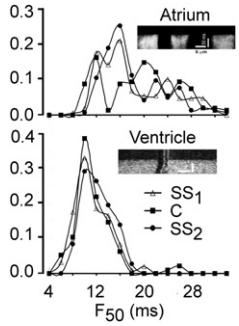
(2 ms/line) across multiple myocytes obtained during sinus rhythm from fluo-4 loaded hearts revealed homogeneous $[Ca^{2+}]_i$ increases in ventricular myocytes, whereas atrial myocytes exhibited areas with delayed transients (see Figure). Histograms of F_{50}values (the time to 50% of peak F/F_0 [where F indicates fluorescence intensity, and F_0 indicates F at rest]) for the subsarcolemmal (SS) and central (C) compartments in ventricular myocytes were largely congruent, whereas the corresponding atrial histograms did not superimpose and exhibited multiple peaks. Thus, major myocyte-to-myocyte differences in the spatial organization of SR Ca^{2+} release exist among *in situ* mouse atrial myocytes, likely reflecting non-uniform t-tubule distribution.



541-Pos

Refractoriness of Ryanodine Receptors During Calcium Alternans in Rabbit Atrial Myocytes

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Electro-mechanical and Ca alternans is a known pro-arrhythmic factor. At the cellular level Ca alternans appears as cytosolic Ca transients of alternating amplitude at regular beating frequency. Direct intra-sarcoplasmic reticulum (SR) [Ca] measurements with the low affinity Ca indicator fluo-5N entrapped in the SR revealed that alternans in diastolic SR content are not a prerequisite for cytosolic Ca alternans, and thus SR Ca content is not the sole determinant of alternans. The goal of this study was to determine whether alternans of the kinetics of recovery from inactivation of ryanodine receptors and refractoriness of release represent a key factor underlying cytosolic Ca alternans. Alternans was induced by electrical pacing (1.6 to 2.5 Hz). After Ca alternans was established, pacing was stopped and the occurrence of spontaneous Ca waves and Ca sparks was quantified. The time interval from cessation of stimulation to the appearance of the first Ca waves was significantly shorter and the frequency of Ca sparks was higher after the small Ca transient compared to the large transient. Application of 0.1 mM caffeine or 10 μM isoproterenol rescued Ca alternans and shortened the rest interval until appearance of Ca waves. Photolysis of caged Ca (DM-nitrophen) to produce photolytically triggered Ca release (PTCR) from the SR was used to probe the refractoriness of SR Ca release during alternans. During the decay phase of the Ca transient PTCR was significantly less during the large Ca transient. During the rising phase of the Ca transient PTCR was greater during the large Ca transient, and was capable of inducing a phase reversal of Ca alternans. We conclude that alternating ryanodine receptor inactivation recovery intervals, together with alternations in SR Ca load, represent key determinants of Ca alternans. (VMS and CL contributed equally).

542-Pos

A Novel Quantitative Explanation of G Protein-Coupled Receptor Modulation of Sinoatrial Cell Automaticity Via Interactions of Ca Clock and Membrane Voltage Clock

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Classical numerical models attribute regulation of normal cardiac automaticity largely to G protein-coupled receptor (GPCR) modulation of sarcolemmal ion currents (membrane clock), in sinoatrial node cells (SANC). While experimental evidence indicates that GPCR modulation of SANC automaticity involves spontaneous rhythmic, Local Ca^{2+} Rreleases (LCRs) (Ca^{2+}clock) from the sarcoplasmic reticulum (SR), the autonomic modulation of a coupled system of $Ca^{2+}$and membrane clocks has not been tested in the context of a dynamic numerical model. **Methods:** We explored the GPCR rate modulation of SANC by using a recent unique numerical model of SANC (Maltsev and Lakatta. Am J Physiol Heart Circ Physiol. 2009;296:H594-615), in which LCR characteristics are graded by the SR Ca^{2+} uptake rate (P_{up}), mimicking phospholamban function regulated by cAMP/PKA signaling. **Results**: The range of physiological chronotropic modulation of SANC by activation of β-adrenergic or cholinergic receptors is well predicted by the model only when the documented changes of ion channels are combined with a simultaneous increase/decrease in P_{up}. A novel mechanism includes changes of diastolic Na^+/Ca^{2+}exchange current (I_{NCX}) that couple earlier/later diastolic Ca^{2+}releases (predicting experimentally defined LCR period shift) of increased/decreased amplitude (predicting changes in LCR signal mass, i.e. the product of LCR spatial size, amplitude, and number/cycle) to the diastolic depolarization and ultimately to the spontaneous rate. Concomitantly, larger/smaller activation of I_{CaL}shifts cell Ca^{2+} balance to support the respective Ca^{2+} cycling changes. **Conclusion**: Our model simulations together with recent experimental results suggest a new paradigm for GPCR heart rate modulation based on the coupled function of Ca^{2+} and membrane clocks in rabbit SANC.

