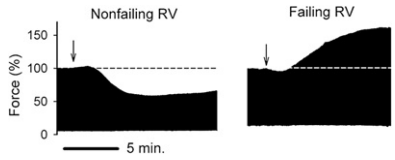
Cardiac Muscle III

3718-Pos

Heart Failure Switches RV Inotropic Responses from Negative to Positive

Guanying Wang, Che-Chung Yeh, Brian C. Jensen, Michael J. Mann, Paul C. Simpson, **Anthony J. Baker**.
VA Medical Center, Univ Calif San Francisco, San Francisco, CA, USA.

Right ventricular (RV) failure is a common but poorly understood disease. It is assumed that an understanding of RV failure can be extrapolated from studies of the left ventricle (LV). **Hypothesis:** RV failure is similar physiologically to LV failure. **Methods:** Using intact RV and LV trabeculae from nonfailing and failing mouse hearts, we measured inotropic (force) responses to alpha-1 adrenergic receptor (α_1-AR) stimulation, and assessed myofilament function using skinned trabeculae. **Results:** Figure shows typical force records from electrically paced trabeculae after α_1-AR stimulation (arrows). For nonfailing RV trabeculae, force decreased $27 \pm 3\%$, mean $\pm$ SEM, n=9. In marked contrast, for failing RV trabeculae, force increased $49 \pm 6\%$, n=21. This switch in inotropic response was paralleled by a switch in the myofilament response to α_1-ARs from decreased Ca^{2+} sensitivity (nonfailing) to increased Ca^{2+} sensitivity (failing). For LV trabeculae, inotropic and myofilament responses to α_1-ARs were not different in nonfailing versus failing hearts. **Conclusions:** In failing RV, the inotropic response to α_1-ARs is switched from negative to positive due to a switch in the myofilament response to α_1-ARs from decreased to increased Ca^{2+} sensitivity. Reject study hypothesis, because RV failure is different physiologically from LV failure.



3719-Pos

In Vitro Motility Studies of C-terminal Myosin Regulatory Light Chain Mutants Implicated in Familial Hypertrophic Cardiomyopathy

William M. Schmidt[1], James Watt[1], Katarzyna Kazmierczak[2], Jeff Moore[1], Danuta Szczesna-Cordary[2].
[1]Boston University School of Medicine, Boston, MA, USA, [2]University of Miami Miller School of Medicine, Miami, FL, USA.

Muscle thick filaments are primarily composed of the hexameric protein myosin, which is responsible for the generation of force and motion. Myosin consists of an N-terminal globular domain which, among other functions, binds to the thin filament and an α-helical domain, which acts as a lever arm to transmit force and motion. Familial hypertrophic cardiomyopathy (FHC), the leading cause of sudden cardiac death among young people, is a pathological thickening of the ventricular walls of the heart that has been caused by single point mutations in sarcomeric proteins, including the myosin regulatory light chain (RLC). Based on our recent work with mutations in the N-terminus of the RLC and the RLC location on the lever arm region, we hypothesized that the RLC-FHC mutations in the C-terminus (P95A, K104E and D166V) may disrupt force generation and contractility. To determine the effects of the FHC-RLC mutations we generated beta isoform myosin bearing mutant light chains by exchanging the porcine cardiac native light chain with recombinant mutant light chains and examined the effects of the mutations with the *in vitro* motility assay. Contrary to our hypothesis, none of the mutants exhibited changes in force production using the frictional loading motility assay, and maximal filament velocity (Vmax) also remained unchanged. Next, we measured regulated actin filament velocity as a function of calcium concentration. The velocity-pCa dependence showed the expected sigmoidal characteristic for all tested mutant myosins, however, the calcium sensitivity (pCa_{50}) increased for K104E and D166V mutants. In addition, D166V and P95A exhibited higher cooperativity. Data for D166V is consistent with previous studies performed in skinned mouse papillary muscle fibers showing an increase in the calcium sensitivity of force and ATPase (Kerrick et al., 2009, FASEB. J. 23:855-65).

