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.

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1206-Pos Board B50

SRP-35 A Putative NAD(P)H Binding Protein Of Skeletal Muscle Sarcoplasmic Reticulum Membrane

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SRP-35 (sarcoplasmic reticulum protein of 35 kDa) is a newly identified integral membrane protein constituent of skeletal muscle sarcoplasmic reticulum. We have deduced the primary structure of the protein from cDNA clones isolated from mouse and rat skeletal muscle cDNA libraries. Primary sequence prediction analysis indicates that the NH2- terminal sequence of SRP-35 encompasses a transmembrane spanning segment or a signal sequence. In addition, SRP-35 is homologous to proteins belonging to the short-chain dehydrogenase/reductases family. Members of this protein family has two domains: the first involved in binding the nucleotide co-factor NAD(P)H, the second responsible for the catalysis of the substrate. SRP35 contains only a putative NAD(P)H binding site. Analysis of tissue distribution of SRP-35 by western blot analysis with affinity purified Ab shows that SRP-35 expression is specific for skeletal muscle since our Ab did not stain any protein in other tissues including heart, brain, liver, kidney, lung, spleen and stomach. Immunohistochemistry of primary cultured mouse myotubes transfected with SRP-35 EGFP construct indicates that SRP-35 is distributed on sarco(endo)plasmic reticulum membranes. Staining of western blot of sarcoplasmic reticulum membrane subfractions isolated from adult mouse skeletal muscle revealed that SRP-35 is associated with heavy sarcoplasmic reticulum. In addition, we found that SRP-35 is an integral membrane protein since it was extracted neither by $NaCO_3$ nor by high salt treatment of isolated sarcoplasmic reticulum membrane, but was solubilised by non-ionic detergent such as CHAPS, DDM and DHPC. We propose that , SRP-35 protein might provide the co-factor to enzymes involved in the generation of local reactive oxygen species within cellular subdomains

1207-Pos Board B51

ATP Release and P2X/P2Y Receptor Activation Account for Slow Calcium Transients Evoked by Electrical Stimulation in Skeletal Muscle Cells

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ATP released to the extracellular medium participates in cell signaling through activation of plasma membrane P2X (ion channels) or P2Y (metabotropic) receptors. Skeletal muscle cells express several P2X and P2Y receptor subtypes and ATP is profusely released during muscle activity. We have previously shown that depolarizing stimuli induce two calcium signals in skeletal myotubes: a fast signal associated with contraction and a slow signal that regulates gene expression. Here we show that extracellular nucleotides released by electrical stimulation are in part responsible for intracellular calcium signals. In rat myotubes, a tetanic stimulus (45 Hz, 400, 1 ms pulses) rapidly increased extracellular levels of ATP, ADP and AMP from 15sec to 3 min, with different half-life times. Exogenous ATP applications induced a dose-dependent increase in intracellular calcium, with an EC_{50} of 7.8 $\pm$ 3.1 μM. Exogenous ADP, UTP and UDP also promote calcium transients. By RT-PCR, we detected mRNA expression for $P2X_{1\text{-}7}$ and $P2Y_{1,2,4,6,11}$ in these cells. Both fast- and slow-calcium signals evoked by tetanic stimulation were partially inhibited by either 10-100 μM suramin (non selective P2X/P2Y blocker) or 2U/ml apyrase (nucleotidase that metabolizes ATP and ADP to AMP). In hemidiaphragm preparations, we demonstrated that apyrase reduces both twitch- and tetanus-evoked increase in tension. Our results suggest that nucleotides released during skeletal muscle activity act through P2X and P2Y receptors to modulate both calcium homeostasis and muscle physiology.

