models to establish whether groups of transcripts for related proteins showed similar patterns of change. Genes whose expression increased progressively throughout the culture period (15/48) included growth factors (Nppa, Nppb, Fgf2, Tgfb1, Tnf), G proteins (Gnai2, Gnaq, Gnas); those whose expression decreased progressively (11/48) included ATP-dependent transporters (Atp1a2, Atp1a3, Atp2a2) and regulatory contractile proteins (Tnni3, Tnnt2). The levels of 9 transcripts, including the caveolar proteins (Cav, Cav2, Cav3), did not change (maximum change < 2- fold). This study shows that even under culture conditions which preserve cellular morphology[1], marked changes in gene expression, many of which are part of "hypertrophy programs", are seen within days.

Supported by the BHF.

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1492-Pos Analysis Of Diffusion Restrictions In Cardiac Muscle Cells

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Board B468

Functional interaction between mitochondria and surrounding ATPases has been found from the experiments on permeabilized heart muscle fibers. According to our earlier analysis, such interaction can be induced by relatively local diffusion restrictions for adenine nucleotides. The specific causes of these restrictions are not known but intracellular structures are speculated to act as diffusion barriers. Based on the proximity of sarcoplasmic reticulum (SR) to mitochondria, we hypothesize that SR not only utilizes ATP but may also act as a diffusion barrier leading to functional coupling of ATPases and mitochondria. The diffusion barriers can be enhanced by cytoskeleton proteins localized near SR. With a 3D finite-element model, we attempted to explore, SR as the first candidate for diffusion barrier. The geometry for the mathematical model was constructed using representative mitochondrial and SR structural organization from confocal and electron microscope images. SR and cytoskeleton proteins were assumed to induce the diffusion restrictions around mitochondria and in planes between neighboring mitochondria. Those restrictions were varied as well as a restriction induced by mitochondrial outer membrane to fit the following set of experimental data: mitochondrial respiration rate dependence on exogenous ADP and ATP; effects of pyruvate kinase and phosphoenolpyruvate additions on respiration. According to our simulations, there are many sets of model parameters that were able to reproduce all experiments considered in this work. However, in all the sets, the permeability of SR network and associated cytoskeleton proteins was very low indicating importance of cytoskeleton proteins in formation of diffusion restrictions. Finally, the layer of free water available for diffusion between mitochondria and SR surrounding mitochondria, is expected to be of the order of 50 nm or less.

1493-Pos Lineage Tracing of Cardiac Explant-Derived Cells

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Board B469

Recent reports suggest that cultured adult cardiac explants produce cells with cardiogenic potential that can form cardiospheres in vitro. The aim of this study was to define the source, morphology and cardiogenic potential of cardiac explant-derived cells using lineage-tracing techniques. The cultured explants produced a heterogeneous population of cells including a distinctive population of small round cardiac-explant derived cells (SEDCs)-fig1.These cells shared some characteristics of cardiac myocytes and survived engraftment in the adult heart. Using MLC2vCRE/ZEG double transgenic, MHCnLAC and Actin-eGFP mice, the SEDCs and other cardiac explant-derived cells from these mice failed to differentiate into cardiac myocytes *in-vivo*, demonstrated by the absence of activation of lineage-restricted reporters and action potential-induced calcium transients. The production of SEDCs was highly dependent on the retention of blood-derived cells or factors in the cultured explant-fig 2. Electron microscopy and immunogold labeling showed that SEDCs were vimentin-positive cells exhibiting phagocytic activity, including uptake of cardiac myocyte sarcoplasmic structures and organelles such as mitochondria-fig3, explaining why they may be positive for cardiac markers on immunohistochemistry. Using lineage tracing, cardiac explant-derived cells are not cardiac progenitors and may acquire the imnunohistochemistical phenotypes of cardiomyocytes by phagocytosis.

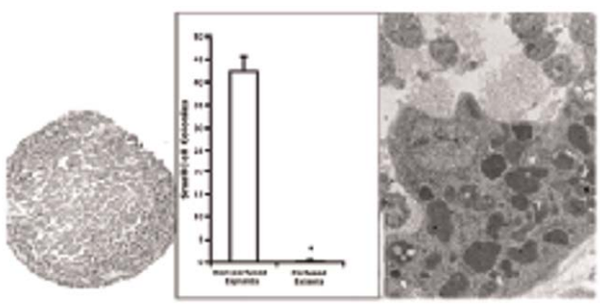

Figure 1 Figure 2 Figure 3

Excitation-Contraction Coupling-I

1494-Pos Resting Concentration of Ca2+ in the Sarcoplasmic Reticulum (SR) of Frog Skeletal Muscle Fibers

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Board B470

The aim of this study was to measure the resting concentration of free Ca in the SR (denoted $[Ca^{2+}]_{SR,R}$) using the low-affinity (K_D = 2.6 mM), membrane-permeable, Ca-indicator dye tetramethylmurexide (TMX). Cut fibers were mounted in a double Vaseline-gap chamber. The external solution was a high-K (relaxing) solution. The K-internal solution contained 20 mM EGTA and a nominally physiological value of $[Ca^{2+}]$ and ~2 mM TMX. After a period of ~1 hour to allow TMX to diffuse to the optical recording site in the central compartment, the surface membrane in the central compartment was permeabilized by exchanging the external solution with relaxing solution containing 0.02% saponin. The aim was to rapidly remove TMX from the myoplasm so that all of the TMX present was in the SR. From the dye-related absorbances, the fraction of TMX bound with Ca (f_{Ca}) increased to a plateau level of 0.213 on average (S.E.M. = 0.017; N=5) giving an average value of $[Ca^{2+}]_{SR,R}$ of 0.71 mM (S.E.M. = 0.07). The value of f_{Ca} just before the saponin treatment was 0.086. (S.E.M. = 0.009). (N.B. that this includes the contribution of Ca-free TMX in the myoplasm. There should have been essentially no CaTMX in the myoplasm at rest owing to the low affinity of TMX.) This value of 0.086 is not significantly different than the value for f_{Ca} of 0.070 (S.E.M. = 0.008; N=6) measured in intact fibers pressure injected with TMX (Konishi & Baylor, 1991, J. Gen. Physiol. *97*:245–270). Assuming that certain properties of cut fiber are similar to those of intact fibers (e.g. the volume of SR relative to that of myoplasm), these results indicate that the physiological value of $[Ca^{2+}]_{SR,R}$ in intact fibers is also near 0.7 mM.

1495-Pos The Contribution of the DHPR in Resting Myoplasmic Calcium Concentration in Skeletal Myotubes

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Board B471

Resting intracellular calcium concentration ([Ca2+]i) in muscle cells is controlled by the calcium ATPases at the plasma membrane and sarcoplasmic reticulum, as well as by the Na+/Ca2+ exchanger. Interestingly the L-type voltage gated calcium channel (Cav1.1) and the ryanodine receptor (RyR) which are the key proteins in EC coupling also play a role in setting [Ca2+]i. In this regard malignant hyperthermia mutations in Cav1.1 and RyR1 alter intracellular Ca2+ regulation in skeletal muscle, suggesting an active participation of both of these calcium channels in preserving a physiological resting calcium concentrations in muscle cells. To test the role of DHPR in resting calcium level we measured [Ca2+]i using double-barreled Ca2+ selective microelectrodes in wild type myotubes, in myotubes that do not express Cav1.1 (mdg), and in mdg cells transduced with Cav1.2. [Ca2+]i was 127±7 nM (mean±SD) in wild type myotubes, 152±16 nM in myotubes expressing Cav1.2 and 102 ± 4 nM in dysgenic myotubes. Incubation of myotubes in low external Ca2+ solution (~7μM + 2 mM Mg2+) reduced [Ca2+]i to ~ 80 nM in all cases. In addition, we evaluated the caffeine dose response curve in Fluo-4AM loaded Wt (Cav1.1), Cav1.2 expressing and mdg myotubes. The threshold for caffeine-induced Ca2+ transients was much lower in myotubes expressing Cav1.2 than Wt (α1S) or mdg myotubes. In summary, the expression of Cav1.2 in mdg myotubes increases [Ca2+]i as well as the caffeine sensitivity.