3720-Pos

Single Molecule Kinetics in the Familial Hypertrophic Cardiomyopathy RLC-R58Q Mutant Mouse Heart

Prasad Mettikolla[1], Rafal Luchowski[1], Shande Chen[1], Zygmunt Gryczynski[1], Ignacy Gryczynski[1], Danuta Szczesna-Cordary[2], Julian Borejdo[1].
[1]Univ of North Texas, Fort Worth, TX, USA, [2]Univ of Miami, Miami, FL, USA.

Familial hypertrophic cardiomyopathy (FHC) is caused by a single-point-mutation in a gene that encodes for the ventricular myosin regulatory light chain (RLC). FHC is a serious heart disease that often leads to a sudden cardiac death of young athletes. The mutation is believed to alter the kinetics of interaction between actin and myosin causing heart to pump blood inefficiently. We report here the direct measurements of this kinetics. The measurements are based on the fact that during contraction a myosin cross-bridge delivers periodic force impulses to actin. Time average of those impulses is the isometric force, and an individual impulse carries the information about kinetics of the interaction. To be able to extract this kinetic information, it is necessary to scale down the experiments to the level of a single molecule. A single molecule of actin of transgenic mouse hearts expressing R58Q mutation associated with a malignant phenotype of FHC was observed by sparse labeling with a fluorophore. The kinetic rate was extracted from impulses by Fluorescence Correlation Spectroscopy (FCS). We show that the kinetic rate is significantly larger in contracting cardiac myofibrils of transgenic R58Q mice than of control transgenic wild type mice. We present evidence that during contraction actin changed orientation at least two times, suggesting that interaction with cross-bridges occurs in 2 or more steps.

3721-Pos

Contraction-Mediated Damage in mdx Mice Trabeculae: A Model for Cardiomyopathy in Duchene Muscular Dystrophy

Ying Xu, Jill A. Rafael-Fortney, Paul M.L. Janssen.
The Ohio State University, Columbus, OH, USA.

Duchene muscular dystrophy (DMD) is an inherited and progressive disease of striated muscle deterioration. Studies on skeletal muscle using an eccentric contraction model have provided great insight of the contraction-mediated damage in skeletal muscle in DMD. Because of different contraction mechanics in the cardiac vs. skeletal muscle, the protocols used to gain understanding in skeletal muscle pathology cannot be readily applied to the myocardium. To investigate contraction-mediated damage in dystrophin-deficient myocardium under physiological conditions, we developed an *in vitro* model of myocardial mechanics-induced injury. We employed computer-programmed protocols to trigger consecutive lengthening ramps during the twitch contraction at 4 Hz in age matched (young and adult) *mdx* and wild type mice right ventricular trabeculae. These ultra-thin muscles possess all major cardiac cell types and their contractile behavior very closely mimics that of the whole heart. In the first group of experiments, 10 lengthening contractions of various (1-10%) magnitude of stretch were performed in trabeculae from 10 week old *mdx* and wild type mice. In the second group, 100 lengthening contractions at each magnitude were conducted in trabeculae from 20 weeks old mice. The peak isometric active developed tension (F_{dev}, in mN/mm^2), diastolic tension (F_{dia}), and force kinetics were measured throughout the protocol, and the rate of decline of these parameters can be taken as a relative measurement of how susceptible the myocardium is to lengthening-induced dysfunction. Our results indicate dystrophin deficient myocardium in older *mdx* mice is more sensitive to severe mechanical stress compared to age-matched wild type controls. This suggests that lengthening contraction in myocardium is an appropriate model to study contraction-mediated damage in DMD associated cardiomyopathy.

