Cardiac Muscle III

3718-Pos

Heart Failure Switches RV Inotropic Responses from Negative to Positive

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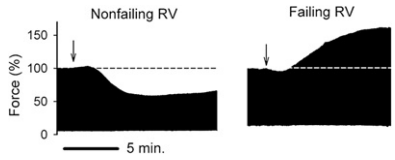
Right ventricular (RV) failure is a common but poorly understood disease. It is assumed that an understanding of RV failure can be extrapolated from studies of the left ventricle (LV). **Hypothesis:** RV failure is similar physiologically to LV failure. **Methods:** Using intact RV and LV trabeculae 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

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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

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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

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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

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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