Experiments were carried out to investigate the possibility that store operated Ca^{2+} entry (SOCE) may be triggered by volatile anaesthetics in malignant hyperthermia susceptible (MHS) human skeletal muscle. Samples of *vastus medialis* muscle were obtained from patients undergoing assessment for malignant hyperthermia (MH) susceptibility using the standardised *in vitro* contracture test. All experiments were performed with institutional Research Ethics Committee approval and informed patient consent, according to the Declaration of Helsinki. Single fibres were mechanically skinned and confocal microscopy used to detect changes [Ca^{2+}] within the re-sealed t-tubules (with fluo-5N) or within the cytosol (with fluo-3). In normal fibres (MHN), exposure to 0.5 mM halothane failed to trigger SR Ca^{2+} release, or to induce depletion of t-tubule Ca^{2+} (n=8). However, in MHS fibres, 0.5 mM halothane induced both SR Ca^{2+} release and a rapid depletion of t-tubule Ca^{2+}, consistent with SOCE (n=8). In ~20% of MHS fibres, SR Ca^{2+} release took the form of a propagating Ca^{2+} wave and this was associated with a corresponding SOCE wave of t-tubule Ca^{2+} depletion. In MHN fibres, both SR Ca^{2+} release and SOCE could be induced by 0.5 mM halothane when the cytosolic [Mg^{2+}] was decreased to 0.2 mM (n=6). In MHS fibres, SOCE was potently inhibited by inclusion of a STIM1 blocking antibody within the re-sealed t-tubules (n=6). These data suggest (i) that in MHS fibres the degree of SR Ca^{2+} depletion induced by a clinically relevant level of volatile anaesthetic is sufficient to induce SOCE and (ii) that STIM1 located within the sarcolemma modulates SOCE.

3704-Pos

Passive Activation of Store-Operated Ca2+ Entry in Myotubes Depends on the Rate of RyR1-Dependent Ca2+ Leak

Viktor Yarotskyy, Robert T. Dirksen.
University of Rochester, Rochester, NY, USA.

In spite of extensive studies of store operated calcium entry (SOCE), the detailed mechanism of SOCE activation in skeletal muscle remains largely unknown. We recently reported that STIM1-Orai1 coupling is required for SOCE activation in myotubes. However, other proteins that control sarcoplasmic reticulum (SR) Ca2+ content may also contribute to SOCE activation. We hypothesized that passive SOCE activation in skeletal muscle depends on the rate of SR Ca2+ leak through the type 1 ryanodine receptor (RyR1). To test this hypothesis, we conducted a series of whole-cell patch-clamp measurements of SOCE current (ISOC) in myotubes obtained from normal and RyR1-null (dyspedic) mice. Myotubes were bathed in external solution containing (in mM): 138 TEA-methanesulfonate, 10 CaCl2, 10 HEPES, 1 MgCl2, 0.1 nifedipine, pH 7.4. The internal patch pipette solution contained (in mM): 140 mM Cs-methanesulfonate, 10 HEPES, 20 Na-EGTA, 4 MgCl2, pH7.4. SOCE was activated by passive SR Ca2+ depletion following intracellular dialysis with 20 mM EGTA. ISOC in myotubes exhibited many hallmarks of SOCE including strong inward rectification and inhibition by La3+, Gd3+, BTP-2, and 2-APB. ISOC current density at −80 mV was significantly ($p<0.01$) larger in normal myotubes (1.05 ± 0.09 pA/pF, n = 33) compared to that from dyspedic myotubes (0.74 ± 0.07 pA/pF, n = 18). Moreover, the speed of ISOC activation was slower in dyspedic myotubes. Specifically, the time to 10%, 50%, and 90% maximal activation were 4.95 ± 0.65 s, 15.3 ± 1.4 s, and 53.9 ± 7.5 s (n = 19), respectively, in normal myotubes and 67.5 ± 11.1 s, 114 ± 13 s, and 159 ± 17 s (n = 18), respectively, in dyspedic myotubes. These results indicate that RyR1 Ca2+ leak promotes passive SOCE activation.

3705-Pos

Expression of Functional Transgenic Alpha1s-DHPR Channels in Adult Mammalian Skeletal Muscle Fibers

Marino G. Di Franco, Marbella Quinonez, Philip Tran, Julio L. Vergara.
UCLA, Los Angeles, CA, USA.

Plasmids encoding for two variants of the α1sDHPR, tagged at the N-terminal with EGFP, were transfected into adult FDB muscles by *in vivo* electroporation. The wildtype variant of the channel (EGFP-α1sDHPR-*wt*) was rendered insensitive to dihydropyridines by site-directed mutagenesis (EGFP-α1sDHPR-*T935Y*). Standard and TPLSM fluorescence microscopy demonstrated that both variants were similarly expressed with high efficiency and targeted to the surface and TTS membranes of the muscle fibers. Functional evaluation of the efficiency of transgenic expression was carried out by characterizing Ca^{2+} currents and SR Ca^{2+} release in single fibers enzymatically isolated from transfected muscles. The fibers were voltage-clamped using a 2-microelectrode configuration and dialyzed internally with solutions containing 30 to 70 mM Cs-EGTA and 20 to 70 mM Cs-MOPS; they were externally bathed with isotonic TEA-Cl containing 2-12 mM Ca^{2+}. Na and Cl currents were blocked with TTX and 9-ACA, respectively. The maximal Ca^{2+} conductance (gCa_{max}), measured in 12 mM Ca^{2+}, was 0.40 ± 0.04 (mean ± SD, n=6) in control fibers isolated from non-transfected animals. This parameter was not significantly different in fibers expressing EGFP-α1sDHPR-*wt* channels (0.42 ± 0.07, n=6). In contrast, fibers expressing EGFP-α1sDHPR-*T935Y* reported a significantly smaller gCa_{max} of 0.27 ± 0.02 (n=5). Interestingly, after treatment with 1 μM of the specific DHPR blocker NP-200, the residual conductance was <5% in control and EGFP-α1sDHPR-*wt* transfected fibers, but 30-70% in fibers transfected with EGFP-α1sDHPR-*T935Y*. Our results suggest that, in adult skeletal muscle fibers, the functional expression of transgenic DHPR channels is done mostly at the expense of the expression of their endogenous counterparts. Supported by NIH grants Supported by NIH grants AR07664, and AR054816.