3722-Pos

Myofilament Force Development is Less Economic in Post-Infarct Remodeled Myocardium

Jolanda van der Velden[1], Nicky M. Boontje[1], Daphne Merkus[2], Ger J.M. Stienen[1], Dirk J. Duncker[3].
[1]Institute for Cardiovascular Research VU University Medical Center, Amsterdam, Netherlands, [2]Experimental Cardiology; COEUR; Erasmus Medical Center, Rotterdam, Netherlands, [3]Experimental Cardiology; COEUR; Erasmuc Medical Center, Rotterdam, Netherlands.

Mismatch between energy supply and demand may limit cardiac performance of the remodeled heart after myocardial infarction. To assess whether economy of myofilament contraction is altered in the post-infarct remodeled myocardium, force and ATP utilization were measured simultaneously in permeabilized muscle strips from pigs with a myocardial infarction (MI). Remote left ventricular subendocardial muscle strips were taken 3 weeks after sham surgery (n=5) or induction of MI (n=5), by ligation of the left circumflex coronary artery. Isometric force and ATP consumption were measured at various [Ca^{2+}] in 17 permeabilized muscle strips in each group.
Three weeks after infarction, significant LV remodeling had occurred, reflected by LV dilation and hypertrophy of the surviving myocardium. LV systolic dysfunction was evident from the significantly reduced ejection fraction. Maximal force was significantly lower in MI (14 ± 1 kN/m^2) compared to sham (29 ± 2 kN/m^2), while maximal ATPase activity was slightly, though not significantly lower in MI (33 ± 5 $\mu M/s$) compared to sham (45 ± 5 $\mu M/s$). Tension cost, the rate of ATP splitting divided by isometric force is a measure of muscle economy. Mean tension cost at maximal activation was almost two-times higher in MI compared to sham ($P<0.05$). In addition, Ca^{2+}-sensitivity (pCa_{50}) of force and ATPase activity were significantly higher in post-MI remodeled myocardium compared to sham. Moreover, Ca^{2+}-sensitivity of ATPase activity was significantly higher than Ca^{2+}-sensitivity of force in MI myocardium

(ΔpCa_{50}=0.08 ± 0.02), while no significant difference was present in sham hearts (ΔpCa_{50}=0.01 ± 0.02).
These measurements indicate that economy of myofilament contraction is reduced in post-MI remodeled myocardium.

3723-Pos
Myosin Heavy Chain Isoform Expression and Contractile Function in Mechanically Unloaded Left Ventricles Following Left Ventricular Assist Device (LVAD) Implantation
Matthew R. Locher, Holly S. Norman, Satoru Osaki, Takushi Kohmoto, Nancy K. Sweitzer, Richard L. Moss.
University of Wisconsin Madison, Madison, WI, USA.
The ventricles of human myocardium normally express low levels of α myosin heavy chain (MHC) on a predominately β MHC background. However, in heart failure the distribution changes to ~100% β MHC with virtually undetectable levels of α MHC, a change that has been associated with contractile dysfunction. In cases of severe failure, surgical implantation of a left ventricular assist device (LVAD) may be used as destination therapy and has been previously associated with improvements in contractile function in single myocytes. Here, we used post-LVAD myocardium in which the heart has been explanted for transplantation to test the hypothesis that mechanical unloading of ventricular myocardium increases contraction kinetics, possibly through the re-expression of α MHC. Measurements of the maximal rate of ATP utilization and isometric force in permeabilized multicellular preparations revealed no significant difference between failing myocardium prior to LVAD implantation (pre-LVAD) and post-LVAD myocardium. Tension cost, which is calculated as the rate of ATP utilization divided by the isometric force, was also similar between groups. For comparison, normal myocardium displayed maximal rates of ATP turnover that were approximately 2.5-fold greater than in pre- and post-LVAD myocardium. SDS-PAGE indicated virtually undetectable levels of α MHC in pre- and post-LVAD myocardium, while protein phosphorylation gels revealed significant differences in the basal level of phosphorylation of myosin binding protein-C, TnT, and TnI between both groups. These results suggest that while mechanical unloading of failing myocardium may not cause a re-expression of α MHC, improvements in contractile function following LVAD implantation may be associated in part with alterations in the phosphorylation status of key regulatory proteins. This work supported by NIH RO1-HL61635 (RLM).

