zebrafish oocytes with a splicing blocker morpholinod designed to bind to RyR3 pre-mRNA. Tails from several fish at 72 hpf (hours post fertilization) were pooled for western blotting. A pan-RyR antibody (34C) reveals three bands, presumably representing RyR3 and RyR1a and b. The slowest band specifically disappears after morpholino injection. Tails from ~50 larvae at 72 hpf were collected for enzymatic dissociation of single muscle fibers. Intact 48 and 72 hpf tails and some dissociated fibers were fixed for EM. The PFJ/JF ratio in EM images of fast fibers in control larvae at 72 hpf was 0.79 ± 0.14, mean ± SEM (7 fish, 70 fibers, 3 triads; 3 experiments) and decreased to 0.03 ± 0.03 (10 fish, 100 fibers, 10 triads; 4 experiments) in injected larvae. To measure Ca2+ sparks, dissociated fibers were adhered to cover slips with matrigel, loaded with Fluo-4, and imaged confocally. 0.3 mM caffeine was used to stimulate sparks, which were readily detected in normal cells but almost absent in morpholino-treated cells. These data identify PFJ as RyR3 and indicate that RyR3 activity is required for the ready-detection of sparks.

644-Pos Board B399
Chemical Uncoupling the DHPR-RyR1 Complex by Substituted Halogenated Biphenyls and Diphenylethers
Yassaman Niknam, Wei Feng, Gennady Cherednichenko, Yao Dong, Issac Pessah.
Vet Med: Molecular Biosciences, UC Davis, Davis, CA, USA.
Ca2+ release units (CRU) of skeletal muscle have two components necessary for excitation-contraction coupling (ECC); the DHPR and RyR1 that physically interact to engage conformational coupling that involves both orthograde and retrograde regulation of Ca2+ dynamics during ECC and SR Ca2+ leak when the muscle is at rest. We previously identified that 2,2’,3,5’,6-pentachlorobiphenyl (PCB95) promotes long-lived stability of the full open state of RyR1 channels (Samso et al 2009). Further investigations PCB95 and its 4- and 5-hydroxy (OH)-derivatives toward rabbit skeletal muscle ryanodine receptor (RyR1) were performed using [3H]ryanodine binding and SR Ca2+ flux analyses. 5-OH metabolites have comparable activity to their respective parent in both assays; however, 4-OH derivatives are unable to trigger Ca2+ release from SR microsomes in the presence of Ca2+-ATPase activity. PCB95 and derivatives are investigated using single channel voltage-clamp and primary murine embryonic muscle cells. Like PCB95, 5-OH-PCB95 quickly and persistently increases channel open probability (Po>0.9) by stabilizing the full-open channel state, whereas 4-OH-PCB95 transiently enhances Po. Ca2+ imaging of myotubes show acute exposure to PCB95 (5μM) potentiates ECC and calcium responses and partially depletes SR Ca2+ stores. Exposure to 5-OH-PCB95 (5μM) increases cytoplasmic Ca2+ leading to ECC failure in 50% of myotubes with the remainder retaining negligible responses. 4-OH-PCB95 neither increases baseline Ca2+ nor causes ECC failure but depletes ECC and calcium responses by 50%. With longer (3 h) exposure to 300nM PCB95, 5-OH-PCB95, or 4-OH-PCB95 decreases the number of ECC responsive myotubes by 22%, 81%, and 51% compared with control by depleting SR Ca2+ and/or uncoupling ECC. The peak dihydropyridine-induced calcium release of PCB95 and chemically related diphenyl ethers differentially influence RyR1 channel gating kinetics, SR Ca2+ leak, and the pattern of ECC impairment, including ECC uncoupling in intact muscle cells. P01 AR52354.

