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Urocortin 2 Regulates Sarcomplasmic Reticulum Calcium via Phosphorylation of Phospholamban and SERCA Activation and Protects against Pro-Arrhythmic Alternans in Cardiac Myocytes from Normal and Failing Hearts

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We tested the effect of the cardioactive peptide Urocortin 2 (Ucn2; 100 nM) on action potential (AP)-induced Ca transients (CaT), sarcomplasmic reticulum (SR) Ca load and cardiac Ca alternans. Experiments were performed on single rabbit atrial and ventricular myocytes from normal and failing hearts. Chronic heart failure (HF) was induced by combined pressure-volume-overload. Ca and mechanical alternans was induced by electrical pacing. Changes in cytosolic [Ca]i and [Ca]SR were monitored with fluorescent indicators, in conjunction with sarcomere length measurements. The average Ca alternans ratio (AR = 1-S/L; S: small amplitude CaT; L: large-amplitude CaT) was 0.51 in normal atrial myocytes, and 0.43 and 0.53 in normal and HF ventricular myocytes, respectively. Ucn2 increased SR Ca load in normal, and to a larger degree in HF ventricular cells, and enhanced AP-induced SR Ca depletion transients. Baseline phosphorylation levels of phospholamban (PLN) at Ser16 were increased in HF myocytes, and Ucn2 stimulation further augmented PLN phosphorylation in normal myocytes (~10-fold) and to a lesser extent (~5-fold) in HF ventricular myocytes. Ucn2 completely abolished Ca alternans in normal myocytes (within 2-3 min) as well as normal and HF ventricular myocytes. In the presence of ABT-724 (1 μM; inhibition of corticotropin-releasing factor receptor; CRFR2) and H89 (1 μM; protein kinase A inhibition) Ucn2 failed to rescue alternans. In conclusion, we propose that Ucn2 rescues cardiac alternans via CRFR2-mediated stimulation of PKA, phosphorylation of PLN and enhanced cytosolic Ca sequestration by SERCA.

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Calcium Mediated Mechanism of Early Afterdepolarizations in LQT2 Ventricular Myocytes

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Background: Loss of function mutations in hERG potassium channels underlie the long QT syndrome (LQT2). LQT2 is associated with fatal ventricular arrhythmias mediated by triggered activity in the form of early afterdepolarizations (EADs). However, the cellular mechanism of EAD formation remains unexplored.

Methods: We have investigated the mechanism of EAD formation in LQT2 ventricular myocytes using a physiologically detailed computational model of calcium (Ca2+) cycling and membrane voltage dynamics. This model bridges the submicron scale of individual couplons of plasmalemmal L-type Ca2+ channels clusters and sarcoplasmic reticulum (SR) Ca2+ release units (CRUs) and the whole cell. We incorporate the novel experimental finding that ryanodine receptors (RyRs) Ca2+ release channels are remodeled in ventricular myocytes isolated from LQT2 transgenic rabbits; RyRs are hyper-phosphorylated leading to enhanced channel activity and hence increased Ca2+ leak.

Results: Computer simulations with RyR hyperactivity modeled as an increased rate of channel opening show that hyperactivity is causally linked to EAD formation. Under stimulation with the β-adrenergic agonist isoproterenol (ISO), LQT2 myocytes with hyperactive RyRs exhibit EADs together with decreased SR load and Ca2+ transient (CaT) amplitude, while myocytes with normal RyR activity exhibit a prolonged action potential without reductions of SR load and CaT amplitude. Simulations show that RyR hyperactivity shortens RyR refractoriness at the CRU level, resulting in late aberrant Ca2+ releases during repolarization. Those releases promote onset of EADs by driving the forward mode Na+-Ca2+ exchanger NCX1 current, which slows repolarization and allows reactivation of L-type Ca2+ current. Modeling predictions are in good agreement with experimental observations, which show that EADs in ISO-stimulated LQT2 myocytes are accompanied by late Ca2+ releases together with decreased SR load and CaT amplitude, and that both late releases and EADs are abolished by inhibition of CaMKII.

