Cardiac contraction is triggered when a membrane depolarisation induces a massive increase in intracellular calcium concentration. This process called ''excitation-contraction (E-C) coupling'' relies on a multimolecular protein complex, the calcium release complex (CRC) organized around the sarcoplasmic reticulum calcium channel, the ryanodine receptor (RyR2). Among the proteins involved in the efficient function of the CRC, calsequestrin, triadin and junctin are sarcoplasmic reticulum proteins able to interact with RyR2 and regulate calcium release.
Mutations in RyR2 and calsequestrin are associated to a rare but fatal cardiac arrhythmia: catecholaminergic polymorphic ventricular tachycardia (CPVT). Nevertheless, variations in these two genes (RYR2 and CASQ2) account so far for only 50 to 70% of the cases, suggesting that other genes are most probably involved. To reveal new genes involved in CPVT, we have based a candidate gene approach on the hypothesis that the pathology could be considered as a disease of the calcium release complex. We therefore searched for variations in the genes encoding proteins of the CRC in a large French cohort of CPVT patients with no detected mutations in RYR2 or CASQ2. We have identified for the first time mutations in the human triadin gene TRDN, and studied the functional consequences of a missense mutation both in a cell model and in vivo after expression in triadin KO mice. Our results confirmed the hypothesis that CPVT can be more generally considered as a defect in the CRC.

2078-Plat
Effects of Autonomic Agents on Ca^{2+} Cycling in Canine Atrial Myocytes during Rapid Pacing
Gary L. Aistrup[1], Hemanth Koduri[1], Aaron Kunamalla[1], Manvinder Kumar[1], Jon Cordeiro[2], Rishi Arora[1], J. Andrew Wasserstrom[1].
[1]Northwestern University, Feinberg School of Medicine, Chicago, IL, USA, [2]Masonic Medical Research Laboratory, Utica, NY, USA.
Atrial fibrillation (AF), or conditions conducive to it, often occur(s) in conjunction with high and/or unbalanced sympathetic/parasympathetic (autonomic) activity, which has profound effects on myocyte Ca^{2+} cycling. Using confocal microscopy, we have begun scrutinizing the effects various autonomic agents–including but not limited to isoproterenol (ISO; *-adrenergic receptor (AR) agonist), phenylephrine (PE; *-AR agonist) norepinephrine (NE, *- and *-AR agonist), and carbachol (CCh; muscarinic cholinergic receptor agonist)–have on Ca^{2+} cycling in isolated canine atrial myocytes paced at cycle lengths (CLs) ranging from 5000-200ms. In general, Ca-transient amplitudes were increased by ISO and NE; decreased by CCh; and varying decreased, increased or unaffected by PE–although PE only increased Ca-transients after PTX-treatment. However, considerable cell-to-cell variability in magnitude/dose-response for such effects was notable. The effects these agents had on irregular Ca-release events (ICREs)–i.e., Ca-alternans triggered-Ca-waves occurring during pacing (t-CaWs), and spontaneous-Ca-waves occurring during a pause after pacing (s-CaWs)–consequent to rapid pacing ($CLs \leq 300ms$) were interestingly distinct. CCh significantly reduced the appearance of all ICREs, yet were often accentuated upon CCh withdrawal. ISO often induced s-CaWs, but suppressed t-CaWs–with Ca-alternans often appearing in their stead. NE mimicked ISO regarding ICREs in some cells, but in others did not suppress and sometimes accentuated t-CaWs. PE accentuating t-CaWs and/or subcellular Ca-alternans in some cells, while suppressing or having no apparent effect on them in others. However, after PTX-treatment, PE mimicked ISO regarding ICREs without s-CaWs induction. These findings not only underscore the complexity of atrial autonomic modulation and its differences with that in ventricle, but also particulars evident only during rapid pacing–i.e., that during or conducive to the onset of AF.

