I-bands. This effect is mediated by the direct interaction of obscurin's Ig2 with a novel isoform of the thick filament associated protein, Myosin Binding Protein-C Slow (MyBP-C slow), that corresponds to transcript variant-1. Variant-1 contains all the structural motifs known to be present in MyBP-C slow (variant-3), but has a unique COOH-terminus consisting of twenty-six amino acids and a new termination codon. RT-PCR showed that variant-1 is abundantly expressed in skeletal muscles during development and at maturity. Quantitative RT-PCR further demonstrated that transcripts containing the novel COOH-terminus are expressed in higher amounts than those lacking it. Three different antibodies to the unique COOH-terminus of variant-1 labeled M-bands and flanking regions in both developing and adult myofibers, suggesting that unlike other forms of MyBP-C that reside in C-zones, variant-1 preferentially concentrates in the middle of the A band. Adenoviral overexpression of obscurin's Ig2 domain and reduction of obscurin via siRNA inhibited the integration of variant 1 of MyBP-C slow into forming M-bands in skeletal myotubes. Collectively, our experiments identify a new ligand of obscurin at the M-band, MyBP-C slow variant-1, and suggest that its interaction with obscurin contributes to the assembly and maintenance of M- and A-bands.

Excitation - Contraction Coupling: Skeletal

1197-Pos Board B41

Functional Identification Of Fiber Types In Enzymatically Dissociated Murine Flexor Digitorum Brevis (FDB) And Soleus Muscles

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Enzymatically dissociated FDB and soleus fibers from mouse were used to compare the kinetics of electrically elicited Ca^{2+} transients of slow and fast skeletal muscle fibers, using the fast Ca^{2+} dye MagFluo4-AM, at 20-22°C. For the case of FDB fibers we found two different morphologies for both single-twitch and tetanic Ca^{2+} transients named morphology type I (MT-I) and morphology type II (MT-II). The kinetic parameters (mean ± s.e.m) of MT-I (11 fibers, 19%) and MT-II (47 fibers, 81%) single-twitch transients were: amplitude (ΔF/F): 0.36 ± 0.03 vs. 0.69 ± 0.03; rise time (ms): 1.44 ± 0.15 vs. 1.01 ± 0.02; half-amplitude width (ms): 10.25 ± 0.92 vs. 3.87 ± 0.12; decay time (ms): 46.15 ± 1.99 vs. 21.08 ± 0.89; and time constants of decay (τ_1 and τ_2, ms): 2.57 ± 0.19 and 33.71 ± 2.29 vs. 1.51 ± 0.05 and 13.19 ± 0.63, respectively; all differences being statistically significant ($p<0.001$). All Ca^{2+} transients parameters of soleus fibers ($n=20$) were not different ($p>0.1$) from those of MT-I FDB fibers. Tetanic responses (100 Hz) of MT-I FDB and soleus fibers showed a staircase shape while the time course of decay followed a single exponential (τ, ms): 73.36 ± 6.82 for FDB and 74.59 ± 6.24 for soleus (both $n=8$). In MT-II FDB tetani no staircase was present, the first peak was larger than the others, and the time course of decay was bi-exponential (Capote et al, J Physiol 2005;564:451). Histochemical and biochemical characterization of both muscles suggest that signals assigned MT-I correspond to slow type I and fast IIA fibers while those assigned MT-II correspond to fast IIX/D fibers. The results point to the importance of Ca^{2+} signaling for characterization of muscle fibers, but also to its possible role in determining fiber function. (FONACIT G-2001000637 and G-2005000372). JCC was supported by Universidad de Antioquia, Colombia.

