1847-Pos Board B617
Different Capacity for Store-Operated Ca^{2+} Entry and Ca^{2+} Extrusion Across the Plasma Membrane of Wild-Type and Dystrophic mdx Mouse Muscle
Tanya R. Cully[1], Joshua N. Edwards[1], Robyn M. Murphy[2], **Bradley S. Launikonis**[1].
[1]University of Queensland, Brisbane, Australia, [2]La Trobe University, Melbourne, Australia.
Store-operated Ca^{2+} entry (SOCE) is a ubiquitously expressed signalling system that is highly specialized in skeletal muscle. ''Deregulated'' SOCE had been proposed as a pathway for Ca^{2+} entry into dystrophic muscle that leads to fibre degradation. We recently showed that this mechanism remains tightly regulated in mdx mouse muscle but the integral SOCE proteins, STIM1 and Orai1, are upregulated 3-fold (Edwards et al., 2010). We now report that the newly identified isoform STIM1L (Darbellay et al., 2011) is upregulated 1.8-fold in mdx muscle. We found SOCE recorded in skinned fibres was 2-fold greater in mdx compared with WT for the same SR Ca^{2+} release amplitude. However cytoplasmic fluo-4 transients in depleted intact fibres showed SOCE in the absence of a sarcoplasmic reticulum (SR) Ca^{2+} pump blocker to be of reduced influx rate in mdx compared to WT fibres. A similar level of SR Ca2+ reloading was detemined in both muscle types following SOCE deactivation. Fura-2 imaging in intact fibres in the presence of 50 μM cyclopiazonic acid (CPA) and no external Ca^{2+} showed that more Ca^{2+} remained in the cytoplasm of mdx compared to WT fibres following SR depletion suggesting that Ca2+ extrusion by the plasma membrane Ca^{2+}-ATPase (PMCA) is restricted in mdx. This helps explain reduced SOCE in intact mdx fibres, as the washout of CPA following SR depletion resulted in the greater amount of trapped cytoplasmic Ca^{2+} re-entering SR to cause a greater degree of SOCE deactivation before externally applied Ca^{2+} could enter the fibre. These results suggest that store-dependent Ca^{2+} influx is greater and PMCA is restricted in its capacity to extrude Ca^{2+} in mdx compared to WT fibres.

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Skeletal Respiratory Muscle in a Model of Chronic Obstructive Pulmonary Disorder
Patrick Robison[1], Erick Hernandez-Ochoa[1], Ramzi J. Khairallah[1], Thomas E. Sussan[2], Christopher W. Ward[3], Shyam Biswal[2], Martin F. Schneider[1].
[1]University of Maryland School of Medicine, Baltimore, MD, USA, [2]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA, [3]University of Maryland School of Nursing, Baltimore, MD, USA.
In Chronic Obstructive Pulmonary Disorder (COPD), mechanical deformations or work overload of the respiratory skeletal muscles may lead to adaptive or maladaptive muscle responses. Sarcomeric deletion, myosin type changes and metabolic alterations have all been observed in respiratory muscles. While the diaphragm has been extensively studied, other respiratory muscles have not been closely examined in this context. Here we apply a new technique for generating primary cultures of intercostal muscle (ITC) fibers to an established mouse model of COPD, thus isolating changes in individual muscle fibers from the general defects in pulmonary function known to occur in COPD. Single ITC fibers were enzymatically dissociated and either loaded with fluorescent calcium indicators or mounted using a novel muscle stretch tool to measure their length-tension relationship as well as passive tension over sarcomere length. Using the ratiometric indicator indo-1, we found a substantial reduction in peak calcium of action potential induced calcium transients in COPD ITC (405/485 ratio; 1.22 vs. 0.95, p=0.003 vs control), but no significant changes in resting calcium concentration (p=0.33). Data taken with MagFluo-4 agree qualitatively with the indo-1 data and show a shortening of the overall decay phase in the ITC fibers of COPD animals. To our knowledge, these data represent the first ex-vivo assessment of the intercostal muscles in COPD. Supported by NIH Grants R01-AR056477, R01-AR055099, T32-AR007592 and T32-HL072751.

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Detection of Sub-Cellular Reactive Oxygen Species in Skeletal Muscle
Poulami Basu Thakur, **George G. Rodney**.
Baylor College of Medicine, Houston, TX, USA.
Our recent studies suggest that sub-cellular site-specific ROS production is likely to play an important role in the response of skeletal muscle to oxidative stress. Using redox active probes we have targeted NADPH oxidase (Nox2), mitochondria, cytosolic glutathione, and the endoplasmic/sarcoplasmic reticulum to investigate these various sources of ROS under physiological and pathophysiological conditions. We show that mechanical stretch increases ROS produced from Nox2. Repetitive contractile activity increases ROS produced from Nox2 and results in activation of the cytosolic glutathione redox cycle. Under conditions that promote ER stress and apoptosis our ER/SR redox probe becomes more oxidized. Understanding the sub-cellular signaling pathways by which oxidants influence muscle function will allow for the development of targeted therapeutic interventions to combat the deleterious effects of hypoxia/reoxygenation, sustained contractile activity as well as skeletal muscle diseases.

