myocytes in comparison to their ventricular counterpart. Furthermore, our laboratory recently found that in atrial cells that underwent rapid atrial pacing (RAP) to simulate pathological effects of atrial fibrillation, the buffering strength was increased. This resulted in smaller central CaT amplitudes and comparable sub-sarcolemmal CaT amplitudes with respect to control cells. In this project we seek to test the effect of buffering in the LCC and its role in the CaT spatiotemporal profile. To elucidate these effects, we performed patch clamp experiments to investigate the differences in the LCC function under different buffering conditions. We incorporated these findings in a minimized spatial computational model to evaluate the impact of buffering and TTs on intracellular Ca^{2+} signals. This ongoing work seeks to investigate the mechanisms that lead to different spatiotemporal profiles of the CaT and how these differences affect atrial cell function. This work should broaden our understanding of atrial cell function in health and disease.

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Ventricular Wall Stress Predicts Disruption of Cardiomyocyte T-Tubule Structure and Ca^{2+} Homeostasis across the Infarcted Heart

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Invaginations of the cellular membrane called t-tubules are essential for maintaining efficient excitation-contraction coupling in ventricular cardiomyocytes. Disruption of t-tubule structure during heart failure promotes dyssynchronous, slowed Ca^{2+} release and reduced power of the heartbeat. While the triggers of t-tubule disruption remain poorly understood, recent evidence suggests that loss of junctophilin-2, a t-tubule anchor, may be critically involved. We presently investigated whether ventricular wall stress following myocardial infarction predicts loss of junctophilin-2, t-tubular structure, and disruption of Ca^{2+} homeostasis. Phase contrast MRI and blood pressure measurements were employed to examine regional ventricular wall stress in the left ventricle of failing, post-infarction, rat hearts in comparison with sham-operated controls. Markedly elevated diastolic blood pressure resulted in increased wall stress in failing hearts, particularly in regions proximal to the infarct where the ventricular wall was thinned (Wall stress $= 61.8 \pm 6.4 kN/m^2$ proximal zone vs $31.6 \pm 4.0 kN/m^2$ sham). Wall stress measurements across failing hearts correlated strongly with junctophilin-2 expression, which was reduced in the proximal but not distal zones. A corresponding disruption of t-tubule organization and density was observed in the proximal zone (t-tubule fraction $= 18.9 \pm 0.4\%$ sham vs $17.1 \pm 0.6\%$ proximal zone), and Ca^{2+} transients were significantly desynchronized and slowed. In agreement with local disruption of cardiomyocyte structure and Ca^{2+} homeostasis, in vivo strain was dramatically reduced in the proximal zone, with near isometric contraction observed. Strain measurements in the distal zone of infarcted hearts were unaltered from equivalent regions in sham. Thus, regional in vivo wall stress effectively predicts cardiomyocyte-level disruption of structure and function in the post-infarction failing heart, which in turn promotes reduced in vivo contractile function. These findings are consistent with a key role of mechanotransduction in signaling cardiomyocyte structural degradation during heart failure.

533-Pos Board B313

Physical Coupling between SERCA2 and PDE3A Regulates SERCA2 Activity in Cardiomyocytes

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Rationale: SERCA2 controls cardiac contractility, and its activity is negatively regulated by the cAMP phosphodiesterase PDE3A through an unknown mechanism. Recent clinical trials have shown upregulation of SERCA2 gene therapy as beneficial in heart failure.
Objective: To examine whether PDE3A is physically associated with SERCA, and to further evaluate whether this protein interaction represent a novel drug target to increase SERCA2 activity.
Methods and results: PDE3 inhibition increased Ca^{2+} transients, SR Ca^{2+} load and SERCA2 activity without altering global cytosolic cAMP levels in field stimulated cardiomyocytes. SERCA2 activity was increased by PDE3 inhibition in cardiomyocytes dialyzed with 5 μmol/l cAMP by patch pipettes. Active PDE3A co-purified and precipitated with SERCA2 from left ventricular myocardium, and proximity ligation assay demonstrated co-localization of PDE3A and SERCA2 in intact cardiomyocytes. A combination of immunoprecipitation and peptide interaction experiments revealed interaction between specific cytosolic regions between amino acids 277 and 493 in PDE3A and amino acid 169 to 216 in SERCA2. By whole cell voltage clamp of intact cardiomyocytes, increased SERCA2 activity was induced by dialysis with disruptor peptides of the SERCA2-PDE3A interaction. TAT-labeled PDE3A-SERCA2 disruptor peptide fragments were further able to increase SERCA2 activity in field stimulated cardiomyocytes. PDE3A-SERCA2 disruptor peptides were able to increase SERCA2 activity in cardiomyocytes in presence of either PKI or Rp-cAMP and without concomitant phospholamban phosphorylation.
Conclusion: PDE3A is physically associated to SERCA2, and this direct interaction regulates SERCA2 activity in cardiomyocytes possibly by direct regulation of SERCA2. Cell permeable disruptor peptides of the PDE3A-SERCA2 protein-protein interaction is able to increase SERCA2 activity and may potentially offer a new therapeutic approach in chronic heart disease.