1198-Pos Board B42

Contribution Of Ryr1 "Leak Channels" To Resting Intracellular Ca2+ In Skeletal Myotubes

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The control of resting free Ca^{2+} ($[Ca^{2+}]_{rest}$) in skeletal muscle is thought to be a balance of channels, pumps (sarcoplasmic reticulum -SR- and plasma membrane ATP-dependent pumps) and exchangers ($Na+/Ca^{2+}$ exchanger). We hypothesized that expression of RyR1 in dyspedic muscle cells, which constitutively lack expression of the skeletal muscle SR Ca^{2+} release, channel, RyR-1, RyR-2, and RyR-3 ($_{Null}$RyR-myotubes) would increase $[Ca^{2+}]_{rest}$ and that this increase would be secondary to passive Ca^{2+} efflux from SR stores mediated by Ry-insensitive leak channels. We explored these mechanisms by measuring $[Ca^{2+}]_{rest}$, using double-barreled Ca^{2+} microelectrodes, in $_{null}$RyR myotubes and myotubes expressing wild-type RyR1 ($_{WT}$RyR1). In addition, changes in $[Ca^{2+}]_{rest}$ produced by several drugs known to modulate the RyR1 channel complex were investigated. We found that $_{WT}$RyR1 myotubes had a 2.0 -fold higher $[Ca^{2+}]_{rest}$ than $_{null}$RyR myotubes. Exposure of both $_{null}$RyR myotubes and $_{null}$RyR myotubes expressing wild-type RyR1 ($_{WT}$RyR1) to 500 μM ryanodine (Ry) or 20 μM (2,6-dichloro-4-aminophenyl) isopropylamine (FLA 365), both of which completely block the caffeine response, had no effects on $[Ca^{2+}]_{rest}$;. However, when $_{WT}$RyR1 myotubes were exposed to a combination of 500 μM Ry and bastadin 5 (B5), $[Ca^{2+}]_{rest}$ was significantly reduced at 23°C in myotubes that express $_{wt}$RyR1 and was reduced to essentially $_{null}$RyR levels at 37°C but had no effect in $_{null}$RyR cells. These results show that expression of RyR1 is responsible for more than half of $[Ca^{2+}]_{rest}$ seen in *Wt* cells and this increase over dyspedic levels is not the result of active gating of the RyR1 channel but instead can be accounted for by RyR1's ryanodine insensitive leak conformation. Supported by NIH PO1 AR052534 (PDA, INP).

1199-Pos Board B43

Prediction of Twitch and High Frequency Local Calcium Dynamics in Mouse EDL Fibers at 15-35°C

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Sites of calcium release, uptake and action are highly organized and densely packed in skeletal muscle cells. This organization suggests an important role for the spatial distribution of organelles and calcium sensitive proteins in muscle function. The current measurement techniques are not able to measure cytosolic [Ca2+] with both high temporal and high spatial resolution. In addition, it is known that all processes are highly temperature dependent. Therefore, the model of Groenendaal et al. [1], that describes local calcium dynamics at physiological frequencies at 35°C, was extended with Q10 correction factors and calcium-fluorescent dye binding kinetics, to predict local calcium dynamics at 15-35°C.

For model validation, simultaneously calcium fluorescent dye and force kinetic measurements were performed. Hereto, murine EDL muscles were isolated and mounted in the set up. Muscle length was adjusted to optimal resting length and stimulation voltage was adjusted to generate maximal force. Rhod-2 AM was loaded in whole muscle at 37°C for 30 minutes or until force was decreased with >25%. Muscles were stimulated at 15-35°C at frequencies between twitch and tetanus.

A significant relation was found between the temperature and the fluorescence characteristics, e.g. decrease in decay time with increasing temperature (one-way ANOVA, p-value<0.05). Model simulations predicted a comparable range. In addition, simulations showed an approximately five-fold difference in calcium gradient throughout the sarcomere between 15 and 35°C.

The validated model is now able 1. to explain calcium fluorescent dye measurements, 2. to translate data at unphysiological to physiological temperature and 3. to predict local calcium dynamics at low and high frequency stimulations at a wide temperature range.

[1] Groenendaal et al. IET Systems Biology, in press.

