarcomere Imaging by Quantum Dots (Qdots) in the Heart

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Numerous studies have been conducted in tissues and cells to elucidate the molecular mechanisms of myocardial contraction. However, because of a number of differences between *in vitro* and *in vivo* conditions, the dynamics of myocardial sarcomere contractions in living animals is not yet understood. In the present study, we developed a novel system allowing for real-time single sarcomere imaging in the living heart. Male Wistar rats were anesthetized with pentobarbital sodium, and median sternotomy was performed under artificial ventilation. Qdots were conjugated with anti- α -actinin antibody and then transfected from the surface of the epicardium of the beating heart, for visualization of the Z-discs. An electron microscopic study confirmed the presence of Qdots in and around the T-tubules and Z-discs in the myocardial cells of the left ventricular wall. Consistent with this, we observed a striated pattern of Qdots (~2 μ m spacing) in the heart under fluorescence microscopy. We are now performing real-time single sarcomere imaging in the beating heart of the rat.

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Single Sarcomere Imaging in Cardiomyocytes with Quantum Dots (Qdots): Physiological Significance of SPOC in Cardiac Beat

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Cardiac sarcomeres exhibit spontaneous oscillations (SPOC) over a broad range of intermediate activating conditions, namely, at pCa ~6.0 (Ca-SPOC), or at the coexistence of MgADP and Pi under the relaxing condition (ADP-SPOC). We have reported that the period of sarcomeric oscillations in skinned myocardium correlates with that of resting heart rate in various animal species [BBRC, 343, 1146-1152 (2006)]. In the present study, we analyzed sarcomeric oscillations in isolated single cardiomyocytes of the rat, by using Qdots conjugated with anti- α -actinin antibody for clear visualization of the Z-lines. First,

we measured the period and amplitude of ADP- and Ca-SPOC at various sarcomere lengths (SLs) in skinned cardiomyocytes, and found that the amplitude of oscillations was inversely related to SL. We also conducted a SL measurement in intact cardiomyocytes at various stimulation frequencies, after transfection of Qdots into the cells. At low frequencies (e.g., 1 Hz), the shortening and relengthening of the sarcomere during a contraction cycle simply reflected the changes in $[Ca^{2+}]_i$. However, an increase in stimulation frequency to the physiological level (~5 Hz) caused a phase shift of shortening and relengthening due to enhancement of the relengthening speed, resulting in the waveform being similar to what was observed during SPOC in skinned myocytes. These findings suggest that the intrinsic auto-oscillatory property of sarcomeres may contribute to the regulation of cardiac beat *in vivo*.

2862-Pos

Modeling of Viscoelastic Properties of Isolated Myocardial Tissue Samples at Different Levels: Cardiomyocytes and Trabeculae

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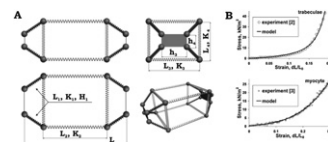
Viscoelastic properties of myocardium play an important role in a heart function. They determine the extent of filling of the heart, its subsequent stroke volume and contraction velocity. We present here the 3D model consisting of elastic springs and linear damping elements on basis of our earlier model [1] (Fig. 1A). Due to changes of geometry the model manifests nonlinear viscoelastic behavior in response to longitudinal stretch. Depending on set of input parameters, the model allows to describe quantitatively nonlinear viscoelastic behavior of both single cardiomyocytes and multicellular samples like trabeculae (Fig. 1B). Model volume stability is an essential condition for model parameter selection because *in vivo* a volume of cardiomyocytes is virtually unchanged. It is significant that viscoelastic parameters of structural elements of the model remain constant all over the range of investigated strains.

Thus, we can describe main viscoelastic properties of myocardial tissue at different organization levels within the basis of the simple mechanical model.

[1] Smoluk, L. et al. 2008. *The FASEB J* 22:756-9.

[2] Granzier, H. and Irving, T. 1995. *Biophys. J.* 68:1027-1044.

[3] Granzier, H. et al. 1996. *Biophys. J.* 70:430-442.



2863-Pos

Radial Force and Lattice Spacing with Multi-Spring Crossbridge Models

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Previous spatially explicit models have used crossbridges consisting of single springs aligned to the axis of the thick and thin filaments. Such one-spring models cannot account for effects of lattice spacing or radial forces generated during axial shortening. We develop crossbridge models with multiple springs to examine how different mechanisms of crossbridge deformations affect radial forces, longitudinal forces, and the effects of changes in lattice spacing. A four-spring crossbridge (4sXB) treats the S2 region and light chain domain (LCD) as linear springs, linked to the thick filament and each other by torsional springs. Changing the rest angle of the S2/LCD linking spring models force generation via a power stroke. A two-spring crossbridge (2sXB) replicates the desired abilities of the 4sXB and is less computationally expensive. Unlike the 4sXB, the length and angle of the 2sXB's springs can be determined for any head position without iterative techniques. Both the 4sXB and the 2sXB use three state kinetics that, at resting lattice spacing, are similar to previous work, easing comparison to previous studies. In contrast to single spring crossbridges, the kinetics of the 4sXB and 2sXB change with lattice spacing. Notably, the axial offset (distance between the thick filament attachment site and myosin-binding site) at which the powerstroke becomes likely to occur varies by more than 5nm over physiological lattice spacings. Both the 4sXB and the 2sXB measure the axial and radial forces generated by during production of axial force. In a typical post-powerstroke position at resting lattice spacing, the axial forces exerted by the 4sXB and the 2sXB differ by approximately 10% while the radial forces are more divergent (differing by as much as 20%), making the choice of crossbridge a critical concern in measurements of radial force. HL65497 (MR), EB001650 (CDW).

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Sarcomere Velocity Regulates the Cross-Bridge Cycling Rate in Cardiac Muscle: a Novel Theory for the Muscle Molecular Motor

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