2079-Plat
Localization and Dynamics of Phosphatidylinositol 4,5-Bisphosphate (PIP_2) in Adult Skeletal Muscle Fibers
Genaro C. Barrientos, Marino DiFranco, Julio L. Vergara.
University of California, Los Angeles, CA, USA.
PIP_2 is a precursor of important second messengers, and by itself is a direct modulator of the activity of ion channels and transporters. We investigated the localization and dynamic changes in PIP_2 levels in live adult muscle fibers by expressing two PIP_2 sensors: an EGFP construct of the pleckstrin homology (PH) domain of the phospholipase delta1 subunit (PH-EGFP), and a construct of the PIP_2 binding domain of the tubby protein (EGFP-tubby). EGFP-tubby has higher affinity and specificity for PIP_2 than PH-EGFP, and has been used to sequester PIP_2 in the plasma membrane. Their respective plasmids (*) were transfected by *in vivo* electroporation of FDB muscles. Two-photon laser scanning microscopy (TPLSM) shows that both EGFP-tubby and PH-EGFP are efficiently expressed in muscle fibers and that they are distributed in a double-banded pattern indicating localization at the transverse tubular system (TTS) membranes (in addition to the sarcolemma). Peak/baseline ratio analysis of TPLSM images suggests that there is a larger proportion of EGFP-tubby than PH-EGFP associated with the TTS membranes. Although the expression of PH-EGFP does not change neither the voltage-dependence nor the amplitude of Ca^{2+} release signals detected with Rhod-5N, the expression of EGFP-tubby apparently left-shifts their voltage-dependence by >10 mV. To further investigate the localization and dynamics of PIP_2 in the TTS, we performed fluorescence resonance energy transfer (FRET) studies. The voltage-dependence of FRET signals, based on the translocation of the lipophilic anion dipicrylamine (DPA), shows that the EGFP tags of both PH-EGFP and EGFP-tubby are anchored within ~6-9 nm of the TTS membrane. (*)The plasmids were kindly provided to us by Dr. Tamas Balla, NICHD, NIH. This work was supported by NIH grants AR047664, AR041802, and AR054816.

2080-Plat
Decreased Fatigue Resistance is an Early Functional Defect in Skeletal Muscles of Mitochondrial-DNA-Mutator Mice
Håkan Westerblad, Niklas Ivarsson, Arthur J. Cheng, Andreas Fahlström, Andres Hernandez.
Karolinska Institutet, Stockholm, Sweden.
Previous studies from our laboratory have shown that weakness, rather than decreased endurance, is the main contractile defect in a mouse mitochondrial myopathy model (skeletal muscle-specific *Tfam* KO mice). Here we used a mouse model with mitochondrial defects induced by knock-in of a proof-reading-deficient version of PolgA. These mice display an increased number of mutations in the mitochondrial DNA (mtDNA) and a premature aging phenotype. In this study we measured force and free cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_i$) in isolated fast-twitch flexor digitorum brevis fibers of 5 month old mice; at this age the mtDNA mutator mice show no general signs of malfunction. In the unfatigued state, there was no difference in force or $[Ca^{2+}]_i$ between mtDNA mutator and control muscle fibers. However, during fatigue induced by repeated tetanic contractions, force and tetanic $[Ca^{2+}]_i$ declined more rapidly in mtDNA mutator than in control muscle fibers. Muscles of mtDNA mutator mice also showed several signs of impaired mitochondrial function: decreased activity of citrate synthase and 3-hydroxyacyl-CoA dehydrogenase and decreased protein expression of PGC-1α and cytochrome c oxidase-1. In conclusion, decreased endurance due to impaired mitochondrial respiration is an early sign of muscle dysfunction in mtDNA mutator mice.

Platform: Protein-Ligand Interactions

2081-Plat
Improving the Accuracy of Knowledge-Based Scoring Functions for Protein-Ligand Interactions by Accounting for Sparse Data in the Training Set
Sam Z. Grinter, Xiaoqin Zou.
University of Missouri, Columbia, MO, USA.
In the derivation of any knowledge-based scoring function, one must decide how to manage sparse features in the training data. Here we present STScore, a distance-dependent set of atomic pair potentials that uses a novel approach to minimize the sparse data problem. The overall approach is to represent the actual potential of mean force, which is unknown, as a random variable whose probability density is determined by the evidence in the training set. This provides a natural way of representing the uncertainty in the potential of mean force (PMF), for each bin and atom pair type. STScore is an average of the PMF and an alternative force-field-based potential, with the weights chosen to minimize the error in the sum. This weighting scheme implies that STScore will give more weight to the force-field potential whenever training data is scarce. We show that STScore effectively combines the two alternatives, exceeding the performance of either potential alone, and leads to improved binding mode and binding affinity predictions.

2082-Plat
Pharmaceutical Applications of the Polarizable Amoeba Potential, Including Protein-Ligand Binding Affinity and Drug Solubility, using the Force Field X Software
Michael J. Schnieders[1], Yue Shi[1], Johnny Wu[1], Jonas Baltrusaitis[2], Wei Yang[3], Pengyu Ren[1].
[1]University of Texas at Austin, Austin, TX, USA, [2]University of Iowa, Iowa City, IA, USA, [3]Florida State University, Tallahassee, FL, USA.
Accurate prediction of protein-ligand binding affinity is essential to computational drug discovery. Although virtual screening has been widely utilized, current approaches are seriously limited by the accuracy of the underlying potential energy model (i.e. force field) that describes atomic interactions. A