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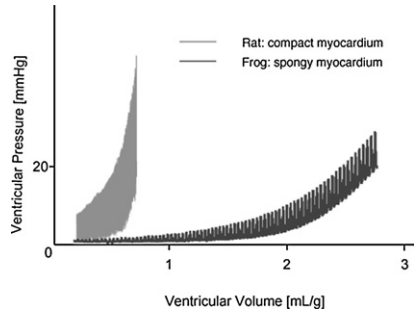
Background: In vertebrates, there are two kinds of myocardium, compacta and spongiosa, which are associated with blood supply systems i.e. coronary and sinusoidal circulation. To characterize the diastolic properties of these two types of ventricles, we analyzed the ventricular end-diastolic pressure-volume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

functions carried out by PKD that can best be explained by a new model that requires specific spatially-resolved subcellular targeting.

3210-Pos Board B257

Higher Aromatase Expression In Female Heart May Underline Its High Estrogen Content Resulting In Cardioprotection

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Estrogen (E2) is a well-known cardioprotective steroid hormone. Although heart has all the machinery to biosynthesize estrogen from testosterone by cytochrome P450 aromatase, little is known about the role of local heart E2 concentration [E2] in cardioprotection. We hypothesized that high heart [E2] in females could be one mechanism for the higher cardioprotection in females. We optimized the radioimmunoassay technique to measure heart [E2] in whole homogenate by diethyl ether extraction. Male mouse hearts have significantly higher E2 levels (35 ± 3 pg/ml, n=6) than plasma (12 ± 0.9 pg/ml, n=5). Heart [E2] in female mice at estrus and diestrus (diestrus 20.2 ± 1.5 pg/ml n=4; estrus 17.2 ± 0.9 pg/ml, n=4) were very similar to plasma [E2]. Interestingly, in the proestrus stage, heart [E2] was extremely high ~ 170 ± 4 pg/ml, almost 3 times higher than plasma [E2]. The final heart [E2] will depend on the testosterone level as well as the efficiency of the aromatase to convert testosterone to E2. As females have much lower levels of testosterone (~40pg/ml at estrus and diestrus and ~240 pg/ml in proestrus) compared to males (2 ng/ml), much higher heart [E2] in females at proestrus compared to male lead us to hypothesize that the aromatase expression/activity is much higher in females than males. We performed real time PCR and western blot analysis to quantify transcript and protein levels of aromatase in male and in female mice at estrus stage, as this stage is under the control of the preceding estrogen peak at proestrus. Aromatase transcript levels were similar in males and females at estrus, but aromatase protein levels were two fold higher in estrus compared to male. We speculate higher aromatase expression in females may underline its high estrogen content, thus resulting in cardioprotection.

3211-Pos Board B258

Glyceollin Attenuates Vascular Contraction By Inhibiting Rhoa/rho Kinase Pathway

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Isoflavones such as genistein and daidzein prevented agonist-induced vascular contraction in isolated rat aortic rings. Glyceollins are derived from the parent isoflavone daidzein through a series of pterocarpan intermediates. We hypothesized that glyceollin attenuates vascular contractions through inhibition of RhoA/Rho kinase pathway. Rat aortic rings were denuded of endothelium, mounted in organ baths and treated with either glyceollin (20 or 100 μM) or vehicle (DMSO) for 60 min after submaximal contraction by NaF (8.0 mM). The phosphorylation level of the myosin light chain (MLC_{20}), myosin phosphatase target subunit 1 (MYPT1) and protein kinase C (PKC)-potentiated inhibitory protein for heterotrimeric myosin light chain phosphatase of 17-kDa (CPI17) were determined by means of the Western blot. Glyceollin not only inhibited vascular contractions induced by NaF (8.0 mM), but also decreased the activation of RhoA and subsequent phosphorylation of $MYPT1^{Thr855}$ and $CPI17^{Thr38}$. These results indicate that glyceollin attenuates vascular contraction by inhibiting RhoA/Rho-kinase signaling.

