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Urotensin-II Induces Vascular Smooth Muscle Cell Proliferation and Creb Phosporylation Through Store Operated Calcium Entry and EGFR Transactivation
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Urotensin-II (U-II) is a vasoactive peptide with many effects in the cardiovascular system. It has been described to induce vascular smooth muscle cell (SMC) proliferation, and is involved in the pathogenesis of atherosclerosis, restenosis, and vascular remodelling; however, its signalling pathway still remains unclear. The aim of this study was to elucidate the role of calcium (Ca^{2+})-dependent signalling and alternative signalling pathways in UII-evoked VSMCs proliferation, focussing our attention on store-operated Ca^{2+} entry (SOCE) pathway and epithelium growth factor receptor (EGFR) transactivation.
We used 5-bromo-2-deoxyuridine (BrdU) labeling, molecular knockdown with small interfering RNA (siRNA) and immunofluorescence to study UII-promoted aortic SMC proliferation. Ca^{2+} mobilization assays were performed to study Ca^{2+} entry in cultured SMC.
We found that UII enhanced intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) which was significantly reduced by classical SOCE inhibitors and by knockdown of essential components of the SOCE such as STIM1, Orai1, or TRPC1. Moreover, UII stimulated SMC proliferation and Ca^{2+}/cAMP response element-binding protein (CREB) activation through SOCE pathway that involved STIM1, Orai1, and TRPC1. Co-immunoprecipitation experiments showed that UII promoted the association between Orai1 and STIM1, and between Orai1 and TRPC1. Additionally, we determined that epithelium growth factor receptor (EGFR) transactivation, extracellular signal-regulated kinase (ERK) and Ca^{2+}/calmodulin-dependent kinase (CaMK) signaling pathways were involved in UII-mediated Ca^{2+} influx, CREB activation and VSMCs proliferation.
Our data show for the first time that UII-induced SMC proliferation and CREB activation requires a complex signalling pathway that involves on the one hand SOCE mediated by STIM1, Orai1 and TRPC1, and on the other hand EGFR, ERK, and CaMK activation.
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Calcium Fluxes, Sparks, and Waves I

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Emergence and Synchronization of the "Calcium Clock" in a 3-Dimensional Model of a Sino-Atrial Node Cell with Explicit Channel Gating
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We constructed a three dimensional stochastic numerical model of propagated spontaneous diastolic calcium release in sino-atrial node cells, using explicit gating of individual calcium channels, without assuming either a discrete sub-membrane compartment or an inactivated state of the RyR. Immunofluorescence staining of SA node cells for RyRs revealed a dense, variable network of clusters, with small clusters intermediate between major ones, located immediately below the surface membrane. The presence of such intermediate "bridging" clusters was crucial to allow propagation of local calcium releases in the presence of 3D diffusion and buffering. When this RyR network was simulated, local calcium releases propagated as partially synchronized wavelets localized near the surface membrane. Calcium in the junctional and free sarcoplasmic reticulum followed a complex pattern of recycling through the interior of the cell, with areas of both depletion and enhancement. These calcium wavelets acted as a partially periodic "calcium clock" that entrained with classical membrane currents to regulate beating rate in response to simulated beta-adrenergic stimulation, as demonstrated experimentally. When the RyR sensitivity was very high or NCX density was low, there was a paradoxical regime in which synchronization was lost, causing sympathetic stimulation to reduce rather than increase beating rate. This regime may be important in heart failure or with RyR mutations.

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Super-Resolution Modeling of Calcium Release in Heart
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Stable calcium-induced calcium release (CICR) is critical for maintaining normal cellular contraction during cardiac excitation-contraction coupling (ECC). The fundamental element of CICR in heart is the calcium (Ca^{2+}) spark, which arises from a cluster of ryanodine receptors (RyR2) that is triggered to produce a local regenerative release of Ca^{2+} from the sarcoplasmic reticulum (SR). Recent work suggests that the RyR2s mediate the majority of SR Ca^{2+} leak via both "visible" release events, Ca^{2+} sparks, and "invisible" non-spark Ca^{2+} release events, Ca^{2+} quarks (Williams et al. BJ 2011). Together, these events create an SR Ca^{2+} leak pathway responsible for balancing the SR Ca^{2+} pump (SERCA) in cardiac myocytes. Here we present a detailed, three-dimensional model of a Ca^{2+} release unit (CRU) in heart that incorporates RyR2 geometries informed by super-resolution STED (stimulated emission depletion) microscopy. The model exhibits spontaneous Ca^{2+} sparks with realistic kinetics and morphology, as well as robust Ca^{2+} spark initiation and termination across a wide range of geometries and conditions. Furthermore, the model captures the details of Ca^{2+} spark and non-spark based leak while still maintaining normal ECC gain. The model predicts that subspace and RyR2 cluster geometry critically influence the CRU's function. Large (40+ RyR2s), densely packed RyR2 clusters exhibited higher Ca^{2+} spark fidelity and overall Ca^{2+} leak, due to enhanced inter-RyR2 coupling via local subspace $[Ca^{2+}]$. We find that the fidelity of a RyR2 cluster can be predicted from either the cluster size or the spectral properties of a simplified RyR2 network. We also show that luminal $[Ca^{2+}]$ dependent regulation of RyR2 is not critical for robust spark termination but decreased RyR2 Ca^{2+} flux due to local luminal depletion is. These results provide insights into the dynamics of CICR in the heart under normal and pathological conditions.

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Anti-Arrhythmic Block of Ryr2 by Flecainide Versus Pro-Arrhythmic Block by Tetracaine in a 3D Model of a Cardiac Cell
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Although excess Ca^{2+} release through RyR2 in diastole is a key factor in arrhythmia, not all RyR2 antagonists have an antiarrhythmic action. Our previous studies comparing the anti-arrhythmic blocking action of flecainide with the pro-arrhythmic blocking action of tetracaine found that flecainide reduced the amount of Ca^{2+} released during localised diastolic release events (Ca^{2+} sparks) but increased their frequency. As a result, flecainide had no effect on overall SR Ca^{2+} leak or SR Ca^{2+} content. However, the likelihood that each Ca^{2+} spark could generate an arrhythmogenic Ca^{2+} wave was reduced because the Ca^{2+} sparks were smaller. In contrast, tetracaine decreased spark-mediated SR Ca^{2+} leak but significantly increased SR Ca^{2+} content, Ca^{2+} released during a spark and frequency of Ca^{2+} waves. These results suggested that the flecainide-induced reduction in Ca^{2+} spark size contributes to its anti-arrhythmic action by reducing the probability of saltatory wave propagation between adjacent Ca^{2+} release sites.
We developed 3D models of Ca^{2+} dynamics, which are linked to whole cell ionic current models of ventricular and sinoatrial node. By including a kinetic model for tetracaine and flecainide block of RyR2, we find that open state block of flecainide stabilises Ca^{2+} release. In contrast, the closed state block by tetracaine has the effect of increasing the positive feedback of Ca^{2+} on SR Ca^{2+} release; the net result is an increase in the amplitude of Ca^{2+} sparks, destabilization of SR Ca^{2+} cycling and cardiac arrhythmia.

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Role of the Inter-RyR Coupling in Cardiac Intracellular Calcium "Clock"
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Cardiac rhythm is generated by the sinoatrial node in the normal heart. It has been recently shown that spontaneous local Ca^{2+} releases from sarcoplasmic reticulum (SR) through Ryanodine Receptors (RyRs) occur during late