affect of OM on the actin-activated power-stroke. These results show that OM has a direct effect on the power-stroke structural transition that drives force generation in muscle, thus providing structural insight into the mechanism of this new potential therapy for heart failure. Our results also highlight the utility of structure-based time-resolved FRET and EPR assays for the discovery and characterization of allosteric enzyme modulators.

2844-Pos Board B536

Impact of Regulatory Light Chain Phosphorylation on the Stiffness of α and β Myosin

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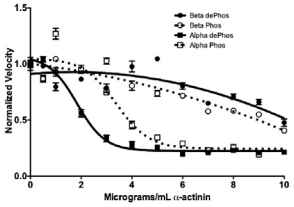
It has been demonstrated that the phosphorylation of the RLC can also aid in the attachment of myosin heads to actin. Moreover, it has been demonstrated that phosphorylation of the RLC is altered during heart failure progression. Here we examined the role of the RLC, and its phosphorylation, on the motility of α and β Myosin. We characterized the strain dependent kinetics of both myosins by analyzing the dependence of velocity on load using increasing amounts of α-actinin up to 10 μg/ml. As expected, α-myosin, both phosphorylated and dephosphorylated, had a faster actin sliding velocity than β-myosin over the majority of α-actinin concentrations, while phosphorylation of the β-myosin had no significant impact on the actin sliding velocity. Interestingly, although phosphorylation of α-myosin significantly reduced the unloaded sliding velocity it, increased velocity under loaded conditions (See Figure). These results suggest that the phosphorylation of the RLC acts at the molecular level to stiffen the lever arm of myosin aiding in both myosin head attachment and power stroke.



2845-Pos Board B537

The Effect of Myosin Regulatory Light Chain Phosphorylation on N47K Mutant Myosin Mechanics

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Familial Hypertrophic Cardiomyopathy (FHC) is characterized by left ventricular hypertrophy that can often be preceded by diastolic dysfunction. The clinical presentation of the disease varies from asymptomatic to progressive heart failure to sudden cardiac death. FHC is caused by mutations in genes that encode for all major sarcomeric proteins. There are 12 known FHC-linked mutations in the myosin regulatory light chain (RLC). The RLC mechanically stabilizes the myosin lever arm, which is crucial to myosin's ability to transmit contractile force. Two FHC mutations, N47K and R58Q, located in the RLC Ca^{2+}-Mg^{2+} site have previously been shown to reduce actin filament velocity under load, stemming from a more compliant lever arm (Greenberg et al., PNAS add details 2010). In contrast, phosphorylation of the RLC can impart stiffness to the myosin lever arm. We hypothesized that phosphorylation of the N47K-RLC may mitigate distinct mutation-induced structural and functional abnormalities. To generate mutant β-myosin, native pig RLC was depleted from porcine cardiac myosin heavy chain and reconstituted with mutant N47K or wild-type human RLC. In the work presented here, *in vitro* motility assays were utilized to investigate the effects of RLC phosphorylation on the N47K-RLC mutant phenotype in the presence of an α-actinin frictional load. Consistent with previous findings, myosin bearing the N47K mutation reduced actin sliding velocity compared to WT when incubated with α-actinin, resulting in a 25% reduction in force production. Phosphorylation of N47K mutant myosin increased sliding velocity and restored force production to WT values. These results point to RLC phosphorylation as a potential target to ameliorate the FHC RLC phenotype at the molecular level. Supported by AHA-12PRE11910009 (AK), 10POST3420009 (PM), NIH- HL071778 & HL108343 (DSC) and HL077280 (JM).

2846-Pos Board B538

Ventricular Myosin Modifies in Vitro Step-Size When Phosphorylated

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Cardiac and skeletal muscle myosins have the central role in contraction transducing ATP free energy into the mechanical work of moving actin in transduction/mechanical coupling. Inheritable cardiomyopathies are more frequently linked to myosin mutations than other sarcomeric proteins. Hereditary skeletal myopathies linked to myosin are less common. They lead to muscle weakness or affect myosin isoforms expressed during development leading to arthrogryposis syndromes. Myosin has a motor domain containing ATP and actin binding sites and light chains stabilized lever-arm that undergoes rotation impelling bound actin. The lever-arm converts torque generated in the motor into linear displacement (step-size). Relative myosin and actin filament sliding is modeled in vitro with a motility assay quantitating actin filament translation over a myosin coated surface. A novel quantum dot super-resolution in vitro motility assay confirmed a 5 nm step-size for fast skeletal myosin while β cardiac myosin (βMys) had multiple unitary steps, most frequently 5 and 8 nm, and a rare 3 nm displacement. The myosin lever-arm is stabilized by bound essential and regulatory light chains (ELC and RLC). RLC phosphorylation at S15 is linked to modified lever-arm mechanical characteristics contributing to disease and to myosin filament based contraction regulation. We have studied the effect of RLC phosphorylation on the step-size of porcine βMys. Phosphorylated βMys has ~85% of the myosin phosphorylated. We find RLC phosphorylation causing the distribution of longest step increasing from 37% to 71%. This dramatic re-distribution of step-sizes provides significant gain in average step-size. The results indicate a mechanism for contraction regulation by step-size adaptation using post-translational modification of the myosin filament via RLC phosphorylation. Research supported by R01AR049277, R01HL095572 and the Mayo Foundation.

