We also investigated how RyR2 mutations associated with catecholaminergic polymorphic ventricular tachycardia (CPVT) influence the dynamics of Ca^{2+} sparks, ''invisible'' non-spark Ca^{2+} leak, $[Ca^{2+}]_i$ transients, and SR Ca^{2+} content. We observe that CPVT mutations can lead to unstable Ca^{2+} spark dynamics, altering SR Ca^{2+} content and promoting $[Ca^{2+}]_i$ signaling instability. Our new model provides significant insights into the dynamics of local control of CICR under physiological and pathological conditions.

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Investigation of Arrhythmogenic Calcium Events by Initiating Local Calcium Release in Cardiomyocytes

Brian M. Hagen, Joseph P.Y. Kao, W. Jonathan Lederer.
BioMET & Physiology, University of Maryland School of Medicine, Baltimore, MD, USA.

In cardiac muscle the sarcoplasmic reticulum (SR) contains the Ca^{2+} that is released during excitation-contraction coupling to initiate cross-bridge cycling, sarcomere shortening and force generation. Under physiological conditions SR Ca^{2+} uptake by the SR/ER Ca^{2+} ATPase is balanced by Ca^{2+} ''leak'' out of the SR through the SR Ca^{2+} release channels (ryanodine receptors, RyR2). Under diverse conditions SR Ca^{2+} overload can develop (i.e. elevation of $[Ca^{2+}]_{SR}$) and this is associated with an increase in the open probability of the RyR2s which leads to SR Ca^{2+} instability. SR Ca^{2+} overload is thus associated with an increased Ca^{2+} spark rate, increased ''invisible'' SR Ca^{2+} leak and the development of a propagating chain-reaction of Ca^{2+}-induced Ca^{2+} release (Ca^{2+} wave) within the ventricular myocyte. This abnormal Ca^{2+} signaling activates the sarcolemmal Na^+/Ca^{2+} exchanger to produce an arrhythmic inward current. In order to investigate SR Ca^{2+} release under these conditions, local Ca^{2+} release was produced by photolysis of a caged paraxanthine (BiNiX, 3-(4,5-bis(carboxymethyl)-2-nitrobenzyl)-paraxanthine), a caffeine-like ''activator'' of RyR2. Using a newly developed photolysis system, laser flashes (354 nm) of different sizes and shapes were rapidly (millisecond) positioned on the focused image plane of a confocal microscope. A spectrum of responses in murine cardiac myocytes was observed ranging from instigating Ca^{2+} sparks to triggering Ca^{2+} waves. Eliciting a fully propagating Ca^{2+} wave required SR Ca^{2+} overload and/or an increased sensitivity of RyR2s to cytosolic Ca^{2+}. This study was uniquely successful in triggering multiple Ca^{2+} waves simultaneously. Creating multiple Ca^{2+} wave fronts in a myocyte enabled the generation of an extrasystole triggered by a delayed after-depolarization. This new experimental technology allows systematic investigation of rare extrasystoles triggered by Ca^{2+} waves.

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The Role of Junctional- and Non-Junctional Ca Release Sites in the Generation of Aberrant Diastolic Ca Release in Myocytes from Post-Myocardial Infarction Hearts

Andriy E. Belevych, Cynthia A. Carnes, George E. Billman, Sandor Gyorke.
Physiology & Cell Biology, OSU, Columbus, OH, USA.

Cardiac Ca signaling is organized into structurally and functionally specialized compartments that include junctional Ca release units (CRUs) coupled to L-type Ca channels (LTCC) and LTCC-free non-junctional CRUs. Little is known about subcellular diferences in patholgic Ca handling and their role in cardiac arrhythmogenesis. We have shown that diminished Ca signaling refractoriness in diseased myocytes contributes to their susceptability for diastolic Ca waves (DCWs). The objective of present study was to define the subcellular determinants of arrhythmogenic DCWs by quantifying functional differences in Ca signaling from anatomically distinct sites (junctional vs. non-junctional) and their relative roles in the genesis of DCWs. We employed high resolution 2D confocal Ca imaging in ventricular myocytes isolated from normal and post-myocardial infarction (MI) arrhythmia-prone canine hearts. Ca release sites were categorized as early- or delayed-response (corresponding to junctional and non-junctional regions, respectively) based on activation time following electrical stimulation. MI (but not control) myocytes exhibited regular DCWs when paced in the presence of isoproterenol. These DCWs predominantly originated from early-response sites. Consistent with this observation, majority of spontaneous Ca sparks also occurred at early-response sites. Interestingly, after stimulated Ca release the restitution time of Ca sparks was similar between control and MI myocytes. Furthermore, application of a two-pulse restitution protocol revealed similar rates of Ca release recovery between control and MI myocytes at the early-response sites. However, the Ca release recovery at late-response sites was markedly abbreviated in MI cells. These results suggest that increased propensity of MI myocytes toward arrhythmogenic DCWs can be attributed to diminished refractoriness of non-junctional CRUs facilitating the transition of Ca sparks at eager junctional sites to self-propagating Ca waves via the recruitment of non-junctional sites.