543-Pos

Ignition Point: A Novel Parameter of Sinoatrial Nodal Cell (SANC) Diastolic Depolarization (DD) Reports the Onset of Spontaneous Local Subsarcolemmal Ca Release (LCR) and Predicts Cycle Length

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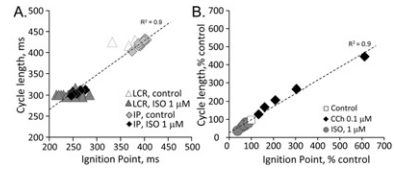
The LCR period, the time from the prior action potential-triggered Ca release to spontaneous LCR occurrence, is a major determinant of the spontaneous cycle length (CL) of SANC. Based on prediction of LCR-activated diastolic inward Na/Ca exchanger current by a recent SANC model (Maltsev&Lakatta, AJP;2009:296;H594-615), we hypothesized that the DD rate change (dVm/dt) would manifest an abrupt transition, i.e. Ignition Point (IP), when LCRs begin to occur. Simultaneous confocal Ca imaging and Vm recordings of rabbit SANC showed that at optimal filtering (60Hz), a dVm/dt threshold (0.15 V/sec) detected an IP that faithfully reported LCR period (Fig.A) both prior to and during β-adrenergic receptor stimulation (ISO). Higher thresholds (e.g. 0.5 V/s, previously used to identify a take-off potential) failed to predict both LCR period and IP. Furthermore, the IP time shifts in response to carbachol (CCh,5 cells) or ISO (7 cells) form a continuum that predicts the concomitant CL (%control of 421 ± 3ms) shifts (Fig.B). Conclusion:IP predicts both LCR period and CL, avoiding ambiguous terms e.g. early or late DD, and linear or nonlinear DD.



544-Pos

Canine Purkinje Cells Exhibit Complex and Rate-Dependent Beat-To-Beat Variations in Calcium Transients

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Purkinje fibers serve a critical role in ensuring the electrical activation of the ventricles, but spontaneous Ca^{2+} release in the Purkinje system is considered a possible trigger of arrhythmias. To understand the underlying mechanisms, we explored the rate-dependence of Ca^{2+} transients in single canine Purkinje cells loaded with fluo3 and imaged with a confocal microscope at room temperature. Ca^{2+} transients were evoked by electrical field stimuli applied at rates ranging from 0.1 to 5 Hz. At slow rates, stimuli induced Ca^{2+} transients that originated at the cell periphery then spread into the cell interior as a large-amplitude propagating Ca^{2+} wave. At faster rates, Ca^{2+} transients were smaller and remained localized to the subsarcolemmal space near the periphery. The origination of Ca^{2+} transients directly under the cell membrane, with or without an accompanying Ca^{2+} wave, is consistent with the lack of transverse-tubules in Purkinje cells. In addition, during steady pacing, the amplitude of local Ca^{2+} transients showed significant and unusual beat-to-beat variability, as neither constant amplitude Ca^{2+} transients nor stable beat-to-beat alternans were observed (n = 27 cells). The degree of variability, quantified as the coefficient of variation (s.d./mean) increased as the pacing rate increased (at 1 Hz, COV = 0.25 ± 0.12; at 3.3 Hz, COV = 0.53 ± 0.21, n=6 cells). The results indicate that fast pacing increases the instability of sarcoplasmic reticulum Ca^{2+} release in Purkinje cells, even though the amplitude of Ca^{2+} release decreases. We speculate that the beat-to-beat variability results from stochastic recruitment of small populations of Ca^{2+} release channel clusters in the small volume near the cell periphery. These results provide insight into Ca^{2+} and electrical instability originating in the Purkinje system, a possible precursor of arrhythmia.

