muscle. This work was supported by the National Institutes of Health and the American Heart Association.

Platform Z: Cardiac Muscle I

1149-Plat
Ventricular Myocyte Morphology in Long Term Culture
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Previously we have introduced a single cell system allowing long term culturing (1 week) of adult rat ventricular myocytes while maintaining their overall morphology, contractile behaviour and calcium-signalling.
Here, we characterize the subcellular morphology of the myocytes, including the Golgi apparatus, endo-/sarcoplasmic reticulum (ER/SR), plasma membrane and mitochondria. Cells were isolated from adult rats following a standard enzymatic procedure.
Organelles were labelled using targeted expression of fluorescent proteins, e.g. dsRed1 fused to the subunit VIII of human cytochrome C oxidase for mitochondria, YFP fused to a GPI-anchor for the plasma membrane, YFP fused to ts045G for the Golgi apparatus and dsRed2 fused to calreticulin for the ER/SR. Complementing this we also applied fluorescent dyes; di-8-ANEPPS for the plasma membrane and MitoTracker Green for the mitochondria. 3-dimensional stacks of individual cells were acquired with a nipkow-disc based confocal microscope.
Using both labelling approaches, the analysis of the plasma membrane illustrated a gradual loss of the t-tubules during culturing with cytosolic membrane fragments being present for extended time periods.
Mitochondria, which are very prominent and densely packed in cardiac myocytes, underwent an apparent fusion of originally isolated mitochondria, possibly reflecting the loss of t-tubules. While the structure of the ER/SR remained unaltered, the Golgi apparatus underwent a significant redistribution during the culturing time from a wide cytosolic distribution to a perinuclear accumulation.
We provide important evidence that cell morphology changes unavoidably occurring in adult cardiac myocytes during long term culture are highly reproducible and thus strongly support the application of such a single cell model in high-content screening applications.
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1150-Plat
Calcium Independent Positive Inotropy By Fast Cardiac Myosin Motor Gene Transfer In Slow Myosin Dominant Ventricular Myocytes
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Current inotropic drug therapies used to boost cardiac muscle performance focus on elevating the amount of calcium that is mobilized for activation of the myofilaments. These calcium based therapies can provide short term benefits, but when administered long term can actually increase mortality due to calcium overload and the development of fatal arrhythmias. Calcium mobilization has also been the key target for current gene therapy strategies (i.e. SERCA2a over-expression, PLN knockdown, S100 protein expression) to treat the failing heart. Here we present a novel form of calcium independent positive inotropy by fast cardiac myosin motor gene transfer. We designed a recombinant adenovirus to express the full length human α-myosin heavy chain (α-MyHC, *MYH6*) gene in rabbit or human ventricular myocytes that endogenously express almost exclusively β-MyHC. Healthy or diseased adult cardiac myocytes were isolated by enzymatic digestion and maintained in primary culture for 48 hours. Highly efficient α-MyHC gene transfer was confirmed by fluorescent immunocytochemistry and Western blotting. In all cases contractility of single cardiac myocytes was measured 48 hours after gene transfer of α-MyHC by measuring sarcomere shortening and intracellular calcium transients. Sarcomere shortening was ~35% greater in cardiac myocytes transduced with the α-MyHC adenovirus when α-MyHC made up ~30% of the total myosin protein. Intracellular calcium transient amplitudes, however, were not affected by α-MyHC gene transfer. In permeabilized myocyte experiments we found that α-MyHC gene transfer did not affect myofilament calcium sensitivity, but did speed the kinetics of myosin cross bridge transitions from weakly-bound to strongly-bound states. We conclude that α-MyHC gene transfer offers a novel form of calcium independent positive inotropy for β-MyHC dominant adult ventricular myocytes.

1151-Plat
Role of Thin Filament Cooperative Activation in Length-dependent Activation in Skinned Porcine Ventricular Muscle
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The basis of the Frank-Starling mechanism of the heart is the intrinsic ability of cardiac sarcomeres to produce greater active force in response to stretch (i.e., length-dependent activation). We have reported that troponin plays a key role in this phenomenon via on-off switching of the thin filament state, in concert with titin-based passive force (Terui et al., *J Gen. Physiol.* 131;275-283:2008). In the present study, we systematically investigated the role of thin filament cooperative activation in length-dependent activation using skinned porcine ventricular muscle at sarcomere lengths of 1.9 and 2.3 μm. MgADP (3 mM) increased Ca^{2+} sensitivity of force and enhanced the speed of contraction, indicating enhanced cooperative activation. MgADP was found to attenuate length-dependent activation, with and without quasi-complete reconstitution of thin filaments with fast skeletal troponin (sTn; from rabbits). Conversely, inorganic phosphate (Pi, 20 mM) decreased Ca^{2+} sensitivity of force and the speed of contraction, indicating reduced cooperative activation. Pi enhanced length-dependent activation, with and without sTn reconstitution. Qualitatively similar results were obtained with MgADP or Pi in rabbit fast skeletal muscle, with higher Ca^{2+} sensitivity of force than in cardiac muscle. Linear regression analysis revealed that the speed of contraction, Ca^{2+} sensitivity of force and length-dependent activation were strongly correlated in both cardiac and skeletal muscle. These results suggest that length-dependent activation is regulated via thin filament cooperative activation, such that the length-dependent increase in the fraction of cross-bridges is less in high cooperative activation states, coupled with a loss of recruitable cross-bridges.

