the procedure. The other electrode was used to dialyze the fiber with a solution containing either 6-15 mM BAPTA, or up to 60 mM EGTA (pCa=7.0), and adjusted to pH=7.0 with 20 or 60 mM MOPS, respectively. Free myoplasmic Ca^{2+} concentration ($[Ca^{2+}]$) changes were measured with the low affinity indicator OGB-5N (200 μM). SR Ca^{2+} release was elicited by either 20 mM caffeine, or 1 mM 4-chloro-methy-phenol (4CmC), added to the external solution. The maximal fluorescence change of the Ca^{2+} indicator was assessed at the end of the protocol by exposing the fibers to saponin (0.1 mg/ml in isotonic $CaCl_2$, pH=7.0 with 20 mM MOPS). To prevent changes in shape of the fibers under these conditions, they were pretreated for 1 min with 1% formaldehyde (in Tyrode). The experiments were conducted at 20 °C. A single model compartment was used to estimate (from the $[Ca^{2+}]$ changes) the total Ca^{2+} released in response to caffeine/4CmC application. We obtained values of SR Ca^{2+} content in the range of 15-27 mM for normal FDB fibers. Interestingly, comparable values were obtained in fibers from *mdx* mice.

1211-Pos Board B55

The Absence of Utrophin Does Not Further the Impairment of Ca^{2+} Release Displayed by *mdx* Muscle

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The double mutant utr$^{-/-}$/mdx mouse has been postulated to be a better model of Duchenne Muscular Dystrophy than *mdx* mouse because it displays a progression of pathological features comparable to that in humans. We previously demonstrated that FDB fibers from *mdx* mice show limitations in action potential (AP) elicited Ca^{2+} release. Here we investigated the properties of the APs and the ability to release Ca^{2+} (30 mM [EGTA], 20ºC) in response to single and trains of APs (20 pulses, at 33Hz and 100Hz) in FDB fibers isolated from control, *mdx* and utr$^{-/-}$/mdx mice. Single APs of normal amplitude but longer duration were recorded in utr$^{-/-}$/mdx fibers, whereas the amplitude of the Ca^{2+} release was ~37% smaller than in normal fibers, but comparable with that found in *mdx* fibers. Fibers from the three strains sustained trains of Ca release at 33 Hz in which the amplitude of individual Ca^{2+} release transients decayed exponentially towards a sustained release amplitude with two time constants (τ_1=10ms, τ_2=200ms). In response to 100Hz trains, the amplitude of Ca^{2+} release in normal fibers decayed still with a double exponential (τ_1=3.5ms, τ_2=41ms) in which the amplitude of the 2nd and 20th transient along the train were ~50% and ~35% that of the first one, respectively. In contrast, fibers isolated from both *mdx* and *utr$^{-/-}$/mdx* mice could be divided in two groups according to their tetanic response to 100Hz trains: approximately 75% of *mdx* and 33% of utr$^{-/-}$/mdx fibers showed a behavior similar to that observed in normal fibers; in the remaining 25% of *mdx* and 67% of utr$^{-/-}$/mdx fibers, respectively, the amplitude of the 2nd Ca^{2+} release transient was ~20% of the first one, and this amplitude was sustained throughout the train.

1212-Pos Board B56

Deficits in Ca2+ Release and in vivo Muscle Strength in Heterozygous I4895T RyR1 Knock-In Mice

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The mutation from isoleucine to threonine of the skeletal isoform of the ryanodine receptor (RyR1) at residue 4898 results in severe Central Core Disease (CCD). Under homozygous expression (IT/IT), we reported a lack of Ca^{2+} release in response to electrical and pharmacological activation despite SR Ca^{2+} store content indistinguishable from control. Here we used heterozygous knock-in mice for the I4895T (IT/+; analogous to human I4898T) RyR1 mutation to determine the effects of the mutation on muscle strength and Ca^{2+} handling in flexor digitorum brevis (FDB) and interosseous muscle fibers.
We compared *in vivo* muscle strength of wild-type (WT) and IT/+ mice. IT/+ mice exhibited significant weakness in both upper body and grip strength assays (4-paw peak grip force: 2400 ± 70 mN, n=8 and 2040 ± 80 mN, n=14 in WT and IT/+ mice, respectively). We also determined the magnitude of action potential- and ligand-evoked Ca^{2+} release in single intact FDB fibers using Ca^{2+} fluorometry. The magnitude of both electrically- and ligand-evoked Ca^{2+} release was significantly reduced in IT/+ fibers. Moreover, the maximum rate of change in mag-fluo-4 fluorescence during the rising phase of the electrically-evoked Ca^{2+} transient was significantly reduced in IT/+ fibers (WT 0.17 ± 0.01 ΔF/F/ms vs IT/+ 0.11 ± 0.01 ΔF/F/ms, n= 53, 56, respectively). Finally, the frequency (1.9 ± 0.5 and 0.8 ± 0.3 events/scan) and Ca^{2+} spark mass (5.9 ± 0.3 and 4.6 ± 0.2 μm^3) of local Ca^{2+} release induced by osmotic shock (440 mOsm with sucrose, 750 lines/sec) were reduced in acutely dissociated IT/+ interosseous fibers compared to that of WT fibers. Together, these findings are consistent with the hypothesis that the IT mutation in the putative RyR1 selectivity filter significantly reduces Ca^{2+} flux through the channel.