3724-Pos
Myofibrillar Protein Expression and Contractility in Neonates and Infants with Congenital Right Ventricular Outflow Obstruction
Fatiha Elhamine, Bodgan Iorga, Gerardus Bennink, Konrad Brockmeier, Robert Stehle, **Gabriele Pfitzer**.
University of Cologne, Koeln, Germany.
In this study we investigated the postnatal developmental changes in sarcomeric protein expression in parallel with contractile parameters in myofibrils isolated from small resections from the right ventricular (RV) outflow tract in 25 patients with Tetralogy of Fallot and related congenital heart diseases (CHD). These CHDs are associated with RV hypertrophy and outflow tract obstruction. The age of the patients ranged from 4 days to 38 months. The resections were procured during surgical correction of the cardiac malformation and would have been otherwise discarded. In the neonate (4 days old), the expression of slow skeletal TnI (ssTnI) and atrial light chain (ALC-1) was ~82% and ~50% respectively and declined to respectively < ~8% and ~3% at the age of 38 months. This down-regulation in ssTnI and ALC-1 expression correlated ($p<0.05$) with the decline in Ca^{2+}-sensitivity from $pCa_{50} = 5.95$ in the neonate to $pCa_{50} = 5.33$ in 38 months old infants. Neither contraction nor relaxation kinetics correlated with ssTnI expression. However, ALC-1 expression correlated positively with the activation kinetics, k_{ACT} and force redevelopment, k_{TR} ($r = 0.62$, $p<0.05$). The time course of relaxation is biphasic with an initial slow quasi-linear decay followed by a fast exponential decay. The rate constant of the fast exponential decay, k_{REL} correlated positively with ALC-1 expression ($r = 0.57$, $p<0.05$). In summary right ventricular hypertrophy associated with congenital heart disease does not prevent the developmental down-regulation of ssTnI and ALC-1 although we cannot exclude that this down-regulation occurs at a slower rate than in healthy infants. The change in ssTnI expression correlates with the expected decrease in Ca^{2+}-sensitivity while ALC-1 expression appears to modulate crossbridge turnover kinetics in agreement with studies in animals.

3725-Pos
Sex Dimorphic Myofilament Function and AMPK Expression in R403Q Hearts
John P. Konhilas.
University of Arizona, Tucson, AZ, USA.
Male mice expressing an autosomal dominant mutation in alpha-myosin heavy chain (R403Q) develop hypertrophic cardiomyopathy characterized by progressive left-ventricular dilation and cardiac dysfunction whereas females do not. We wished to determine whether these sex dimorphisms were due to underlying differences in myofilament contractile function. Therefore, we determined the sensitivity of the myofilaments to Ca^{2+} in demembranated cardiac trabeculae (CT) from wild-type (WT) and R403Q male and female mice (10-12 months of age). We demonstrate that the R403Q mutation did not affect Ca^{2+}-sensitive tension development in CT from males. While Ca^{2+}-sensitivity was greater in both male WT and R403Q CT compared to WT females, they were less sensitive to Ca^{2+} than CT from female R403Q hearts. We also determined rates of tension redevelopment (k_{tr}) following a release-restretch protocol in CT from WT and R403Q male and female hearts at the same age. CT from R403Q male hearts exhibited an enhanced k_{tr} compared to WT males. The k_{tr} in WT female CT was similar to WT males. The k_{tr} in R403Q female CT measured between WT and R403Q males. We hypothesized that the sex dimorphisms in myofialment function reflect an increase in the energetic cost of contraction when expressing the R403Q mutation. Therefore, we measured levels of Adenosine monophosphate-activated kinase (AMPK), a central sensor of the cellular energy state. Total AMPK protein levels were significantly increased in 10-12 month male R403Q hearts compared to WT controls. Female R403Q hearts showed the opposite: total AMPKα expression was lower compared to WT controls. We conclude that (1) the increased Ca^{2+}-sensitivity may provide sufficient contractile support in female R403Q hearts maintaining a compensated state, and (2) the increased AMPK expression in male R403Q hearts is indicative of an increased energetic demand caused by the mutation.

