Subcellular fractionation of a complex proteome (cardiac tissue) allows for enrichment of a subset of low abundant proteins. This permits more in depth analysis of the proteome by reducing the complexity. Adult rat cardiac tissue was made ischemic by ligating the left anterior descending coronary artery in vivo for 1 hour with no reperfusion, and the healthy remote area was differentiated from the ischemic tissue by staining with Evans Blue dye after harvesting the heart. A series of differential centrifugation steps produced nuclear, mitochondrial, cytoplasmic, microsomal, and sarcomeric fractions of rat ischemic and remote healthy tissue. The sarcomeric fractions of the remote vs ischemic cardiac tissue were digested with trypsin, and the peptides were labeled with isobaric tags for relative quantitation (iTRAQ). The labeled peptides were then fractionated with an Agilent 3100 OFFGEL fractionator, which separated the peptides in 12 fractions based on their isoelectric point from pH 3-10. The 12 fractions from the OFFGEL were run on a Dionex U-3000 nano LC coupled to a ThermoFinnigan LTQ running in PQD (pulsed Q dissociation) mode to detect the low mass reporter ions of the isobaric tags on the peptides. The peptides were analyzed with MASCOT (MatrixScience), and Scaffold v2.5.2 Q+ with a minimum of two unique peptides and a level of confidence set at 95%. Five-fold more proteins were identified when the labeled digests were fractionated with OFFGEL compared to no fractionation prior to LC-MS/MS. With a one hour ischemic event, we found approximately 11% of the detected proteins in the sarcomeric fraction had changed at least 1.5 fold. Therefore, this in-solution method incorporating sub-proteomic fractionation in conjunction with OFFGEL separation may be an approach for discovery of relative protein changes in cardiac tissue.

3742-Pos

Contractile Function is Altered by Regulation of HO-1 Activity in Single Adult Rat Cardiomyocytes

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Heme oxygenase-1 (HO-1) is a membrane protein upregulated in response to oxidative stress that confers cardioprotection. The therapeutic potential of HO-1 can be assessed by examining cardiomyocytes survival and function using cobalt protoporphyrin (CoPP) or tin protoporphyrin (SnPP) to increase or inhibit HO-1 activity, respectively. Whether altered HO-1 function affects cardiomyocyte contractility, however, is unknown. Thus, we determined the effects of CoPP (n=11), SnPP (n=15), or PBS (control, n=15) on *in vitro* single intact adult cardiomyocyte contraction and relaxation using video microscopy and calcium imaging (IonOptix) at 0.5, 1 and 2Hz. At 0.5Hz there was no difference in the rate or magnitude of shortening between groups, and all cells had similar fractional shortening (FS) normalized to peak calcium (FS/Ca^{2+}). FS/Ca^{2+} was, however, greater at 1Hz and 2Hz in SnPP-treated cells, while CoPP- and PBS-treated cells maintained similar FS/Ca^{2+} relationships across frequencies ($p<0.05$). Intriguingly, while SnPP-treated cells increased contraction at faster pacing, only 53% of those cells contracting at 0.5Hz beat synchronously at 1Hz, compared to 100% of CoPP-treated cells and 80% of PBS-treated cells. This difference became more pronounced at 2Hz, as only 26% of SnPP-treated cells and 60% of PBS treated cells able to follow stimulation, while 100% of CoPP-treated cells continued to beat synchronously. Similarly, although time to 90% relaxation was not different between groups at 0.5Hz, it was significantly faster in CoPP- vs. SnPP-treated cells at 2Hz ($p<0.05$). These results suggest that increasing HO-1 activity via CoPP treatment maintains cell viability under stress, while SnPP- induced HO-1 inhibition reduces survivability at higher pacing frequencies. The mechanisms behind this action are unknown, but may be partially due to HO-1-induced calcium desensitization of the myofilament. Supported by NSF GRFP (SGN) and NIH HL086709 (MA, MR).

