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

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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.

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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).

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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.

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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.