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787-Pos Board B556
Microscopic Heat Pulses Induce Ca^{2+}-Independent Contraction of Cardiomyocytes
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Laser irradiation has developed into a novel technique of non-invasive stimulation in cardiac and neural tissues. However, physical parameters for the laser irradiation-induced cardiac contractions have not been clarified, because various physicochemical reactions, such as photochemical and photothermal effects, are triggered in this process. Here we studied the effects of laser-induced local temperature changes on the functions of isolated cardiomyocytes. We demonstrated previously that a microscopic heat pulse (ΔT = 0.2°C for 2 sec) induces a Ca^{2+} burst in cancer cells (HeLa cells) at a body temperature (Tseeb et al., *HFSP J.*, 2009), with the mechanism similar to that of rapid cooling contracture in skeletal and cardiac muscles. In the present study, we generated microscopic heat pulses by focusing infrared laser light in extracellular solution near adult rat cardiomyocytes. We found that a microscopic heat pulse (ΔT = 5°C for 0.5 sec) induces contractions at basal temperature of 36°C. At 25°C, larger ΔT was required to induce contractions. When 2.5 Hz heat pulses were repeatedly applied, we observed oscillatory contractions of cardiomyocytes. Different from contractions induced by electric stimulation, Ca^{2+} transients were not detected during the contraction. Likewise, heat pulses induced contractions of skinned cardiomyocytes in Ca^{2+}-free solution in the presence of ATP. These results demonstrate that heat pulses can regulate cardiac contractions without any involvement of Ca^{2+} dynamics, by directly activating the actomyosin interaction. Hence, our microheating technique may be useful for stimulating the beating of failing hearts without causing abnormal Ca^{2+} dynamics.

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Conformational Dynamics of a "Titratable" Cardiac Inotrope
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Ischemic heart disease is a leading cause of morbidity and mortality in the world. To help gain insight into mechanisms and forge new experimental therapeutics we are using time-resolved fluorescence resonance energy transfer (TR-FRET) to detect structural changes within the cardiac troponin complex that occur during acute ischemic insult. During an ischemic event, penultimate to cell death, the sarcomere undergoes acidification. In cardiac myocytes, this decrease in pH uncouples calcium homeostasis from contractility. We have shown that the neonatal cardiac isoform of TnI, ssTnI, exhibits reduced pH-sensitivity compared to the adult cTnI isoform, however ssTnI confers deleterious effects at baseline in the context of an adult myocyte. Functional studies demonstrated that this pH-insensitivity stems from ssTnI residue H132. Introduction of a histidine at the homologous position in cTnI (A164H) mitigates the pH-sensitivity of the calcium-force relationship in cardiac myocytes. We are now establishing a TR-FRET approach to detect structural changes within the troponin complex in functional thin filaments with the goal of elucidating the structural dynamics that control pH-sensitive calcium-activated force production in the heart. We have engineered labeling sites designed to detect Ca^{2+} and pH sensitive structural changes in cTnI and cTnC. We will discuss our progress on utilizing a novel high-throughput fluorescence lifetime plate-reader that allows high-precision detection of time-resolved fluorescence.

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Streptococcal Anti-Phospholipid Antibody, MAb10F5, Binds Greatest in the Cardiac Valve Region
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An autoimmune related inflammation occurring after group A streptococcal infection is believed to be behind the development of rheumatic heart disease. The group A streptococcal conservative M protein sequence when injected into mice generates a promiscuous antibody referred to as mAb10F5. Injection of the M protein sequence into Lewis rats results in heart manifestations and injection of mAb10F5 which is against M protein sequence into Lewis rats results in the display of the antibody in the heart, especially in the valve region. The binding of mAb10F5 in the heart resembles that of anti-cardiac myosin with binding in all three regions of the heart examined (artial, valve and ventricular apex) during the first 72 hrs after injection. However, mAb10F5 remains significantly bound in the valve region at 72 hrs while binding of anti-cardiac myosin binding decreases. MAb10F5 is an IgG2b antibody and mouse IgG2b has been shown to promote autoimmunity in the mouse. However, injection of just mouse IgG2b into Lewis rats leads to little binding of antibody in the heart. The binding that does occur is again greatest in the valve region, but dissipates after the first 24 hrs and is virtually negative by 72 hrs. This is significantly different from mAb10F5 which remains significantly bound in this region at the 72 hr time point (p = 7.95E-16, ANOVA). Thus, though IgG2b may have initial autoreactivity in the first 24 hrs, it does not remain bound in the heart valve region. Therefore, mAb10F5 which is associated with the streptococcal conservative M protein sequence demonstrates a more specific reactivity to this region of the heart which may be a factor in the development of valvulitis.