3726-Pos
Intralipid Protects Cardiac Function of Late Pregnant Mice against Ischemia/Reperfusion Injury
Jingyuan Li, Andrea Ciobotaru, Soban Umar, Parisa Partownavid, Siamak Rahman, Mansoureh Eghbali.
UCLA, Los angeles, CA, USA.
Female mouse hearts show better functional recovery after ischemia/reperfusion (I/R) injury compared with males. However, the vulnerability of isolated late pregnant (LP) hearts to I/R injury is unknown. Here we investigated the susceptibility of isolated mouse hearts in LP and postpartum (PP) to I/R injury. Isolated hearts (Langendorff) from female mice in diestrus stage (NP), LP, one day PP (PP1) and 7 day PP (PP7) were subjected to 20 minutes of global normothermic (37°C) ischemia followed by 40 minutes of reperfusion. The heart function was recorded throughout the experiments and infarct size was assessed by triphenyltetrazolium staining at the end of reperfusion. Although the function was similar in all 4 groups before ischemia, the functional recovery of LP hearts at the end of reperfusion was significantly lower compared to NP hearts; the rate pressure product (RPP) was reduced from 12926 ± 1479mmHg*beats/min in NP to 1614 ± 438mmHg*beats/min in LP mice. Interestingly, the RPP recovered partially in PP1 to 4716 ± 584mmHg*beats/min and almost fully back to NP levels one week PP. Consistent with the functional recovery findings, the infarct size was markedly larger in LP (59.7 ± 5.2%) compared with NP (15.2 ± 0.8%). The infarct size was restored partially in PP1 and fully back in PP7. Recently we have observed that Intralipid can protect the male mouse heart against I/R injury. To test whether Intralipid can improve the heart function in LP mice, 1% Intralipid was applied to isolated LP hearts at the onset of reperfusion. Intralipid treatment significantly improved the cardiac function of LP mice (RPP=11565 ± 1599mmHg*beats/min) and reduced the infarct size (17 ± 1.1%) to similar values as in NP. In conclusion, isolated LP hearts have high vulnerability to I/R injury and postischemic treatment with Intralipid can protect the heart against I/R injury.

3727-Pos
Intralipid Induces Cardioprotection against Ischemia-Reperfusion Injury by Inhibiting the Mitochondrial Permeability Transition Pore Opening Via the PI3K/AKT Pathway
Siamak Rahman, Jean Chrisostome Bopassa, Jingyuan Li, Soban Umar, Andrea Ciobotaru, Parisa Partownavid, Mansoureh Eghbali.
UCLA, Los angeles, CA, USA.
Acute myocardial infarction is a major cause of mortality, and the best hope of salvaging viable myocardium is by rapid cardiac reperfusion. A novel cardioprotective drug which could be applied at the time of reperfusion after acute infarction would be ideal. Here we tested the hypothesis that administration of Intralipid at the onset of reperfusion protects the heart against ischemia reperfusion (I/R) injury. Isolated hearts (Langendorff) from male mice were subjected to 20 minutes of global normothermic (37°C) ischemia followed by 40 minutes of reperfusion with Krebs Henseleit buffer (CTRL) or with additional 1% Intralipid (ILP). Postischemic treatment with Intralipid significantly improved the cardiac function; the rate pressure product (RPP) was increased from 3432 ± 334mmHg*beats/min in CTRL to 15405 ± 1011mmHg*beats/min in ILP. Consistent with the higher functional recovery in ILP, the infarct