1213-Pos Board B57

Malignant Hyperthermia Mutation Alters Excitation-coupled Ca2+ Entry In MH RyR1-R163C Knock-in Myotubes

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Malignant hyperthermia (MH) is a pharmacogenetic disorder of skeletal muscle triggered insusceptible individuals by inhalation anesthetics and depolarizing skeletal muscle relaxants. This syndrome has been linked to a missense mutation in the type 1 ryanodine receptor (RyR1) in more than 50% of cases studied to date. We have examined how the R163C MH-RyR1 mutation alters the Ca^{2+} transient during $[K^+]_e$ depolarization using Fluo4 and excitation-coupled Ca^{2+} entry (ECCE) using manganese-quench of Fura2 Wt and $RyR1_{R163C}$ knock-in myotubes. Exposure of WT and $RyR1_{R163C}$ myotubes to low Ca^{2+} solution ($8.7x10^{-6}$ M) and then to high $[K+]_e$ did not modify initial Ca^{2+} transient ($Ca^{2+}{}_{peak}$), but dramatically altered the time course of the Ca^{2+}-transient, making the duration shorter and the rate of decay faster in all genotypes. However, these changes were more evident in $RyR1_{R163C}$ than Wt myotubes. The rate of Mn^{2+} quench of Fura2 associated with K^+ depolarization (ECCE), was membrane potential dependent and always greater and faster in $RyR1_{R163C}$ myotubes than in Wt. Incubation of Wt and MH $RyR1_{R163C}$ myotubes with 15 μM ryanodine overnight, to block RyR1 Ca^{2+} release, enhanced the amplitude but did not significantly change in the rate of ECCE in either genotype. However, the increment in amplitude was greater in Wt than $RyR1_{R163C}$ knock-in myotubes. We conclude that the pre-existing conformational change caused by the $RyR1_{R163C}$ MH mutation alters the properties ECCE as consequence of significant changes in the retrograde signaling between RyR1 and DHPR making it less sensitive to the conformational change caused of ryanodine.

1214-Pos Board B58

Malignant Hyperthermia and Heat Stroke in Calsequestrin-1 Knockout Mice

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Malignant hyperthermia (MHS) and environmental heat stroke (EHS) in humans present as similar life threatening crises triggered by volatile anesthetics and strenuous exercise and/or high temperature, respectively. Many families (70-80%) diagnosed with MH susceptibility (MHS), and a few with EHS, are linked to mutations in the gene that encodes the type 1 ryanodine receptor (RYR1) located in the sarcoplasmic reticulum (SR) of skeletal muscle. However, mutations in the RYR1 gene are not found in all MH families, suggesting that alternative genes remain to be identified. Here we investigated whether a MH/EHS-like phenotype results from deficiency in skeletal muscle calsequestrin (CASQ1), a SR Ca^{2+}-binding protein that modulates RYR1 function. Exposure of CASQ1-null mice to halothane or heat stress triggers lethal episodes characterized by elevated core temperature, whole body contractures, and severe rhabdomyolysis. Both heat- and halothane-induced episodes are prevented by prior dantrolene administration, the standard antidote used to treat MH episodes in humans. *In vitro* studies indicate that CASQ1-null muscle exhibits increased contractile sensitivity to caffeine, temperature-dependent increases in resting Ca^{2+}, and an increase in the magnitude of depolarization-induced Ca^{2+} release. These findings validate CASQ1 as a candidate gene for linkage analysis in MH/EHS families where mutations in RYR1 are excluded.

1215-Pos Board B59

Expression Of Calsequestrin-1 (CS1) In CS1-null Mice: Restoration Of Ca^{2+} Release Unit Architecture And Amplitude Of Ca^{2+} Transient In Fast-twitch Muscle Fibres

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Amplitude of calcium (Ca^{2+}) transients and width of the Sarcoplasmic Reticulum (SR) lumen in Ca^{2+} release units (CRUs) are significantly reduced in Calsequestrin1 (CS1)- null mice, moreover increase in fatigue resistance is characteristic of the CS1-null-model (*Paolini et al 2007*). We extend the study of the null model at molecular level: decrease in expression of CS2, Triadin, Sarcalumenin were detected in CS1-null FDB muscles in comparison to wild type (wt) and differential FDB (null/wt) expression of 13400 mRNAs was assayed by microarray profiling. To rescue the CS1-null phenotype, exogenous mouse CS1 was expressed in adult null-FDBs by *in vivo* DNA electrotransfer. CS1 expression and correct targeting to CRUs was verified by confocal