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

Norbert Weiss, Tina Andrianjafiniony, Sylvie Dupre-Aucouturier, Dominique Desplanches, **Vincent Jacquemond**.
University Lyon 1, UMR CNRS 5123, Villeurbanne, France.

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

Benjamin L. Prosser[1], Erick O. Hernández-Ochoa[1], Richard M. Lovering[1], Danna B. Zimmer[2], Martin F. Schneider[1].
[1]University of Maryland, Baltimore, MD, USA, [2]Texas A and M University, College Station, TX, USA.

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

James D. Pickering, Ed White, **Derek Steele**.
University of Leeds, Leeds, United Kingdom.

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

Jackson Taylor, Zhong-Ming Wang, Ramón Jiménez-Moreno, María Laura Messi, Osvaldo Delbono.
Wake Forest University School of Medicine, Winston-Salem, NC, USA.

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

Céline Berbey, **Bruno Allard**.
University Lyon 1, CNRS, Villeurbanne, France.

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.