645-Pos Board B400
Altered Ion Channel Properties of Ryanoide Receptor from Heart Muscle Lacking Calstabin2
Nathalie Saint, Albano C. Meli, Valerie Scheuermann, Alain Lacampagne.
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The cardiac ryanodine receptor (RyR2) is the main channel for the release of intracellular calcium during excitation-contraction coupling in cardiac muscle. Calstabin2 (i.e. FKBP12.6), a member of the FK506-binding protein family, has high affinity to RyR2, stabilizing RyR2 in the closed state and reducing its activity. The goal of the present work was first to examine the ion channel properties of RyR2 from calstabin2−/− mice reconstituted into lipid bilayers in resting condition. The second aim was to evaluate the effect of adding recombinant calstabin2 on RyR2 ion channel properties. RyR2 channels from 9 months old calstabin2 deficiency mice exhibited an increase of opening probability compared to RyR2 from wild-type mice in conditions of low free cytosolic Ca2+ of 150 nM to approximate the conditions in the heart during diastole. The activity of RyR2 calstabin2-/- channels was also higher with 350 nM/L. and 700 nM/L cytosolic Ca2+ . When channels were activated by higher cytosolic Ca2+ concentration (> 700 nM/L free Ca2+ ), no differences in open probability were found between RyR2 calstabin2–/– and WT channels. Addition of 1 mM Na+ or 1 mM Na+ + 1 mM ATP decreased the number of long openings to the maximum conductance in both native and calstabin2-/- channels with a prevalent subconductance activity for the depleted calstabin2 RyR2 channels. Addition of calstabin2 did neither increase nor decrease RyR2 calstabin2-/- open probability and had no effect in preventing the sub-conductance activity. This suggests that in vitro addition of calstabin2 is not sufficient to restore the RyR2/calstabin2 complex. This conclusion should further be supported by the biochemical analyzes of RyR2 macromolecular complex remodeling, performed along with age-dependency.

646-Pos Board B401
Modulation of DHPR Inactivation by RyR1 Activity in Mouse Skeletal Muscle Fibers
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Malignant hyperthermia is a potentially fatal hypermetabolic state originating from excessive release of calcium stored in the sarcoplasmic reticulum (SR) of skeletal muscle. In most cases, MH susceptibility results from mutations in the type 1 ryanodine receptor (RyR1). In previous work (Andronache et al., PNAS 2009) we reported that heterozygous murine carriers of MH mutation Y524S (human Y522S) exhibit changes in steady state inactivation of the dihydroprididine receptor (DHPR), the sensor of the transverse tubular (TT) membrane potential. Availability curves were left shifted along the voltage axis suggesting that a feedback signal from RyR1 modulates DHPR inactivation. In the present study we investigated the hypothesis that junctional fluctuations of free Ca2+ concentration are involved in the feedback mechanism. We performed two-electrode voltage clamp experiments on enzymatically isolated toe muscle fibers of both WT and mutant mice (Y524S+/−) and measured L-type Ca2+ current and optical signals from fluorescent Ca2+ indicators. To test the hypothesis we applied conditions that would modify junctional Ca2+ levels. Millimolar concentrations of caffeine led to a left shift in the availability curve for L-type current indicating that drug-induced RyR1 hyperactivity can mimic the effect of the mutation. On the other hand, internal dialysis with an artificial solution containing 10 mM of BAPTA to effectively reduce the local Ca2+ transients near open RyR1 channels had little effect on the difference in steady state inactivation between WT and mutant fibers. We conclude that the altered inactivation depends on RyR1 hyperactivity but does not require the continuous presence of local Ca2+ fluctuations within the junctional gap separating TT and SR.