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1496-Pos Importance Of NCX In The Regulation Of Ca^{2+} Homeostasis In Skeletal Muscle

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Board B472

Repetitive stimulation of mouse muscle fibres poisoned with ciclopiazonic acid (CPA), causes a gradual increase in the basal $[Ca^{2+}]_i$, measured with Fura-2, accompanied by a progressive decrease in Ca^{2+} transient amplitude, measured with MagFluo-4 (1). In the present work we have further explored this effect using enzymatically dissociated FDB muscles, from rats of different ages. In the presence of 5 μM CPA the initial amplitude of MagFluo-4 transient, expressed as ΔF/F was 0.33 ± 0.027 (mean±sem, n=10) in normal ringer, 0.28 ± 0.014 (mean±sem, n=9) in 0Na solution and 0.22 ± 0.015 (mean±sem, n=4) in 0Na0Ca solution. For fibers from juvenile (20 days old) animals the two time constants of decay of the calcium signals in the three solutions were τ_1=2.20±0.04, τ_2=31.93±2.28 (mean±sem, n=10); τ_1=2.06±0.23, τ_2=67.35±6.57 (mean±sem, n=7) and τ_1=1.68±0.06, τ_2=39.63±4.6 (mean±sem, n=5) for Control, 0Na and 0Na0Ca conditions respectively. During repetitive stimulation at 0.2 Hz in normal solution, the transient amplitude was not appreciably affected, while in the presence of CPA(5μM), it diminished progressively. After 40 episodes the amplitude of Ca^{2+} transient diminished by about 71.4% in NaCa normal solution, while in 0NaCa or 0Na0Ca solutions, the amplitude decreased by about 22.7% and 30.5% respectively. The rates of decay of the transient amplitude during repetitive stimulation were $9.03*10^{-4}s^{-1}$ (control), $6.09*10^{-4}s^{-1}$ (0NaCa) and $4.4*10^{-4}s^{-1}$ (0Na0Ca). In all cases the slow decay phase of the Ca^{2+} transients was practically abolished. The decrease of the transient amplitude observed under these conditions has been explained in terms of the increase in the basal Ca^{2+} concentration (1). However, the present results indicate that this effect is modulated by external Na^+ and Ca^{2+}, suggesting the possibility of a participatory role of the Na-Ca exchange mechanism.

(FONACIT G-2001000637).

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1497-Pos Functional Evaluation of Transgenic Expression of Rat Mutant Sodium Channels in Murine Skeletal Muscle Fibers

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Board B473

We engineered a novel construct of the rat skeletal voltage sensitive sodium channel Mu1 (tagged with EGFP between domains II and III) to carry a mutation on the glutamate 403 residue (to glutamine, E403Q). This mutation has been previously reported to eliminate the sensitivity to TTX of this channel (Stephan et al., 1994, JMB *137*:1–8). Transfection of this construct in FDB mouse muscles was carried out using *in vivo* electroporation. Protein expression in muscle fibers was examined with TPLSM, demonstrating that most of the EGFP fluorescence is found in restricted areas at subsarcolemmal regions of the fibers. In addition, several fibers showed fluorescence sarcomeric banding patterns. Electrophysiological experiments evidenced the presence of TTX-insensitive Na currents after the endogenous Na channels were fully blocked in the presence of 500 nM [TTX]. The remaining TTX insensitive Na currents had slower kinetics than the endogenous ones. Their percentage contribution with respect to the total Na current (without TTX) ranges from 7 – 20%, depending on the level of transgenic protein expression. We take advantage of this mutant channel to assess the relative expression of the Na channel at the surface and TTS membranes and to evaluate the effective levels of expression of functional membrane proteins in skeletal muscle following *in vivo* transfection protocols.

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1498-Pos Properties Of The Tubular System Network In Skeletal Muscle

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Board B474

The tubular (t-) system in skeletal muscle acts as conduit for extracellular fluid and appears to be a functional longitudinally connected network, as shown in skinned fibres (Posterino et al, 2000). To examine the properties of the t-system network we used toad and rat muscle. Intact bundles were exposed to solution containing either Cascade Blue or purified 500- or 2000-kDa fluorescein dextran (29 and 55 nm in diameter, respectively) and imaged on a 2-photon microscope. A strong fluorescence band immediately outside the fibre was observed in all cases. Only Cascade Blue and 500-kDa fluorescein dextran showed dye entry into the t-system. Isolated intact fibres were exposed to 500-kDa dextran and rhod-2 salt and then mechanically skinned to trap fluorescent dye in the sealed t-system. Confocal imaging showed the dextran and rhod-2 had access to the entire regular t-system. However, only rhod-2 had access to the longitudinal connections. FRAP experiments with rhod-2 in the sealed t-system showed full recovery in 30 min but 500-kDa dextran showed only partial recovery from bleaching in > 1 hr. Field stimulation of skinned fibres from rat showed an action potential (AP)-induced Ca^{2+} release uniformly along the length of the scanning line positioned parallel to the long axis of the fibre, indicating stimulation elicited APs at each t-tubule, which in turn propagated radially across the fibre. Spontaneously activating Ca^{2+} release was imaged at a consistent angle to the scanning line, indicating APs propagated longitudinally along the fibre at a constant rate of 7.4 ± 1.3 mm/s ($n = 8$). In conclusion, the t-system network is excitable and has a lumen that is fully connected that can evenly distribute small molecules. Furthermore, the t-tubules have a significantly larger diameter than the longitudinal connections.

1499-Pos 3D-Reconstruction of the Structural Association between Triads and Mitochondria in Skeletal Fibers

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Board B475

Skeletal muscle can vary considerably its metabolism during activity. Since cell metabolism is controlled by Ca^{2+} signals, the precise positioning of mitochondria next to the Ca^{2+} release sites may be of great importance for the proper functioning of muscle fibers. In adult skeletal muscle, mitochondria are mostly located in proximity of triads, structures formed by the close apposition of SR and T-tubules, which mediate excitation-contraction coupling releasing Ca^{2+} during muscle activation (calcium release units, CRUs). Using electron microscopy, we have identified short strands (or tethers, ~10 nm long) connecting specifically the outer membrane of mitochondria to the SR, on the opposite side to the T-tubules (Boncompagni and Protasi, 2007; *Biophys J. 92*:A313). Tethers frequency increases during post-natal maturation, suggesting that these structures may have a crucial role in the progressive targeting of mitochondria next to triads. The SR-mitochondria association is sufficiently strong that treatment of FDB fibers with hypotonic solution results in stretching of the SR vesicle in correspondence of tethers. Using electron tomography, we have reconstructed the tri-dimensional architecture of triad/mitochondria interface providing additional information on the structural relationship between these two myoplasmic organelles. SR surrounds the mitochondria with lateral sacks/tubules and it is closely associated to it (*tethered*)

also in mitochondrial regions closer to the Z line, on the side opposite to the triad. The molecular nature of the physical linkage is not yet identified and for now we can only speculate as to the possible structural importance that these small bridges may have in holding SR and mitochondria together.

