The N-terminal domains of cMyBPC inhibit the activation of the steady state ATPase myosin by f-actin. However, in the presence of native cardiac thin filaments.the N-terminal domains C1C2 and C0C2 of mouse and human cMyBPC produce biphasic effects on the steady state ATP hydrolysis of cardiac myosin-S1. That is, ATPase is activated at low ratios of cMyBPC-N-terminal domain to thin filament and is inhibited by higher ratios similar to the effects observed with f-actin. These data suggest that low ratios of cMyBPC N-terminal domains activate thin filaments in a mechanism similar to that of rigor myosin-S1 but higher ratios inhibit the ATPase rate by competing with myosin-S1-ADP-Pi binding to actin and thin filaments. Effects also appear species-dependent since the C0C1 domains of human cMyBPC produce similar effects as C1C2 and C0C2 on ATPase in the presence of thin filaments, but mouse C0C1 does not produce significant activation.

Cellular Pathways & Networks

811-Pos Board B580

Meclofenamate Sodium, a Non-Steroidal Anti-Inflammatory Drug, Directly Interacts with the Proteasome and Causes Cell Death in H9c2 Cardiac Cells

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In the United States, 30 billion dosages of non-steroidal anti-inflammatory drugs (NSAIDs) are consumed annually to relieve fever, pain, and inflammation. While NSAIDs are generally considered safe, some including meclofenamate sodium (MS) have been reported to increase the risk of cardiovascular events. Because the impairment of the proteasome, a multi-catalytic complex that breaks down 60-80% of proteins in the mammalian cell, has been associated with heart failure, the effects of MS on the proteasome activity were investigated. MS at 30 micromolar inhibited purified 20S proteasome activity by 40% and 26S proteasome activity in H9c2 rat cardiac cells and mouse heart tissue lysates by 30-40%. MS was also shown to decrease the cell proliferation rate and muscle differentiation of H9c2 cells. To investigate the mechanism of action of MS, H9c2 cells were treated with MS and the fluorescent proteasome inhibitor MV151 that bind to beta 1, beta 2, and beta 5 enzymatic sites of the proteasome. MS, in a concentration-dependent manner, competed with MV151, indicating that MS may exert its inhibitory effects by directly binding to the proteasome's active sites. At the mRNA level, 100 micromolar MS increased the expression of genes involved in stress response and antioxidant metabolism, such as glutathione S-transferase omega. When H9c2 cells were pre-treated with 3 millimolar tempol, a membrane-permeable radical scavenger, then with 200 micromolar MS for 24hr, tempol recovered the beta 5 26S proteasome activity by 20-40%. However, tempol and other antioxidants such as N-acetyl cysteine and L-ascorbic acid (vitamin C) alone inhibited the proteasome activity by 20-50%. MS does not increase the caspase-3 activity at 10-30 micromolar concentrations, suggesting that apoptosis is not involved in the decreased cell viability in MS treated H9c2 cells.

812-Pos Board B581

The Intrinsic Hyperexcitability of SMA Motor Neurons is a Result of Dysfunctional Spinal Circuitry

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Spinal muscular atrophy (SMA) - the most common genetic cause of death in infancy - is a motor neuron disease caused by reduced expression of the survival motor neuron (SMN) protein. It is currently unknown why reduction in SMN, leads to selective motor deficits and muscle weakness. Using the SMA-Δ7 mouse model, we have previously reported that the sensory-motor circuit is dysfunctional early in the disease and precedes any motor neuron loss. Motor neurons in the L1 segment, innervating proximal muscles, are abnormally hyperexcitable and more affected than L5 motor neurons, innervating distal hindlimbs early after birth. To test whether this hyperexcitability is either due to synaptic dysfunction or SMN deficiency *per se* in motor neurons, we recorded intracellularly from L5 motor neurons at a time (P4) when there was no significant functional synaptic impairment.

