Cardiac Muscle Regulation II

2999-Pos Board B429
Cardiac Over-Expression of Creatine Kinase Improves Function in Failing Myocytes

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Aims: Abnormal energy metabolism contributes to heart failure (HF) and the failing heart is energy starved. Here we tested whether augmented CK energy metabolism improves myocyte dysfunction in experimental HF.

Methods and Results: We tested the response to the β-agonist isoproterenol (2.5 μM, ISO) in cardiomyocytes isolated from wild-type (WT) mice and mice over-expressing cardiac myosin light chain kinase (cMLCK) from sham and HF (8 wk transverse aortic constriction, TAC) hearts, to dissect whether over-expressing CK-M or CK-mito might alter myocyte function at baseline or after an increase in energetic demand. At baseline, there were no differences in sarcomere fractional shortening (FS) or whole Ca2+ transient amplitude in response to ISO among sham WT, CK-M or CK-mito myocytes. However, ISO impact on FS, Ca2+ transient, time to 50 Ca2+ decay, and sarcomere re-lengthening were all reduced in WT TAC hearts, consistent with prior reports. Conversely, over-expressing CK-M or CK-mito rescued ISO-induced inotropy in TAC myocytes. No sizable differences in ISO response were noticed in cells obtained from sham WT, CK-M or CK-mito hearts. To test whether over-expressing CK-M or CK-mito confers a degree of protection against acute oxidative stress, non-TAC myocytes were exposed to H2O2 (50 μM for 10 min). The interval between the beginning of H2O2 superfusion and the appearance of an irreversible arrhythmia was measured. WT and CK-M myocytes showed a similar response (530 ± 87a vs. 370 ± 60b, n=5), whereas in CK-mito this interval was prolonged (580 ± 74a).

Conclusions: Over-expressing CK-M and CK-mito under failing-TAC conditions improves myocyte contraction and relaxation, likely through preserved Ca2+ handling; however, only the up-regulation of CK-mito can effectively buffer ROS, especially those of mitochondrial origin.

Acute Ablation of Cardiac Myosin Light Chain Kinase Decreases Cardiac Performance

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Cardiac myosin light chain kinase (cMLCK) phosphorylates a single site in the regulatory light chain (RLC) of myosin to increase Ca2+ sensitivity of myofilament contractions. A constellation of contractile protein phosphorylations in the presence of G-actin can occur and produce a better tolerance to acidosis. The results suggest that CAIII may function in regulating intracellular pH, its physiological function in muscle is unclear. Mice lacking CAIII showed lower than wild type intracellular pH in skeletal muscle cells during fatigue treatment. To further understand the role of CAIII in muscle functions and stress adaptation, we developed transgenic mice overexpressing CAIII in the heart under the control of a cloned myosin heavy chain promoter for phenotype comparisons with wild type mouse hearts that are CAIII negative. Three months old transgenic mice showed normal cardiac phenotypes under non-stress conditions. Cardiac function was examined using ex vivo working heart preparations under normal and low pH conditions to investigate CAIII function in pH regulation of cardiac muscle. With equilibration of 5% CO2 generating pH 7.4 in normal Kreb’s perfusion buffer, 10% CO2 was used to lower pH to 7.0. Functional data showed that transgenic and wild type hearts had similar pumping functions under normal pH. Perfused with low pH buffer, heart functions of both groups were decreased. In comparison with wild type controls at low pH, CAIII transgenic mouse hearts showed higher left ventricular pressure development and systolic and diastolic velocities under both baseline conditions and increased afterload stress, indicating a better tolerance to acidosis. The results suggest that CAIII may function in compensating for intracellular pH under acidic conditions, a tractive novel approach to develop new treatment of chronic congestive heart failure.

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Cardiac Remodeling in the Mouse Model of Marfan Syndrome Develops Independently from Aortic and Valvular Abnormalities

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Marfan syndrome (MFS) is a multisystem disorder of connective tissue caused by mutations in fibrillin-1. Heart involvement in the Marfan syndrome patients includes cardio-root dilatation, valvular insufficiency, and myocardial dysfunction; it remains unclear, however, whether alterations in myocardium are triggered by valvular and aortic pathology or they develop independently. We evaluated the age-dependent cardiac remodeling and left ventricular dysfunction in the mouse model of MFS known as Fbn1309G/+ -/- mouse (Marfan HT mouse) using echocardiography, pressure-volume loop analyses and a number of histological and biochemical techniques.

Marfan HT mice of 2-4 month demonstrated a hypertrophic cardiac remodeling accompanied by predominant decline of diastolic function and increased TGF-β canonical (p SMAD2/3) and non-canonical (pERK ½ and pMAPK38) signaling. Hypertrophic mycardium among older HT mice (6-14 months) was associated with two distinctly different phenotypes manifesting either dilated or constricted LV chamber. Dilatation of LV chamber was accompanied by biochemical evidence of greater mechanical stress, including elevated ERK1/2 phosphorylation and brain nutrient peptide expression in comparison with constricted heart. Diastolic dysfunction in the older HT mice was combined with significant systolic impairment. The aortic valve regurgitation was registered in 20% of constricted group and 60% of dilated, while mitral insufficiency was observed in 40% of constricted group and 100% of dilated. In Marfan HT mice, extracellular matrix abnormalities were not associated with the increase of intestinal fibrosis and myocardial hypertrophy. In the mouse model of fibrillin-1 haploinsufficiency the early onset of hypertrophic cardiac remodeling and dysfunction is not consequent to functional valvular abnormalities, but it is likely to result from deficient mechanosensing and transmission of mechanical forces.

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Transgenic Over-Expression of Carbonic Anhydrase III in Cardiac Muscle Demonstrates a Mechanism to Resist Acidosis

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Carbonic anhydrase III (CAIII) is an abundant protein in skeletal muscle, liver and adipose cells. A cytosolic enzyme that catalyzes conversions between CO2 and H2O, CAIII is a key factor in pH regulation of cardiac muscle. With equilibration of 5% CO2 generating pH 7.4 in normal Kreb’s perfusion buffer, 10% CO2 was used to lower pH to 7.0. Functional data showed that transgenic and wild type hearts had similar pumping functions under normal pH. Perfused with low pH buffer, heart functions of both groups were decreased. In comparison with wild type controls at low pH, CAIII transgenic mouse hearts showed higher left ventricular pressure development and systolic and diastolic velocities under both baseline conditions and increased afterload stress, indicating a better tolerance to acidosis. The results suggest that CAIII may function in compensating for intracellular pH under acidic conditions, a tractive novel approach to develop new treatment of chronic congestive heart failure.

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Myocardial Interstitial Serotonin and its Major Metabolite, 5-Hydroxyindole Acetic Acid Levels Determined by Microdialysis Technique in vivo Rat Heart

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Aims: The aim of this study was to elucidate myocardial interstitial serotonin (5-HT) kinetics in the heart, including 5-HT reuptake and enzymatic degradation to 5-hydroxyindole acetic acid (5-HIAA) via monoamine oxidase (MAO). Methods: Using microdialysis technique in anaesthetized rats, we simultaneously monitored myocardial interstitial levels of 5-HT and its major metabolite, 5-HIAA, in the left ventricle and examined the effects of local administration of a MAO inhibitor, pargylene, or a 5-HT uptake inhibitor, fluoxetine.