1152-Plat
Length-Dependent Active Tension Development In Single Intact Cardiomyocytes, Isolated From Different Regions Of Guinea Pig Heart
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Introduction: Information on the force-length relation of intact myocytes from different regions of the heart is scarce. We therefore studied myocytes, isolated from apical and basal areas of guinea pig left and right ventricles (cell numbers: LV_A 22, LV_B 29, RV_A 11, RV_B 12). **Methods:** Force-length relations were measured by attaching carbon fibers to myocytes, allowing application of diastolic stretch while measuring passive and active force.[1] Cells were kept at $36 \pm 1°C$ and paced at 2 Hz. Recorded forces were normalized to cell cross-sectional area and used to construct end-diastolic, end-systolic and active tension-length relations (EDTL, ESTL and ATL=ESTL-EDTL; respectively). In addition, the ratio of the slopes of ESTL and EDTL was used as a cross-section independent factor to characterise the Frank-Starling Gain (FSG) in individual cells. **Results:** For all tissue regions ESTL, EDTL and, hence, ATL, are linear over the range of end-diastolic sarcomere lengths studied (1.88-2.15 μm). Plotting the slope values of ATL vs. EDTL for all cells shows a positive correlation (slope 1.29, R^2=0.24, 74 cells). In addition, FSG is larger than one for all cells studied: RV_B 3.91 ± 0.54), RV_A (2.84 ± 0.30), LV_B (2.77 ± 0.17), and LV_A (3.20 ± 0.24). **Conclusions:** Using the carbon fiber technique, it is possible to probe length-dependence of passive and active tension at the single cell level, without the interference of extracellular structures. The Frank-Starling Gain varies between the different regions of the heart and the positive correlation between ATL and EDTL confirms that in intact cells passive force bearing structures (such as titin) are likely to play a role in modulating length dependent activation.
[1] Iribe et al, Force-length relations in isolated intact cardiomyocytes subjected to dynamic changes in mechanical load. AJP 2007/292:1487-1497.

1153-Plat
Sarcomere Length Dependence Of The Force-pCa Relationship In Cardiac Muscle Is Influenced By Properties Of Thin Filament Regulatory Units
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Myocardial performance is tightly regulated by sarcomere length (SL), which is thought to be due at least in part to concurrent changes in myofilament lattice spacing and the relative proximity (and binding probability) of myosin heads to actin. Furthermore, crossbridge binding enhances Ca^{2+} binding to troponin (Tn) in cardiac muscle, a unique form of cooperative thin filament activation

whose mechanism is currently unknown. Experiments here were designed to test the hypothesis that the properties of Tn are important in determining the SL dependence of force in cardiac muscle. We compared trabeculae exchanged with WT cTn vs. cTn containing a mutant (L48Q) cTnC with enhanced Ca^{2+} affinity. L48Q cTnC caused a left-shift in the force-pCa relationship at long (2.3 μm) SL as compared to WT cTnC. Interestingly, L48Q cTnC effectively eliminated SL-dependence of the force-pCa relationship, via a much larger left-shift at short SL, while SL dependence of F_{max} was unaffected. This suggests that SL-dependence of cardiac force development can be greatly influenced by the properties of native cTn, perhaps by limiting crossbridge binding, and that this effect is likely most important at shorter SL. Furthermore, increasing the Ca^{2+} binding and/or cTnC-cTnI interaction properties of cTn (such as with L48Q cTnC) can reduce or eliminate this limitation. Ongoing experiments will determine whether TnI phosphorylation can restore SL dependence by decreasing Tn Ca^{2+} affinity, thereby reducing myosin access to actin binding sites. This will also be tested by exchange with I61Q cTnC-Tn, a mutant with reduced Ca^{2+} affinity. Overall, these results imply that the cardiac length-force relationship is governed, at least in part, by properties of thin filament regulatory units. Support appreciated from NIH R01 HL 65497 (MR) and T32 HL07828 (FSK).