1500-Pos Evidence for a Close Spatial Arrangement of Intracellular Domains of the Skeletal Muscle DHPR Using Measurements of Fluorescence Resonance Energy Transfer (FRET) Between CyPet and YPet in a NonDestructive Measuring Variant

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Board B476

The putative intracellular portions of DHPRs, namely the C-terminal and the N-terminal tail, and the loops connecting the putative intra-membrane domains, play important functional and structural roles. Examples are the β-subunit recruiting function of the I/II loop, the bi-directional signalling function of the II/III loop during ec-coupling, the influence of the III/IV loop on RyR1 mediated Ca^{2+}-delivery, and the multiple involvements of the carboxyl tail (e.g. channel targeting and inactivation). These channel parts are likely to act in concert (e.g. during the inactivation process), which would require their spatial proximity. We engineered cDNAs encoding fluorescently double-labelled DHPR constructs where one the above mentioned structures was tagged with CyPet and another one with YPet. A CyPet-YPet fusion construct, either free or attached to the DHPR N-terminus, served as control. Confocal fluorescence microscopy indicated correct targeting of the constructs upon expression in dysgenic myotubes. CyPet was selectively excited using a 440nm diode laser and the fluorescence intensity ratio of YPet related emission to that emerging solely from CyPet (I_{cy}/I_y) served as a measure for the degree of FRET. We find a high ratio (strong FRET) for the control constructs, although a smaller one for the N-terminally attached CyPet-YPet fusion. Less energy transfer was observed between the N-terminus and C-terminus of the channel. However, strong energy transfer was also measured between the intracellular loops, providing evidence for a close spatial arrangement or even interaction of these domains. In view of the implication of these structures in skeletal muscle ec-coupling, it would be important to investigate whether the observed spatial proximity is self-organized or whether it results from physical coupling to the RyR1.

1501-Pos A Limited Role Of The $Ca_V1.1$ β Subunit C-terminus For Skeletal-type Excitation-contraction Coupling

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The C-terminus of the $Ca_V1.1\beta$ subunit has repeatedly been suggested to play an essential role in skeletal-type excitation-contraction coupling (ECC). In particular a unique hydrophobic heptad repeat motif (L478,V485,V492) in the C-terminus has been proposed to be a crucial determinant (Sheridan et al., 2004, Biophys. J.). We expressed zebrafish and rabbit wild-type β_{1a} and corresponding mutants, in which the LVV motif was substituted by AAA (β_{1a}AAA) in the paralyzed zebrafish β_1-null mutant *relaxed*. This system allows immunocytochemical, biophysical and ultrastructural studies on channel function in cultured myotubes with the advantage to test the implications of observed *in-vitro* changes *in-vivo* by larval motility analysis. Surprisingly, both β_{1a} AAA constructs could quantitatively restore α_{1S} triad expression as well as intracellular Ca^{2+} transients. But most importantly, they fully rescued the motile phenotype in otherwise paralyzed *relaxed* larvae. Thus, our results indicate that the β_{1a}-specific C-terminal heptad repeat is not required for skeletal-type ECC.

To test in our system the contribution of the C-terminus to skeletal-type ECC, rabbit β_{1a} with a C-terminal truncation of 35 residues ($\beta_{1a}\Delta35$) was expressed in zebrafish *relaxed* larvae. Deletion mutant $\beta_{1a}\Delta35$ showed a significant reduction of larval movement intensity, but only to approximately a half. In contrast, an ancestral β subunit (housefly, β_M) was able to restore α_{1S} triad expression but was unable to recover functional ECC. As β_M is lacking all three non-conserved regions, i.e., the N-terminus, the linker region and the C-terminus (latter comparable to $\beta_{1a}\Delta35$), our results indicate that the C-terminus of β_{1a} plays a critical role, but is not the exclusive determinant of skeletal-type ECC in β_{1a}. Further studies will reveal the additional role of the other non-conserved β_{1a} regions in skeletal-type ECC.

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1502-Pos Rem Inhibits Skeletal Muscle EC Coupling by Reducing the Number of L-type Ca^{2+} Channels at Triad Junctions

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Board B478

In skeletal muscle, the L-type voltage-gated Ca^{2+} channel (or 1, 4-dihydropyridine receptor; DHPR) serves as the voltage sensor for

excitation-contraction (EC) coupling. Members of the RGK (Rem, Rem2, Rad, Gem/Kir) family of Ras-related monomeric GTP-binding proteins inhibit both cardiac and neuronal voltage-gated Ca^{2+} channels. In this study, we examined the effects of Rem on EC coupling and the function of the skeletal muscle L-type Ca^{2+} channel. Depolarization-induced myoplasmic Ca^{2+} transients of myotubes expressing YFP-Rem were reduced in comparison to transients of control myotubes. This impaired EC coupling was not a consequence of altered function of the type 1 ryanodine receptor (RyR1), or of reduced Ca^{2+} stores, since the application of 4-chloro-*m*-cresol, a direct RyR1 activator, elicited Ca^{2+} transients in YFP-Rem expressing myotubes which were not distinguishable from those in control myotubes. Whole-cell patch clamp measurements demonstrated that the magnitude of L-type Ca^{2+} current was reduced by ~75% compared to that of normal myotubes or myotubes expressing YFP only. Potentiation of the L-type current by ±BAY K 8644 (5 μM) in YFP-Rem-expressing myotubes was similar to that observed in control myotubes. Measurements of immobilization-resistant charge movement indicated that YFP-Rem reduced the number of functional L-type Ca^{2+} channels in the plasma membrane to an extent sufficient to account for the decrease in Ca^{2+} current. Taken together, our results suggest that chronic upregulation of Rem negatively inhibits skeletal muscle EC coupling by reducing the *total* number of L-type Ca^{2+} channels in the plasma membrane.

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1503-Pos Intracellular Calcium Concentration Modulate Inactivation Of Voltage Sensor In Skeletal Myotubes

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The effect of intracellular [Ca^{2+}] on inactivation process was studied in Fluo 4-AM loaded myotubes from mice expressing wild type RyR1 (MHN) or heterozygous (HET) and homozygous (HOM) "knock-in" mice with a R163C RyR1 mutation associated with malignant hyperthermia (MHS). Reduction of extracellular Ca^{2+} or incubation with 20 μM SKF 96365, an inhibitor of Ca^{2+}entry, did not change peak amplitude of the K^+-induced Ca^{2+} transient, but was associated with a significant reduction in the area under the curve of the Ca^{2+} transient as a result of an increased rate of decay (all genotypes). The effect of genotype on Ca^{2+} transient decay was MHS-HOM >>MHS-HET>MHN. The time course of the transient's decay appears to be linked to Ca^{2+} entry during membrane depolarization associated with excitation- coupled Ca^{2+} entry (ECCE). The rapid decline of the Ca^{2+} transient associated with exposure to low Ca^{2+} or SKF 96365 solutions represents an accelerated inactivation of the voltage sensor of the DHPR rather than Ca^{2+} depletion in the sarcoplasmic reticulum, since caffeine was still able to induce a vigorous intracellular Ca^{2+} release when the K^+-induced Ca^{2+} transient had declined to baseline. These results show that RyR1 MH R163C mutation enhances the magnitude of ECCE. Furthermore it suggests that a decrease in myoplasmic [Ca^{2+}] (by removing extracellular Ca^{2+} or preventing its entry) at the inner rather than the outer side (*Rios and Pizarro 1991*) of the DPHR modulates the inactivation process of the voltage sensor as shown by the reduction of the time course of K^+-induced Ca^{2+} transient.