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FRET Assay for Single Molecule Measurements of Troponin Activation
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We seek to understand the energetics and design principle of the $TnTmA_7$ switch and the basis for cooperative activation. Single molecule studies provide the needed resolution, revealing conformational substates in a model-independent manner. Multiple single-cysteine mutants of cardiac TnI (cTnI) and cardiac TnC (cTnC) were engineered to generate an assay to resolve Ca^{2+}- and myosin-dependent conformational substates of cTn in single molecule FRET experiments. Thin filaments were reconstituted from F-actin, Tm, and cTn, which consisted of donor (AlexaFluor 546)-labeled cTnI, acceptor (ATTO655)-labled cTnC, and wt cTnT. Optimized buffer conditions provide stably associated thin filaments at a 1 nM concentration. Complex stability was confirmed by fluorescence cross-correlation spectroscopy (FCCS). In the most promising cTn construct, Ca^{2+} saturation increases the TE by 14%.

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PKA Effects on Cardiac Troponin and Myofibril Relaxation
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Protein kinase A (PKA) phosphorylation of myofibrillar proteins constitutes an important pathway for beta-adrenergic modulation of cardiac contractility and relaxation. PKA targets the N-terminus of cardiac troponin I (cTnI), cardiac myosin-binding protein C (cMyBP-C) and titin. We recently reported that PKA phosphorylation of cTnI alone, or in combination with cMyBP-C, is sufficient to suppress cardiac contractility and the sarcomere length dependence of contraction. Troponin C (cTnC) interaction with cTnI (C-I) is a critical step in contractile activation that may be modulated by cTnI phosphorylation. Here we tested the hypothesis that altering C-I interactions by PKA, or by phosphomimetic mutations in cTnI, directly affects myofilament relaxation. Using a fluorescent probe coupled to cTnC (C35S IANBD), the Ca^{2+} binding affinity to troponin complex (cTn) and C-I interaction affinity were monitored. Ca^{2+} binding to cTn (pCa_{50}) was significantly decreased by PKA, when both whole cTn or cTnI alone were phosphorylated (pCa_{50} = 0.3 and 0.1 units, respectively). PKA phosphorylation of cTnI also weakened C-I interaction in the presence or absence of Ca^{2+}, with a greater effect for the former. Rat ventricular myofibrils were isolated and endogenous cTn was exchanged with either wild type (WT), nonphosphorylatable (S23/24A cTnI) or phosphomimetic (S23/24D cTnI) recombinant cTn. Mechanics were monitored at maximum and submaximal Ca^{2+}. PKA treatment of WT or S23/24D cTnI exchange resulted in a significantly increased slow phase relaxation rate ($k_{REL,slow}$) and decreased duration of slow phase relaxation ($t_{REL,slow}$). These effects were largest at submaximal Ca^{2+}. In contrast, exchange with S23/24A cTnI resulted in no effects on relaxation kinetics. Together these data suggest that weakened C-I interaction affinity from PKA phosphorylation results in increased myofilament relaxation kinetics. These effects may enhance early phase diastolic relaxation during beta-adrenergic stimulation. Supported by AHA7400069 (VR), AHA2090056 (DW), HL65497 (MR).

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N-Terminal Truncated Cardiac Troponin I Enhanced the Contractility of Isolated Cardiomyocytes
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Cardiac TnI has a unique N-terminal extension that is a heart-specific regulatory structure not present in skeletal muscle TnI. Previous studies have