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Ablation of Major PKA and/or Camkii Phosphorylation Sites in the RyR2 Channel Differentially Affects the Susceptibility of Mice to Vagotonic Atrial Fibrillation

Roberto Ramos Mondragón, Emmanuel Camors, Patricia P. Powers, Héctor H. Valdivia.
Center for Arrhytmia Research, Michigan University, Ann Arbor, MI, USA.

Rationale: To date, three major phosphorylation sites in the cardiac Ca^{2+} release channel/ryanodine receptor (RyR2) have been determined to undergo phosphorylation in vivo. Ser2808 (mouse nomenclature) is phosphorylated by both CaMKII and PKA, while Ser2814 and Ser2030 appear to be exclusively phosphorylated by CaMKII and PKA, respectively. Independent studies have shown that increased levels of RyR2 phosphorylation at S2808 and/or S2814 increase the vulnerability of mice and other mammals (including humans) to atrial fibrillation (AF). However, the role of S2030 has not been determined yet in experimental animals. Methods: We generated mice with single (S2808A and S2030A) and double (S22808A/S2030A) ablation of PKA sites, and mice with double ablation of CaMKII sites (S2808A/S2814A). Homozygous PKA- and CaMKII-phosphorylation deficient mice were subjected to rapid atrial pacing via an intracardiac catheter and their susceptibility to vagotonic (50 ng/g carbachol) AF was compared against WT littermates. Results: The CaMKII-phosphorylation deficient S2808A/S2414A double mutant mice, but not the single mutant S2808A mice, decreased their propensity to AF compared to WT mice. Surprisingly, the phospho-mutant S2030A mice displayed increased vulnerability to AF and this phenotype was not prevented by altering the phosho-state of S2808, as it was observed in the S2030A/S2808A mice. Interestingly, AF episodes lasting more than 15 min were more frequent in the CaMKII-phosphorylation deficient S2808A/S2814A mice. Conclusions: CaMKII-phosphorylation of S2814 increases significantly the incidence of non-sustained periods of AF, while PKA-phosphorylation of S2030 seems to prevent AF. Finally, the phosphorylation of S2808 does not play an important role in the prevention and/or duration of AF in this model.

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Increased Serca Pump Expression is Associated with Slow Termination of Calcium Sparks and Delayed Local Recovery in Vascular Smooth Muscle Cells of Hyperthyroid Rats

Miyamin J. Miranda-Saturnino, David R. de Alba-Aguayo, Martha Mercado-Morales, Agustín Guerrero-Hernández, **Angelica Rueda**.
Biochemistry, Cinvestav-IPN, Mexico, Mexico.

In vascular smooth muscle cells of cerebral arteries (VSMCs), spontaneous and repetitive local Ca^{2+} signals produced by the Ca^{2+} channel/Ryanodine Receptor (RyRs), known as Ca^{2+} sparks, are involved in the regulation of vascular myogenic tone. However, the molecular mechanisms behind the frequency and properties of Ca^{2+} sparks in VSMCs are still under study. Considering that in VSMCs, spontaneous Ca^{2+} sparks are generated at preferred subcellular locations, we have analyzed the spatio-temporal properties of repetitive same-location Ca^{2+} sparks, its time-dependent recovery (or restitution) and the regulation that luminal Ca^{2+} might exert in this phenomenon by using the experimental model of hyperthyroid rat (T_3).

Ca^{2+} sparks recorded in VSMCs from T_3 rats showed similar frequency, amplitude, and Ca^{2+} flux compared with those recorded in control cells. However, Ca^{2+} spark properties such as duration, size, time-to-peak, and k decay were significantly increased in T_3 cells; and as a consequence, spark-mediated Ca^{2+} leak was augmented by 3.3-fold. In both experimental groups, amplitude recovery of repetitive Ca^{2+} sparks was an all-or-none phenomenon and did not show any time-dependency. Histogram distribution of spark-to-spark delays from T_3 cells showed a 4-fold reduction in shorter delays (0-200 ms), with the shortest spark-to-spark delay of 115.9 ms in T_3 cells and of 67.2 ms in control cells. Although SERCA pump expression was increased in vascular tissue from T_3 rats, both basal Ca^{2+} concentration and intracellular SR Ca^{2+} load, determined by caffeine, remained unchanged. Our data suggest that in VSMCs of T_3 rats spontaneous Ca^{2+} sparks decrease SR Ca^{2+} content not only by increasing its frequency but also by delaying its termination to counteract SR Ca^{2+} overload resulting from the overexpression of SERCA pump.

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Ultrafast Calcium Wave in Cultured Vascular Smooth Muscle Cells

Jean-Jacques Meister[1], Jairo Camilo Quijano[2].
[1]Swiss Federal Institute of Technology, Lausanne, Switzerland, [2]Politécnico Colombiano JIC, Medellin, Colombia.

Communication between vascular smooth muscle cells (SMCs) allows control of their contraction and so regulation of blood flow. The contractile state of SMCs is regulated by cytosolic Ca^{2+} concentration ($[Ca^{2+}]i$) which propagates as Ca^{2+} waves over a significant distance along the vessel. In this work, we