100%O_2 (Normo) or 100%N_2 (Hypo). We recorded XRD patterns and LV pressure (LVP) at end-diastolic LVP (EDP) of 0 and 20 mmHg. **Results**: Under Normo, increasing EDP significantly increased the developed LVP (EDP0: 104 ± 26mmHg vs. EDP20: 141 ± 25mmHg, $p<0.01$). Under Hypo, developed LVP and its duration did not show significant differences compared with those in Normo. However, increasing EDP under Hypo significantly decreased developed LVP (72 ± 13mmHg vs. 96 ± 16mmHg, $p<0.01$). The minimum value of the (1,0)/(1,1) intensity ratio (I_{min}) provided by the XRD analysis was used as an index of AMI. I_{min} showed a significantly negative correlation with developed LVP regardless of Normo or Hypo. The diastolic myosin filament lattice spacing (MFL) calculated from the diffraction angle of the (1,0) equatorial reflection would be reduced by increasing EDP. In contrast to a significantly positive MFL-I_{min} correlation under Normo, we observed the significantly negative MFL-I_{min} correlation under Hypo. We confirmed that the duration of Ca^{2+} transient was slightly longer but the amplitude of Ca^{2+} transient was unchanged under Hypo. **Conclusion**: These novel findings suggest that under Hypo the probability of AMI decreases even though the MFL was reduced with increasing preload. This is an underlying mechanism for reduced cardiac contractile performance under hypoxia.

3201-Pos Board B248

Influence Of Acidic pH On The Rate Of Force Development In Cardiac Muscle

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Cellular acidosis, a consequence of myocardial ischemia, reduces the Ca^{2+} sensitivity of cardiac contraction and maximal Ca^{2+} activated force in cardiac muscle. These effects are similar to that seen in fast (psoas) and slow (soleus) skeletal muscle with reduced pH. Previous studies have also demonstrated no effect of low pH on the rate of force redevelopment (k_{tr}) at maximal Ca^{2+} activation in slow and fast muscle fibers, but that k_{tr} is slowed at submaximal Ca^{2+} activation. However, it is unknown whether low pH affects calcium dependence of k_{tr} in cardiac muscle. To characterize the influence of acidic pH on k_{tr} we have measured Ca^{2+} activation of skinned cardiac trabeculae at pH 7.0 and 6.5. As in skeletal muscle, reduced pH significantly decreased isometric force in cardiac muscle at all levels of Ca^{2+} activation ($\Delta pCa_{50} = 0.73$). Interestingly, in contrast to skeletal muscle, k_{tr} at low pH in cardiac trabeculae was significantly faster at both maximal (pH 7 = 5.1 ± 0.5 s^{-1}, pH 6.5 = 6.9 ± 0.3 s^{-1}) and half-maximal (pH 7 = 3.0 ± 0.3 s^{-1}, pH 6.5 = 6.6 ± 0.2 s^{-1}) Ca^{2+} activation. This is consistent with previous studies showing increased force redevelopment in cardiac muscle when force is inhibited with phosphate, vanadate, or reduced sarcomere length. Our results support the idea that k_{tr} is negatively correlated to the size of the cross-bridge pool available for recruitment to cooperative activation of the thin filament. Force inhibition such as that seen with lower pH may reduce the cross-bridges available for recruitment, which would reduce this slowing effect and speed force redevelopment.. Supported by NIH R01 HL 65497 (MR), T32 HL07828 (FSK) and NSERC Discovery (TEG).

3202-Pos Board B249

Role of Strongly-Bound Crossbridges in Cooperative Cardiac Thin Filament Activation

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Experimental evidence indicates that activation of cardiac thin filaments is enhanced by strongly-bound myosin crossbridges and that crossbridge binding is in turn cooperatively regulated by end-to-end interactions between adjacent tropomyosins. We examined the impact of crossbridge binding and nearest-neighbor tropomyosin interactions on thin filament activation using a computational model.

We represented individual thin filament regulatory units (RUs) with the model of McKillop and Geeves [Biophys J, 1993 65(2)] wherein RUs are found in blocked (B, non-permissive), closed (C, permissive), and open (M, permissive with crossbridge) states. The B to C transition was assumed to depend upon Ca2+ concentration. Nearest-neighbor RU interactions were represented by causing transitions of the individual RU model to depend on the status of neighbors. Ensembles of N interacting RUs were modeled as Markov networks generated by considering all possible unique configurations of individual RUs (B, C, or M) within a chain.

