parenchyma. To assess the impact of SAH on global cytosolic Ca^{2+} and arteriolar diameter, intact freshly-isolated parenchymal arterioles (PAs; 30-60 μm diameter) were loaded with the ratiometric Ca^{2+} indicator fura-2. At physiological intravascular pressure (40 mmHg), PAs from SAH animals exhibited significantly elevated cytosolic Ca^{2+} (349 ± 10 nM vs. 248 ± 14 nM) and developed significantly greater (~2-fold) myogenic tone compared with PAs from sham-operated animals. The L-type voltage-dependent Ca^{2+} channel (Ca_V) blocker nimodipine caused ~90% reduction in Ca^{2+} and tone in PAs from both groups suggesting elevated PA Ca^{2+} following SAH results from enhanced L-type Ca_V activity. Increased Ca_V activity may reflect Ca_V upregulation or membrane potential (V_M) depolarization of arteriolar smooth muscle. When V_M was clamped at K^+ equilibrium potential using elevated extracellular K^+ (60 mM; −22 mV), Ca^{2+} and tone were similar between groups, suggesting surface Ca_V density is unchanged by SAH. To examine whether suppression of voltage-dependent K^+ channel (K_V) activity contributes to V_M depolarization, outward K^+ currents were measured in isolated PA myocytes using conventional whole cell patch clamp electrophysiology. Currents sensitive to 4-aminopyridine, a K_V channel blocker, were reduced by ~70% (at +40 mV) in myocytes from SAH animals compared with controls. Taken together, our results suggest decreased K_V channel activity causes V_M depolarization, increased Ca_V activity, elevated cytosolic Ca^{2+} and enhanced constriction of PAs following SAH. Impaired regulation of PA diameter may contribute to local ischemia and neurological deterioration following SAH.

Calcium Fluxes, Sparks & Waves I

536-Pos

Nitric Oxide Can Mediate Beta-Adrenergic- and CaMKII-Dependent Spontaneous Ca^{2+} Waves in Cardiac Myocytes, Independent of PKA Activation

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Increased diastolic SR Ca leak can initiate spontaneous Ca waves (SCaWs). SCaWs activate inward Na/Ca exchanger current causing an arrhythmogenic delayed afterdepolarization. Here we examine SCaWs in ventricular myocytes isolated from rabbit hearts. Myocytes did not exhibit SCaWs at baseline conditions, but 43% did when exposed to isoproterenol (ISO). This ISO-induced increase in activity was reversed by inhibition of CaMKII by KN93, but not with PKA inhibition by H89. At similar $[Ca]_{SRT}$ (121 μM) myocytes treated with ISO plus KN93 had significantly fewer SCaWs versus those treated with ISO or ISO plus H89 (0.2 ± 0.28 vs. 1.1 ± 0.28 & 1.29 ± 0.39 SCaWs $cell^{-1}$, respectively). We attribute this increase in activity to the previously characterized CaMKII-dependent increase in RyR-dependent leak. We also find that SR Ca leak is increased by the nitric oxide (NO) donor, SNAP; and this NO-dependent effect is also completely reversed by KN-93. We also show the increase in leak to be dependent on nitric oxide synthase 1 (NOS1) activity. At comparable SR Ca load (132 μM) ISO treated myocytes have significantly higher leak vs. control (8.4 vs. 3.8 μM). The ISO-induced leak (at constant SR Ca load) was attenuated by the NOS1 inhibitor, SMLT, but not the NOS3 inhibitor, L-NIO (3.5 vs. 6.8 μM). Moreover, ISO causes an upward trend in myocyte [NO] (sensed by the NO-dependent dye, DAF-2 A), and the NOS inhibitor, L-NAME significantly attenuated the development of SCaWs. Together this data suggests a novel pathway in which β1-adrenergic receptor activation stimulates NO production via NOS1, which in turn activates CaMKII to increase RyR gating, SR Ca leak, SCaWs and delayed afterdepolarizations.

537-Pos

On the "spark Frequency Vs. Leak Rate" Relationship in Ventricular Myocytes: A Study in the Rabbit

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Recent studies have linked heart disease to mutated or hyperactive ryanodine receptors (RyRs) in ventricular myocytes, renewing the interest on how the sarcoplasmic reticulum (SR) Ca release process works during diastole. We addressed two questions of potential clinical relevance: (1) whether the SR Ca leak rate (J_{leak}) can be entirely explained by Ca sparks, and (2) whether the spark-dependent fraction of J_{leak} can be varied upon RyR phosphorylation. We used confocal microscopy to simultaneously measure J_{leak} and Ca sparks in Fluo 4-loaded rabbit ventricular myocytes. Control cells (C; n = 47) were paced at 0.5 Hz, while isoproterenol-treated cells (I; n = 14, [Isoproterenol] = 125 nM) were paced at 0.25 Hz to match the SR loads (C = 136.4 ± 5.6 μmoles/l cytosol; I = 126 ± 9.2 ; P = 0.47 in *t*-test with Welch correction). J_{leak} was quantified as in Shannon et al. (2002; *Circ Res* 91:594-600), but using a lower K_m for the forward rate of uptake in the I group. Although J_{leak} did not significantly differ among the groups (C = 10.87 ± 0.93 μM/s; I = 13.12 ± 2.53 ; P = 0.42), the spark frequency was more than doubled in the isoproterenol-treated cells (C = 1.21 ± 0.15 sparks * $(100\ \mu m)^{-1}$ * s^{-1}); I = 2.82 ± 0.51; P = 0.0082). These findings point to an increase in the spark-dependent fraction of J_{leak} upon RyR phosphorylation (for a given SR load), while suggesting an enhancement of Ca-induced RyR coupling relative to the influence of stabilizing RyR couplers.

