1709-Plat
Crosstalk between RyR2 Oxidation and Phosphorylation Contributes to Cardiomyopathy in Mice with Duchenne Muscular Dystrophy
George G. Rodney[1], Qiongling Wang[1], Guoliang Wang[1], Xander H.T. Wehrens[1,2].
[1]Molecular Physiology & Biophysics, Cardiovascular Research Institute, Baylor College of Medicine, Houston, TX, USA, [2]Department of Medicine/ Cardiology, Baylor College of Medicine, Houton, TX, USA.
Patients with Duchenne muscular dystrophy (DMD) are at risk of developing cardiomyopathy and cardiac arrhythmias. Studies in a mouse model of DMD revealed that enhanced sarcoplasmic reticulum (SR) Ca^{2+} leak contributes to the pathogenesis of cardiac dysfunction. In view of recent data suggesting the involvement of altered phosphorylation and oxidation of the cardiac ryanodine receptor (RyR2)/ Ca^{2+} release channel, we hypothesized that inhibition of RyR2 phosphorylation in a mouse model of DMD can prevent SR Ca^{2+} leak by reducing RyR2 oxidation. Confocal Ca^{2+} imaging and single RyR2 channel recordings revealed that inhibition of either S2808 or S2814 phosphorylation, or inhibition of oxidation could normalize RyR2 activity in *mdx* mice. Moreover, genetic inhibition of RyR2 phosphorylation at S2808 or S2814 reduced RyR2 oxidation. Production of reactive oxygen species (ROS) in myocytes from mdx mice was reduced by both inhibition of RyR2 phosphorylation or the ROS scavenger 2-mercaptoproppionylglycin (MPG). Finally, it was shown that ROS production in *mdx* mice is proportional to the activity of RyR2-mediated SR Ca^{2+} leak. We conclude that increased reactive oxygen species (ROS) production in the hearts of *mdx* mice drives the progression of cardiomyopathy. Inhibition of RyR2 phosphorylation can suppress SR Ca^{2+} leak in *mdx* mouse hearts in part by reducing RyR2 oxidation.

1710-Plat
Secretoneurin, a Novel Endogenous CaMKII Inhibitor, Augments Cardiomyocyte Calcium Handling and Inhibits Arrhythmogenic Calcium Release
Anett H. Ottesen[1], Cathrine R. Carlson[2], Andrew G. Edwards[2], Ole J.B. Landsverk[3], Rune F. Johansen[4], Morten K. Moe[5], Magnar Bjørås[4], Mats Stridsberg[6], Torbjørn Omland[1], Geir Christensen[2], Helge Røsjø[1], **William E. Louch**[2].
[1]Division of Medicine, Akershus University Hospital, Lørenskog, Norway, [2]Institute for Experimental Medical Research, Oslo University Hospital Ullevål, Oslo, Norway, [3]Department of Pathology, Oslo University Hospital Rikshospitalet, Oslo, Norway, [4]Department of Microbiology, Oslo University Hospital Rikshospitalet, Oslo, Norway, [5]Division of Diagnostics and Technology, Akershus University Hospital, Lørenskog, Norway, [6]Department of Medical Sciences, Uppsala University, Uppsala, Sweden.
Secretoneurin (SN) is the functional fragment of secretogranin II, a granin protein that is increased in heart failure patients and associated with mortality. In non-cardiac cells, SN has been shown to alter Ca^{2+} homeostasis. We presently investigated the effects of exogenous SN treatment on Ca^{2+} handling in heart. SN was observed to be rapidly taken up into intact hearts, and internalized into cardiomyocytes via endocytosis. Bioinformatic analyses suggested potential interactions between SN and calmodulin (CaM), and also the catalytic region of Ca^{2+}/CaM-dependent protein kinase II δ (CaMKIIδ). These putative interaction sites were confirmed by employing pull-down, immunoprecipitation, and Biacore analyses. SN attenuated CaMKIIδ activity in a dose-dependent manner, and reduced autophosphorylation of CaMKIIδ in Langendorff-perfused hearts, both in the absence and presence of isoproterenol. SN also reduced basal and isoproterenol-induced CaMKIIδ-dependent ryanodine receptor phosphorylation, and CaMKIIδ-dependent phosphorylation of phospholamban. In isolated cardiomyocytes, exogenous SN (2.8 μmol/L) increased the magnitude and kinetics of cardiomyocyte Ca^{2+} transients and contractions. In agreement with reduced CaMKIIδ-dependent ryanodine receptor phosphorylation, Ca^{2+} spark frequency and dimensions were reduced, and sarcoplasmic reticulum Ca^{2+} content was increased. Augmentation of Ca^{2+} transients occurred despite reduction in the magnitude of L-type Ca^{2+} current, as expected following CaMKIIδ inhibition. During challenge with isoproterenol, SN treatment reduced the frequency of arrhythmogenic Ca^{2+} waves. Similarly, in patch-clamped cells stimulated with action potentials, the appearance of delayed afterdepolarizations and spontaneous beats was inhibited by SN. In conclusion, SN is a novel CaMKIIδ inhibitor that inhibits ryanodine receptor Ca^{2+} leak, resulting in augmented sarcoplasmic reticulum Ca^{2+} content, Ca^{2+} transients, and contractions with reduced occurrence of arrhythmogenic Ca^{2+} waves and delayed afterdepolarizations. These findings suggest that increased SN levels during heart failure may be compensatory in the most severely ill patients.

