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$^{2+}$], 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$^{2+}$]$^\text{SR}$, signaling instability. Our new model provides significant insights into the dynamics of local control of CICR under physiological and pathological conditions.

1631-Pos Board B361
Investigation of Arrhythmogenic Calcium Events by Initiating Local Calcium Release in Cardiomyocytes
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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$^{2+}$]) 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$^{2+}$ wave propagation was studied by photolysis of a tetracaine derivative (BINX, 3,4,5-bis(carboxyethyl)-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 RyR2 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 afterdepolarization. This new experimental technology allows systematic investigation of rare extrasystoles triggered by Ca$^{2+}$ waves.

1632-Pos Board B362
The Role of Junctional- and Non-Junctional Ca Release Sites in the Generation of Arrhythmic Diastolic Ca Release in Myocytes from Post-Myocardial Infarction Hearts
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Cardiac Ca$^{2+}$ 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 differences in pathologic Ca handling and their role in cardiac arrhythmogenesis. We have shown that diminished Ca$^{2+}$ signaling refractoriness in diseased myocytes contributes to their susceptibility 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. DCW release sites were categorized as early- or delayed-response (corresponding to junctional and non-junctional regions, respectively) based on activation time from T3 rats, both basal Ca$^{2+}$ concentration and intracellular SR Ca$^{2+}$ load, determined by caffeine, remained unchanged. Our data suggest that in VSMCs of T3 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.

1633-Pos Board B363
Ablation of Major PKA and/or CamkkII Phosphorylation Sites in the RyR2 Channel Differentially Affects the Susceptibility of Mice to Vagotonic Atrial Fibrillation
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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 CamkkII and PKA, while Ser2814 and Ser2030 appear to be exclusively phosphorylated by CamkkII 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 (S2808A/S2030A) ablation of PKA sites, and mice with double ablation of CamkkII sites (S2808A/S2814A). Homozygous PKA- and CamkkII-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 CamkkII-phosphorylation deficient S2808A/S2814A double mutant mice failed the single low-dose carbachol test but not the single high-dose test. Surprisingly, the phospho-mutant S2030A mice displayed increased vulnerability to AF and this phenotype was not prevented by altering the pho-sho-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 CamkkII-phosphorylation deficient S2808A/S2814A mice. Conclusions: CamkkII-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.

1634-Pos Board B364
Increased Serca Pump Expression is Associated with Slow Termination of Calcium Sparks and Delayed Local Recovery in Vascular Smooth Muscle Cells of Hyperthyroid Rats
In vascular smooth muscle cells of cerebral arteries (VSMCs), spontaneous and repetitive local Ca$^{2+}$ sparks are involved in the regulation of vascular myogenic tone. However, the mechanical 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.

1635-Pos Board B365
Ultrastable Calcium Wave in Cultured Vascular Smooth Muscle Cells
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In vascular smooth muscle cells of cerebral arteries (VSMCs), spontaneous and repetitive local Ca$^{2+}$ sparks are involved in the regulation of vascular myogenic tone. However, the mechanical 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$_3$).

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Ultrafast Calcium Wave in Cultured Vascular Smooth Muscle Cells
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have characterized an intercellular ultrafast Ca\textsuperscript{2+} wave observed in cultured A7r5 cell line and in primary cultured SMCs (pSMCs) from rat mesenteric arteries. This wave, induced by local mechanical or local KCl stimulation, had a velocity around 15 mm/s. Combination of precise alignment of cells with fast Ca\textsuperscript{2+} imaging and intracellular membrane potential recording, allowed us to analyze rapid [Ca\textsuperscript{2+}]i dynamics and membrane potential events along the network of cells. The rate of [Ca\textsuperscript{2+}]i increase along the network decreased with distance from the stimulation site. Gap junctions or voltage-operated Ca\textsuperscript{2+} channels (VOCCs) inhibition suppressed the ultrafast Ca\textsuperscript{2+} wave. Blockage of the voltage-operated Ca\textsuperscript{2+} (VOCA) or ryanodine receptors (RyR) did not affect the Ca\textsuperscript{2+} response. Mechanical stimulation induced a membrane depolarization that propagated and that decayed exponentially with distance. Our results demonstrate an electrometric spread of membrane depolarization that drives a rapid Ca\textsuperscript{2+} entry from the external medium through VOCCs, modeled as an ultrafast Ca\textsuperscript{2+} wave. This wave may trigger Ca\textsuperscript{2+} release from intracellular stores that drives observed slower Ca\textsuperscript{2+} waves ex vivo and in vivo.

