deep transient store depletion which in turn switched on SOCE in a wild type cell. The peak of the Ca^{2+} transient (triggered by the entry of the external Ca^{2+} into the cytoplasm following the internal store depletion) was normalized to the peak of the SR Ca^{2+} release transient (1.06 ± 0.11, n=17 vs 0.8 ± 0.08, n=9). On isolated FDB fibers under voltage clamp, we found that the voltage dependence of the normalized fluorescence of the calcium transients in response to membrane depolarizations ranging between −60 and +30 mV, with a 10 mV increments were well fitted with a Boltzmann distribution (V0.5: -23.22 ± 1.35 mV vs -24.15 ± 0.77 mV with respective k values of 6.14 ± 1.15 vs. 6.93 ± 0.65). Fatigue inducing protocols are being currently tested. Collectively, these data point toward altered Ca^{2+} homeostasis in the compact mice. Understanding the mechanisms behind them may help developing new strategies in muscle atrophy, ageing and wasting diseases. To our knowledge, we are the first to employ Mstn(Cmpt-dl1Abc) mice and perform a comparative study to characterize their Ca^{2+} homeostasis in light of SOCE.

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STIM1 Regulates Sarcoplasmic Reticulum CA^{2+}-ATPase 1A (SERCA1a) in Skeletal Muscle

Keon Jin Lee[1], Changdo Hyun[1], Jin Seok Woo[1], Chang Sik Park[2], Do Han Kim[2], Eun Hui Lee[1].
[1]The Catholic Univ. of Korea, Seoul, Korea, Republic of, [2]GIST, Gwangju, Korea, Republic of.

Stromal interaction molecule 1 (STIM1) mediates Ca^{2+} movements from the extracellular space to the cytosol through a store-operated Ca^{2+} entry(SOCE) mechanism in various cells including skeletal muscle cells. In the present study, to reveal the unidentified functional role of the STIM1 C-terminus from 449 to 671 amino acids in skeletal muscle, binding assays and quadrupole time-of-flight mass spectrometry were used to identify proteins binding in this region along with proteins that mediate skeletal muscle contraction and relaxation. STIM1 binds to sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 1a (SERCA1a) via this region (called STIM1-SBR). The binding was confirmed in endogenous full-length STIM1 in rabbit skeletal muscle and mouse primary skeletal myotubes via co-immunoprecipitation assay and immunocytochemistry. STIM1-knockdown in mouse primary skeletal myotubes decreased Ca^{2+}-uptake from the cytosol to the sarcoplasmic reticulum (SR) through SERCA1a only at micromolar cytosolic Ca^{2+} concentrations, suggesting that STIM1 could be required for the full activity of SERCA1a possibly during the relaxation of skeletal muscle. Various Ca^{2+} imaging experiments using myotubes expressing STIM1-SBR suggest that STIM1 is involved in intracellular Ca^{2+} distributions between the SR and the cytosol via regulating SERCA1a activity without affecting SOCE. Therefore, in skeletal muscle, STIM1 could play an important role in regulating Ca^{2+} movements between the SR and the cytosol.

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Accelerated Activation of SOCE Current in Myotubes from Two Mouse Models of Anesthetic- and Heat-Induced Sudden Death

Viktor Yarotskyy[1], Feliciano Protasi[2], Robert T. Dirksen[1].
[1]Department of Pharmacology and Physiology, University of Rochester, Rochester, NY, USA, [2]Center for Research on Ageing & Department of Neuroscience and Imaging, Università Gabriele d'Annunzio, Chieti, Italy.

Store-operated calcium entry (SOCE) channels play an important role in Ca^{2+} signaling. Recently, excessive SOCE was proposed to play a central role in the pathogenesis of malignant hyperthermia (MH), a pharmacogenic disorder of skeletal muscle. We tested this hypothesis by characterizing SOCE current (ISkCRAC) magnitude, voltage dependence, and rate of activation in myotubes derived from two mouse models of anesthetic- and heat-induced sudden death: 1) type 1 ryanodine receptor (RyR1) knock-in mice (Y524S/+) and 2) calsequestrin 1 and 2 double knock-out (dCasq-null) mice. ISkCRAC voltage dependence and magnitude at −80 mV were not significantly different in myotubes derived from wild type (WT), $Y524S/^{+}$ and dCasq-null mice. However, the rate of ISkCRAC activation upon repetitive depolarization was significantly faster at room temperature in myotubes from Y524S/+ and dCasq-null mice. In addition, the maximum rate of ISkCRAC activation in dCasq-null myotubes was also faster than WT at more physiological temperatures (35-37°C). Azumolene (50 μM), a more water-soluble analog of dantrolene that is used to reverse MH crises, failed to alter ISkCRAC density or rate of activation. Together, these results indicate that while an increased rate of ISkCRAC activation is a common characteristic of myotubes derived from Y524S/+ and dCasq-null mice and that the protective effects of azumolene are not due to a direct inhibition of SOCE channels.