3743-Pos

Study of O-Glcnacylation of Contractile Proteins in Cardiac Myofibrils by Enzymatic Labelling

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Phosphorylation occurs on serine and threonine residues and plays important roles in the regulation of contractile proteins. In heart failure changes in levels of phosphorylation are reported in a number of cardiac sarcomeric proteins. *O*-linked-*N*-acetylglucosamine (*O*-GlcNAc) modification is another possible posttranslational modification on serine and threonine residues and recent publications reported mapping of *O*-GlcNAc modification sites in some rat contractile proteins, including myosin heavy chain (MHC), actin, cardiac troponin I and myosin light chains (MLC1 and MLC2). *O*-GlcNAc modification on normal donor hearts (49yr F and unknown) and hypertrophic obstructive cardiomyopathy myectomy samples (33yr M and 42yr M) were studied. Cardiac myofibrils were isolated and the *O*-GlcNAc groups labelled using an enzymatic labelling system in the presence of PUGNAc (inhibits *O*-GlcNAc removal enzyme *O*-GlcNAcase) and protease inhibitors. This method allows coupling of an azido modified *N*-acetylgalactosamine (UDP-GalNAz) to *O*-GlcNAc using the mutant enzyme Y289L β1,4-galactosyltransferase (Y289L GalT). The labelled groups were detected by reacting the azide group with an alkyne bearing the tetramethylrhodamine (TAMRA) fluorescent tag for direct imaging following SDS-PAGE. The gel was post-stained with a total protein stain for analysis with densitometry. The labelling process showed no impact on myofibril protein profiles when native and labelled myofibrils were compared. Preliminary results showed that *O*-GlcNAcylation profiles vary between samples with a total of 7 proteins identified. Strong TAMRA signals from α-actinin and MLC1 were observed in all the four myofibrils samples. In three of the samples the proteins actin, tropomyosin (Tm) and myosin binding protein-C (MyBP-C) were positively labelled. MHC and desmin *O*-GlcNAcylation were observed in one of the four subjects. This enzymatic labelling method will be investigated further for possibility of quantification and methods for mapping sites of these modifications with mass spectrometry will be explored.

3744-Pos

Cannabinoid (CB) Receptors are not Involved in A-955840 Induced Negative Inotropic Effects in Isolated Cardiac Myocytes

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A-955840, a selective CB_2 agonist, has been shown to elicit concentration-dependent decreases in cardiac contractility in the anesthetized dog (decreased maximal velocity of left ventricular pressure development [LV dP/dt max]). However, it is unknown whether this represents a direct effect or a response dependent on other factors (such as autonomic tone and neurohumoral factors) present in vivo. This study examined if A-955840 had a direct effect on contractility of isolated cardiac myocytes, and if so to determine the potential involvement of CB_1 and CB_2 receptors. Contractility was assessed in vitro using percent changes in maximal shortening velocity of sarcomeres (dL/dt max) and fractional shortening of sarcomere length (FS) in rabbit left ventricular myocytes. A-955840 reduced dL/dt max and FS in a reversible and concentration-dependent manner with an IC_{50} of 11.4 μg/mL (based on dL/dt max) which is similar to the IC_{50} value of 5.5 μg/mL based on the effects of A-955840 on LV dP/dt max in anesthetized dogs. A-955840 (4.0 μg/mL) reduced myocyte contractility (%FS) to a similar extent in the absence and presence of a CB_2 antagonist, SR-2 (24.0 ± 3.4 vs. 23.1 ± 3.0 %, n=5) or a CB_1 antagonist, rimonabant (18.8 ± 2.3 vs. 19.8 ± 2.7 %, n=5). A-955840 (4.0 μg/mL) also reduced L-type calcium current of rabbit ventricular myocytes (1.05 ± 0.11 vs. 0.70 ± 0.12 nA, n=5, $P<0.01$). These results suggest that A-955840 exerts direct negative inotropic effects on isolated rabbit ventricular myocytes, which is mediated by neither CB_1 nor CB_2 receptors, and consistent with off-target negative inotropy mediated by inhibition of the cardiac L-type calcium current.

3745-Pos

Effects of Development of Compensatory Hypertrophy on Force Frequency and Beta-Adrenergic Responses

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Background: The progression to heart failure involves two steps: compensatory hypertrophy, a process where contractile function is enhanced and myocyte size is enlarged, is followed by decompensatory hypertrophy and myocardial weakening. In our current study we aim to understand the temporal resolution of the development of compensatory hypertrophy through the analysis of functional and molecular changes. At present, we are assessing the impact of these changes on contractile modulators through analysis of effects on frequency and beta-adrenergic regulation.