538-Pos

Regulation of Sarcoplasmic Reticulum Calcium Leak by Cytosolic Calcium in Rabbit Ventricular Myocytes

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Diastolic sarcoplasmic reticulum (SR) Ca leak determines Ca content and, therefore, the amplitude of action potential-induced global Ca transients in ventricular myocytes. However, the pathways and properties of SR Ca leak have been poorly described. Here, we studied the effects of cytosolic [Ca] ($[Ca]_i$) on SR Ca leak in permeabilized rabbit ventricular myocytes. Using confocal microscopy we simultaneously measured intra-SR free Ca ($[Ca]_{SR}$) with fluo-5N and cytosolic Ca sparks with rhod-2, and monitored SR Ca leak as the change in $[Ca]_{SR}$ over time after complete SERCA inhibition with thapsigargin (10 μM). Increasing $[Ca]_i$ from 150 to 250 nM significantly increased SR Ca leak (by ~30%) over the entire range of $[Ca]_{SR}$. This increase in SR Ca leak associated with an increase in Ca spark frequency. Further increasing $[Ca]_i$ to 350 nM led to rapid $[Ca]_{SR}$ depletion due to the occurrence of spontaneous Ca waves. In contrast, lowering $[Ca]_i$ to 50 nM markedly decreased SR Ca leak rate (by ~60%) and nearly abolished Ca spark activity. When the ryanodine receptor (RyR) was completely inhibited with ruthenium red (50 μM), changes in $[Ca]_i$ between 50 and 350 nM did not produce any significant effect on SR Ca leak, showing that changes of $[Ca]_i$ over a physiological range alter SR Ca leak solely by regulating RyR activity. However, decreasing $[Ca]_i$ to a lower, non-physiologically level (5 nM) activated additional SR Ca leak pathway(s) that were insensitive to RyR or SR Ca ATPase inhibition. In summary, $[Ca]_i$ plays an important role in regulating SR Ca leak by activating RyR and preventing Ca leak through unspecified pathways.

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Dynamic Changes of Calcium in Sarcoplasmic Reticulum of Rabbit Atrial Myocytes

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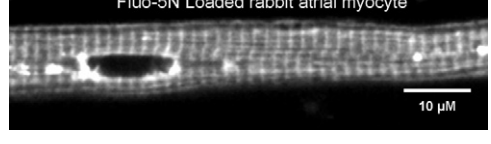
With each contraction in atrial muscle, Ca^{2+} is released from the sarcoplasmic reticulum (SR) through ryanodine receptors and then reaccumulated by the activity of the SR Ca^{2+} ATPase (SERCA). Recent experiments by us and others have examined SR Ca^{2+} concentration ($[Ca^{2+}]_{SR}$) using low affinity fluorescent Ca^{2+} sensors (e.g. fluo-5N) in ventricular myocytes (Brochet et al. PNAS 2005 102(8) 3099-104) however, similar characterization in atrial cells is missing. Here we examine $[Ca^{2+}]_{SR}$ dynamics in healthy adult rabbit atrial myocytes using fluo-5N. Fractional SR Ca^{2+}release at room temperature was 30% (n=12), where caffeine was used to determine 100% release. Restoration of $[Ca^{2+}]_{SR}$ by SERCA was consistent with the decline of $[Ca^{2+}]_i$ with each beat (1 Hz). The interconnectivity of the cell-wide SR and nuclear envelope and endoplasmic reticulum was characterized using fluorescence recovery after photobleaching (FRAP). Additional diverse modulations of atrial cell $[Ca^{2+}]_{SR}$ will be discussed.



540-Pos

Spatiotemporal Profiles of Sarcoplasmic Reticulum Ca^{2+} Release in Mouse Atrial Cardiomyoctes *in situ*

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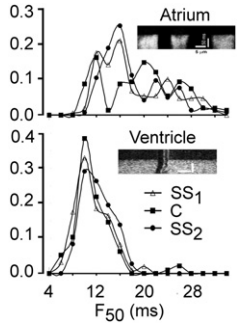
Previous studies in atrial cardiomyocytes isolated from various mammalian species have demonstrated poorly developed transverse(t)-tubules and inhomogeneities of sarcoplasmic reticulum (SR) Ca^{2+} release, but little is known of mice. Here, we examined the t-tubular organization and action potential-evoked Ca^{2+} release in *in situ* myocytes, using confocal microscopy of Langendorff-perfused hearts. Imaging of ANNINE-6plus-stained sarcolemmal membranes revealed paucity of t-tubules in atrial, but densely and regularly spaced t-tubules in ventricular myocytes. However, both myocyte types exhibited regular, striated appearance of type 2 ryanodine receptor distribution. Line-scans

(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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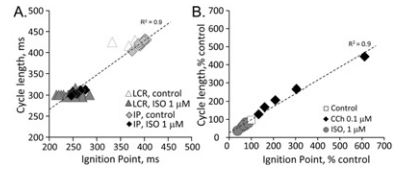
The LCR period, the time from the prior action potential-triggered Ca release to spontaneous LCR occurrence, is a major determinant of the spontaneous cycle length (CL) of SANC. Based on prediction of LCR-activated diastolic inward Na/Ca exchanger current by a recent SANC model (Maltsev&Lakatta, AJP;2009:296;H594-615), 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