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Inward Rectifier Potassium Channels in Mammalian Skeletal Muscle Fibers

Julio L. Vergara, Marbella Quiñonez, Carl Yu, Marino DiFranco.
Physiology, UCLA School of Medicine, Los Angeles, CA, USA.

We combined electrophysiological, optical, immunohistochemical and transgenic expression approaches to study the K inward rectifier current (IKir) in muscle fibers (FDB) from adult mice. Dissociated fibers were stained with the potentiometric dye di-8-ANEPPS and voltage clamped (at V_K) using two-microelectrodes. Extracellular [K] ranged from 4 to 96mM; intracellular K content was equilibrated with that of the micropipettes (160mM). Ionic currents other than IKir were eliminated using specific blockers and (for subtraction) IKir was blocked with 1 mM Ba, or by replacing the external solution to one containing TEA. IKir records in response to 100 ms hyperpolarizations showed rapid onsets followed by slow decays which were more prominent at large hyperpolarizations. Voltage changes at the transverse tubular system (TTS) were optically monitored and compared with radial cable model simulations incorporating a constant field/blocking particle approximation of Kir channels. Magnitude, rectification, and [K]-dependence of IKir were well-predicted using an average PK_{IR} value of $\sim 6 \times 10^{-6}$ cm/s while assuming equal density of Kir channels at the surface and TTS membranes. Western blots for Kir2.2 in crude muscle extracts and microsomal preparations reported a single protein band (~50kD). This band was also found for Kir2.1 in both preparations, but an additional band of ~55kD was seen in microsomal preparations. Two-photon imaging of muscles immunostained with specific antibodies demonstrated that both Kir isoforms are present at the surface and TTS membranes. Likewise, transfection of muscle fibers with plasmids encoding either EGFP tagged-Kir2.1 or Kir2.2 resulted in overexpression of each isoform in both membranes and an increased IKir up to ~4-fold those in control fibers. As expected, transfected fibers showed an increased threshold for AP, but unlike controls, they exhibited repetitive APs when stimulated with small long-lasting (~500ms) current pulses. Supported by NIH/NIAMS AR047664, AR041802, and AR054816.

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STIM1 Enhances SR Ca^{2+} Refilling through Activating SERCA2a in Rat Ventricular Myocytes

Guiling Zhao[1], Didier X.P. Brochet[1], Tianyu Li[2], Paul Rosenberg[2], W. Jonathan Lederer[1].
[1]Center for Biomedical Engineering and Technology, Department of Physiology, University of Maryland School of Medicine, Baltimore, MD, USA, [2]Department of Medicine, Ion Channel Research Group, Duke University School of Medicine, Durham, NC, USA.

In cardiac ventricular myocytes, the function of "stromal interaction molecule 1" (STIM1), an ER/SR Ca^{2+} sensor, is still a mystery regarding its localization, mobilization, and Ca^{2+} signaling regulation. Here, adult rat ventricular myocytes freshly isolated or in primary culture were examined. Endogenous STIM1 was found by immunofluorescence and shown to be distributed mainly within the Z-disk. Using mcherry tagged expression of STIM1, we found that re-distribution of STIM1 does not occur after SR Ca^{2+} depletion (using thapsigargin, 2 μM) nor does SR Ca^{2+} depletion affect STIM1 movement within the SR when evaluated with fluorescence recovery after photobleaching (FRAP). Consistent with this result, native protein electrophoresis showed that STIM1 exists mainly as an oligomer, which is not altered upon SR Ca^{2+} depletion. Additionally, no store-operated Ca^{2+} entry (SOCE) or Ca^{2+} release-activated Ca^{2+} current (ICRAC) was observed in control or STIM1 overexpressing ventricular myocytes. The overexpression of STIM1 did, however, have dramatic consequences in ventricular myocytes: The SR Ca^{2+} content did increase as did SR Ca^{2+} leak. Parallel investigations indicated that STIM1 physically binds to phospholamban (PLN), suggesting that overexpressed STIM1 may activate SERCA2a by regulating PLN and this may underlie the increase in SR Ca^{2+} content. The molecular signaling and regulatory pathways that may be involved in these pathways are discussed.