and whole heart experiments, inherently predispose toward arrhythmia and may explain the susceptibility of HCM sufferers to SCA.

1145-Plat

Normal and Abnormal Ca Cycling during Rapid Pacing and the Development of Triggered Waves in Dog Left Atrial Myocytes

J. Andrew Wasserstrom[1], Jonathan M. Cordeiro[2], Benjamin Toren[1], Manvinder S. Kumar[1], Aaron Kunamalla[1], James E. Kelly[1], Rishi Arora[1], Gary L. Aistrup[1].
[1]Northwestern University, Chicago, IL, USA, [2]Masonic Medical Research Laboratory, Utica, NY, USA.

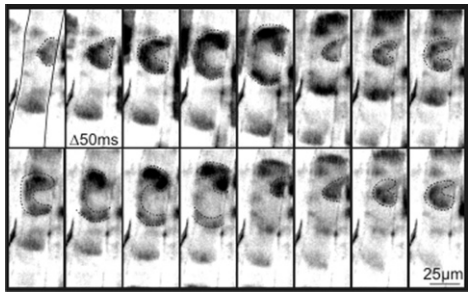
Although there have been a number of studies of Ca cycling in atrial myocytes, few have investigated the effects of rapid pacing on Ca cycling, as would occur during atrial fibrillation (AF). We investigated subcellular Ca cycling during rapid pacing in left atrial myocytes using high resolution confocal imaging in order to study rate-dependent changes in Ca cycling that might occur during AF. Myocytes were isolated from dog left atrium, loaded with fluo-4AM and studied during pacing at basic cycle lengths ranging from 5000-200msec (36^{o}C). Myocytes showed a positive force-frequency relationship and normal rapid restitution. However, at BCL $\leq$300, Ca transient magnitude abruptly decreased[GLA1] and multiple Ca waves developed during rapid pacing. Many waves were very large in magnitude and comparable to that inducible by caffeine. These triggered waves only develop during stimulation and are absent at rest. Triggered wave frequency and velocity both increased with rate and with increasing diastolic Ca levels. We also found this highly unusual Ca wave behavior in myocytes of intact dog left atrium during rapid pacing, underscoring the fact that this form of Ca wave behavior is not unique to isolated myocytes and occurs in normal atrium. Our results demonstrate that Ca cycling becomes highly abnormal at high heart rates and is characterized by highly fragmented Ca waves that occur at a high frequency. It is likely that these forms of Ca dysregulation could contribute to Ca overload and triggered activity during AF.

1146-Plat

Calcium Spark and Wave Behavior in the Intact Rat Heart

Brian M. Hagen, W.J. Lederer.
University of Maryland, Baltimore, Baltimore, MD, USA.

Spontaneous Ca^{2+} release from the sarcoplasmic reticulum and subsequent activation of Ca^{2+}-sensitive conductances have been postulated as mechanisms to develop arrthymogenic ectopic ventricular beats. Previous studies investigating subcellular Ca^{2+} signaling in the whole-heart have been limited by low temporal and/or spatial resolution. To overcome these obstacles we used an ultra-fast confocal microscope to image the epicardial surface of a Langendorff-perfused heart loaded with the Ca^{2+} indicator fluo-2. Myocardium displayed normal Ca^{2+} transients when paced at low frequencies, and sub-cellular alternans at high frequencies (>5Hz). Importantly a pause in the pacing protocol unmasked the development of Ca^{2+} sparks and spark-triggered Ca^{2+} waves. The abundance of pause-dependent Ca^{2+} sparks and waves increased with pacing frequency. Occasionally during the pause after rapid pacing, spiral Ca^{2+} waves could be observed in a small population of myocytes. Attached figure shows a single myocyte (cell borders indicated by a solid line, top-left) with time; fluorescence depicted on inverted grayscale. The dotted line highlights the spiral Ca^{2+} wave. This study provides new information on rate-dependent Ca^{2+} instability in normal rat hearts and lays the foundation for investigations of Ca^{2+} instabilities due to genetic and acquired heart diseases.



1147-Plat

Characteristics and Mechanisms of Fluid Pressure-Induced Ca^{2+} Waves in Atrial Myocytes

Sun-Hee Woo, Joon-Chul Kim.
Chungnam National University, Daejeon, Korea, Republic of.