2847-Pos Board B539

A13T Mutation in the Regulatory Light Chain Associated with Cardiac Hypertrophy Imposes Differences in Kinetics of Healthy and Diseased Ventricles

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The effect of A13T (alanine to threonine) hypertrophic cardiomyopathy mutation in the myosin regulatory light chain (RLC) was examined in working ex-vivo myofibrils from the hearts of transgenic (Tg) mice. Myofibrillar actin was labeled with a fluorescent dye. A small volume within the I-band (?1 fL), containing on average 3-4 actin molecules, was observed by confocal microscopy. The myofibrils were cross-linked with EDC [1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide] to prevent shortening during muscle contraction. Working myosin cross-bridges cause actin to undergo the cyclic fluctuations of orientation, which were measured by recording the polarization of fluorescent light emitted by the actin-bound fluorophore. The autocorrelation function of fluctuations of polarized fluorescence contains information about the kinetics of motion, which were found to be very different for left vs. right ventricles. The center of distribution of orientations of transition dipoles during contraction were very different for the Wild Type (WT) and mutated (MUT) ventricles, but their skewness and kurtosis were the same. The distribution of orientations measured in contracting WT myofibrils could be fitted by at least two Gaussians reflecting a pre- and post power stroke states of the myosin cross-bridges. However, the distribution of MUT myofibrils showed only one Gaussian relationship suggesting that the hypertrophic phenotype associated with the A13T-RLC mutation might be characterized by a loss of the pre-power stroke state.

2848-Pos Board B540

The K104E Mutation of the Myosin Regulatory Light Chain Alters Kinetics and Distribution of Orientations of Cross-Bridges in Transgenic Cardiac Myofibrils

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Cross-bridge (XB) kinetics and the degree of order were examined in contracting myofibrils from the ex-vivo left ventricles of transgenic (Tg) mice expressing Familial Hypertrophic Cardiomyopathy (FHC) Regulatory Light Chain (RLC) mutation K104E. Since the kinetics and degree of order are best studied when an individual XB makes a significant contribution to the

overall signal, the number of observed XBs was minimized to ~20. Autofluorescence and photobleaching were minimized by labeling the myosin lever arm with a relatively long-lived red-emitting dye containing a chromophore system encapsulated in a cyclic macromolecule. We show that the K104E mutation, when compared with Wild Type (WT) ventricles, had significant effect on both the kinetics of the interaction between actin and myosin and on the degree of order of the myosin lever arm. In particular, the K104E mutation increased the rate of XB binding to thin filaments and the rate of execution of the power stroke, while decreasing the rate of XB dissociation from actin. This implies that the mutated ventricle may be prone to decreased maximal tension and increased muscle relaxation time suggesting a potential for diastolic dysfunction in patients. Mutated XBs were significantly better ordered during steady-state contraction and during rigor but mutation had no effect on the degree of order in relaxed myofibrils.

2849-Pos Board B541

Measuring Work Loops in Intact Isolated Cardiac Myocytes by Controlling Pre- and Afterload using a New Generation Force Transducer

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A recent exciting development in cardiac research is the ability to do mechanical experiments on intact isolated cardiac myocytes. Here we mimic cardiac PV loops by imposing a 'pre-load' and 'after-load' on the myocyte. For these experiments we developed a new generation force transducer as currently available force transducers were not sufficiently sensitive or had insufficiently stable base line levels to achieve force control. A cantilever with a spring constant of 7 Nm is interrogated using an interferometer via an optical fiber. The resolution of the interferometer/probe system is 2nm, resonance frequency $>$ 2 kHz and force resolution $<$ 10 nN. As the probe is small enough to be fully submerged in water, variations in solution level have no effect on the force measurement, resulting in a stable baseline (drift $<$ 50 nN over a 10 minute period at 21C). Software was written to take in the signal from the force transducer, process it and return a signal to a linear motor that could stretch or shorten the myocyte in order to control force levels. Using this software we were able to achieve a two-sided force clamp (setting 'pre-load' and 'afterload') to measure work loops and re-create the Frank-Starling relation at the single cell level. Experiments show that in isolated cardiac mouse myocytes residual active force at the end of diastole limits the effective work the mycoyte can produce. Small concentrations of BDM, thought to inhibit strong crossbridge formation by stabilizing weak cross bridges, allow the myocyte to relax at end-diastole, shifting the pre-load-sarcomere length relation upwards. The resulting increase in length dependent activation outweighs the effects of crossbridge inhibition, leading to strongly increased mechanical work per contractile cycle.