545-Pos

Gap-Junction Uncoupling Paradoxically Increase Synchronization of Spontaneous Calcium Release in the Intact Heart

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Intracellular calcium (Ca) dysregulation associated with cardiac disease has been linked to mechanisms of ventricular arrhythmias. We have previously shown that spontaneous calcium release from an aggregate of many cells in

situ (an SCR) occurs in a synchronized fashion; however, the mechanism responsible for synchronizing SCR activity in coupled myocardium is unknown. Since others have reported that Ca can diffuse through gap junctions (GJ), we hypothesize that uncoupling cells by blocking GJ will desynchronize, and attenuate SCR activity. Methods: To test this hypothesis high resolution optical mapping of Ca (Indo-1AM) from the anterior surface of the Langendorff perfused guinea pig heart (n=5) was performed in hearts under high Ca conditions ([Ca2+]e=5.5mM), with and without carbonoxolone (CBX, 50μM) to reduce GJ coupling. Endocardial cryoablation were performed to eliminate Purkinje fibers and cytochalasin-D (7μM) was administered to remove motion artifact. Fifteen seconds of rapid pacing (350-160 ms cycle length) followed by a pause was used to induce SCR activity. Results: In all preparations, SCR activity was observed across the entire mapping field before and after CBX. With CBX, the amplitude of SCR activity increased (+14.8%, $p < 0.05$) and its time to peak occurred earlier (−11.2%, $p < 0.01$) compared to no CBX. CBX also decreased the range of local SCR time to peaks across the mapping field (−17.2%, $p < 0.05$), suggesting that uncoupling myocytes synchronizes spontaneous calcium release across cells. There was no statistical difference in the occurrence of triggered activity before and during CBX. Conclusions: These results demonstrate that the occurrence of spontaneous calcium release in tissue (an SCR) does not require Ca diffusion though GJs. In fact, spontaneous calcium release in tissue is paradoxically enhanced during GJ inhibition.

546-Pos

Synchronization of Spontaneous Calcium Release Waves Among Myocytes in Intact Heart Determines the Magnitude of Delayed Afterdepolarizations and Triggered Activity

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Intracellular Ca waves occur as the result of spontaneous Ca release (SCR) during Ca overload. Ca waves activate Na-Ca exchange, causing delayed afterdepolarizations (DAD) which can achieve threshold and produce triggered extrasystoles. It is not known how these single cell events depolarize enough myocytes in intact heart to produce a triggered beat. We combined experimental observations with computer simulations to explain how SCR synchronization among myocytes brings a critical tissue mass to threshold. Confocal microscopy was used to measure SCR waves in groups of myocytes in the LV epicardium of rat hearts loaded with fluo-4AM. Contraction was abolished with cytochalasin-D and blebbistatin. Raising extracellular [Ca] and rapid pacing protocols were used to increase sarcoplasmic reticulum (SR) Ca load and induce Ca waves. As Ca load increased, the number of myocytes giving waves increased. Both the wave latency and the variability (SD) of wave latency decreased with increasing Ca load. Similar results were obtained in isolated rat ventricular myocytes, indicating that the reduction in latency interval and variability represent intrinsic properties of SR release in Ca overload. Computer simulations demonstrate that decreasing wave latency and variability determine the rate and magnitude of increased cytoplasmic [Ca] and therefore determine the timing and magnitude of the DAD. The synchrony of SCR waves among myocytes therefore determines the likelihood of achieving threshold and producing a triggered beat. These results demonstrate that intrinsic properties of SR Ca release are responsible for Ca wave synchronization during Ca overload, causing DADs to reach threshold and produce triggered arrhythmias.

547-Pos

Looking at the Trigger for CICR During Rat Cardiac Action Potentials

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It is generally accepted that cardiac CICR is triggered by L-type Ca2+ channel activation during the action potential. At the microscopic level, CICR is revealed by calcium sparks whose activation by single L-type Ca2+ channels has been demonstrated. In this study, we have tried to visualize the Ca2+ influx (that triggers CICR) during normal action potentials and when the L-type Ca2+ channel is partially blocked and/or during the application of an L-type Ca2+ channel gating modifier FPL64176. Using Fluo-4 and high speed confocal line scanning we have detected the rise in Ca2+ that precedes SR Ca2+ release. When ~90% of L-type Ca2+ channels are blocked with 10 uM nifedipine, L-type Ca2+ influx is seen as an increase in fluorescence of ~2 %/ms, which is 8% of the rate of rise of Ca2+ associated with Ca2+ sparks. This is associated with a latency for Ca2+ spark activation of typically 9 ms; assuming a Kd for fluo-4 of 800 and a resting Ca2+ of 65nM the trigger in these conditions equates to a current of ~1 nA for a 30 pL cell. An unexpected finding was that FPL64176 did not dramatically decrease the latency for Ca2+ spark activation, as might be expected if many short L-type Ca2+ channel openings are required to activate CICR. In addition, no 'sparklets' were observed as might be expected if L-type Ca2+ channels are located almost exclusively in the junctional space and Ca2+ sparks activate with minimal delay.