647-Pos Board B402
Ca2+ Influx Mediated by Reverse Mode of Na+/Ca2+ Exchanger Is Enhanced in Malignant Hyperthermia Skeletal Muscle
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1Department of Molecular Biosciences, University of California at Davis, Davis, CA, USA, 2Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA, USA.
Malignant hyperthermia (MH), is potentially fatal pharmacogenetic disorder of skeletal muscle, which has been associated to intracellular Ca2+ dysregulation. The Na+/Ca2+ exchange (NCX) is a bidirectional transporter that normally extrudes Ca2+ from the cell (forward mode), but also brings Ca2+ into the cell (reverse mode) under special conditions such as intracellular Na+ accumulation or membrane depolarization. Resting intracellular Ca2+ concentration ([Ca2+]i) and intracellular Na+ concentration ([Na+]i) were elevated in MH susceptible (MHS) swine and mouse muscles compared to WT (MHN) muscle fibers. Lowering extracellular Na+ induced an elevation of [Ca2+]i in both MHN and MHS swine and rodent muscle fibers that could be prevented by removal of extracellular Ca2+. Local application of KB-R7943, a nonselective NCX blocker, reduced [Ca2+]i in both MHN and MHS muscle fibers and the magnitude of the elevation of [Ca2+]i observed during a MH episode. YM-2444769 an NCX blocker that preferentially inhibits NCX3 reverse mode did not reduce resting [Ca2+]i in MHN, but it does in MHS muscle fibers, and decreased the amplitude of the elevation of [Ca2+]i induced by halothane in these muscles. These results demonstrate the existence of a functional NCX in skeletal muscle, which appears to be enhanced in MHS muscle fibers. In addition, they represent the first evidence that NCX-reverse mode- contributes to the dysfunction of [Ca2+]i and [Na+]i and intracellular Ca2+ elevation induced by halothane in MHS muscle.
Funding: NIH AR43140, AR052534 (PDA, IRL)

648-Pos Board B403
Myofilament Ca2+ Desensitization is Associated with Reduced L-Type Ca2+ Channel Activity Mediated by Neuronal Nitric Oxide Synthase in Left Ventricular Myocytes from Murine Hearts
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Seoul National University, College of Medicine, Seoul, Republic of Korea.
Myofilament Ca\(^{2+}\) desensitization (Myo-Ca\(^{2+}\)-Desens) has been shown to affect intracellular Ca\(^{2+}\) homeostasis and therefore may regulate Ca\(^{2+}\)-sensitive ion channels in the plasma membrane. Recently, we provided direct evidence to show that neuronal nitric oxide synthase (nNOS or NOS1) was responsible for Myo-Ca\(^{2+}\)-Desens and the increase in systolic Ca\(^{2+}\) transient amplitude [Ca\(^{2+}\)i] in LV myocytes from angiotensin II (Ang II)-induced hypertensive rats. So far, whether and how Myo-Ca\(^{2+}\)-Desens affects L-type Ca\(^{2+}\) current (ICa) is not known. Here, we analyze the effects of Myo-Ca\(^{2+}\)-Desens by means of pharmacological, physiological and pathological tools on ICa in LV myocytes from hypertensive rats. Intracellular Ca\(^{2+}\) chelator, 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA, 10 mM), completely reversed BDM-reduction of ICa.

Interestingly, NOS1 inhibition with a selective inhibitor, SMTC, did not affect [Ca\(^{2+}\)i], but restored ICa in the presence of BDM, suggesting that nNOS mediates ICa inhibition with Myo-Ca\(^{2+}\)-Desens. Next, increasing stimulation frequency (2Hz, 4Hz and 8Hz) shifted the relaxation phase of sarcomere length-[Ca\(^{2+}\)i] relationship to the right and increased systolic and diastolic [Ca\(^{2+}\)i] and reduced ICa in rats. SMTC increased ICa at 2 and 4 Hz. Similarly, myofilament Ca\(^{2+}\) sensitivity was reduced in LV myocytes from hypertensive rats at 2Hz and no further change was observed at 4 and 8 Hz. Surprisingly, although ICa was reduced at all 3 frequencies, SMTC failed to affect ICa despite that it reversed Myo-Ca\(^{2+}\)-Desens.