1200-Pos Board B44

Effects of Changes in Extracellular Concentration of Na^+ and K^+ ($[Na^+]_o$, $[K^+]_o$) on the Ca^{2+} Release Elicited by High Frequency Stimulation. Implications for Muscle Fatigue

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Changes in $[Na^+]_o$ and $[K^+]_o$ occurring during high frequency stimulation has been proposed as a cause of muscle fatigue. We investigated this hypothesis by measuring the Ca^{2+} release elicited by short high frequency trains (100Hz, 10 pulses) in rested frog semitendinosus fibers exposed to various $[Na^+]_o$ or $[K^+]_o$. Myoplasmic $[Ca^{2+}]$ changes (Ca^{2+} transients) elicited by action potentials (AP) were estimated from Ca^{2+}-dependent OGB-5N fluorescence changes. Segments of fibers, stretched to 4.5-5μm, were mounted in an inverted double grease-gap chamber placed in an inverted microscope equipped for epifluorescence. Fibers were held at -100mV and stimulated with 0.5ms current pulses. Normal Ringer solution contained (mM): 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 10 MOPS, 10 dextrose, pH=7.2 with NaOH. $[K^+]_o$ ($[Na^+]_o$) was increased (reduced) by equimolar replacement with Na^+ (N-methyl-D-glucamine). Fibers were loaded (30min) with a solution containing (mM): 110 aspartate, 5 ATP-K_2, 5 Na_2-creatine-phosphate, 20 MOPS, 0.05-0.1 EGTA, 5 $MgCl_2$, 0.2 OGB-5N, pH=7.2 with KOH. A complex interaction of the effects of changing $[K^+]_o$ or $[Na^+]_o$ on membrane potential, AP overshoot and duration, and Ca^{2+} release was found. Using normal Ringer, the amplitude of Ca^{2+} transients elicited by single pulses increased with depolarizations up to -65mV. Raising $[K^+]_o$ had a dual effect on Ca^{2+} release. Ca^{2+} transient's amplitude increased between 2.5 to 10 mM, and decreased markedly for higher $[K]_o$. Potentiation of Ca^{2+} release, but not depression, could be reverted by current injection. This suggests a depolarization independent effect of K^+ ions on Ca^{2+} release.

Halving $[Na^+]_o$ did not affect Ca^{2+} release elicited by single stimulation, but impaired the release in response to repetitive stimulation. Further reduction of $[Na^+]_o$ to 1/3 of normal highly reduced Ca^{2+} release.

1201-Pos Board B45

Intracellular Ca^{2+} Homeostasis In Rat Fast-Twitch Skeletal Muscle Fibers During Disuse atrophy

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Skeletal muscle atrophy is presumed to be associated with changes in Ca^{2+} signalling pathways but whether or not intracellular Ca^{2+} homeostasis is critically affected in that situation and if so how, remains unclear. Furthermore, this question has so far been essentially addressed in slow-twitch muscle and there is very limited related information concerning fast-twitch muscle. Here we characterized properties of *flexor digitorum brevis* (FDB) muscles following a 2 week period of hindlimb suspension. This protocol resulted in a 19 % reduction in FDB muscle weight and in a corresponding 18 % reduction in fiber diameter. Fibre type distribution remained however unchanged with 12 % of type I, 84 % of type IIa, and 4 % of type IIb fibres. Voltage-clamp measurements showed that the slow Ca^{2+} current yielded essentially identical properties in control and atrophied fibers. In voltage-clamped fibres loaded with the Ca^{2+} dye indo-1, neither the resting $[Ca^{2+}]$ level nor the peak change in $[Ca^{2+}]$ elicited by 5-100 ms-long membrane depolarization from -80 to + 10 mV, significantly differed between control and atrophied fibers. However, the rate of $[Ca^{2+}]$ decay after the end of a pulse was reduced by 30-50% in the atrophied fibres (e.g. rate constant of decay was 13.6 ± 1 and 8.8 ± 0.9 s^{-1} in 20 control and 24 atrophied fibres, respectively, following a 20 ms-long pulse). This effect appears to be consistent with a reduced contribution of both saturable and non-saturable components of myoplasmic Ca^{2+} removal. Still, western blot analysis showed that the amount of two major components of Ca^{2+} removal, parvalbumin and type 1 sarco-endoplasmic reticulum Ca^{2+}-ATPase, was not reduced in the atrophied FDB muscles.

