muscles. We hypothesize that nifedipine, reduces basal ATP release thereby decreasing purinergic receptor activation, which in turn reduces [Ca^{2+}]r and muscle destruction in mdx skeletal muscles. Funding: FONDECYT 1110467, Proyecto Anillo ACT1111 and AFM14562 (EJ, MC), NIH AR43140, AR052534 (PDA), U-INICIA VID 2011, U-INICIA 02/12M (MC).

Excitation-Contraction Coupling II

3683-Pos Board B411

TRIC-A Prevents Store-Overload Induced Calcium Release Through Interaction with the Cardiac Ryanodine Receptor

Xinyu Zhou[1], Ki Ho Park[1], Pei-hui Lin[1], Mingzhai Sun[1], Zui Pan[1], Miyuki Nishi[2], S.R. Wayne Chen[3], Hiroshi Takeshima[2].
[1]The Ohio State University, COLUMBUS, OH, USA, [2]Kyoto University, Kyoto, Japan, [3]University of Calgary, Calgary, AB, Canada.

TRIC-A and TRIC-B are trimeric intracellular cation channels located at the sarcoplasmic reticulum (SR) or endoplasmic reticulum (ER) of multiple cell types. These channels regulate the permeability of K ions across the SR/ER and consequently the movement of Ca ions during excitation-contraction coupling. Previously we showed that genetic ablation of TRIC led to compromised K-permeability and Ca release across the SR membrane, supporting the hypothesis that TRIC could function as counter-ion channels that allows the flow of K ions into the SR during the acute phase of Ca release. In the absence of TRIC, overload of Ca inside the ER/SR causes instability of Ca storage and release, leading to stress-induced dysfunction of multiple tissues. Spontaneous Ca waves, also called store overload-induced Ca release (SOICR) mediated by the type 2 ryanodine receptor (RyR2), evoke ventricular tachyarrhythmia in individuals with heart failure. Our biochemical studies revealed that the carboxyl-tail domain of TRIC-A could interact with the RyR channel, suggesting the possibility that TRIC-A may directly regulate the Ca release activity. We found that TRIC-A, but not TRIC-B, prevented the appearance of SOICR in HEK293 cells expressing RyR2. Cytosolic Ca measurement by Fura-2 and ER luminal Ca measurement by D1ER revealed that expression of TRIC-A in HEK293 cells could prevent overload of Ca inside the ER by targeting the RyR2 channel function. Such effect was translated into suppression of SOICR. These effects appeared to be specific for TRIC-A, as co-expression of RyR2 with TRIC-B did not affect SOICR. Together, our data suggest that functional interaction between TRIC-A and RyR can modulate the Ca release process from internal stores and regulate Ca homeostasis across the ER/SR.

3684-Pos Board B412

Nadph Oxidase-Induced Oxidative Stress Impairs Autophagy in *Dystrophic Skeletal Muscle*

Rituraj Pal, James A. Loehr, Shumin Li, Michela Palmieri, Marco Sardiello, **George G. Rodney**.
Baylor College of Medicine, Houston, TX, USA.

Duchenne muscular dystrophy (DMD) is a fatal degenerative muscle disease, attributed to a defect in the gene that encodes dystrophin. Emerging evidence implicates oxidative stress may impair autophagy in DMD patients and *mdx* mice, a model of DMD, by activating cytotoxic mediators. However, the specific source of ROS and the mechanism(s) of impaired autophagy have not yet been elucidated in dystrophic muscle. Therefore, understanding the interaction between oxidative stress and defects in autophagy is pivotal as we seek effective therapeutic targets in DMD. Here we demonstrated that nicotinamide adenine dinucleotide phosphatase (NADPH oxidase or Nox2)-induced oxidative stress was linked to impaired autophagy in *mdx* mice through Nox2-dependent superoxide production, Src kinase activation and further NOX2 activation via $p47^{phox}$ phosphorylation. The defect in autophagy was accompanied by persistent activation of Src kinase, which activated the autophagy repressor mammalian target of rapamycin (mTOR) via PI3K/Akt phosphorylation. Inhibition of Nox2 or Src kinase reduced oxidative stress and partially rescued the defective autophagy in *mdx* mice. We also have genetically down-regulated Nox2 activity in the *mdx* mouse to further corroborate that NADPH oxidase was the main source of oxidative stress, which impaired autophagy in DMD. Our data highlights novel pathogenic aspects of DMD and proposes NADPH oxidase as a potential therapeutic target.

3685-Pos Board B413

Local Redox Modifications in Skeletal Muscle Differentially Affect Sarcoplasmic Reticulum Calcium Release and Muscle Force Generation

Arthur J. Cheng, Joseph D. Bruton, Håkan Westerblad, Johanna T. Lanner.
Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden.