There is intriguing clinical evidence for a predisposition to atrial fibrillation by regurgitant jets of blood in patients with mitral valve incompetence. To understand molecular and cellular basis for the fluid pressure (FP)-induced atrial arrhythmias we used fluid-jet that mimics the regurgitant jet in the valve disease, and investigated local and global Ca^{2+} signaling in single rat atrial myocytes. FP of ~7 dyne/cm^2 enhanced Ca^{2+} sparks and Ca^{2+} microwaves with higher potency in the peripheral sites than in the central sites. Higher strength of FP (12-16 dyne/cm^2) elicited global Ca^{2+} waves with two different spatiotemporal patterns: one with longitudinal propagation and the other with transverse propagation. Atrial cells exposed to FP showed one of the two waves or both. The FP-induced transverse wave was similar to action potential (AP)-triggered Ca^{2+} wave. FP-induced longitudinal waves was significantly slower than the transverse waves and always originated from focal Ca^{2+} signal occurring at a central site located at one fifth of the cell length. Both types of FP-induced waves were dramatically inhibited by the blocker of inositol 1,4,5-trisphosphate receptors (IP_3Rs), 2-APB (3 μM). U73122 (5 μM), the inhibitor of phospholipase C (PLC), also suppressed both types of FP-induced waves, while its inactive analogue U73343 did not inhibit them. A slight gradual increase in the background Ca^{2+} level and enhancement of spark occurrences were continued to be observed in 2-APB- or U73122-treated cells under FP. Inhibition of Na^{+}-Ca^{2+} exchange (NCX) using KB-R7943 (0.5 μM) eliminated FP-induced transverse waves but significantly enhanced FP-elicited longitudinal waves. These results suggest that FP may activate PLC and IP_3Rs, thereby triggering focal Ca^{2+} release-derived global waves. We also propose that peripheral Ca^{2+} increase during FP may activate the forward mode of NCX, triggering AP-induced transverse wave.

1148-Plat

Stretch Dependent XROS Signaling: Rapid Mechanotransduction in Heart

Benjamin L. Prosser[1], Christopher W. Ward[2], Brian Hagen[1], Ramzi J. Khairallah[1], W. Jonathan Lederer[1].
[1]University of Maryland School of Medicine, Baltimore, MD, USA, [2]University of Maryland School of Nursing, Baltimore, MD, USA.

Control of cardiac Ca^{2+} release is critical for the regulation of contraction and maintenance of electrical activity. We have recently identified a provocative new signaling pathway, termed ''X-ROS'' signaling, that regulates normal Ca^{2+} release in healthy heart cells, but which may drive pathologic Ca^{2+} release in disease (Prosser et al., *Science*, 2011). The novel signaling arises from the physiological lengthening of heart cells (i.e. a stretch), such that occurs during diastolic filling. This stretch triggers the generation of reactive oxygen species (ROS) by the enzyme complex NADPH oxidase-2 (NOX2). NOX2 is found at the surface sarcolemmal membrane and in the transverse tubules of heart cells, closely opposed to Ca^{2+} release channels (ryanodine receptors, or RyR2s) in the junctional sarcoplasmic reticulum. A stretch-dependent process activates NO**X**2 production of **ROS** (''X-ROS'' signaling), which reversibly oxidizes nearby RyR2s. X-ROS oxidation ''tunes'' the sensitivity of the RyR2s, resulting in an increase in the Ca^{2+} spark rate and enhancement of Ca^{2+} signaling. This process is defective in the fatal muscle disease Duchenne Muscular Dystrophy (DMD), and likely contributes to abnormal Ca^{2+} signaling in DMD.
Our recent results suggest that during a sustained stretch of a cardiomyocyte, there is a rapid, yet transient (~15s) elevation of ROS production that subsides over the duration of stretch. However, during repetitive cyclical stretch, such as occurs during the cardiac cycle, a new level of steady-state ROS production is achieved and maintained. This suggests that the level of steady state ROS generation in the cell may be graded by diastolic length, or pre-load. This finding has key implications for the role of redox signaling and oxidative stress in cardiac patho/physiology. Current work is aimed at investigating how this process operates in the intact, beating heart.

1149-Plat

Sarcoplasmic Reticulum Calcium Depletion Waves in Vascular Smooth Muscle Cells: An Inside View of Spatiotemporal Calcium Regulation

Mitra Esfandiarei, Yohan Choi, Arash Y. Tehrani, Jeremy G. Hoskins, **Nicola Fameli**, Cornelis van Breemen.
University of British Columbia, Vancouver, BC, Canada.

We present results from a novel study of Ca^{2+} waves in vascular smooth muscle cells using a sarcoplasmic reticulum (SR)-targeted Ca^{2+} indicator that specifically binds to the luminal protein calsequestrin.
Agonist-stimulated waves of elevated cytoplasmic calcium concentration regulate blood vessel tone, and vasomotion in vascular smooth muscle. Previous studies employing cytoplasmic calcium indicators revealed that these calcium waves are generated by a combination of inositol 1,4,5-trisphosphate (IP3) and calcium-induced calcium release (CICR) from the SR. Our findings confirm that these waves are due to regenerative CICR by the receptors for IP3 (IP3R). The main new finding from our bservations is a transient elevation in luminal SR Ca^{2+} concentration ([Ca^{2+}]_SR) both at the site of wave initiation, just before regenerative Ca^{2+} release commences, and at the advancing wave front, during wave propagation. This strongly suggests a role of [Ca^{2+}]_SR in activation of IP3R.
In addition, we find that these depletion waves are characterized by a decreasing velocity as they progress. We developed a quantitative diffusional model to analyze this finding and conclude that the gradual decrease in the velocity of the SR Ca^{2+} depletion wave, observed in the absence of external calcium, indicates continuity of the lumen of the sarcoplasmicreticulum network.
Finally, our observation that the depletion wave was arrested by the nuclear envelope may have implications for selective Ca^{2+} signalling.