(1Hz, room temperature) into nucleoplasmic and cytoplasmic [Ca^{2+}]. There was a significant difference in diastolic (121 ± 24nM *vs* 149 ± 35nM; 99 ± 17nM *vs* 121 ± 26nM) and systolic (420 ± 148nM *vs* 364 ± 102nM; 787 ± 172nM *vs* 491 ± 157nM) [Ca^{2+}] between cytoplasmic and nucleoplasmic compartments in mouse and rat cells, respectively (both n=15; P<0.01).
The results reveal that, in cardiac myocytes, the Ca^{2+}-dependent fluorescent properties of Fluo-4 differ between cytoplasm and nucleoplasm and that significant differences between cytoplasmic and nucleoplasmic [Ca^{2+}] exist during diastole as well as systole.

1543-Pos
Control of Ca Release Synchrony by Action Potential Configuration in Murine Cardiomyocytes
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Spatially non-uniform or ''dyssynchronous'' sarcoplasmic reticulum (SR) Ca release has been reported in cardiomyocytes from failing hearts. Using a murine model of congestive heart failure (CHF) following myocardial infarction, we investigated whether altered action potential (AP) configuration promotes release dyssynchrony. We observed that APs (1 Hz) were prolonged in cardiomyocytes isolated from the viable septum of CHF hearts, compared to and sham-operated controls (SHAM). Representative AP recordings were included in a detailed computational model of the Ca dynamics in the dyad. The model predicted reduced driving force for L-type Ca current and more dyssynchronous opening of ryanodine receptors during stimulation with the CHF AP than the SHAM AP. These predictions were confirmed in isolated cardiomyocyte experiments, when cells were alternately stimulated by SHAM and CHF AP voltage-clamp waveforms. However, when a train of like APs was used as the voltage stimulus, the SHAM and CHF AP produced a similar Ca release pattern. In this steady-state condition, both modeling and cell experiments showed that greater integrated Ca entry during the CHF AP lead to increased SR Ca content. We modeled the effect of increased SR Ca content by increasing the Ca sensitivity of the ryanodine receptor, which we observed increased the synchrony of ryanodine receptor activation. Thus, at steady-state, Ca release synchrony was maintained during the CHF AP as greater ryanodine sensitivity offset the de-synchronizing effects of reduced driving force for Ca entry. Our results suggest that dyssynchronous Ca release in failing mouse myocytes results from alterations such as T-tubule re-organization, and not electrical remodeling.

1544-Pos
Imaging of the Ryanodine Receptor Distribution in Rat Cardiac Myocytes with Optical Single Channel Resolution
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We have applied a new optical super-resolution technique based on single molecule localisation to examine the peripheral distribution of a cardiac signalling protein, the ryanodine receptor (RyR), in rat ventricular myocytes. Using high-resolution antibody labeling data we show that the new imaging approach, termed localization microscopy, can give novel insight into the distribution of large proteins, with optical single channel resolution. We present, to our knowledge, the first direct data showing evidence for a two-dimensional array-like arrangement of RyRs in cardiac muscle. Morphological analysis of peripheral RyR clusters in the surface membrane revealed a mean size of ~14 RyRs per cluster, almost an order of magnitude smaller than previously estimated. Clusters were typically not circular (as previously assumed) but elongated with an average aspect ratio of 1.9. Edge-to-edge distances between adjacent RyR clusters were often less than 50 nm suggesting that peripheral RyR clusters may exhibit strong inter-cluster signalling. The cluster size varied widely and followed a near-exponential distribution. We show that this distribution is compatible with a stochastic cluster assembly process and construct simple cluster growth models that generate size distributions very similar to our experimental observations. Based on the placement and morphology of RyR clusters we suggest that calcium sparks may be the result of the concerted activation of several clusters forming a functional 'supercluster' whose gating is controlled by both cytosolic and sarcoplasmic reticulum luminal calcium levels. The new imaging approach can be extended to other cardiac proteins and should yield novel insight into excitation-contraction coupling and the control of cardiac contractility.

