with methyl-β-cyclodextrin (MCD) and then measured SOCE in these myotubes. Reduced SOCE was observed following MCD extraction, similar to the limited SOCE we previously observed in young m2/s−/− and wild-type aged muscle fibers. Further studies will determine if mutation of conserved residues in the MARVEL domain of MG29 modulate SOCE function in, and the lipid composition of, skeletal muscle cells.

1355-Pos Board B306 Differential Role of Calcequstrin Isomers on Calcium Entry in Skeletal Muscle FDB Fibres Francesco Zorzato1, Barbara Mosca2, Leda Beergamelli1, Giorgia Vallee1, Alessandra Nori1, Susan Treves1, Feliciano Protasi1, Pomeo Volpe1, 1Life Science and Biotechnology, University of Ferrara, Ferrara, Italy, 2Department of Anesthesiology University Hospital Basel, Basel, Switzerland, 3Sciene Biomediche, University of Padova, Padova, Italy, 4Department Anesthesiology University Hospital Basel, Basel, Switzerland, 5CeSI & DNICS, University of Chieti, Chieti, Italy.

JP45 is a sarcoplasmic reticulum membrane protein interacting with Cav1.1 and calcequstrin (CASQ). The JP45/CASQ complex enhances Cav1.1 calcium activity to promote calcium entry to maintain an adequate level of releasable SR calcium during tetanic contraction. In this study, we investigated FDB fibres from JP45/CASQ1 double KO (DKO), JP45/CASQ2 double KO (DKO) and JP45/CASQ1/CASQ2 triple KO (TKO) mice to assess the role, if any, of JP45 and CASQ2 on calcium entry. Mag-Fluo-4 was used to monitor rapid Ca2+ transients evoked by a 0.5 ms pulse at supramaximal field stimulation. In the presence of 1.8 mM Ca2+ in the extracellular solution, the peak calcium signal observed in FDB fibres from WT and JP45/CASQ2 DKO was similar, in JP45/CASQ1 DKO and JP45/CASQ1/CASQ2 TKO FDB fibres, peak calcium was lower compared to WT. The half time of the decay of the Ca2+ transient in FDB fibres from JP45/CASQ1 DKO, JP45/CASQ2 DKO and JP45/CASQ1/CASQ2 TKO was significantly slower compared to WT. Such a difference disappeared when Ca2+ was substituted by 100 μM L-α-aspartate in the extracellular medium. The effect of La3+ was more evident upon stimulation of FDB fibres with repetitive 0.5 ms pulses at 100 Hz. The calcium signal evoked by a train of pulses at 100 Hz for 300 ms was strongly inhibited by La3+ in fibers from JP45/CASQ1 DKO and JP45/CASQ1/CASQ2 TKO, but negligibly affected in WT and in JP45/CASQ2 DKO. Mn2+ quenching experiments in fura-2 loaded FDB fibres from JP45/CASQ1 DKO and JP45/CASQ1/CASQ2 TKO show that calcium influx during tetanic stimulation is blocked by 50 μM nifedipine an inhibitor of Cav1.1 calcium currents. These results show that ablation of CASQ2 as well as the time evolution of SR Vm and local changes in intra-SR/cytosolic ion concentrations. Our results show that RyR Ca2+ influx is indeed electrically balanced by K+, Cl− and Mg2+ counter-ions. When RyRs are open, Mg2+ initially moves into the SR and then surprisingly moves back out. Redundant pathways exist to carry K+ counter-current (RyR and TRIC) during SR Ca2+ release. When RyRs close, the K+ must exit through TRIC channels to maintain resting SR Vm at 0 mV. Our analysis indicates TRIC channels are essential for restoring SR K+ re-equilibration that is needed to re-establish the normal trans-SR Ca2+ driving force.

1357-Pos Board B308 A Compartment Model to Investigate the Roles of SR Membrane Channels during E-C Coupling Claudio Berti, Michael Fill, Dirk Gillespie. Molecular Biophysics and Physiology, Rush University Medical Center, Chicago, IL, USA.