1636-Pos Board B368
Altered CamKII and ROS Microdomains Favor Sparks in Orphaned RyR After Myocardial Infarction
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In ventricular myocytes, ryanodine receptors (RyRs) are typically organized at the Z-line. The RyR2 complexes are the main source of Ca\textsuperscript{2+} sparks in these tissues. In the absence of RyRs or Ca\textsuperscript{2+} release, the mechanical load on the heart cell to its calcium signaling sensitivity. However, an increase in RyR2 sensitivity alone is insufficient to explain a persistent increase in Ca\textsuperscript{2+} sparks, implicating additional stretch-dependent players in the sustained elevation of calcium signaling sensitivity with increased mechanical stress. We investigated three potential players based on their previous links to mechanical stress and modulation by RyR2. First, we explored the role of nitric oxide (NO), Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II (CaMKII), and gap junctional coupling (i.e., the mechanical load on the heart cell to its calcium signaling sensitivity). Our results indicate that the increase in the proarrhythmogenic Ca\textsuperscript{2+} waves at the onset of reperfusion are mainly mediated by a CaMKII-dependent phosphorylation.

1639-Pos Board B369
The Coupled-Pacemaker Clock System of Sinoatrial Nodal Cells Regulates Both the Action Potential Rate and Rhythm
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Normal automaticity of the sinoatrial-node cells (SANC) is regulated by integrated molecular functions within a system of two clock-like oscillators: the sarcoplasmic reticulum, acting as a “Ca\textsuperscript{2+} clock,” rhythmically discharges diastolic local-Ca\textsuperscript{2+} releases (LCRs) beneath the cell surface membrane; LCRs activate an inward Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger current (INX) that prompts the “Membrane clock,” the ensemble of other sarcolemmal-electrogenic molecules, to generate action potentials (APs). Crosstalk between the two clocks regulates SANC spontaneous AP cycle length (CL). We determine whether clock crosstalk also regulates the rhythm of AP CL in response to perturbation that induce brady-cardia via specific inhibition of either membrane or Ca\textsuperscript{2+}-clock functions.

We employed ivabradine (IVA, 3, 10 and 30\mu M) which directly inhibits membrane clock ion channel functions, but has no direct effect on Ca\textsuperscript{2+} clock functions. The IVA-induced increase in the AP CL, however, lowers Ca\textsuperscript{2+} influx, which reduces Ca\textsuperscript{2+}-activated calmodulin-Ac-cAMP/PAK signaling, affecting Ca\textsuperscript{2+} and phosphorylation-dependent functions that drive the coupled clock system. Direct and specific inhibition of SERCA2 by cyclopiazonic acid (0.5 and 5\mu M) reduces the magnitude and delays the occurrence of the LCR-Ca\textsuperscript{2+} signal, leading to a reduction in Ca\textsuperscript{2+}-activated calmodulin-Ac-cAMP/PAK signaling, and delayed and reduced Ca\textsuperscript{2+} activation of INX and a prolongation of the AP CL. Importantly, prolongation of LCR period (IVA by 16 ± 2 to 46 ± 5\% CPA by 17 ± 2 to 53 ± 5\%, AP CL (IVA by 13 ± 4 to 36 ± 8\%, CPA by 45 ± 4 to 84 ± 8\%), and the increase in AP CL variability (IVA by 8 ± 10 to 190 ± 50\%, CPA by 36 ± 17 to 183 ± 50\%) are proportional to each other. The tight inter-relationships among these variables in response to specific perturbation of either the M or the Ca\textsuperscript{2+} clock, therefore, reflect clock crosstalk.

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