1202-Pos Board B46

Decreased Skeletal Muscle Intramembrane Charge Movement And Contractile Activation In S100A1 Knock Out Mice

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S100A1, a calcium binding protein expressed in skeletal and cardiac muscle, modulates Ca^{2+} signaling. We have previously shown that isolated flexor digitorum brevis (FDB) skeletal muscle fibers from S100A1 knock out (ko) mice exhibit reduced Ca^{2+} transients with delayed onset in response to single action potentials compared to wild type (wt) FDB fibers (Prosser et al, 2008). Utilizing the potentiometric dye di-8-ANEPPS, we now show no differences in action potential properties between wt and ko fibers. Using whole cell voltage clamp of single isolated fibers we examined surface and transverse tubule membrane electrical properties in wt and ko fibers. Preliminary non-linear capacitive current measurements indicate that maximum charge moved (Q_{max}) is less in ko than wt fibers (wt Q_{max} = 43.6 ± 3.4 nC/μF, ko Q_{max} = 32.0 ± 3.5 nC/μF; $p < .05$). The temporally delayed, steeply voltage dependent component of intramembrane charge movement at intermediate depolarizations (Qγ) is less pronounced in ko than in wt fibers, consistent with a steeper voltage dependence of charge moved (wt k = 7.2 ± .5, ko k = 10.8 ± 1.2, $p < .05$). The midpoint voltage of charge moved was not different (wt Vhalf = -26.1 ± 2.8 mV, ko Vhalf = -28.5 ± 1.5 mV). Both the amplitude and the voltage dependence of Cav1 Ca^{2+} currents were similar in wt and ko fibers. In vivo maximal specific force (force normalized to muscle mass) was significantly decreased in ko compared with wt muscles (wt P_o = 1.52 ± .09 g/mg, ko P_o = 1.11 ± .09 g/mg; $p < .05$). These results are consistent with muscles lacking S100A1 having decreased charge moved during membrane depolarization, causing depressed SR Ca^{2+} release, and ultimately decreased force generation.

1203-Pos Board B47

DHPR Activation Is A Prerequisite For SR Ca^{2+} Release Induced By Increased Osmolarity In Isolated Rat Skeletal Muscle Fibres

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Rat *flexor digitorum brevis* (FDB) fibres were superfused with isoosmotic Tyrode's solution before exposure to either hyperosmotic (405 mOsm) or hypoosmotic (254 mOsm) solutions, and the effects on cell volume, membrane potential (E_m) and intracellular Ca^{2+} ($[Ca^{2+}]_i$) determined. Solutions were made hyperosmotic by addition of sugars or divalent cations, and hypoosmotic by reducing $[NaCl]_o$. Under control conditions, FDB fibres typically exhibited a low resting E_m of -60.1 ± 2.91, n=67. All hyperosmotic solutions induced a sustained decrease in cell volume, membrane depolarisation (by ~14-18 mV, n=40) and SR Ca^{2+} release. However, sugar solutions caused a pronounced increase in global $[Ca^{2+}]_i$, while solutions made hyperosmotic by addition of divalent cations induced only LCR. Decreasing osmolarity caused an increase in cell volume and membrane hyperpolarisation (15.04 ± 1.85 mV, n=8), while $[Ca^{2+}]_I$ was unaffected. However, on return to the isoosmotic solution, restoration of cell volume and E_m was accompanied by LCR. With all protocols, SR Ca^{2+} release was markedly inhibited by the RyR1 inhibitor tetracaine or the dihydropyridine receptor (DHPR) inhibitor nifedipine. Inhibition of sarcolemmal Cl^- channels with 9-anthracene carboxylic acid (9-AC) or the Na/K/2Cl (NKCC) co-transporter with furosemide, both induced a marked hyperpolarisation of E_m to -79.2 ± 3.7 mV (n=8) and -89.2 ± 1.7 mV (n=17), respectively. In the presence of furosemide, the depolarisation associated with hyperosmotic shrinkage was reduced by 48.3 ± 2.3 % (n=7). These findings suggest that (i) tonic activation of the NKCC co-transporter and consequent effects on $[Cl]_i$ underlie the low resting E_m in FDB fibres and (ii) the initiation of LCR that accompanies a decrease in cell is caused by membrane depolarisation, which may be linked to increased activity of the NKCC co-transporter.