1545-Pos
Synchronization of Spontaneous Stochastic RyR Activation in Ventricular Myocytes (VM) by Camp or Disengagement of Phospholambam (PLB) From SERCA2
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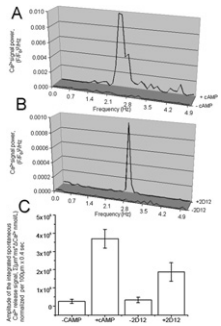
Stochastic RyR activation underlies Ca^{2+} sparks in VM. Here we show that in saponin ''skinned'' VM, bathed in 100 nM Ca^{2+} at 35°C, cAMP converts this stochastic spontaneous RyR activation (Ca^{2+} sparks-confocal linescan imaging) into synchronized, rhythmic RyR activation (Fig. A) about an average dominant frequency 2.2 ± 0.13 Hz (n=3). Of note, cAMP does not alter the SR Ca^{2+} load assessed by the rapid application of caffeine (107 ± 17.3 nM Ca^{2+} n=9 prior and 121 ± 19.3 nM Ca^{2+} n=3 during cAMP exposure). When Ca^{2+} pumping into SR is selectively accelerated by a PLB antibody (2D12, 0.013 mg/ml) that disengages PLB from SERCA2, stochastic RyR Ca^{2+} release becomes rhythmic (Fig. B) about an average dominant frequency of 2.6 + 0.21 Hz (n=5). The amplitude of the integrated spontaneous Ca^{2+} release signal during any given epoch increases when stochastic RyR activation becomes synchronized, i.e. converted to rhythmic activation (Fig. C). This cAMP-generated rhythmicity of spontaneous RyR activation of VM mimics rhythmic spontaneous diastolic Ca^{2+} releases in sinoatrial nodal pacemaker cells which have a basal high level of intrinsic cAMP-dependent signaling.



1546-Pos
Subcellular Mechanisms of Early Impaired Calcium Homeostasis with Chronic Beta$_1$-Adrenergic Stimulation in Mice
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Chronic beta-adrenergic stimulation leads to heart failure (HF). In mice overexpressing beta1-adrenoceptors (TG), increased diastolic Ca load in cardiomyocytes at early age is pivotal for the development of HF. The mechanisms underlying intracellular Ca dysregulation are unclear. We examined cytosolic Ca transients (Fluo4-AM, field stimulation), Na-Ca-exchanger (NCX) function and protein expression, cytosolic Na (SBFI) and T-tubular structures (Di8-ANEPPS) in cardiomyocytes from young (8-16 wks) TG mice and wildtype (WT) littermates.
Results: Systolic [Ca] amplitude was unchanged, time to peak [Ca] (140 ± 5 vs. 127 ± 3 ms) and [Ca] decay (time constant, tau, 223 ± 16 vs. 182 ± 9 ms) were significantly prolonged in TG vs. WT. Diastolic Ca leak from the SR (quantified as tetracaine-sensitive change in diastolic [Ca] or diastolic Ca spark frequency) was unchanged. However, cytosolic Ca removal by NCX during coffein application was significantly slower (tau, 3683 ± 337 in TG vs. 2304 ± 272 ms in WT), indicating reduced forward mode NCX activity. NCX protein expression was unchanged. Preliminary results indicate increased cytosolic [Na] in young TG. Furthermore, confocal line scans revealed delayed (> 15 ms until half-maximal) systolic Ca release in 24.7 ± 2.6 (TG) vs. 4.6 ± 1.4% (WT) of the intracellular regions (n=32 and 31 cells, resp., p<0.01). The extent of dyssynchronous Ca release correlated with time to peak systolic [Ca] (R=0.51, P<0.001) and was associated with a lower density and increased irregularity of T-tubules in TG (22.8 ± 1.6% of cell volume in TG vs. 26.1 ± 2.5% in WT). In summary, in early HF remodeling with chronic beta1-adrenergic stimulation, slowed cytosolic Ca clearance isnot related to increased diastolic SR Ca leak but associated with decreased NCX forward mode activity, which may be related to increased cytosolic [Na]. Reduced T-tubule density with dyssynchronous, slowed systolic Ca release additionally contribute to increased cytosolic Ca load.

1547-Pos
Rational Design and Structrual Analysis of Ca^{2+} Biosensor and Application in Skeletal Muscle Cells
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Quantitative and real-time detection of Ca^{2+} signaling in internal Ca^{2+} store sarcoplasmic reticulum (SR) of skeletal muscle cells is essential to explore