Methods: Trabeculae are excised from the right ventricular free wall of New Zealand White rabbits. Thin linear muscle preparations are then suspended in culture media at high preload and stimulated to contract for up to 24 hours at 1 Hz. At 0-2, 6-8, 12, 18, or 24 hours the effects on either frequency-dependent or beta-adrenergic regulation are measured. Data is analyzed in real-time through customized Labview software.

Results: We have shown that during the progression of compensatory hypertrophy the beta-adrenergic response shifts to the left indicating a higher sensitivity with increasing culture time. Changes in frequency from 1 to 4 Hz of trabeculae cultured from 0-24 hours lead to an increase in developed force of 37% at 0-2 hours (n=5), 53% at 6-8 hours (n=7), 20% at 12 hours (n=6), 30% at 18 hours (n=6) and a decrease of 69% at 24 hours (n=4). This suggests that the

contractile reserve decreases with consideration of protein characteristics governing the force-frequency relationship. However, the molecular alterations involved in the beta-adrenergic response lead to an increase in sensitivity. By following contractile function over time, and assessing the impact of physiologically relevant modulators of function, we will obtain a temporal resolution of cardiac function in its transition from the healthy to the diseased state.

3746-Pos

A Novel Pleiotropic Effect of Statins: Enhanced Cardiomyocyte β2-Adrenoceptor Responsiveness

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Pleiotropic effects of statins on endothelial cells, vascular smooth muscle cells and fibroblasts are well-established and contribute to reduced cardiovascular morbidity and mortality. Here we test whether these effects extend to the cardiomyocyte. Adult rat ventricular cells were maintained in culture +/- 10 μM simvastatin (SIMV). After 48h, shortening and $[Ca^{2+}]_i$ responses to β1-adrenoceptor stimulation were identical in SIMV and control cells, but a marked SIMV potentiation of the β2 response was seen (Figure 1). Statins mediate their effects through cholesterol-dependent and -independent pathways. Caveolae are cholesterol-dependent signalosomes that limit the magnitude of β2, but not β1, responses in the adult cardiomyocyte. Therefore we surmised that SIMV's effects could be mediated through caveolar disruption. Indeed, the mean density of caveolae (visualised by electron microscopy) was reduced in SIMV-treated cells (0.63 ± 0.08 vs. 0.86 ± 0.11 μm^{-1}; $P<0.05$; n=9 cells from 3 hearts). This is the first demonstration of effects of statins on the β responsiveness of the adult cardiomyocyte. These data suggest a novel mechanism for the beneficial effects of statins in heart failure - enhancing the contractile reserve of the failing heart, through effects on the caveolar signalosome.

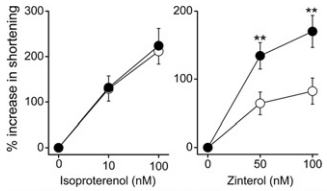

Figure 1. Simvastatin (●) selectively enhances the inotropic response of the cardiac myocyte to β2, but not β1, adrenoceptor stimulation compared with controls (○). Similar effects on $[Ca^{2+}]_i$ responses to β-stimulation were seen. Data are from n=24-28 cells; 7 hearts. ** $P<0.01$ vs control; Student's t-test.

Intracellular Cargo Transport

3747-Pos

Real Time Visualization of Axonal Transport of GTPase Rab7 in Rat Embryonic Dorsal Root Ganglia

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Charcot-Marie-Tooth (CMT) neuropathy, characterized by severe sensory neuron loss, is the most common inherited disorder of the peripheral nervous system. Several GTPase Rab7 protein mutants, mainly targeted to the highly conserved amino acid, have been identified in CMT type 2B. Exact mechanism of how such point mutations cause malfunction of neurons is not well understood. Here, we studied how those Rab7 mutations affect their axonal transport in primary rat dorsal root ganglia neurons. Real time fluorescence imaging revealed that Rab7-containing endosomes engage in bi-directional transport in axons, similar to that of TrkA receptors in the same culture. However, the speed of Rab7 transport is significantly slower than that of TrkA. In addition, there is a clear variation in the speed of axonal transport between wild-type Rab7 and mutated Rab7 proteins. Our work suggested that point mutations of Rab7 proteins could potentially cause or contribute to CMT2B neurodegenerative disease by regulating its axonal transport process.