The model-generated steady-state force-pCa curve (N=6) possessed a Hill coefficient of 3.0. Hill coefficients fit separately to portions of the curve below and above half activation were 3.2 and 2.4, respectively. Rate of force redevelopment following rapid slack/restretch (ktr) showed strong dependence on activation level (ktr=2.6 s-1 at pCa 6.0 vs. 9.3 s-1 at pCa 4.3). Increasing the cross-bridge duty cycle in the model increased myofilament Ca2+ sensitivity but had an opposite effect on Ca2+ sensitivity of ktr. Simultaneous matching of reported force and ktr sensitivities required a duty cycle of 30%. Increasing N toward 26 (a realistic filament length) tended to improve the fit with experiments. These results suggest that cycling crossbridges act through nearest-neighbor interactions along the thin filament to 1) increase myofilament Ca2+ sensitivity, 2) cooperatively enhance activation, and 3) slow the rate of force redevelopment at low levels of activation.

3203-Pos Board B250

Kinetics of ADP Release From Cycling Cross Bridges In Contracting Skinned Cardiac Muscle Monitored With A Fluorescent Probe

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Kinetics of ADP release from cycling cross bridges were studied in Ca activated skinned papillary muscle by displacement of fluorescent ADP bound to the cross bridges in AM*ADP state(s) by non-fluorescent ADP photogenerated from caged ADP. A strip of glycerinated papillary muscle (100um, 2mm) from guinea pig left ventricular in Ca-free solution was loaded with a mixture of non-fluorescent ATP (1mM), fluorescent 3′-amino-deoxy ATP (aminoATP) (50μM) and 5 mM caged ADP in the presence of an ATP back-up system. At the plateau of force at pCa5.8 the muscle was rapidly transferred into the photolysis trough filled with silicone oil and irradiated by a 437 nm laser pulse. Alternatively, a muscle loaded with only fluorescent amino ATP (50μM) was allowed to contract at pCa4.5 in oil and develop rigor with amino ADP bound to the cross bridges. Following photolysis of caged ADP the kinetics of force and fluorescent transients were found markedly different in contracting and rigor muscles. In contracting muscle the force and fluorescence both increased following caged ADP photolysis, while in rigor muscle the photolysis induced an increase in fluorescence, but decrease in force. Kinetics of ADP release estimated by the rate of fluorescence increase was significantly slower in contracting muscle than that in rigor: 2-4 s-1 vs 18-20s-1, suggesting that at least two different AM*ADP states exist during ATP hydrolysis by cycling cross bridge in contracting papillary muscle. Supported by NIH grant R03 AR05 2885 for A.K.

3204-Pos Board B251

Mechanoenergetics of Actomyosin Interaction Analyzed by Cross-Bridge Model

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We present a mathematical model of actomyosin interaction, as a further development of actomyosin model that links mechanical contraction with energetics (Vendelin et al, Annals of Biomedical Engineering: 28, 2000). The new model is a three-state Huxley-type model, with two strong binding states and one weak binding state, for cross-bridge interaction and a model of calcium induced activation. The force produced by the attached cross-bridge in strong binding state is assumed to be elastic and depends linearly on the axial distance z along the myosin and actin filaments between the equilibrium position of the myosin head and the nearest actin binding site. The model is self-consistent and is based on T. Hill formalism linking free energy profile of reactions and mechanical force.

In several experimental studies it has been shown that the dependency between oxygen consumption and stress-strain area is linear. Additionally, the relation between stress-strain area and oxygen consumption is the same for isometric and shortening contractions. In this work, we analyzed free energy profiles of actomyosin interaction by changing free energies of intermediate states and free energies of activation of different reactions.

In our simulations we replicated the linear dependence between oxygen consumption and stress-strain area together with other important mechanical properties of cardiac muscle such as developed stress dependence on the sarcomere length and force-velocity relationship.