We employed the *in vitro* intact spinal cord preparation. Whole-cell patch clamp recordings revealed that the intrinsic properties of SMA L5 motor neurons were not significantly different than wild type (WT) age-matched counterparts. The passive membrane property of input resistance was on average 72.7 ± 18.1 MΩ in WT (n=10) and 53.9 ± 21.7 MΩ in SMA (n=5) motor neurons. Similarly, the time constant was 1.9 ± 0.5 ms in WT and 2.0 ± 0.7 ms in SMA. Furthermore, the active membrane properties of: Rheobase (WT: 0.7 ± 0.2 nA; SMA:0.8 ± 0.2 nA), $V_{threshold}$ (WT: -31.6 ± 3.7 mV; SMA: -33.6 ± 2.6 mV), action potential amplitude (WT: 72.8 ± 5.2 mV; SMA: 73.2 ± 3.6 mV) and rate of rise (WT: 48.8 ± 6.2 mV/ms; SMA: 40.2 ± 10.0 mV/ms) were not significantly different (t-test).

These results indicate that the intrinsic hyperexcitability of SMA motor neurons stems from dysfunctional spinal circuits and raise the possibility that SMA may be a disease of motor circuits acting via non-cell autonomous mechanisms.

813-Pos Board B582

Aldose Reductase Inhibition or Activation of Transketolase Offset Adverse Metabolic Remodeling Improving Function in Type 2 Diabetes Myocytes Exposed to Hyperglycemia

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In type 2 diabetes (T2DM), hyperglycemia (HG) and increased sympathetic drive may alter mitochondria energetic/redox properties, decreasing the organelle's functionality. These perturbations sustain basal low-cardiac performance and limited exercise capacity. We have recently reported that improving the redox/energetic balance of T2DM (*db/db*) murine cardiomyocytes/hearts with GSH or palmitate (Palm) preserves contractile performance under high-energy demand imposed by combined HG and β-adrenergic stimulation. To further understanding the metabolic basis of Palm salutary action, we first applied metabolomics to Langendorff-perfused T2DM murine hearts subjected to energy/redox stress conditions. In the absence of Palm, HG activates polyol pathways (sorbitol, glycerol, xylitol) triggering flux limitations in glycolytic and pentose phosphate (PP) pathways. The metabolite profile under Palm reveals that this fatty acid (FA) reverses the detrimental action of HG by decreasing polyol accumulation and increasing flux through PP, glycolytic, and beta-oxidation pathways, with a concomitant improvement in cardiomyocyte contraction. To confirm this observation, next we inhibited the polyol or activated the PP pathways. Preincubating HG+ISO-treated myocytes with the aldose reductase inhibitor Zopolrestat (1μM) improved the adrenergic inotropic response, in parallel with the re-activation of glycolysis and oxidative phosphorylation. These same effects were observed pre-incubating HG+ISO challenged myocytes with the activator of transketolase benfotiamine (50μM). Present data indicate that in T2DM hearts HG/ISO regimen unveils a status of mitochondrial dysfunction that favors adverse intracellular redox/energetic conditions and a metabolic remodeling that implies a glucose shunt to polyol pathways and an apparent blockade of glycolysis. These changes appear to be relieved by the exogenous administration of the FA Palm.

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Intracellular Transport Control of Signaling Networks

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Many cellular networks rely on the regulated transport of their components to transduce extracellular information into precise intracellular signals. The dynamics of these networks is typically described in terms of compartmentalized chemical reactions. There are many important situations, however, in which the properties of the compartments change continuously in a way that cannot naturally be described by chemical reactions. Here, we develop an approach based on transport along a trafficking coordinate to precisely describe these processes and we apply it explicitly to the TGF-β signal transduction network, which plays a fundamental role in many diseases and cellular processes. The results of this newly introduced approach accurately capture the distinct TGF-β signaling dynamics of cells with and without cancerous backgrounds and provide an avenue to predict the effects of chemical perturbations in a way that closely recapitulates the observed cellular behavior.

815-Pos Board B584

Import of Short Peptides in Yeast as a Bistable Switch

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Multistability in biological systems is an important concept that is believed to underlie phenotypic diversity in microorganisms as well as tissue differentiation at the multicellular level. Despite the importance of the subject, understanding of this phenomenon derives from a very small group of extensively studied examples. We are working to identify novel biological networks that exhibit bistability in nutrient uptake. In particular, we are investigating uptake