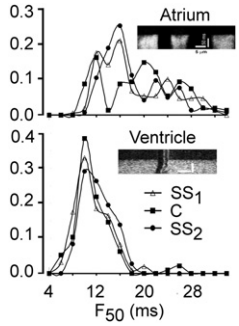
(2 ms/line) across multiple myocytes obtained during sinus rhythm from fluo-4 loaded hearts revealed homogeneous $[Ca^{2+}]_i$ increases in ventricular myocytes, whereas atrial myocytes exhibited areas with delayed transients (see Figure). Histograms of F_{50}values (the time to 50% of peak F/F_0 [where 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.

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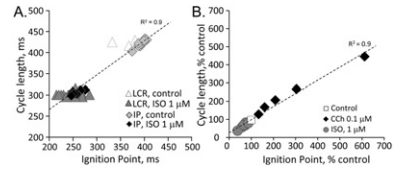
The LCR period, the time from the prior action potential-triggered Ca release to spontaneous LCR occurrence, is a major determinant of the spontaneous cycle length (CL) of SANC. Based on prediction of LCR-activated diastolic inward Na/Ca exchanger current by a recent SANC model (Maltsev&Lakatta, AJP;2009:296;H594-615),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