548-Pos

Modeling the Effects of Genetic Manipulations of Calsequestrin on Local Calcium Release and Depletion in Cardiac Myocytes

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Cardiac calsequestrin (CASQ2), a Ca buffer localized to the junctional SR (jSR) of cardiac myocytes, is known to bind to the RyR-triadin-junctin complex, participate in the luminal regulation of RyRs, and modulate Ca spark activity. To investigate the functional role of CASQ2 during spontaneous Ca sparks, we constructed a hybrid CTMC-ODE stochastic simulation of a Ca release site model composed of 100 Lee-Keener RyRs [J. Theor. Biol. 253:668-679, 2008] that includes Ca activation, Ca inactivation, CASQ2-RyR binding, and the dynamics of myoplasmic and luminal domain Ca and buffer concentrations. Myoplasmic and network SR [Ca] were determined by balancing the simulated average release flux and reuptake mediated by SERCA. The model reproduces average properties of spontaneous sparks in normal myocytes including spark amplitude, blink nadir, and junctional SR recovery time. Parameter studies were performed to interpret the effects of known arrhythmogenic CASQ2 mutants [Terentyev et al., Biophys. J. 95(4):2037-2048, 2008] on average spark properties. Increases in the total amount of CASQ2 resulted in increased spark amplitudes and increased jSR recovery times (observed in myocytes overexpressing wildtype CASQ2); shallower nadirs were observed in simulations but not experiment. Increasing the Kd of CASQ2 and Ca binding decreases spark amplitude and jSR recovery time (observed in myocytes expressing the dominant negative mutation CASQ2-DEL that suppresses CASQ2-Ca binding); in simulations (but not experiment) release-reuptake balance leads to increased network SR [Ca]. Increasing the Kd of CASQ2 and RyR binding is associated with decreased network SR [Ca], as in myocytes expressing CASQ2-R33Q, a variant with dominant negative effects on interactions of CASQ with RyRs. The implications of these results to mechanism of release regulation by CASQ2 will be discussed.

549-Pos

Loss of Calsequestrin (Casq2) in the Heart Increases Spark Frequency and Alters Spark Properties

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Lack of Casq2 causes spontaneous Ca2+ releases from the sarcoplasmic reticulum (SR) and catecholaminergic-polymorphic ventricular tachycardia. We tested the hypothesis that lack of Casq2 alters elementary Ca2+ release events (Ca2+ sparks) by comparing spark properties of ventricular myocytes isolated from wild-type (WT) and Casq2 null (Casq2-/-) mice. Sparks were recorded in line-scan mode and analyzed with SparkMaster. Spark mass was calculated as amplitude×1.206×FWHM3. SR Ca2+ load was measured by rapid application of caffeine. In intact Casq2-/- myocytes stimulated with 100nM isoproterenol, spark amplitude and spark width (FWHM) increased compared to WT (0.77 ± 0.019 vs. 0.39 ± 0.02 Δ(F/Fo), and 2.4 ± 0.03 vs. 1.3 ± 0.05μm, 1095 and 105 sparks respectively), resulting in larger spark mass (20 ± 1.4 vs. 2.2 ± 0.4 Δ(F/Fo).μm3). Time-to-peak and spark duration (FDHM) were 2.5-fold longer and spark frequency was 4-fold higher in Casq2-/- myocytes (2.4 ± 0.2 vs. 0.5 ± 0.08 sparks×100μm-1×s-1). Spark-mediated leak (spark mass×spark frequency) was much larger (47.6 vs. 1.2 Δ(F/Fo) μm3). In saponin-permeabilized myocytes, spark-mediated leak and spark frequency were also higher in Casq2-/- myocytes (249.2 vs. 171.3 Δ(F/Fo)μm3, and 9.5 ± 1 vs. 4.2 ± 1 sparks×100μm-1×s-1, 587 and 333 sparks for Casq-/- and WT respectively), but the differences between Casq-/- and WT were less pronounced compared to intact myocytes. This may be a consequence of the increased spark-mediated SR Ca2+ leak resulting in significantly decreased SR Ca2+ load in permeabilized Casq2-/- cells (−25% at baseline and −31% with 50μM cAMP, n= 10-16 myocytes per group).
Conclusions: Lack of Casq2 in cardiac myocytes increases the spark frequency and the spark-mediated leak. This is still observed in permeabilized cells despite decreased SR Ca2+ load, suggesting that this is due to a primary Casq2