2178-Plat
Activity of Both PKA and Camkii is Required for Maximal RyR Sensitivity Under Beta-Adrenergic Stimulation
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In cardiac myocytes, calcium sparks exhibit time-dependent refractoriness such that triggering of a second spark soon after an initial spark has terminated is improbable. Recent studies in rat ventricular myocytes suggested that spark amplitude recovery is controlled by local sarcoplasmic reticulum (SR) refilling whereas refractoriness of spark triggering depends on both refilling and ryanodine receptor (RyR) sensitivity.
Here we examined spark refractoriness in mouse ventricular myocytes by exposing Fluo-3 loaded quiescent cells to 50 nM ryanodine, recording sparks with a confocal microscope, and analyzing the repeated sparks that were produced at singular RyR clusters.
Beta-adrenergic stimulation accelerated spark amplitude recovery and decreased median spark-to-spark delay compared to control. Spark amplitude recovery was also accelerated/decelerated, respectively, by either activating or inhibiting PKA with forskolin or H89. Spark-to-spark delays were not affected by forskolin but increased in H89. Experiments performed on S2808A mice with a mutated PKA phosphorylation site corroborate these results.
Forskolin results suggest that during beta-adrenergic stimulation pathways other than PKA can be activated. Beta-adrenergic stimulation in the presence of PKA or CaMKII blockers (H89 and KN93, respectively) were studied. In both conditions, time constant of amplitude recovery was increased and spark-to-spark delay was slightly higher than control. Employing a mathematical model allowed us to gain further insight into experimental results. Under control conditions SR refilling is enhanced due to activation of SERCA via endogenous PKA activity. Additional activation of either PKA or CaMKII is sufficient to accelerate spark amplitude restitution through faster refilling. However, inhibition of either pathway prevents RyR sensitivity to be increased. Thus, activity of both kinases is necessary to explain the changes in RyR gating observed during beta-adrenergic stimulation.

2179-Plat
Critical Requirements for the Initiation of a Cardiac Arrhythmia in Heart: Cell Number
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Many features of an intact functioning heart provide a margin of safety with respect to the generation of cardiac arrhythmias. We have been investigating Ca^{2+} dependent arrhythmias and the requirements for such pathological activity including features that focus on the margin of safety. One of the primary issues is the number of cells involved in the initiation process. If too few cells are needed to initiate the arrhythmia, arrhythmias will be rampant. If too many cells are needed, then it is difficult to explain how known arrhythmias occur. Thus the balance is one of the critical parameter to be estimated to validate the model and also to develop a useful set of computational tools to examine lethal cardiac arrhythmias. It has been suggested in published work that, a large number (~800,000) of ventricular myocytes be depolarized to overcome the electrotonic load of the heart. In our recent work we have re-examined this question in light of a revised stochastic model of electrical and Ca^{2+} activity. We have found that as few as 12 myocytes may be needed to initiate an ectopic cardiac arrhythmia. This reduction of over 4 orders of magnitude reflects important improvements in the modeling. These changes include the stochasticity of the Ca^{2+} modeling, improved assumptions regarding the ionic currents in heart cells and updated geometries. We will present and discuss these critical changes and the justification for them. Our new findings regarding the initiation of spontaneous arrhythmias are in line with functional data from the heart. This new finding suggests a mechanism by which the generation of arrhythmias is possible while still maintaining a high safety margin.

2180-Plat
Structural and Functional Alteration of RyR Clusters After Remodeling in Persistent Atrial Fibrillation
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In chronic atrial fibrillation (AF), abnormalities in Ca^{2+} release from RyR have been implicated as major factors contributing to arrhythmia and contractile dysfunction, but the relation to RyR organization remains unknown. Using STED microscopy we examined RyR cluster morphology in isolated atrial myocytes from sheep with persistent AF (N=6, 16-23 weeks of AF) and age matched control (Ctrl) animals; in parallel experiments we measured Ca^{2+} sparks in permeabilized myocytes. STED measurements revealed RyR clusters typically contained 15 contiguous RyR and on average this did not differ between AF and Ctrl. However, the nearest neighbor distance between clusters was reduced in AF. Grouping of clusters within 150 nm as functional Ca^{2+} release units (CRU) indicated that in AF these units exhibit increased fragmentation, with more clusters per functional unit. Measurement of Ca^{2+} sparks in permeabilized myocytes revealed a >50% increase in spark frequency and a higher prevalence of macrosparks. Spark time-to-peak (TTP) and duration were also increased, but width was reduced. Measurement of the intrinsic cellular buffer capacity showed this was reduced in AF. Using computational modeling it was found that the increased TTP and duration can be ascribed to the increased number of clusters per functional grouping in AF. The observed increased CRU fragmentation and reduction in Ca^{2+} buffering, can increased occurrence of sparks and macrosparks in AF. In conclusion, ultrastructural reorganization of RyR clusters within the functional units contributes to overactive Ca^{2+} release with increased chance of propagating events in AF.

2181-Plat
Subcellular Origin and Tissue-Wide Synchronization of Abnormal Ca Release in the Genesis of Ca-Dependent Atrial Arrhythmia
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Introduction. Abnormal diastolic Ca release (DCR) from the sarcoplasmic reticulum has been implicated in both ventricular as well as atrial fibrillation (AF). Atrial cells lack the extensive T-tubule network that facilitates Ca signaling in ventricular myocytes. How the distinct structural organization of atrial cells affects the genesis of AF is currently unknown.
Methods and Results. We used rapid 2D confocal imaging to map Ca changes in atrial single myocytes and tissue preparations derived from CASQ2 knockout (CASQ2.KO) mice manifesting Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT). Isolated CASQ2.KO atrial cells showed frequent local and cell-wide DCR events when exposed to isoproterenol. DCR predominantly originated nearly simultaneously at several ''eager'' sites localized at cell periphery. The distribution of latencies to local DCR events in CASQ2.KO cells displayed a left-ward shift consistent with abbreviated RyR2 refractoriness associated with CPVT. This abbreviated RyR2 refractoriness translated into highly synchronous spontaneous Ca oscillations across the intact atrial tissue. Conditional overexpression of SERCA2a in CASQ.KO mice exacerbated proarrhythmic Ca oscillations by increasing the proportion of interior eager release sites and enhancing DCR synchronicity in both isolated atrial cells and atrial tissue preparations.
Conclusions. Our results suggest that aberrant DCR involves a certain set of eager Ca release sites that 1) only in part coincide with sites involved in normal EC coupling at cell periphery and 2) act as "pacemakers" for spontaneous Ca oscillations present in atrial cells. Due to abbreviated release site refractoriness, DCR is synchronized between neighboring cells thereby leading to tissue-wide Ca oscillations that may form basis for AF. SERCA2a overexpression exacerbates arrhythmic Ca oscillations by further enhancing DCR synchronicity.

2182-Plat
Hyperphosphorylation of RyRs Underlies Triggered Activity in Transgenic Rabbit Model of LQT2 Syndrome
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Loss-of function mutations in HERG potassium channels are associated with ventricular tachycardia and sudden cardiac death caused by stress. We aimed to investigate changes in Ca homeostasis in cardiomyocytes derived from LQT2 hearts and to determine whether these changes contribute to