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1504-Pos S100A1 Binds the Calmodulin Binding Site of RyR1 and Positively Regulates EC Coupling in Skeletal Muscle: Functional Studies

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Board B480

S100A1, a 21 kD dimeric Ca^{2+} binding protein, enhances cardiac Ca^{2+} release and contractility, but its role in skeletal muscle has been less well defined. Additionally, the precise molecular mechanism underlying S100A1 modulation of SR Ca^{2+} release in striated muscle has not been fully elucidated. Here, utilizing a transgenic S100A1 KO mouse line, we demonstrate a direct role of S100A1 in EC coupling in murine FDB skeletal muscle fibers. PMT recordings with indo-1 show no difference in resting fluorescence emission ratio, indicating no alteration in resting $[Ca^{2+}]_i$ in S100A1 deficient mice. However, the absence of S100A1 leads to a decreased global Ca^{2+} transient following electrical excitation, as measured using indo-1 or fluo-4 Ca^{2+} indicator dyes. Adenoviral expression of S100A1 in cultured S100A1 KO fibers restored the peak amplitude of the indo-1 Ca^{2+} transient to that of WT fibers. High-temporal resolution confocal microscopy revealed a 31.6% decrease in peak ΔF/F0 and a 38.6% increase in the duration of the rising phase of the Ca^{2+} transient in S100A1 KO fibers. We propose that S100A1 binds to a novel site on the cytoplasmic face of RyR that is conserved throughout striated muscle and corresponds to a previously identified calmodulin binding site. Studies with isolated SR vesicles at physiological Ca^{2+} concentrations reveal that S100A1 competes with calmodulin for binding to this site with similar low micromolar affinities. NMR spectroscopy provides structural evidence for the basis of this interaction between S100A1 and RyR. Taken together, these data suggest that S100A1 plays a significant role in EC coupling in skeletal muscle, primarily through specific interactions with the calmodulin binding domain of RyR1.

1505-Pos Sarcolipin Depresses Skeletal Muscle Contractility In Nebulin KO Mice

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Board B481

Recently, we have generated a novel nebulin KO mouse model. The underlying mechanisms for depressed contractility in nebulin KO mice involve reduced thin filament lengths. Altered Ca^{2+} homeostasis might also be involved, as gene expression analysis revealed that sarcolipin (SLN, a potent SERCA-inhibitor) is upregulated in nebulin KO mice. Therefore, we investigated SLN protein expression in nebulin KO mice, as well as SR Ca^{2+} uptake, myofiber Ca^{2+} transients, and muscle contractile performance. Western blotting was performed on soleus, EDL, and quadriceps. Ca^{2+} transport activity of quadriceps SR vesicles was assayed, and Vmax was determined. FDB fibers were enzymatically dissociated, and Ca^{2+} transients during field stimulation were determined using Fura2FF. Finally, we determined intact soleus contractility, and Ca^{2+}-activated maximum force in skinned soleus fibers. In KO mice, SLN protein expression was highly upregulated in soleus, EDL and quadriceps, SERCA was slightly downregulated, and phospholamban and calsequestrin were comparable between both groups. Ca^{2+} uptake of isolated SR vesicles was reduced in nebulin KO mice across a range of Ca^{2+} levels; Vmax was reduced by >3 fold. Ca^{2+} release upon field stimulation was comparable between FDB fibers from KO and wt mice. However, rate of Ca^{2+} reuptake was significantly reduced in KO fibers, as indicated by increased values for tau (i.e. the time course of transient decay). Twitch and tetanic force generation were reduced in KO mice (60 and 53% respectively), and tetanic half relaxation time was increased in nebulin KO mice. Finally, maximum Ca^{2+}-activated force was reduced by ~36% in nebulin KO mice (note the significantly larger *tetanic* force reduction in KO). These data demonstrate that altered Ca^{2+} homeostasis plays an important role in muscle dysfunction of nebulin KO-induced skeletal. In addition, the present study suggests a functional relation between nebulin and SLN.

1506-Pos Post-tetanic Calcium Transients Induced By Electrostimulation In Adult Skeletal Muscle Fibers

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Board B482

Tetanic electrical stimulation induces two separate calcium signals in rat skeletal myotubes, a fast one dependent on RyRs and related to myotube contraction, and a late, slow signal, dependent on dihidropyridine receptors (DHPR) and IP3Rs and related to transcriptional events. Until now, however this slow calcium signal has been hard to show in adult muscle fibers.

We have monitored intracellular calcium kinetics in isolated adult muscle fibers loaded with Fluo-3. Flexor digitorum brevis (FDB) muscle from 4–6 weeks-old BalbC mice were dissected, individual fibres dissociated by enzymatic digestion of the whole muscle and plated on matrigel coated cover slips in standard culture medium.

When stimulated with 6 s trains of 0.3 ms pulses at 45 Hz we observed the onset of a fast calcium tetanus (associated with contraction) and a second, slower signal very similar to those described in rat primary myotubes. The slow signal (more than the fast one) was inhibited by 25 μM nifedipine, suggesting a role for DHPR in its onset. Experiments in the absence of extra-cellular Ca^{2+} show that this slow signal has at least two components: an earlier one dependent on and a later one independent of extracellular Ca^{2+}.

In adult mice muscles, we found that different types of IP3R are differentially expressed in different types of muscle fibers, supporting the idea that different Ca^{2+} kinetics for the slow signal may exist in different types of muscle fibers. These results support our hypothesis that kinetics of Ca^{2+} transients mediated by IP3R may have a role in the activation of specific transcriptional programs of slow and fast-type muscles fibers

1507-Pos Role Of Mitochondria In Slow Calcium Transients Induced By Electrical Stimulation In Skeletal Myotubes

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Board B483

Mitochondria interconnected network senses cytoplasm calcium content and modulates intracellular calcium homeostasis. In skeletal myotubes, electrical field stimulation induces two calcium signals in cytoplasm, nucleus and mitochondria: a fast signal associated with excitation-contraction coupling and a slow signal that has a role in regulation of gene expression. Here, we analyzed in more detail the role of mitochondria on cytoplasm and nuclei slow calcium signals.

Fluo-3 fluorescence of electrically stimulated (400 pulses, 45 Hz) skeletal myotubes was evaluated. Uncoupling of mitochondria using carbonyl cyanide m-chlorophenylhydrazone (CCCP) or mitochondria calcium release blockage with the Na^{+}/Ca^{2+} calcium exchanger inhibitor CGP37157, did not preclude the slow calcium signal to appear. While CCCP completely uncouples mitochondria, judged from the drop in mitochondria membrane potential (evaluated using tetramethyl rhodamine), the slow calcium transient becomes significantly longer, not returning to basal levels in the experimental time. CGP37157 treated myotubes also displayed significantly larger and longer slow calcium transients, accompanied by a decrease in mitochondria membrane potential.