Our results suggest that Myo-Ca\(^{2+}\)-Desens reduces ICa in rat LV myocytes. nNOS mediates the effect in normal but not in hypertensive rat hearts.

649-Pos Board B404
RGK Proteins Inhibit slow, Depolarization-Dependent Ca\(^{2+}\) Entry into Cultured Myotubes
Christin F. Romberg, Donald Boccolari, Ulises Meza, Roger A. Bannister. University of Colorado Denver-AMC, Aurora, CO, USA.

Three physiological functions have been described for the skeletal muscle 1,4-dihydropyridine receptor (CaV1.1): 1) voltage-sensor for excitation-contraction (EC) coupling, 2) L-type Ca\(^{2+}\) channel, and 3) voltage-sensor for slow, depolarization-dependent Ca\(^{2+}\) entry. Members of the RGK (Rad, Rem, Rem2, Gem, Kir) family of monomeric GTP-binding proteins are potent inhibitors of the former two functions of CaV1.1. However, it is not known whether the latter function that has been attributed to CaV1.1 is subject to modulation by RGK proteins. The purpose of this study was to determine whether Rad, Gem and/or Rem inhibit the slowly activating, persistent Ca\(^{2+}\) current that is dependent on the voltage-sensing capability of CaV1.1. To investigate this possibility, Venus fluorescent protein-fused RGK proteins (V-Rad, V-Rem and V-Gem) were overexpressed in otherwise normal mouse myotubes and the abilities of each of these V-RGK proteins to inhibit EC coupling, L-type Ca\(^{2+}\) current and depolarization-induced Ca\(^{2+}\) entry in myotubes were assessed using electrical field stimulation, whole-cell voltage-clamp and Ca\(^{2+}\) imaging, respectively. As shown previously for YFP-Rem, both EC coupling and L-type current density were substantially attenuated in developing myotubes expressing either V-Rad or V-Gem. The reductions in L-type current and EC coupling were paralleled by reductions in depolarization-induced Ca\(^{2+}\) entry (89%, 99% and 91% for V-Rad, V-Rem and V-Gem, respectively, relative to control). Thus, we provide the first evidence of modulation of this enigmatic type of Ca\(^{2+}\) entry peculiar to skeletal muscle. Moreover, the similar inhibitory effects of RGK proteins on both L-type current amplitude and slow Ca\(^{2+}\) entry provide further evidence that both modes of Ca\(^{2+}\) flux utilize a common pathway. Supported by AG038778 (to RAB).

650-Pos Board B405
Expression of the Embryonic Cav1.1 Splice Variant in Adult Mice Alters Excitation-Contraction Coupling but Does not Cause Dystrophic Myotonia
Asaf Z. Cohen, Tanya R. Cully, Bradley Launikonis, Donald Beqollari, Ulises Meza, Roger A. Bannister. University of Colorado Denver-AMC, Aurora, CO, USA.

Previously, we discovered that zebrafish, as well as all higher teleost fish, lack skeletal muscle, which is not (immediately) required for ECC, is still enigmatic. Interestingly, NOS1 inhibition with a selective inhibitor, SMTC, did not affect [Ca\(^{2+}\)i], but restored ICa in the presence of BDM, suggesting that nNOS mediates ICa inhibition with Myo-Ca\(^{2+}\)-Desens. Next, increasing stimulation frequency (2Hz, 4Hz and 8Hz) shifted the relaxation phase of sarcomere length-[Ca\(^{2+}\)i] relationship to the right and increased systolic and diastolic [Ca\(^{2+}\)i] and reduced ICa in rats. SMTC increased ICa at 2 and 4 Hz. Similarly, myofilament Ca\(^{2+}\) sensitivity was reduced in LV myocytes from hypertensive rats at 2Hz and no further change was observed at 4 and 8 Hz. Surprisingly, although ICa was reduced at all 3 frequencies, SMTC failed to affect ICa despite that it reversed Myo-Ca\(^{2+}\)-Desens.

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