During excitation-contraction (E-C) coupling, efficient Ca2+ release by ryanodine receptor (RyR) channels from the sarcoplasmic reticulum (SR) requires that counterions move into the SR. These counterions fluxes affect both to effectively clamp the SR membrane potential (Vm) near 0 mV, sustaining the trans-SR Ca2+ driving force. Likewise, Ca2+ uptake by sarcoplasmic reticulum Ca2+-ATPase (SERCA) pumps is facilitated by the existence of counterion fluxes. The SR also contains TRIC (SR K+ channels) and CI channels in addition to RyRs and SERCA pumps. Thus, SR Vm during and between RyR-mediated Ca2+ releases is determined by multiple channels whose function spatially and temporally varies. The contribution of each channel to SR Vm control is unclear and debated. We used a compartment model to examine counterion (K+, Cl− and Mg2+) fluxes across the SR membrane during E-C coupling. The model allowed us to study the roles of the different ion channels, as well as the time evolution of SR Vm and local changes in intra-SR/cytosolic ion concentrations. Our results show that RyR Ca2+ influx is indeed electrically balanced by K+, Cl− and Mg2+ counter-currents. When RyRs are open, Mg2+ initially moves into the SR and then surprisingly moves back out. Redundant pathways exist to carry K+ counter-current (RyR and TRIC) during SR Ca2+ release. When RyRs close, the K+ must exit through TRIC channels to maintain resting SR Vm at 0 mV. Our analysis indicates TRIC channels are essential for restoring SR K+ re-equilibration that is needed to re-establish the normal trans-SR Ca2+ driving force.

1358-Pos Board B309 Model of a Propagating Action Potential (AP) in a Twitch Skeletal Muscle Fiber Mounted in a Double-Vaseline-Gap Chamber - Currents Involved in Shaping the AP Fatouros Touma1, Gabor Gyurkovics1, Cedric R.H. Lamboly2, Paul C. Pape2, 1Physiologie et biophysique, Université de Sherbrooke, Sherbrooke, QC, Canada, 2Victoria University Institute of Sport, Exercise, and Active Living, Melbourne, Australia.

Following the peak of the action potential (AP) in a twitch fiber from frog, a very rapid repolarization occurs to an approximately constant plateau level (the “after potential”) ~30 mV more positive than the resting potential (RP). Our results include time- and voltage-dependent gating of the Na and K currents based on the Hodgkin-Huxley (HH) models. The assumed properties of the Na, K, and leak conductances for the surface membrane are from Table 1B of the above reference. The specific conductances for these currents in the T-system were assumed to be the same as those for the surface. An access resistance for the T-system was adjusted to give an ~1-ms delay between the surface AP and that in the T-system. The model results indicate that the “after potential” can be explained by Na current resulting from recovery from inactivation - a new explanation for the “after potential” to our knowledge. Modelled APs with a Ca-activated K current, I_{Ca}^{K}, replacing the HK K current in the model are consistent with measured APs at different Ca concentrations (Lamboly et al., 2013; Biophys. J. 104(2):290a). The model supports a relatively straightforward way for analyzing these results leading to our conclusion that I_{Ca}^{K} is the main determinant of the rapid repolarization phase of the AP. Consequently, I_{Ca}^{K} comprises a very important negative feedback mechanism for controlling Ca2+ release from the sarcoplasmic reticulum.

1359-Pos Board B310 CaMKII-Dependent Phosphorylation of RyR2 Causes Domain Unzipping and Reduced Calmodulin Binding, But Dantrolene Reverses These Effects Hirotoshi Uchinomiya1, Yi Yang2, Jose L. Puglisi1, Ye Chen-Izu1, Razvan L. Cornea2, Xander H.T. Wehrens3,4, Donald M. Bers1. 1Department of Pharmacology, University of California Davis, Davis, CA, USA, 2Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN, USA, 3Cardiovascular Research Institute, Baylor College of Medicine, Houston, TX, USA, 4Department of Molecular Physiology and Biophysics, Department of Medicine (Cardiology), Baylor College of Medicine, Houston, TX, USA.

Increased diastolic SR Ca2+ (Ca) leak via cardiac ryanodine receptors (RyR2) can cause cardiac arrhythmias and dysfunction in heart failure (HF). CaMII-dependent RyR2 phosphorylation (at S2814) is thought to be a critical...