Fluo-3 fluorescence signals are normally higher in the nuclear region compared to the cytoplasm, but this difference disappears in cells treated with CGP37157, reinforcing the idea of mitochondria buffering capacity around the nuclei.

We conclude that slow cytoplasm transients after tetanus do not depend on calcium release from mitochondria, but it was also evident that mitochondria exert an important role as a calcium buffer for these signals and are needed for slow calcium signal termination.

1508-Pos The Effect Of Mitochondrial Poisoning By FCCP On Capacitative Ca^{2+} Entry In Mouse Skeletal Muscle Fibers

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Enzymatically dissociated mouse FDB muscle fibers, loaded with Fura-2 AM, were used to test whether mitochondrial poisoning with the uncoupler FCCP affected the capacitative Ca^{2+} entry in this preparation. Fibers were treated with 40 μM BTS, to block contraction and minimize movement artifacts, and 10 μM ciclopiazonic acid (CPA) to block the Sarcoplasmic Reticulum (SR) Ca^{2+} ATPase activity. Under these conditions, fibers were repetitively exposed to high K^+ (150 mM) or 4CmC (400 μM), in the absence of extracellular Ca^{2+}, to deplete SR Ca^{2+} stores. After store depletion, readmission of Ca^{2+} (5 mM) in the external medium caused an increase in the basal Ca^{2+} concentration, $[Ca^{2+}]_i$, which initially proceeded at a rate of 0.84 ± 0.15 nM/s and later at a rate of 5.64 ± 1.96 nM/s (mean ± sem, n=12). Normally, after reaching $[Ca^{2+}]_i$ values higher than 400 nM, external Ca^{2+} was withdrawn to avoid massive contraction of the fibers. Ca^{2+} entry was much reduced in fibers exposed to 2 μM FCCP for 2 min, after depletion of SR Ca^{2+}: in 9 fibers the initial rate of Ca^{2+} increase was 0.34 ± 0.05 nM/s (mean ± sem) and a steady plateau of 161.3 ± 14.1 nM was reached in 217 ± 19 s. The results show that mitochondria disruption affects Ca^{2+} entry through the store operated pathway, indicating that they may have an important role for the activation of this mechanism.

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1509-Pos P311 Regulates Store-operated Calcium Entry In Skeletal Muscle

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Board B485

Recent advances in non-excitable cells have defined Orai1 as a pore-conducting unit of store-operated Ca^{2+} entry (SOCE) located on the plasma membrane, and STIM1 as the Ca^{2+} sensor located on the endoplasmic reticulum. Previous works from our group and others demonstrated the existence of SOCE in skeletal muscle cells; however, the molecular regulators of SOCE in muscle development are still unclear. Here we show that P311 is an essential player in SOCE that could regulate the established components of the SOCE machinery in muscle cells. The discovery of P311 originated from our findings that SOCE is developmentally regulated in skeletal muscle, as SOCE is almost absent in C2C12 myogenic cells at the myoblast stage and is induced upon differentiation into myotubes. Using a combination of DNA microarray screening and shRNA silencing approaches, we found that mRNA for P311 was significantly upregulated in C2C12 myotubes compared with C2C12 myoblasts, and silencing of P311 led to complete ablation of SOCE in C2C12 myotubes. P311 is an 8 kDa cytosolic protein coded by a gene also known as the pentylenetetrazol-related gene *PTZ-17*. Western blot confirmed that P311 is up-regulated during C2C12 myoblasts differentiation. Overexpression of exogenous P311 in C2C12 cells resulted in marked enhancement of SOCE. Together, our data suggest that P311 may be a potent cytosolic regulator of SOCE.

1510-Pos Comparison of the Spatially-Averaged Myoplasmic Calcium Transient (Δ[Ca]) Elicited by an Action Potential (AP) in Fast-Twitch Fibers of Normal and *Mdx* Mice

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In a study of Δ[Ca] evoked by an AP in enzymatically-dissociated FDB fibers of adult mice, Woods et al. (J. Physiol. 557, 2004) detected two abnormalities in *mdx* fibers (22 °C; fibers not containing mmolar [EGTA]):

1. peak amplitude was ~0.55 times normal;
2. full-duration at half-maximum (FDHM) was ~6 times normal (48 vs. 8 ms).

We have made similar comparisons using intact, rather than enzymatically-dissociated, fibers (16 °C). An individual fiber within a manually-dissected bundle from an EDL muscle was microinjected with furaptra, and Δ[Ca] was measured as described by Baylor and Hollingworth (J. Physiol. 551, 2003). In 17 normal fibers, the amplitude and FDHM of Δ[Ca] were 18.5 ± 0.6 μM and 5.0 ± 0.2 ms, respectively (mean ± SEM); in 12 *mdx* fibers, the corresponding values were 14.2 ± 0.6 μM and 4.7 ± 0.2 ms. The difference in amplitude is significant ($P<0.001$; two-tailed *t* test) whereas the difference in FDHM is not ($P=0.3$). At present, we do not understand the source of the large FDHM in the *mdx* fibers of Woods et al. Computer modeling (cf. Baylor and Hollingworth, J. Gen. Physiol. 130, 2007) indicates that a ~23% reduction in δ[Ca] amplitude is consistent with a ~16% reduction in the peak rate of sarcoplasmic reticulum Ca release (if the release time course is unchanged). Additional modeling in *mdx* fibers is in progress to investigate possible alterations in other variables, including the time

course of SR Ca release, resting [Ca], the concentration of parvalbumin, and the binding and pumping of Ca by the SR Ca pump.

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1511-Pos Structural and Functional Evaluation of Branched Myofibers in Young and Old *Mdx* Mice

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Board B487

Skeletal muscle function is dependent on the organization of skeletal muscle structure. In dystrophic (dy/dy) muscle, malformed myofibers have been shown to be weaker and prone to injury when stimulated (Head et al, *J Physiol*, 1990). This concept was further strengthened by recent findings which show that, in the extensor digitorum longus muscle (EDL) of dystrophic (*mdx)* mice, susceptibility of whole muscle to injury from lengthening contractions is linked to the extent of myofiber branching within the muscle (Chan et. al., *Am J Physiol-Cell*, 2007). We examined the morphology of enzymatically-isolated muscle fibers of the EDL and flexor digitorum brevis (FDB) from young (2–3 months) and old (8–9 months) *mdx* and control mice (C57BL10). In young *mdx* EDL, 6% of the myofibers had visible malformations (i.e., inter-fiber splitting, branched ends, mid-fiber appendages). In contrast, 70% of myofibers in old *mdx* mice contained visible malformations. In the *mdx* FDB, malformation occurred in only 4% of the young myofibers and 52% within old myofibers. Age-matched controls did not display the altered morphology of *mdx* muscles. The membrane-associated (e.g., beta-spectrin) and cytoplasmic (e.g., desmin) cytoskeletal structures appear normal in the malformed *mdx* myofibers, and despite multiple branches, all had a single neuromuscular junction (TRITC-BTX). In *mdx* FDB's with significantly branched ends, an assessment of global, electrically evoked Ca^{2+} signals (Indo1PE-AM) revealed a 23.3 ± 4.8% decrease in amplitude and a 14.2 ± 6.2% increase in the decay time constant in myofibers with significant branching. No alteration in the basal myoplasmic [Ca^{2+}] (i.e., Indo Ratio) was seen in malformed vs. normal *mdx* myofibers. In summary, aging *mdx* myofibers develop morphological malformations that are likely associated with alterations in myofiber Ca^{2+} signaling.