1711-Plat
Large-Scale, Automated Calcium Spark Analysis using iSpark Reveals Functional and Spatial Remodeling During Cardiac Hypertrophy
Qinghai Tian[1], Laura Schröder[1], Aline Flockerzi[1], Andre Zeug[2], Lars Kaestner[1], **Peter Lipp**[1].
[1]Institute for Molecular Cell Biology, Saarland University, Homburg/Saar, Germany, [2]Institute for Cellular Neurophysiology, Medizinische Hochschule Hannover, Homburg/Saar, Germany.
Understanding of cardiac Ca signaling is driven by advancements in imaging and analysis tools. The availability of fast line scanning allowed the identification of Ca sparks that revolutionized our appreciation of cardiac Ca signaling in the healthy and diseased heart. Ultra-high-speed confocal imaging of three dimensional (2D over time) Ca spark properties yields novel high content data about such signals whose characteristics eventually determine the performance of the entire heart. The full potential of such data remains concealed owing to the lack of comprehensive, fully automated and unbiased analysis tools. We have developed an intelligent software tool (iSpark) employing fully automatic, self-adaptive and unbiased algorithms to investigate Ca sparks in cardiac myocytes from healthy animals and mice with tiered stages of hypertrophy. Long-term recording of Ca sparks with high speed 2D over time confocal imaging of permeabilized ventricular myocytes produced high content spark data. With iSpark we explored 670,000 individual events and revealed that their subcellular arrangement, amplitude and frequency were substantially altered with the severity of cardiac hypertrophy. While line scanning of our data failed to show any correlation, iSpark-based analysis demonstrated a malfunctional microscopic Ca signaling strongly correlating with the disorganization of T-tubules and the severity of hypertrophy. The availability of highly sophisticated analysis tools, such as iSpark, substantially fosters large-scale data exploration. iSpark-based analysis of large-scale Ca spark data revealed a causal contribution of distinct subcellular remodeling of Ca handling to the progression of cardiac hypertrophy.

1712-Plat
Regulation of Calcium Clock-Mediated Pacemaking by Inositol-1,4,5-Trisphosphate Receptors in Mouse Sinoatrial Nodal Cells
Nidhi Kapoor[1], Andrew Tran[1], Jeanney Kang[1], Rui Zhang[1], Kenneth D. Philipson[2], Joshua I. Goldhaber[1].
[1]Heart Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA, [2]Department of Physiology, University of California, Los Angeles, CA, USA.
Sinoatrial node (SAN) automaticity is attributable to the interplay of several membrane currents such as funny current (I_f) and the Na-Ca exchanger (NCX) current activated in response to ryanodine receptor (RyR) mediated Ca release from the sarcoplasmic reticulum (SR). Whether another SR Ca release channel, the inositol-1,4,5-triphosphate receptor (IP_3R), can influence automaticity in SAN is controversial, in part due to the confounding influence of periodic Ca flux through the sarcolemma accompanying each beat. We used atrial-specific NCX knockout (KO) SAN cells to study IP_3 signaling in a system where periodic $[Ca]_i$ cycling persists despite the absence of depolarization or Ca flux across the sarcolemma. We recorded confocal line scans of spontaneous Ca release in WT and NCX KO SAN cells, in the presence or absence of an IP_3R blocker (2-APB) or during inhibition of phospholipase C by U73122. We found that superfusion with 2APB (2 μM) decreased the frequency of Ca transients in WT by 82.7% ($n=9$, $p<0.05$) and Ca waves in NCX KO by 69.9% ($n=10$, $p<0.05$). Similar results were also obtained with U73122 (1 μM). Alternatively, increased IP_3 production induced by phenylephrine (PE; 10 μM) increased Ca transient frequency in WT ($n=8$. $P> 0.05$) and Ca wave frequency in KO cells ($n=9$, $p <0.05$) that was reversed by 2-APB. To determine if IP_3Rs exert their modulatory effect on pacemaking via RyRs, we recorded Ca transients during application of PE in the continued presence of ryanodine at a blocking concentration (100 μm) that does not deplete SR stores. Under these conditions PE was unable to restore Ca transients. Thus, Ca release from IP_3Rs can modulate the ''Ca clock'' processes that regulate pacemaker frequency in the murine SAN via Ca-induced Ca release through the RyRs.

1713-Plat
Structural Studies of IP3R by Cryoem
Qiu-Xing Jiang, Hui Zheng, Marc Llaguno.
Cell Biology MC9039, UT Southwestern Medical Center, Dallas, TX, USA.
Inositol 1,4,5-trisphosphate receptors (IP3Rs) play important roles in a battery of cellular activities. Structural study of the receptors is therefore very important for understanding how they are gated by their natural ligands and are modulated by their intracellular partners. In the past several years, multiple groups have generated very disparate reconstructions of the type 1 IP3R.