Aims: Reactive oxygen species are implicated in reducing muscle force production through oxidative cellular damage in skeletal muscle following repetitive fatiguing muscle contractions. We investigated whether shifting cellular redox status from an oxidized to reduced state could improve recovery of force production in skeletal muscle following fatigue. Methods: Mechanically-dissected intact single fibers from flexor digitorum brevis muscles of C57/BL6 mice were fatigued at physiological temperature (32 °C) with brief 150 ms duration 70 Hz tetani every 1 s for a total of 60 contractions. Fibers were superfused with the reducing agent dithiothretrol (DTT 1 mM, n = 10) for 20 min after fatigue-induced force loss was established. Results: The reducing agent, DTT, temporarily improved low-frequency (30 Hz) contractile force by 65% by increasing myofibrillar Ca^{2+} sensitivity without affecting sarcoplasmic reticulum (SR) Ca^{2+} release during recovery from fatigue. Intriguingly, addition of the oxidizing agent tert-butyl hydroperoxide (T-BOOH 10 μM, n=7) also temporarily improved 30 Hz force by 47% without affecting SR Ca^{2+} release. We then determined whether antioxidants and inhibitors of free radical production could prevent in the first place the oxidative damage associated with fatigue. We found that a mitochondria specific antioxidant (SS-31) and nitric oxide synthase inhibitor (L-NAME) recovered fatigue-induced impairments in SR Ca^{2+} release without affecting 30 Hz force. Conclusion: Antioxidants and inhibition of free radicals improves Ca^{2+} release during recovery, but has no effect on improving low-frequency force production. On the other hand, acute change in intracellular milieu using redox agents improves force development but does not affect Ca^{2+} release. Thus, proteins involved in SR Ca^{2+} release and myofibrillar contractile proteins show different dependency on the local redox micro-environment.

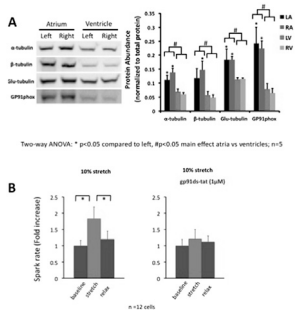
3686-Pos Board B414

Stretch-Dependent Sub-Cellular Ca^{2+} Signaling in Atrial Myocytes

Maura Greiser, Benjamin L. Prosser, Ramzi Khairallah, Chris W. Ward, W. Jon Lederer.
University of Maryland, Baltimore, MD, USA.

Atrial myocytes undergo stretch during diastole as do ventricular myocytes. We have identified a novel mechanism that links cellular stretch in ventricular myocytes to the tuning of Ca^{2+} release from the sarcoplasmic reticulum (SR). This mechanism, "X-ROS signaling", depends on NOX2 (NADPH oxidase) to generate reactive oxygen species (ROS), which increases the triggering sensitivity of ryanodine receptors (SR Ca^{2+} release channels) to [Ca^{2+}]i. Stretch mediates X-ROS signaling through microtubules, which appear to interact with NOX2 to enable it to generate ROS.

We evaluated X-ROS signaling in murine atrial myocytes. Ca^{2+} sparks were recorded before, during and after stretch. Ca^{2+} spark frequency increased during stretch and returned to pre-stretch values during relaxation. Interestingly, microtubule density was higher and protein expression levels were increased in atria compared to ventricles (Fig 1 A). Similarly, protein expression of the catalytic NOX2 subunit gp91phox was higher in atria compared to ventricles (Fig 1 A). Inhibition of gp91phox with the inhibitory peptide gp91ds-tat prevented stretch-induced Ca^{2+} spark increases in atrial myocytes (Fig 1 B). Thus, X-ROS signaling is important in atrial myocytes and may contribute to physiological regulation of Ca^{2+} signaling and to Ca^{2+} dependent arrhythmogenesis in disease.



3687-Pos Board B415

Amelioration of Ischemia-Reperfusion Induced Muscle Injury by the Recombinant Human MG53 Protein

Hua Zhu[1], Janet L. Roe[2], Ki Ho Park[1], Tao Tan[1], Pei-hui Lin[1], Jianjie Ma[1], Thomas J. Walters[2].
[1]Department of Surgery, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA, [2]Extremity Trauma and Regenerative Medicine, US Army Institute of Surgical Research, Fort Sam Houston, TX, USA.

Acute compartment syndrome (CS) in skeletal muscle is caused by lack of blood supply due to the rise of compartment pressure after various injuries. CS requires timely surgical treatment, known as fasciotomy, to reduce the compartmental pressure and re-establish blood circulation. In previous studies, we found that MG53 plays an important role in membrane repair in multiple tissues. Here we test whether recombinant human MG53(rhMG53) protein can protect muscle injury associated with CS. With our suture-induced hindlimb CS mouse model, we found that the *mg53-/-* muscle released more creatine kinase (CK) into blood circulation as compared to wild type control