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1512-Pos Alterations in the Dystrophin Glycoprotein Complex (DGC) are Associated with Ca^{2+} Release Impairment in Skeletal Muscle Fibers

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We have previously shown that Ca^{2+} release is depressed in *mdx* mice. Here, we have further studied this process in *mdx* mice and in a transgenic mice that overexpress >10-fold sarcospan (Tg-SSPN); both conditions are primarily associated with alterations of the DGC. AP-elicited Ca^{2+} release was measured in enzymatically isolated fibers from mdx and SSPN-Tg FDB muscles. OGB-5N was used as the Ca^{2+} indicator and a two-microelectrode amplifier was used for the electrophysiological measurements. *Mdx* fibers were internally dialyzed with 2, 5, 10, 20 or 30 mM EGTA adjusted to pCa~7. Ca^{2+} release was elicited by either single or repetitive stimulation. Tg-SSPN fibers were dialyzed with 20 mM EGTA. Ca^{2+} release fluxes were calculated from the Ca^{2+} transients using a kinetic model that took into account the Ca^{2+} binding properties of intracellular buffers. For all the [EGTA]s tested, Ca^{2+} release in *mdx* fibers was smaller than that in control fibers; maximal differences were observed at 2 and 30 mM EGTA. In response to repetitive stimulation, *mdx* fibers displayed further depression of the amplitude of individual Ca^{2+} transients along the train than those from control mice; furthermore, the inter-pulse [Ca^{2+}] seemed to remain higher in *mdx* fibers. In contrast the APs did not report differences between *mdx* and control fibers. Ca^{2+} release was also depressed (up to 70 %) in Tg-SSPN mice compared with controls; also, as occurred in *mdx* muscle fibers, no significant changes were observed in the APs from Tg-SSPN. Nevertheless, unlike in *mdx* mice, two-photon imaging of Tg-SSPN fibers stained with di-8-ANEPPS showed abnormalities in the architecture of transverse tubular system.

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1513-Pos Gene Expression Upon Electrical Stimuli In Normal And Dystrophic Muscle Cell Lines

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Board B489

Skeletal muscle responds to exercise through changes in gene expression that modify fiber characteristics and muscle mass. Slow calcium signals, associated to IP3 receptors can be elicited in cultured muscle cells upon electrical stimulation and have been shown to be involved in regulation of gene expression.

The aim of this study was to compare the pattern of gene expression in normal (RCMH) and dystrophic (RCDMD) human skeletal muscle cell lines comparing rest and depolarization conditions, to establish possible alterations in the adaptive response of skeletal muscle cells to a model of exercise.

We used 22 K mouse oligonucleotide microarrays to analyze total RNA from RCMH (n=5) and RCDMD (n= 4) myotubes obtained 4h post-electrical stimulation (400pulses, 1ms, 45Hz). cDNA prepared from control and depolarized samples was labeled with Alexa-555 and Alexa-647 dyes prior to microarray hybridization. Loess normalization followed by statistical analysis using an unad-

justed P-value ≤ 0.01 as cut off, resulted in 132 and 150 differentially expressed genes in RCMH and RCDMD myotubes, respectively. The main differences in the transcriptional response were observed for genes involved in "regulation of actin cytoskeleton" and "Wnt signaling pathway" in RCMH cells, and "natural killer cell mediated cytotoxicity" and "MAPK signaling pathway" in RCDMD cells.

We focused our interest on 44 common genes differentially expressed in both cell lines upon electrical stimulation. Particularly different amongst these cell lines was Neuregulin-1 gene expression, a growth factor that potentiates myogenesis and induces utrophin gene expression, a homolog of dystrophin, in skeletal muscle cells.

1514-Pos Altered Properties of Voltage-Activated Ca^{2+} Release Flux in Skeletal Muscle of a Transgenic Mouse Heterozyous for the Y522S Mutation in RyR1

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Board B490

Malignant Hyperthermia (MH) is a life-threatening hypermetabolic condition induced by certain trigger agents and resulting from excessive Ca^{2+} release in skeletal muscle of susceptible individuals. To study characteristics of voltage-activated Ca^{2+} release in MH muscle, we used transgenic mice (Y522S+/−) heterozygous for one of the human ryanodine receptor mutations (Chelu et al. 2006, FASEB J, *20*:329). Fura-2 fluorescence transients elicited by step depolarization in voltage-clamped muscle fibers of adult Y522S+/− mice were converted to Ca^{2+} release flux using a removal model fit procedure (Ursu et al. 2005, J. Physiol, *562*:347). Previous studies indicated a higher sensitivity of Ca^{2+} release to voltage activation in MH muscle revealed by a left-shift of the activation curve. MH muscle is also more sensitive to caffeine, a well known RyR1 agonist used in diagnostic tests to identify MH susceptibility. This drug at concentrations of 2, 5 and 10 mM likewise induced a left shift in the voltage dependence of Ca^{2+} release which was stronger in Y522S+/− fibers. Studying the release flux time course, we found a higher peak relative to its plateau value in fibers of mutant mice and a faster rise to the peak at a given voltage. To investigate recovery from the rapid inactivation following the release flux peak, a double pulse protocol was applied consisting of a conditioning pulse to +50 mV (20 ms) and a subsequent test pulse. The time interval between pulses was varied from 10 to 690 ms. The time course of peak recovery was slower in fibers of mutant animals compared to controls. We tentatively attribute these changes in Y522S+/− fibers to alterations in Ca^{2+}-induced Ca^{2+} release and Ca^{2+}-dependent inactivation of the mutant ryanodine receptors.

1515-Pos Enhanced Excitation-Coupled Calcium Entry in Myotubes Expressing Malignant Hyperthermia Mutation R163C is Attenuated by Dantrolene

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Board B491

Dantrolene is the drug of choice for the treatment of the pharmacogenetic syndrome malignant hyperthermia (MH), a life-threatening complication of general anesthesia and other clinical conditions in humans and animals. MH is associated with acute increase in intracellular Ca^{2+} and contracture of skeletal muscle. The muscle relaxant properties of dantrolene have been closely correlated with its ability to reduce Ca^{2+} rises in the myoplasm however the exact mechanisms by which dantrolene interrupts the MH episode and promotes skeletal muscle relaxation remain unclear. In this study we demonstrate that one mechanism of dantrolene's action is to block excitation coupled calcium entry (ECCE) in both adult mouse FDB fibers and primary myotubes. Block of ECCE is independent of either inhibition of RyR1 channels reconstituted in BLM or activation of SR Ca^{2+} release triggered by EC coupling. The inhibitory potency of dantrolene on ECCE is consistent with the drug's clinical potency for reversing the MH syndrome, and is incomplete (by ~70%) as predicted by its efficacy as a muscle relaxant. This effect on ECCE is dose-dependent (IC_{50}=4.2 μM). Conventional capacitative calcium entry was not affected by dantrolene. Using the Mn^{2+} quench technique, we report the novel finding that myotubes isolated from mice heterozygous and homozygous for the MH susceptibility mutation R163C in RyR1 show significantly enhanced ECCE rates. The enhanced ECCE in MH cells could be restored to those measured in wild type cells after exposure to clinical concentrations of dantrolene. We propose that gain of ECCE function is an important etiological component of MH susceptibility and possibly contributes to the MH episode. We identify inhibition of ECCE as new mechanism for dantrolene's clinical efficacy as a muscle relaxant.

