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.

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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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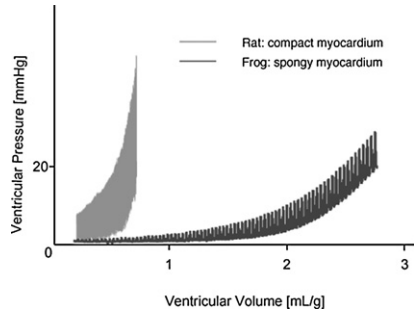
Background: In vertebrates, there are two kinds of myocardium, compacta and spongiosa, which are associated with blood supply systems i.e. coronary and sinusoidal circulation. To characterize the diastolic properties of these two types of ventricles, we analyzed the ventricular end-diastolic pressure-volume 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