3706-Pos

An Important Fraction of the Mammalian Skeletal Muscle Chloride Conductance is Located in the Transverse Tubules

Alvaro Herrera, Marino DiFranco, **Julio L. Vergara**.
UCLA School of Medicine, Los Angeles, CA, USA.

The actual density of chloride (ClC1) channels in the surface and transverse tubular system (TTS) membranes of mammalian skeletal muscles is still unknown. To investigate this issue, we simultaneously recorded fluorescence signals and chloride currents (ICl) in enzymatically dissociated FDB muscle fibers, stained with the potentiometric indicator di-8-ANEPPS, and voltage-clamped using a 2-microelectrode configuration. The external solution contained (in mM) 150 TEA-Cl, 15 CsMOPS, 2 $CaCl_2$, 0.5 $CdCl_2$, and 200nM TTX. Internally, the fibers were equilibrated with a solution containing 60 CsCl, 40 CsEGTA, 40 CsMOPS, and 5 $MgCl_2$ and voltage-clamped at the chloride equilibrium potential (−20mV). gCl was maximally activated by a pulse to +60mV (150ms) and its voltage-dependence calculated from 9-ACA-sensitive tail currents (measured at the onset of a pulse to −100mV) after 200ms test pulses (−100 to +80mV in amplitude). Boltzmann distributions fitted to the data (n=8) yielded: gCl_{max}=-2.1 ± 0.4 S/F, or 8.1 ± 1.5 mS/cm^2; $V_{1/2}$=73 ± 11 mV and k=24 ± 4 mV. The amplitude (in detaF/F) of di-8-ANEPPS fluorescence transients recorded at the onset of the test pulses were plotted as a function of the pulse amplitudes. In the presence of 9-ACA, the deltaF/F vs. voltage relationship was linear over the entire range of pulse amplitudes explored (slope= −0.124 ± 0.015 /100mV), whereas in the presence of ICl the slope of the linear dependence was less steep. For hyperpolarizing pulses, associated with large instantaneous inward currents, the slope was −0.099 ± 0.03 /100mV; for depolarizing pulses (smaller positive currents) the slope was −0.11 ± 0.025 /100mV. The differential attenuation of the average TTS voltage change in the presence of ICl was predicted by a radial cable model provided that ~30% of the total gCl was in the TTS. Supported by NIH grants Supported by NIH grants AR07664, and AR054816.

3707-Pos

Skeletal Muscle Fibers of Cold-Acclimated Mice Display Increases in Basal Calcium, Mitochondrial Content and Fatigue Resistance

Håkan Westerblad, Takashi Yamada, Joseph D. Bruton.
Karolinska Institutet, Stockholm, Sweden.

Mammals initially generate heat by repetitive muscle activity (shivering) when exposed to a cold environment. Shivering can later be replaced by heat generated in brown adipose tissue by activation of uncoupling protein-1 (UCP1). Interestingly, adaptations in skeletal muscles of cold exposed animals are similar to those obtained with endurance training. We studied the function of non-shivering flexor digitorum brevis (FDB) muscles of wild-type (WT) and UCP1-KO mice kept at room temperature (24°C) or cold-acclimated (4°C) for 4-5 weeks. Myoplasmic free [Ca^{2+}] ([Ca^{2+}]$_i$; measured with indo-1) and force were measured under resting conditions and during fatigue induced by repeated tetanic stimulation in intact single fibers. We observed no differences between fibers from WT and UCP1-KO mice. On the other hand, muscle fibers from cold-acclimated mice showed increases in basal [Ca^{2+}]$_i$ (~50%), tetanic [Ca^{2+}]$_i$ (~40%), and SR Ca^{2+} leak (~four-fold) as compared to fibers from room-temperature mice. Muscles of cold-acclimated mice also showed increases in expression of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), citrate synthase activity (reflecting increased mitochondrial content), and fatigue resistance. In conclusion, cold exposure induces changes in FDB muscles similar to those observed with endurance training and we propose that increased basal [Ca^{2+}]$_i$ has a key role in these adaptations.

3708-Pos

Allele Specific Gene Silencing in Autosomal-Dominant Skeletal Myopathies

Ryan E. Loy[1], Mohammed A. Mostajo-Radji[2], John D. Lueck[1], Robert T. Dirksen[1].
[1]University of Rochester, Rochester, NY, USA, [2]Rochester Institute of Technology, Rochester, NY, USA.

Central Core Disease (CCD) and Malignant Hyperthermia (MH) are linked to single amino acid substitutions in the skeletal muscle Ca^{2+} release channel, the type 1 ryanodine receptor (RyR1). We focus on two autosomal dominant (AD)