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1516-Pos Time Course Of K+-induced Calcium Transients In Malignant Hyperthermia (R163C) Myotubes

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The effect of raising extracellular K^+ was investigated in Fluo 4-AM loaded myotubes from non-MH mice (expressing wild type RyR1

(MHN) or heterozygous (HET) and homozygous (HOM) "knock-in" mice with a MH R163C mutation (MHS). Exposure of MHN and MHS myotubes to high K^+ solutions elicited Ca^{2+} transients whose peaks were similar but the magnitude was greater in MHS than in MHS and dependent on $[K^+]_e$. The pharmacological threshold for K^+ induced Ca^{2+} transients was less in MHS than MHN. The Ca^{2+} signal showed an initial peak that was followed by spontaneous decay in all groups of myotubes, in spite of the fact that the plasma membrane remained depolarized. However, this decay was slower in MHS than MHN myotubes, and as consequence the duration of the transients was longer in MHS than MHN. The spontaneous decay of the Ca^{2+} signal after K^+ depolarization was not due to exhaustion of Ca^{2+} in the sarcoplasmic reticulum (SR) and/or RyR1 inactivation in MHN or MHS myotubes since caffeine was able to induce a robust intracellular Ca^{2+} release at the end of the K-induced Ca^{2+} transients in all of the genotypes. We conclude that K^+-induced Ca^{2+} transients in MHN and MHS myotubes are voltage, time and genotype dependent. MHS myotubes exhibit a lower threshold for K^+-induced Ca^{2+} transients than MHN myotubes. The spontaneous decay of the Ca^{2+} signal during a K^+ depolarization is a sign of DHPR inactivation rather than a Ca^{2+} depletion of the SR or inactivation of RyR1. The onset of the inactivation process is slower in MHS (greater in HOM than HET) than MHN myotubes.

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1517-Pos Store Operated Ca^{2+} Entry In Skeletal Muscle From Humans Susceptible To Malignant Hyperthermia

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Board B493

Store-operated Ca^{2+} entry (SOCE) has been shown to occur following pharmacological depletion of SR Ca^{2+} in mechanically skinned skeletal fibres. While SOCE does not appear to be significant following physiological SR Ca^{2+} depletion (e.g. during a twitch or tetanus) it may have a role in pathological forms of SR Ca^{2+} release. In the present study, SOCE was studied in muscle fibers from patients susceptible (MHS) or not susceptible (MHN) to malignant hyperthermia (MH). Most cases of MH are caused by RyR1 mutations, which confer increased sensitivity to activation of the channel by volatile anesthetics or caffeine. Samples of human vastus medialis muscle were obtained with ethical approval from patients under investigation for MH susceptibility. Single fibers were exposed to fluo-5N before mechanical skinning under paraffin oil, thereby trapping the dye in the resealed t-system. In both MHS (n=4) and MHN (n=6) fibers, induction of a maximal SR Ca^{2+} release (induced by 20 mM caffeine/ 0.05mM Mg^{2+}) caused depletion of t-system Ca^{2+}, apparent as a sustained reduction in Fluo-5N fluorescence. Under conditions designed to mimic anesthetic induction, 0.5 mM halothane induced SR Ca^{2+} release and coincident depletion of t-system Ca^{2+} in all MHS fibers (n=4). In contrast, none of the MHN fibers responded to 0.5 mM halothane (n=6). These results

(i) demonstrate the presence of SOCE in human skeletal muscle and
(ii) suggest that SOCE might contribute to the sustained rise in $[Ca^{2+}]_i$ that occurs during an MH episode.

1518-Pos Functional Changes in Skeletal Muscle of a Mouse Model for Huntington's Disease

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Board B494

Huntington's disease (HD) is a fatal neurodegenerative disorder resulting from an expanded CAG repeat coding for an elongated polyglutamine stretch in the mutant huntingtin protein. Expression of mutant huntingtin is not restricted to the brain but also found in other tissues including muscle. Skeletal muscle of HD patients and of R6/2 mice, the best studied transgenic animal model for this disease, shows atrophy and characteristic changes in the overall gene expression pattern. In this study, we searched for functional changes in muscles of R6/2 mice. Isometric force was recorded in both slow (m. soleus) and fast twitch muscle (EDL). While the time to peak of twitch contractions remained almost unchanged the half time of twitch and tetanus relaxation was found to be significantly increased in EDL. The force-frequency relation was shifted to lower frequencies. No corresponding alterations were observed in soleus muscle, indicating that changes in contractile properties are confined to the fast-type muscle fibers of R6/2 mice. To investigate properties of voltage-activated Ca^{2+} release, we derived Ca^{2+} fluxes from fura-2 fluorescence records in voltage-clamped fast muscle fibers (m. interosseus). In responding fibers of R6/2 mice, the dependence of peak Ca^{2+} release on membrane voltage was minimally changed. On the other hand, localized intracellular Ca^{2+} transients ("sparks") triggered by hyperosmotic solutions (Wang et al., Nat. Cell Biol. *7*:525, 2005) and measured using fluo-4 and confocal line scanning revealed a significantly increased duration (FDHM). The observed changes might result from a reduced rate of calcium removal from the myoplasmic space that could for instance be expected based on a reported reduced expression of the Ca^{2+} binding protein parvalbumin.

1519-Pos The Importance of SERCA2 in Skeletal Muscle Function and Fatigue Development

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Board B495

Fatigue is defined as the inability to maintain force or power output during sustained or repetitive contractions. It is hypothesized that fatigue can be linked to intrinsic alterations in the sarcoplasmatic reticulum (SR) limiting Ca^{2+} release and hence activation of the contractile apparatus and force output. Contraction ends and the muscle relaxes when SR Ca^{2+} ATPase (SERCAs) pumps cytosolic Ca^{2+} back into the SR refilling the Ca^{2+-}store. A reduction in SERCA pump rate or activity in muscles after exercise has been reported. SERCA proteins exist as 3 family members. SERCA1, expressed in fast twitch skeletal muscle fibers, SERCA2, expressed in slow twitch skeletal muscle fibers and the heart and SERCA3, expressed in various non-muscle tissues. Loss of SERCA1 results in exercise-induced impaired muscle relaxation in humans (Brody's disease). We would expect loss of SERCA2 to be seriously detrimental to slow twitch skeletal muscle. We therefore generated *Serca2*$^{flox/flox}$*MLC-1f*$^{wt/cre}$ (MLC1f-SERCA2KO) mice, in which the *Serca2* gene is deleted in skeletal muscle. *Serca2* gene excision was detected in soleus, extensor digitorum longus, tibialis anterior, gastrocnemius muscles and the diaphragma, but not in the heart or in non-muscle tissue. MLC1f-SERCA2KO mice appeared overall normal. In soleus muscle, *Serca2* mRNA and SERCA2 protein were reduced to 12 ± 2 % (SEM) and 6 ± 2 % of control values, respectively. SERCA1 and SERCA3 mRNA or protein expression levels were very low and unaltered. *In situ* experiments showed that there was no difference between MLC1f-SERCA2KO and control soleus muscles in the maximum tetanic force generation and fatigue development. We conclude that contrary to expectations MLC1f-SERCA2KO soleus muscle shows normal contractile properties in spite of almost complete loss of SERCA2 protein suggesting that impaired Ca^{2+} sequestering by the SR cannot be a primary cause of fatigue.