3748-Pos

Modeling Cytoskeletal Dynamics and Vesicle Movements in Growing Pollen Tubes

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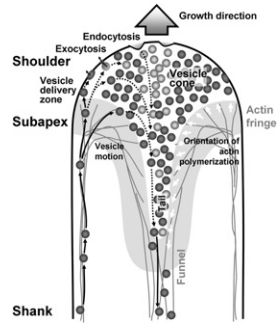
Intracellular cargo transport is a crucial process in growing plant cells. Since cellular expansion in walled cells entails the continuous assembly of new wall material, enormous amounts of polysaccharides need to be delivered to the growth site. In the rapidly elongating pollen tube the spatio-temporal movement pattern of exocytotic vesicles is precisely targeted and controlled by the continuously polymerizing actin cytoskeleton in the subapical region of the cell. Remarkably, the cone-shaped target region at the apical pole of the cylindrical cell does not contain much filamentous actin. We model the vesicular trafficking using as boundary conditions the expanding cell wall and the actin array forming the subapical actin fringe. Dynamic advancement of this actin fringe was obtained by imposing a steady shape and constant polymerization rate of the actin filaments. Letting vesicle flux into and out of the apical region be determined by the orientation of the actin microfilaments was sufficient to generate a flow that corresponds in magnitude and orientation to that observed experimentally. This model explains how the cytoplasmic streaming pattern in the apical region of the pollen tube can be generated without the presence of filamentous actin.



3749-Pos

Velocities of Microtubule-Based Motors in Living *Chlamydomonas*

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Reports in the published literature suggest that the velocities of vesicle transport in living neurons are discrete and quantal (multiples of a fundamental velocity), with the instantaneous velocity being dependent upon the number of molecular motors driving transport (Shtridelman *et al*., *Cell Biochem. Biophys*, 2008). We similarly observed discrete changes in the velocity of microspheres undergoing saltatory transport on the flagella of *Chlamydomonas*, and that these velocities appeared to be dependent upon location along the flagellum. We therefore studied the movements of adherent microspheres on flagella, driven by the intracellular motors kinesin-2 and dynein-2, to determine whether transport is driven at multiple, discrete velocities and whether they are spatially correlated. We measured separately the translational velocities of unconstrained and optically trapped microspheres as a function of position along the flagellum. The velocities of unconstrained microspheres were on average about two-fold higher than those of trapped microspheres. Unconstrained microsphere velocities in the anterograde and retrograde directions were not spatially correlated except at turn-around points near the beginning and end of the flagellum where velocities were consistently lower. Histograms of these data showed a broad distribution of velocities and suggested no strong evidence for quantized velocities. For trapped microspheres, for a given anterograde or retrograde transport event we often saw at least two discrete velocities; however, any two transport events can have different 'slow'and 'fast'velocities. Thus even when velocities are measured from a single microsphere at a specific position on a flagellum, combining multiple velocity histograms results in an apparently non-quantized, broad distribution of velocities. What causes the abrupt change between discrete velocities during movements of a microsphere is yet unknown.

3750-Pos

How the Flagellum Measures Its Length

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The intraflagellar transport (IFT) particle injector controls eukaryotic flagellar length. The injector works by restricting the availability of new material for growth of the organelle, analogous to a fuel injector controlling the speed of a single piston engine by limiting fuel in the piston. Using quantitative TIRF microscopy and computational image processing we measure GFP-tagged IFT proteins KAP and IFT27 in Chlamydomonas reinhardtii flagella over a range of cellular and flagellar states (i.e. regenerating cell vs steady state cell and short flagellum vs long flagellum). From measuring the IFT particles in the flagellum, we then back-calculate the behavior of the IFT particle injector. We then derive mathematical models for the system that controls the IFT particle injector, finding that our data are consistent with a two-state time of flight model and not a diffusing signal or a constant IFT particle number model as previous studies have suggested. These results indicate that the group of proteins responsible for the injector behavior includes a two-state protein, such as a GTPase, that travels the length of the flagellum to measure the flagellar length. A mutant in this putative protein with either a constitutive excited state or a constitutive ground state would then have abnormally long or abnormally short flagella respectively. Our results further indicate that the flagellar length is set by the flagellum rather than the cell, which implies that the organelle can self-regulate, to some extent independent of the cell.

3751-Pos

On the Movement of Cargo Driven by Molecular Motors and the Asymmetric Exclusion Processes

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