2850-Pos Board B542

Temperature and Transmural Region Influence Functional Measurements in Unloaded Left Ventricular Cardiomyocytes

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Intact cardiomyocytes are increasingly being used to investigate the molecular mechanisms of contraction and to screen new therapeutic compounds. The function of the cardiomyocytes is often measured from the calcium transients and sarcomere length profiles. We studied the role of experimental temperature and transmural region on indices of function in freshly isolated, unloaded cardiomyocytes. Intact cardiomyocytes were isolated from the sub endocardium, mid myocardium and sub epicardium of 3 month old Sprague-Dawley rats. Myocytes from each region were studied at 25, 31, and 37°C. Cytosolic calcium transients were measured using Fura-2 fluorescence while sarcomere length shortening and relengthening profiles were measured using high speed video capture. For both the calcium transients and sarcomere length profiles, the time to peak and the time to half relaxation decreased significantly with increasing temperature. Increasing temperature also raised the minimum and maximum calcium levels of all cells. Of note, there was a reduced normalized standard deviation (standard deviation divided by the mean) at higher temperatures for calcium fluorescence amplitudes, time to peak calcium, and rates of sarcomeric shortening and relengthening. The amplitudes and minimum of the calcium transients were significantly dependent on transmural region, and several sarcomere length parameters exhibited statistical interactions between temperature and transmural region. Together, these results show that biological variability can be reduced by performing experiments at 37°C rather than at room temperature, and by isolating cells from a specific transmural region. Adopting these procedures will improve the statistical power of subsequent analyses and increase the efficiency of future experiments.

2851-Pos Board B543

A Novel Method for Isolating and Culturing Human Cardiomyocytes from Cryopreserved Tissues

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Current animal models fail to accurately mimic human heart biology. We have developed a novel method to isolate viable human cardiomyocytes (HCMs) from cryopreserved heart transplant donors. The Sydney Heart Bank is an effective and unique resource for isolating HCMs from donors as young as 2 months to 65 years. Preliminary data were collected from 2 donors aged 4 and 59 years. Left ventricle (LV) samples were frozen (−196°C) after being excised by transplant surgeons. LV tissues stored for 48 and 30 months, respectively, were then warmed gradually over 24 hours to 37°C. 300 μm thick cryosections were digested with collagenase type B and D [Mollova et al.. 2013 PNAS. 110:1446], gently agitated and then plated. Cell viability of isolated HCMs was evaluated using CellTracker CMFDA and calcein-AM. Our findings showed that isolated HCMs are metabolically active and viable. HCMs that were metabolically challenged using FCCP (trifluorocarbonylcyanide phenylhydrazone) increased their oxygen consumption. Immunohistochemical analyses using antibodies against cardiac markers showed that isolated HCMs express sarcomeric alpha-actinin, connexin43 and cardiac troponin T. Addition of BDM (2,3-butanedione monoxime, an inhibitor of myosin ATPase activity) allowed a much higher yield of viable HCMs (20-25% compared to 5% in the absence of BDM). However, the use of BDM in these culture seems to inhibit contractility of freshly isolated HCMs. Current studies are investigating optimal culture conditions to control contractility and viability of HCMs that will be used to replace current animal models. Taken together, our studies show that viable HCMs can be isolated and cultured from LV samples and may be used to investigate cardiomyocyte biology and pathology.

2852-Pos Board B544

Physiological Contractility of Cardiomyocytes in the Wall of Mouse and Rat Azygos Vein

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We recently demonstrated the abundant presence of cardiomyocytes in the wall of thoracic veins of adult mouse and rat (Kracklauer, Feng, Jiang, Lin, Lin, Jin, FEBS J. 280:880-91, 2013). The highly differentiated morphology and myofilament protein expression of the venous cardiomyocytes suggested contractile functions. Here we further investigated the contractility of mouse and rat azygos venous rings in comparison with that of atrial strips and ventricular papillary muscle. X-gal staining of transgenic mouse vessels expressing lacZ under cloned cardiac troponin T promoter demonstrated that the venous cardiomyocytes are discontinuous from atrial myocardium and longitudinally aligned in the wall of thoracic veins perpendicular to the vessel axis. Histology study showed striation patterns in the venous cardiomyocytes, which indicate encirclement orientation of myofibrils in the vessel wall. Mechanical studies found that mouse and rat azygos veins produce strong cardiac type twitch contractions when stimulated by electrical pacing in contrast to the weak and slow smooth muscle contractions induced using 90 mM KCl. The twitch contraction and relaxation of rodent azygos veins further exhibited cardiac type beta-adrenergic responses. Quantitative characterization showed that the contractions of venous cardiomyocytes are slightly slower than that of atrium muscle but significantly faster than that of ventricular papillary muscle. These novel findings indicate that the cardiomyocytes in rodent thoracic veins possess fully differentiated cardiac muscle phenotype despite their anatomical and functional separation from the heart.