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1208-Pos Board B52

Role Of K_{ATP} Channels During Fatigue And Metabolic Inhibition In Chicken Slow Skeletal Muscle

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The slow-twitch muscle fibers in contrast to the fast-twitch muscle fibers are fatigue-resistant. During fatiguing exercise, the ATP cost of contraction is reduced. This reduction in ATP can be sensed by K_{ATP} channels (ATP-sensitive potassium channels), which couple the metabolic state of the cell to its electric activity, causing its activation. Therefore, it has been proposed that K_{ATP} participate in fatigue in the fast-twitch skeletal muscle besides to ischemia in nervous system. In slow-twitch muscle fibers there are few studies related to muscle fatigue phenomena. Thus, in this study we designed an *in vitro* model for fatigue in chicken slow-twitch skeletal muscle to investigate the role of K_{ATP} channels in the fatigue process. We studied the effects of glibenclamide, which blocks K_{ATP} channels, on twitch and tetanus tension in the anterior *latissimus dorsi* slow muscle induced to fatigue. The results show that glibenclamide increases tension in the fatigued muscle. Also, the slow muscle was exposed to metabolic poisoning by cyanide, a condition in which the ATP formation is inhibited and when its intracellular concentration is diminished K_{ATP} channels are activated which in turn produces a reduction in muscle tension. The addition of glibenclamide in these conditions abolished the effect produced by cyanide. Moreover, we studied the possible role of intracellular calcium by studying the effects of glibenclamide on the contractures evoked by caffeine, which is known that releases calcium from sarcoplasmic reticulum. In these conditions glibenclamide increases tension. Thus, we are showing evidences of the role of K_{ATP} channels in the fatigue process, since glibenclamide increases twitch and tetanus tension in chicken fatigued slow muscle and during metabolic inhibition. These effects could be mediated by an increase in the calcium release from sarcoplasmic reticulum.

1209-Pos Board B53

Insulin Resistant Skeletal Muscle: Mitochondria Structure, Dynamics And Calcium Homeostasis

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Oxidative metabolism operated by mitochondria is altered in insulin resistant skeletal muscle, and these alterations are crucial for phenotype development. Mitochondria interconnected network is required for cell calcium homeostasis, whereas calcium modulates oxidative metabolism as well as mitochondria fission and fusion process. Skeletal muscle fibers, present two subpopulations of mitochondria: subsarcolemmal (SSM) and intermyofybrillar (IMF) mitochondria. SSM electron chain activity is decreased in insulin resistant and diabetic patients; moreover, mitochondria fusion protein Mfn2 is repressed in obese, hyperinsulinemic rats. We evaluated the structure-function relation between mitochondria dynamics and excitation-contraction coupling (ECC) associated calcium transients.

We developed an insulin resistance animal model based on high fat feeding of C57BL/6J mice and we studied mitochondria form skeletal muscle fibers. Proteins associated to mitochondria fission machinery, Drp-1 and Fis1 presented elevated levels in total muscle extracts from insulin resistance animals, as well as particular subsarcolemmal accumulation, evaluated using inmunohistochemistry. Furthermore, we developed an image processing method to quantify mitochondria network continuity in both SS and IMF mitochondria.

When we analyzed calcium transients associated to ECC in flexor digitorum brevis (FDB) fibers, we found a differential effect of insulin. Fibers from control animals, presented a decrease in calcium transients amplitude after cells were treated with insulin; however, fibers from insulin resistant animals, presented unaltered calcium transients.

Taken together, these evidences indicate that there is a differential distribution of mitochondria fission machinery proteins in control and insulin resistant skeletal muscle fibers, and that ECC associated calcium transients show differential susceptibility to insulin in control versus insulin resistant fibers that may be due to altered mitochondria structure adaptation.

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1210-Pos Board B54

Sarcoplasmic Reticulum Calcium Content in Normal and Dystrophic Mammalian Skeletal Muscle Fibers

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The evaluation of the maximal amount of releasable Ca^{2+} (Ca^{2+}-content) that is stored in the sarcoplasmic reticulum (SR) of skeletal muscle fibers is a topic of great importance in muscle physiology. We have developed a method to quantitatively estimate the SR Ca^{2+} content in isolated mammalian muscle fibers based on Ca^{2+} dependent fluorescence measurements performed in the presence of high concentrations of Ca^{2+} buffers, and model calculations of Ca^{2+} flux underlying the fluorescence changes. Single fibers were enzymatically isolated from murine FDB muscles and impaled with two microelectrodes. One electrode, filled with 1M KCl, was used to record the membrane potential throughout