1520-Pos Failure to Generate Action Potential in Aging Muscle Fibers under Physiological Recording Conditions

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Board B496

The tetanic force of intact muscle fiber contractions significantly decreases in aged compared to young mice and is often partially fused. This study examined the passive electrical properties and action-potential generation in enzymatically dissociated, current-clamped *flexor digitorum brevis* muscle fibers from young (3–6 month) and old (21–24 month) FVB mice. Electrotonic and action potentials were recorded in response to single sub- and suprathreshold current pulses of 0.5 ms duration. Repetitive suprathreshold stimulation at 40, 75, and 100Hz with 10s intervals was applied for as long as the cell was viable. Approximately 50% of the fibers from aging mice exhibited frequency-dependent failures in action-potential generation at 100Hz, a typical frequency for fast-twitch motor unit function, but not at lower frequencies, which underscores the physiological relevance of our findings. To analyze the underlying mechanism for this alteration in aging muscle fibers, we examined the voltage-dependence of activation and inactivation for the sodium and delayed rectifier potassium channels in the whole-cell and cell-attached configurations of the patch-clamp.

Supported by NIH/NIA

1521-Pos Effects Of Hyperosmotic Agents On Intracellular Ca^{2+} And Cell Morphology In Rat Skeletal Muscle Fibers

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Board B497

Skeletal muscle swells as a consequence of intense muscular activity and then returns to its original volume following cessation of exercise. Recent work on isolated flexor digitorum brevis skeletal muscle fibres has shown that cell swelling (induced by hypoosomotic solutions) has little effect on $[Ca^{2+}]_i$, while cell shrinking (induced by hyperosmotic solutions) causes a rise in $[Ca^{2+}]_i$. However, previous studies have used differing methods to increase osmolarity including addition of sugars or $CaCl_2$. We have compared the effects of increasing extracellular osmolarity, using either $CaCl_2$ or sucrose. The concentrations were adjusted to produce the same change in cell volume. The effects of reduced osmolarity (achieved by decreasing $[NaCl]_e$ from 140.3 mM to 55 mM) was also investigated. Rat flexor digitorum brevis muscle fibers were isolated enzymatically and loaded with Fura-2 AM. In some experiments, the structure of the t-tubule was investigated using di-8-ANNEPS, combined with confocal microscopy. Switching from control (298mOsm) to a hyperosmotic-sucrose (404mOsm) solution caused a robust rise in cytocolic $[Ca^{2+}]$. However, the amplitude of the response to hyperosmotic-$CaCl_2$ (404mOsm) was only 9.6 ±0.03% of that induced by sucrose ($n=8$; $P<0.001$), despite a similar change in cell volume. Preincubation with the RyR1 inhibitor tetracaine (1 mM) reduced the sucrose response to 3.6 ± 0.06%, ($n=6$; $P<0.001$), suggesting the rise in cytocolic Ca^{2+} reflects SR

Ca^{2+} release. Exposure to the hypoosmotic (254mOsm) extracellular solution elevated $[Ca^{2+}]_i$ by only 7.3 ± 0.04% (n=8; P<0.001) of the response to sucrose. Assessment of t-tubule integrity revealed that exposure to hyperosmotic-sucrose, but not hyperosmotic-Ca^{2+} or hypoosmotic solution, generated visible t-tubule disruption. The mechanism underlying the marked difference in response to sucrose or Ca^{2+} is not presently known.

1522-Pos MG53 Is An Essential Component Of The Acute Membrane Repair Machinery

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An elemental process in cell biology involves dynamic membrane remodeling and repair, which mediates efficient cellular signaling and maintains cell integrity. Membrane repair defects have been linked to numerous disease states including muscular dystrophy, heart failure and neurodegeneration. Repair of the plasma membrane damage requires intracellular vesicular trafficking to injury sites, a process that is thought to involve entry of extracellular Ca through the damaged plasma membrane. While there is significant interest in establishing the mechanisms underlying plasma membrane repair, the molecular machinery that facilitates vesicle translocation during acute membrane repair has not been fully resolved. Here we show that mitsugumin 53 (MG53), a novel muscle-specific tri-partite motif (TRIM) family protein, is a critical component of the acute cell membrane-repair machinery. MG53 interacts with phosphatidylserine to associate with intracellular vesicles, which display dynamic trafficking to and fusion with sarcolemmal membranes. Mice null for MG53 exhibit progressive muscular dystrophy with defective membrane-repair capacity. Acute injury of the sarcolemmal membrane leads to exposure of the cell interior to an external oxidized environment that produces oligomerization of MG53, followed by recruitment of MG53-containing vesicles to patch the injury site. Mutation of a critical cysteine residue in MG53 that disrupts oligomerization of MG53 leads to impairment of repairsome formation at the sites of membrane damage. Together, our dada demonstrate that muscle membrane repair involves two physiological steps that require oxidation-mediated nucleation of MG53 at the injury site followed by vesicle accumulation leading to repairsome formation.

1523-Pos Blebbistatin: Use As A Myocardial Excitation-Contraction Uncoupling Agent

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Blebbistatin (BLEB) is a recently discovered compound that specifically inhibits myosin II ATPase activity. If specific, this agent could be used to uncouple myofilament contractility from the electrical events that lead to cytosolic Ca^{2+} release in the cardiac myocyte. We investigated the mechanical and structural actions of BLEB inhibition in intact and skinned rat cardiac trabeculae, single rabbit psoas myofibrils and in intact isolated rat myocytes. Application of BLEB (10 Î¼M) reduced twitch force in intact trabeculae without altering the orientation or position of the myosin heads under diastolic conditions. BLEB reduced contractile force in skinned trabeculae without affecting tension dependent myofilament ATPase activity (index of cross-bridge cycling kinetics). Application of BLEB to skinned trabeculae (overnight) or intact trabeculae (>15 min/dose) in the dark reduced Ca^{2+} activated force (EC_{50}=0.38+/−0.03 ÃŽÂ¼M skinned fibers; EC_{50}=3.92+/−0.73 ÃŽÂ¼M intact fibers). While the rapid (<5ms) application of BLEB to single Ca^{2+} activated rabbit myofibrils immediately reduced force this rate of force decline depended on the [BLEB]. Two-photon line scan microscopy in isolated myocytes (0.5 μM BLEB, >1hr) with Indo1-AM ratiometric 2-photon analysis revealed no significant impact of BLEB on the Ca^{2+} transient (ΔCa^{2+} 0.033+/−0.002 vs. 0.034+/−0.003; μM, τCa^{2+} 263+/−3 ms vs. 278+/−3 ms; control vs. BLEB resp.; n=31–39). We conclude that BLEB specifically uncouples cardiac myofilament activation from Ca^{2+} activation without affecting the cross-bridge cycling kinetics, EC-coupling, or structural parameters. However, the compound is very sensitive to light, a property that severely limits its application to mechanistic physiological studies.

Excitation-Contraction Coupling - II

1524-Pos Efficient Excitation-Contraction Coupling (ECC) in Adult Rabbit Ventricular Cardiomyocytes Requires Brain-type Na Channels, Na/Ca Exchanger (NCX) and L-type Ca Channels

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