3205-Pos Board B252

Increasing Heart Rate Decreased Actin-Myosin Interaction in Isolated Beating Rat Whole Heart

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Background: Heart rate (HR) is one of the determinant factors of cardiac performance. Failing human myocardium shows negative force frequency relation, whereas normal one shows positive relation. **Purpose**: To test the effect of HR on actin-myosin interaction (AMI) in beating rat hearts those have negative

force-frequency relation by X-ray diffraction (XRD) analysis at third-generation synchrotron facility. **Methods**: Seven isolated isovolumically contracting rat hearts were paced at 120, 240, and 300bpm after complete heart block, mounted so that the X-ray beam (15.0keV) passed the left ventricular (LV) free wall, and perfused with Tyrode solution bubbled with 100% O_2. LV volume were adjusted through water filled thin latex balloon inserted into LV cavity so that end-diastolic LV pressure (LVP) was 0 mmHg. The amount of AMI was evaluated by the minimum value of the intensity ratio of inner (1,0) and outer (1,1) equatorial reflections (I_{min}) provided by anaysis of XRD. Between three different HRs, we compared the amount of AMI and LVP. We also measured frequency-dependent changes in Ca^{2+} transient in sliced myocardial preparations at 0.5, 1.0, and 2.0Hz. **Results**: In all hearts, we did not observe incomplete relaxations. As increasing HR at 120, 240, and 300bpm, LVP significantly decreased (66 ± 18, 51 ± 16, and 47 ± 18mmHg, respectively) and I_{min} also significantly increased (0.93 ± 0.16, 1.20 ± 0.11, and 1.56 ± 0.18, respectively), indicating a significant decrease of the amount of AMI. The durations of Ca^{2+} transient at 20% developed level at stimulating frequency of 0.5, 1.0, and 2.0Hz were significantly shortened (233 ± 25, 206 ± 34, and 171 ± 28ms, respectively). **Conclusion**: Increasing HR reduces the AMI. Absence of incomplete relaxations indicates intact intracellular Ca^{2+} handling. These results may derive from shortening the period of Ca^{2+}-myofilament interaction with increasing HR.

3206-Pos Board B253

Regional Nonuniformity of Contraction in the Left Ventricular Free-wall

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The function of the heart is characterized by nonuniform wall motions working coordinately to generate a smooth and effective pump action; however, the functional importance of these heterogeneous motions are largely unknown. To bridge the understanding of the basis for regional variations in contraction, we have analyzed left ventricular free-wall motion using echocardiography and sonomicrometry and quantified the expression of protein levels and post-translational modifications using gel electrophoresis. Porcine myocardium was used for investigation and a stress test, i.e. dobutamine infusion, was performed to amplify transmural contractile gradients during beta-adrenergic stimulation. Here we report greater segmental shortening, strain and strain rate in the endocardium compared to the epicardium in both the longitudinal and circumferential directions ($p<0.05$), but not in the radial dimension, at baseline and during dobutamine infusion. The gradient of strain and shortening mirrors the expression of the myosin heavy chain isoforms, alpha- and beta-MyHC, across the wall, i.e., there is more alpha-MyHC in the epicardium. We propose that differences in expression of specific protein isoforms in healthy, control myocardium is directly related to the shorter period of stretch in the epicardium during the heart cycle, or stretch activation, and that differences in myosin heavy chain isoform content is a direct determinant of the strain differential. This work supported by NIH RO1-HL61635 (RLM) and T32-HL07936 (HSN).

3207-Pos Board B254

Polygenic Modulation of Cardiac Dysfunction in Drosophila Assessed by High-speed Video Imaging, Motion Detection Analysis and Fluorescent Microscopy

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Hypertrophic (HCM), dilated (DCM) and restrictive (RCM) cardiomyopathies are cardiac disorders often resulting from contractile protein mutations. They are frequently dominantly inherited and are characterized by a high degree of clinical heterogeneity proposed to result from modifying genetic factors. For example, HCM patients with multiple causal mutations present a more severe phenotype compared to single-mutation carriers. We employed high-speed digital video imaging and novel motion detection software to characterize in vivo cardiac structure and performance of homozygous Drosophila mutants, quantitatively assessing cardiac diameters, contractile periodicities, fractional shortening and rhythmicity parameters. Fly hearts expressing myosin with depressed or enhanced biomechanical properties exhibited hallmarks of human DCM or RCM respectively. To determine if the Drosophila cardiac phenotypes exhibit dominant modes of inheritance we studied the effects of heterozygotic expression of the myosin mutations. Interestingly, both mutations induced dominant cardiac dilatory responses. This suggests the homozygotic RCM-like phenotype is initiated by a unique cardiac remodeling pathway not activated in the presence of a wild-type myosin gene copy. We also used live-cell imaging and fluorescent microscopy to measure normalized cardiac tube area, in order to investigate polygenic effects of specific sarcomeric mutations on the severity of cardiac phenotypes in double heterozygotes. Combining a dilation-inducing troponin I mutation with the reduced function myosin mutation resulted in a dilatory cardiac phenotype at advanced age, which was more severe than that observed in single heterozygotes. However, combining the troponin mutation with the increased function myosin mutation appeared to prevent the cardiac dilation characteristic of the single heterozygotes. This suggests molecular combinations of certain mutations may have cardioprotective effects. Thus, Drosophila may serve as an effective in vivo tool for identifying and studying genetic enhancers and suppressors of cardiac dysfunction.