3212-Pos Board B259

17β-Estradiol Attenuates Vascular Contraction Through Inhibition Of RhoA/Rho Kinase Signaling Pathway

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It is now well-known that 17β-estradiol has an endothelium-independent, non-genomic vasorelaxant action. In the present study, we hypothesized that 17β-estradiol attenuates vascular contraction by inhibiting RhoA/Rho kinase signaling pathway in rat aorta. Rat aortic rings were denuded of endothelium, mounted in organ baths, and contracted with 30 nM U46619 or 8.0 mM NaF 30 min after pretreatment with 17β-estradiol (30 and 100 μM) or vehicle. We measured the amount of GTP RhoA and the level of phosphorylation of the myosin light chain (MLC_{20}), myosin phosphatase targeting subunit 1 (MYPT1) and PKC-potentiated inhibitory protein for heterotrimeric MLCP of 17 kDa (CPI17). Pretreatment with 17β-estradiol not only inhibited U46619- or NaF-induced vasocontractions and the phosphorylation of MLC_{20} but also inhibited activation of RhoA. 17β-Estradiol also decreased the level of phosphorylation of $MYPT1_{Thr855}$ and $CPI17_{Thr38}$, downstream effectors of Rho-kinase. In conclusion, 17β-estradiol attenuates vascular contraction, at least in part, through inhibition of RhoA/Rho kinase signaling pathway.
Key Words: 17β-estradiol, RhoA, Rho kinase, CPI-17, MYPT1, vasorelaxation

3213-Pos Board B260

Modulation Of Cardiac Na+/H+ Exchange Activity By Muscarinic Agonists, Nitric Oxide and Cyclic GMP

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Na^+-H^+ exchange (NHE) is the principal acid-extrusion mechanism in cardiac myocytes. Its activity has been linked to myocardial ischaemia-reperfusion injury, arrhythmia and the development of cardiac hypertrophy. NHE is modulated acutely by intracellular pH (pH_i), as well as through phosphorylation by kinases. Nitric oxide (NO) is an important regulator of cardiac function. It is synthesised by NO synthases (NOS), which are activated by muscarinic (M_2) receptors. NO targets proteins, partly via protein kinase G, which is activated by cyclic GMP (cGMP). We studied the effect of this regulatory pathway on NHE activity in rat ventricular myocytes. Myocytes were loaded with the acetoxymethyl-ester of carboxy-SNARF-1 (a pH-reporter dye) and superfused with Hepes-buffer at 37°C. Applying a 4min, 20mM NH_4Cl prepulse deposits an intracellular acid-load that stimulates NHE. The membrane-permeant cGMP analog 8Br-cGMP (20μM), the NO donor sodium nitroprusside (1mM) and the M_2 agonist carbachol (100μM) reduced NHE activity (*vs* paired controls). At a common pH_i of 6.6, NHE inhibition was 26%, 29% and 18%, respectively ($P>0.05$). We also transfected adult myocytes with the nNOS gene using an adenoviral system (5×10^{10} viral particles, incubated overnight) to increase NO production capacity. To confirm successful gene-transfer, eGFP was transfected in separate experiments and fluorescence was detected in >90% of cells. NHE activity at pH_i=6.6 was not significantly different in nNOS-transfected cells (*vs* sham-transfected cells). However, on addition of carbachol (100μM), NHE activity was reduced by 50%. These findings illustrate an important role for the NO/cGMP pathway in modulating pH_i homeostasis.
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3214-Pos Board B261

Colocalization Of RyR And $Ca_v1.2$ In Ventricular Myocytes Is Independent Of The Physical Orientation Of The Cell

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We have investigated the effect of cell orientation on the observed colocalization of the ryanodine receptor (RyR) with the L-type calcium channel ($Ca_v1.2$) in adult rat ventricular myocytes. Cells were embedded in 2% agarose[1] and visualised with a 60X 1.2 NA water immersion objective on an Olympus FV1000 confocal microscope; all images were deconvolved before analysis. We imaged cells oriented both parallel and perpendicular to the coverslip. We found that colocalization between RyR and $Ca_v1.2$ in the two orientations was not significantly different from each other, and similar to values previously reported[2]. Cells oriented perpendicular to the coverslip provided additional details of the colocalization: The colocalized region between RyR and $Ca_v1.2$ was often surrounded by an area of RyR fluorescence, implying that the calcium channels within a dyad cover a smaller area than do the RyR. RyR that had no corresponding $Ca_v1.2$, and are presumed to be extra-dyadic, were distributed in the Z disk with no discernable pattern.
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3215-Pos Board B262

Blocking the Late Sodium Current Reduces Intracellular Sodium Accumulation During Sodium Pump Inhibition

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Impairment of Na^+/K^+-ATPase activity reduces sodium efflux and leads to an increase in intracellular Na^+ (Na^+_i). We tested the hypothesis that Na^+ accumulation caused by ouabain, a Na^+/K^+-ATPase inhibitor, would be reduced by concurrent inhibition of the late sodium current (I_{NaL}). We measured Na^+_i, high energy phosphates, and chemical driving force ($\Delta G_{\sim ATP}$) in isolated guinea pig hearts in real time with ^{23}Na- and ^{31}P- NMR. Hearts were pre-