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Enriching Satellite Cells with α2δ1 Promotes Differentiation
Tammy Tamayo, Jesús García.
UIC, Chicago, IL, USA.
Cell transplants into skeletal muscle of patients with muscular dystrophy are limited by donor cell attachment, migration, and survival in the host tissue. In animal models, despite HLA matching and a reduction of the host's immune response, few donor cells are retained in the host muscle. Enriching cells for a surface marker that enhances ability of the cell to attach, migrate, and survive will result in improved cell retention. The purpose of this study was to determine whether α2δ1-enriched primary satellite cells were better candidates for cell transplant than satellite cells without this surface marker. The α2δ1 subunit is part of the L-type calcium channel but appears earlier than the other subunits. We isolated satellite cells from the hind limb muscles of neonatal mice and separated four subpopulations of cells based on the presence or absence of α2δ1 and a marker of quiescence, CD34, by fluorescence activated cell sort (FACS). Satellite cells enriched with α2δ1 survived in heat deactivated media past day 19, while there was no evidence of attachment or survival of cells without α2δ1. In addition, cells that were positive for α2δ1 and negative for CD34 demonstrated the most robust myogenesis out of all four subpopulations. Enhanced myogenesis of this subpopulation was determined by morphology, the pattern of expression of myogenic transcription factors, and the development of excitation-contraction coupling as demonstrated by the presence of L-type calcium currents and calcium transients. As the data relate to differentiation and survival, these results suggest that cells enriched with α2δ1 and without CD34 will demonstrate greater cell retention and force generation after satellite cell transplant, which is a strong candidate for therapy in muscular dystrophy.
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The $Ca_V\beta1$ Subunit Regulates Gene Expression in Muscle Progenitor Cells
Jackson Taylor[1], Tan Zhang[1], Laura Messi[1], Jiang Qian[1], Cristina Furdui[1], Claudia Hereñú[2], Osvaldo Delbono[1].
[1]Wake Forest University School of Medicine, Winston-Salem, NC, USA, [2]National University of La Plata, La Plata, Argentina.
CaVβ subunits are traditionally considered constituents of CaV complexes (CaV1or2, CaVβ, and CaV α2/δ), where they localize at the plasma membrane and serve to regulate channel expression and gating properties. Recent publications also show CaVβ subunit localization in the nucleus. This phenomenon has been observed under a variety of conditions (different cell types, β subunit isoforms, co-expressed proteins, etc). However, the mechanisms responsible for CaVβ subunit nuclear shuttling, as well as a physiological role for this nuclear localization, remain major questions. Ongoing work in our laboratory has shown that muscle progenitor cells (myoblasts) express CaVβ1 protein (but not CaV1 subunits) in both the cytoplasm and nucleus and that the loss of CaVβ1 expression impairs proliferation in these cells. To better understand the mechanisms that link CaVβ1 nuclear localization with control of proliferation, we have conducted large-scale screening experiments designed to identify which genes are directly regulated by CaVβ1, as well as its protein binding partners. To test if CaVβ1 may regulate gene expression, we conducted microarray experiments on RNA extracted from wild type, heterozygous, and CaVβ1 -null mouse primary myoblasts. A number of gene transcripts were found to be differentially regulated based on the relative amount of CaVβ1 expression. To identify specific CaVβ1 target genes, we performed chromatin immunoprecipitation -on-a-chip experiments to locate which promoter regions CaVβ1 bound to across the entire mouse genome. Nuclear binding partners of CaVβ1 were screened using affinity purification of CaVβ1a-YFP from myoblast nuclear fractions coupled with mass spectrometry. Finally, the importance of CaVβ1 in embryonic myogenesis was explored in CaVβ1 -null mice. Our results support the idea of CaVβ subunits acting as transcription factors and regulating gene expression independently from CaV's, and suggest these functions may be particularly important to progenitor cell growth.

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Mechanism of HMG-CoA Reductase Inhibitor-Induced Contractile Dysfunction in Rat Cultured Skeletal Muscle
Kazuho Sakamoto, Syoko Tanaka, Masaya Yamamoto, Anna Mizuno, Tomoyuki Ono, Satoshi Waguri, Junko Kimura.
Fukushima Medical University, Fukushima, Japan.
HMG-CoA reductase inhibitors (statins), cholesterol-lowering drugs, cause contractile dysfunction of skeletal muscles as an adverse effect. We investigated the mechanism underlying this effect in cultured myofibers isolated