3208-Pos Board B255

Diastolic Mechanical Properties of Vascular and Avascular Hearts

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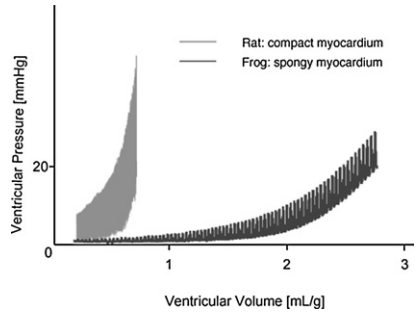
Background: In vertebrates, there are two kinds of myocardium, compacta and spongiosa, which are associated with blood supply systems i.e. coronary and sinusoidal circulation. To characterize the diastolic properties of these two types of ventricles, we analyzed the ventricular end-diastolic pressure-volume relationships (EDPVR) in rat and frog heart that include integrated expression of chamber geometry and passive material properties of myocardial wall.
Methods: Pressure of rat left ventricule and frog ventricule was recorded to obtain EDPVRs under isovolumic contractions with increases of ventricular volume to ~10 mmHg. The curvature changes of EDPVRs were described by nonlinear function. ($EDP = \alpha \bullet EDV^6 + \beta$).
Results: Ventricular volumes were normalized by ventricular weights. The volumes from rat and frog ventricles that provided pressure of 10 mmHg were 0.6 and 2.5 mL/g respectively. EDPVRs from rat and frog showed common shape (see Figure). The values of α were 349 ± 39 and 0.677 ± 0.120 (n = 3) in rats and frogs respectively.
Discussion: Frog spongy ventricles showed higher expandability than rat left ventricles composed of compact myocardium. Compact myocardium with coronary circulation might trade ventricular expandability in return for higher contractility.



3209-Pos Board B256

Novel Functions of Protein Kinase D in Cardiac Excitation-Contraction Coupling

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While the dynamic function of protein kinase D (PKD) has remained enigmatic, recent work has shown that PKD phosphorylates the nuclear regulators HDAC5/7 and CREB in the heart and has been implicated in the maintenance of cellular dysfunction that develops in heart failure. Here we significantly extend our understanding of PKD signaling in heart by investigating the cytosolic targeting of PKD in adult rat ventricular myocytes (ARVMs) using a molecular genetic approach to drive adenovirus-dependent expression of wild type (wt), constitutively active (ca) or dominant negative (dn) PKD in cultured ARVMs. Confocal imaging reveals a significant distribution of PKD in a non-nuclear, striated-reticular pattern in steady-state ARVMs with changes in PKD spatial distribution as PKD activity changes. Consistent with an established role of PKD in targeting cardiac troponin I, caPKD expression led to a marked decrease in contractile myofilament Ca^{2+} sensitivity. Steady-state Ca^{2+} transients were markedly increased in dnPKD cells and are explained in part by a marked increase in sarcoplasmic reticulum (SR) Ca^{2+} load. In addition, changes in the cardiac Ca^{2+} current (I_{Ca}) and behavior of the phosphatase inhibitor calyculin A (CalyA) support a role for PKD as a dynamic regulatory kinase of the L-type Ca^{2+} channel (LTCC). Whole-cell voltage clamp studies illustrate a marked increase in I_{Ca} throughout the entire voltage range in caPKD cells. Dynamic analyses of I_{Ca} reveal that, unlike control cells, the Ca^{2+} current in caPKD cells was maximally activated and did not further increase after phosphatase inhibition, while there was a loss of the CalyA stimulatory response in dnPKD cells. Taken together with our new findings, work to date suggests a complex collection of