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Multiscale Consequences of Spontaneous Calcium Release on Cardiac Delayed Afterdepolarizations

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Spontaneous calcium (Ca) release in cardiac myocytes can produce delayed afterdepolarizations (DADs) that promote arrhythmias. In these studies, we combined experimental and computational approaches to investigate quantitatively how Ca release features at subcellular, cellular, and tissue scales influence DADs. Spontaneous Ca release events and corresponding DADs were measured experimentally from isolated rabbit ventricular myocytes exposed to elevated extracellular Ca (2.7 mM) and isoproterenol (0.25 μM), and were compared between lower and higher total intracellular Ca levels following 1 or 5 paced beats at 400 ms intervals, respectively. At the subcellular scale, Ca waves emerged simultaneously from an average of 1.36 (low) and 2.5 (high) sites per Ca release event. Consistent with the criticality-based theory of Ca wave emergence, Ca wave numbers were proportional to sarcoplasmic reticulum (SR) Ca levels that produced INCX with ~71.1 pA/pF×ms (low) and ~106.0 pA/pF×ms (high) integrated current densities upon rapid exposure to caffeine (10 mM). At the cellular scale, whole-cell calcium transients had peak amplitudes of 0.462 F/F0 (low) and 0.717 F/F0 (high) and full widths at half maximum (FWHMs) of 309.0 ms (low) and 180.0 ms (high). Resultant DADs had peak amplitudes of 1.41 mV (low) and 6.03 mV (high) and FWHMs of 302.3 ms (low) and 140.3 ms (high). Ca wave latencies in single myocytes, which determine Ca release synchrony in cardiac tissue, were 0.996 s (low) and 0.416 s (high). Computer simulation analyses implementing various combinations of the experimentally measured factors suggest that greater numbers of Ca waves, increased SR Ca release, and greater synchrony are associated with larger DAD amplitudes in cardiac tissue. Simulations also suggest that the subcellular number of Ca waves has the largest impact on DAD amplitude in tissue.

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FRET-Based RyR-Selective Detection Reveals No Significant Competition between CaM and S100A1 Binding to RyRs

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Using fluorescence resonance energy transfer (FRET), we directly tested the hypothesis that S100A1 competes with calmodulin (CaM) for binding to intact, functional ryosynode receptors type 1 (RyR1) and II (RyR2) from skeletal and cardiac muscle, respectively. This hypothesis is largely based on competition assays using isolated sarcoplasmic reticulum (SR) membranes from skeletal muscle and evidence that S100A1 binds to peptides corresponding to one of the proposed CaM binding domains of RyR1. However, competition between S100A1 and CaM for RyR binding has not been directly detected. We targeted a donor-labeled FKBP12.6- (D-FKBP) to the cytoplasmic domain of RyR1 or RyR2 in SR vesicles isolated from pig skeletal or cardiac muscle. FRET was detected as a decrease of D-FKBP fluorescence in the presence of 100nM acceptor-labeled CaM (A-CaM) and used to index CaM binding to RyR. Upon pre-incubating SR with [S100A1] ranging from 0.01 to >100 μM, we found partial inhibition of FRET, with μM K, for both skeletal and cardiac SR. By comparison, FRET was completely inhibited by unlabeled WT-CaM (K, ~100nM), indicating that A-CaM and WT-CaM bind RyR with similar affinities and at the same site. Similar results were obtained using co-sedimentation assays conducted under similar experimental conditions, to detect competition between S100A1 and CaM binding to SR membranes. Taken together, these results indicate that CaM-RyR binding may not be significantly competed by S100A1 under normal physiologic conditions. Furthermore, structural analysis of FRET data suggests that S100A1 allosterically interacts with RyR-CaM binding. Initial results from a complementary FRET approach, using acceptor-labeled S100A1, further support the conclusion that S100A1 does not significantly compete with CaM-RyR binding in skeletal or cardiac muscle.

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Calmodulin Potentiates RyR2 Block and Ca Wave Suppression by Flecainide

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