1154-Plat

Effect of pH and Electrostatic Interactions on Myofilament Lattice Volume

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Changes interfilament lattice spacing is a major determinant of force production in striated muscle with reductions in force generation being observed with both expansion and compression of the lattice spacing relative to the normal physiological values. Previous studies have concluded that lattice spacing depends complexly on the balance of outward repulsive forces and inward attractive and compressive forces between thick and thin filaments. Since lattice spacing has important implications on force generation, we examined the effects of alterations in filament charge, induced by changes in intracellular pH (by the rapid application and withdrawal of 30 mM NH4Cl), on lattice spacing in intact twitching cardiac trabeculae. Since we observed changes in sarcomere length in response to changes in intracellular pH and since changes in sarcomere length induce changes in lattice spacing as a result of the isovolumic volume behavior of intact sarcomeres, sarcomere length was maintained at a fixed value (~2.2 μm) during the pH interventions. Lattice spacing increased ($p < 0.05$) following NH4Cl wash in (measured after 5 minutes) when the pH is estimated to increase to ~7.8 (Balnave 2000 J. Physiol.) to 36.9 +/-0.2. Following the washout of NH4Cl (which is estimated to decrease pH to ~6.5 (Swietach 2005 J. Physiol.)) lattice spacing increased to 37.6+/−0.4 elevated above ($P < 0.05$) the space observed at control pH. Since the isoelectric point for myofilaments is ~5 (Naylor, Biophys J, 1985), our findings suggest that, in addition to electrostatic, van der Waals' and osmotic forces, other pH-sensitive forces are also critical determinants of the lattice spacing, possibly the M-protein, myomesin or other M-line proteins which are postulated to hold thick filaments together in the M-band of striated muscle.

1155-Plat

Molecular and Functional Characterization of a Novel Cardiac Specific Human Tropomyosin Isoform

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Previous work using human cardiac RNA identified a novel tropomyosin (TM) isoform designated as TPM1-kappa. We developed a TPM1-kappa specific antibody and quantified the levels of TPM1-kappa protein in the hearts of normal and various cardiomyopathy patients. Our study reveals that TPM1-kappa protein is expressed in the human heart and its level is differentially regulated during cardiomyopathic conditions. To investigate the role of TPM1-kappa in sarcomeric function, we generated several lines of transgenic (TG) mice overexpressing TPM1-kappa in the hearts. Immunohistochemical studies show the incorporation of the TPM1-kappa protein in the myofilaments but no significant pathological alterations are observed in these TG mice. Hearts that express TPM1-kappa protein exhibit significant decreases in rates of contraction and relaxation when assessed by ex vivo work-performing cardiac analyses. Studies on skinned fiber bundles demonstrate decreased myofilament calcium sensitivity with no change in maximum developed tension. Additional biophysical studies, including circular dichroism and in vitro actin-binding assays using recombinant TM proteins demonstrate less stability and weaker actin-binding affinity of TPM1-kappa as compared to TPM1-alpha. Energetic analyses of the structural models of TPM1 kappa and alpha isoforms reveal regions which decrease the overall stability of the TPM1 kappa/kappa and kappa/alpha coiled-coil dimers. This functional analysis of TPM1-kappa provides a possible mechanistic explanation for the isoform switch that is observed in cardiomyopathy patients.

1156-Plat

SPOC: A Functional Assay of Failing and Non-Failing Human Cardiomyocytes

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SPontaneous Oscillatory Contractions, known as SPOCs, are well characterized for skeletal muscle fibers but less so for cardiac muscle. A survey of SPOCs on mammalian hearts from rat to cow showed that, like myosin ATPase activity, SPOC contractions exhibit a linear relationship relative to body size. SPOCs exhibit slow, travelling waves of relaxations that can be induced under precise solvent conditions. These are observed by light microscopy and the time-resolved images are analysed with high spatial and temporal resolution for up to 1 h. Here we examine the functional performance of failing and non-failing human heart samples snap frozen at -200°C (liquid nitrogen) within minutes of clamping the coronary arteries. Thus, it is possible to study the contraction and relaxation of cardiomyocytes from hearts that were also used in transcriptomics studies. With our large bank of frozen human heart tissue we can study the effects of: (1) end-stage heart failure on multiple heart samples from a wide range of patients; and (2) aging on contractile performance using multiple samples of non-failing hearts. We will report preliminary findings from end-stage heart failure patients with familial and non-familial dilated cardiomyopathy, and from un-used donor hearts aged 9-65 years.