1204-Pos Board B48

Age Dependent Expression of $Ca_V\beta_{1a}$ Subunit in Aging Skeletal Muscle

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Ca^{2+} release from the sarcoplasmic reticulum (SR) into the cytosol is a crucial part of excitation-contraction (E-C) coupling. E-C uncoupling, a deficit in Ca^{2+} release from the SR, is thought to be responsible for at least some of the loss in specific force observed in aging skeletal muscle. E-C uncoupling may be caused by alterations in the expression of voltage-dependent calcium channel α_{1s} ($Ca_V1.1$) and β_{1a} ($Ca_V\beta_{1a}$) subunits. In addition to its classical role augmenting $Ca_V1.1$ trafficking and function, overexpression of $Ca_V\beta_{1a}$ has recently been implicated by our laboratory as a negative regulator of $Ca_V1.1$ membrane expression. While previous studies have found $Ca_V1.1$ expression declines in old rodents, $Ca_V\beta_{1a}$ expression had not been examined in aging models. We conducted western blot analyses to examine $Ca_V\beta_{1a}$ expression in mouse skeletal muscle at four age groups across the animal's lifespan. Investigation of $Ca_V\beta_{1a}$ expression was further segregated by muscle compartment location, fiber type composition, and mouse strain. Our results show a substantial increase of $Ca_V\beta_{1a}$ expression both early and very late in life of FVB mice, regardless of fiber type or location. In order to examine the specific subcellular location where this increased quantity of $Ca_V\beta_{1a}$ resides, we performed immunocytochemistry on dissociated single FDB fibers. Additional western blot analyses were performed on isolated subcellular fractions from young and old animals. Together with previous data from our laboratory showing decline in $Ca_V1.1$ as a result of both, aging and $Ca_V\beta_{1a}$ artificial overexpression, these studies suggest a novel role of $Ca_V\beta_{1a}$, and that endogenous overexpression of $Ca_V\beta_{1a}$ during old age may contribute to the coincident loss of specific force in skeletal muscle.

1205-Pos Board B49

Divalent Cation Current And Influx Investigated By The Mn2+ Quenching Method In Resting And Active Voltage-Controlled Mouse Skeletal Muscle Fibres

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Ca2+ ions are known to enter skeletal muscle cells at rest and during activity. Except for the well characterized voltage-gated Ca2+ entry through L-type channels, the pathways involved in these Ca2+ entries remain elusive in adult muscle. The present study aimed at investigating Ca2+ influx at rest and during activity using the method of Mn2+ quenching of fura-2 fluorescence on enzymatically isolated mouse muscle cells under voltage control. The rate of quenching induced by Mn2+ influx was found to be dependent on external [Mn2+] and on membrane potential. At -80 mV replacement of Mg2+ by Mn2+ gave rise to an outward current associated with an increase in the cell input resistance. Calibration of the fura-2 response in ionomycin-permeabilized cells indicated that the Mn2+ influx was too small to be resolved as a macroscopic current. Partial depletion of the sarcoplasmic reticulum (SR) induced by train of action potentials in the presence of the SR-ATPase inhibitor cyclopiazonic acid led to a slight increase in the resting Mn2+ influx but was not associated with a change in cell input resistance and membrane potential. Trains of action potentials per se considerably increased Mn2+ entry. The measurement of the voltage dependence of the Mn2+ influx induced by depolarization steps in the presence or absence of the L-type channel blocker Cd2+ indicated that Mn2+ influx induced by depolarization occurred through L-type channels and through a parallel distinct and electrically silent voltage-gated pathway which may provide 30 % of the global Mn2+ influx at +30 mV.