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Purified IgGs from Type 2 Diabetes with Atrial Fibrillation Induce Intracellular Calcium Release in Cardiomyocytes through IP3 Pathway

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Higher risk of developing life threatening cardiac arrhythmias, such as atrial fibrillation (AF) has been found in type 2 diabetes mellitus (T2DM), but the mechanism underlying the association is largely unknown. Our previous studies showed that circulating IgGs from certain advanced T2DM patients displayed anti-endothelial effects and often induced intracellular Ca release in endothelial cells. The present study is to test the hypothesis that circulating IgGs from T2DM patients with AF perturb intracellular Ca homeostasis in atrial cardiomyocytes which in turn trigger AF. Protein A-purified IgGs were obtained from a cohort of T2DM subjects with AF and subjects without AF or other cardiovascular complications. The data show that IgG (1 μg/mL) from T2DM AF patients caused acute intracellular Ca release in cultured mouse atrial cardiomyocytes HL-1 cells compared to control groups ($p < 0.001$). The IgG-mediated Ca release was insensitive to verapamil (20 μM), mibefradil (25 μM) or BTP-2 (5 μM), indicating that the Ca release is not through voltage-gated Ca channels or store-operated Ca entry. On the other hand, application of Xestospongin C (10 μM) or 2-APB (100 μM), two membrane-permeable IP3 receptor antagonists, or knockdown of IP3 receptor, significantly decreased Ca release stimulated by the IgGs from T2DM AF patients ($p < 0.01$). Taken together, our data suggest that circulating IgGs alter intracellular Ca homeostasis in cardiomyocytes through IP3 pathway in T2DM AF patients. Our studies may provide the mechanistic understanding on a causal role of circulating IgGs in T2DM patients with AF and other symptomatic cardiac conduction diseases. The IgGs may serve as a biomarker for identifying a subset of T2DM patients with increased risk for development of AF.

535-Pos Board B315

Stretch Activated Channel Activation can Promote or Suppress Cardiac Alternans

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Alternans has been linked to the onset of ventricular fibrillation and ventricular tachycardia, leading to life-threatening arrhythmias. Experimentally electromechanical alternans can be either concordant (Long/Short APD corresponds to a Large/Small Ca transient on alternate beats) or discordant (Long/Short APD corresponds to a Small/Large Ca transient). Our previous studies have shown that these phenomena depend on the underlying instability mechanisms (voltage driven or Ca driven) and bi-directional coupling between voltage and Ca cycling. The Large Ca transients also cause larger contractions which can activate mechanically (or stretch) activated currents (SACs). Here, we show how SAC activation can feed back to alter cardiac alternans, using a physiologically detailed rabbit AP and Ca cycling model coupled with SAC (either as Cl current or nonselective cation current). We found that (1) SAC suppresses electromechanically concordant alternans, because SAC shortens longer APD more than the shorter APD and limits the difference between successive APDs, (2) SAC enhances electromechanically discordant alternans, because SAC shortens the Short APD more than Long APD which amplifies the difference of two successive APDs. Qualitatively similar results were found for Cl-selective and non-selective cation current (and also Ca-activated K current). These results suggest that mechano-electric feedback can play an important role in development and stability of cardiac alternans.

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A Novel Role for B-Type Natriuretic Peptide and Phosphodiesterase 2A in Cardiac Sympathetic Neurons from Prehypertensive Rats

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Natriuretic peptides (NPs) play a pivotal role in the regulation of intravascular volume by modulating blood vessel tone and renal function. Elevated B-type

natriuretic peptide (BNP) is regarded as an early compensatory response to hypertension and heart failure, although the use of a recombinant BNP agonist in clinical trials has proved disappointing. However previous data shows that BNP decreases cardiac sympathetic neurotransmission by attenuating activation of neuronal calcium signaling via a cGMP-PKG pathway. Emerging evidence suggests that phosphodiesterase 2A (PDE2A) is upregulated in heart failure. Therefore we tested whether PDE2A was directly involved the efficiency of BNP modulation of Ca^{2+} handling in cardiac sympathetic neurons from pre-hypertensive spontaneously hypertensive rats (SHRs).
Cardiac stellate ganglia were enzymatically isolated and cultured. Neuronal calcium current was measured using whole cell configuration of the patch-clamp technique. [Ca^{2+}]i transient was measured by ratiometric fluorescence imaging. BNP significantly reduced the magnitude of the Ca^{2+} transients and calcium current in normotensive Wistar-Kyoto (WKY) rats, but not in SHR sympathetic neurons. PDE2 inhibitor Bay60-7550 restored the capacity of BNP to reduce [Ca^{2+}]i in the SHR. Overexpression of PDE2A using a viral vector (Ad.CMV-mCherry.PDE2A) on the sympathetic neurons abrogated the response to BNP in the WKY. This was reversed by PDE2 inhibition. Interestingly, overexpression of dnPDE2A (a catalytically-dead mutant of PDE2A) using a viral vector (Ad.CMV-mCherry.dnPDE2A) rescued the BNP inhibition of the calcium handling from the SHR.
These data demonstrate that attenuation of [Ca^{2+}]i and the neuronal calcium current by BNP is impaired in the SHR, and this may be associated with apparent over activity of PDE2A. Our results suggest that neuronal PDE2A may play a potential role as a pharmacological target to restore the efficacy of BNP to decrease sympathetic neurotransmission.

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Reduced Heart Rate in Mice Harboring an SR Luminal Ca^{2+} Sensor Mutation (E4872Q) is Linked to Abnormal Ca^{2+} Release and Pacemaker Function in Isolated Cardiocytes Derived from the Mutant RyR Clone

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Rationale: The coupled-clock theory of cardiac pacemaker normal automaticity integrates numerous facets of pacemaker cell Ca^{2+} cycling and electrophysiology. Details of intrinsic RyR molecular mechanisms that regulate spontaneous RyR activation to generate local Ca^{2+} releases (LCRs) of the ''Ca^{2+} clock'', that drive normal automaticity, however, have not been elucidated.
Objective: We hypothesized that spontaneous RyR activation to generate LCRs of the ''Ca^{2+} clock'' will be attenuated in cells harboring mutant SR luminal Ca^{2+} sensor (E4872Q).
Methods and Results: We measured the spontaneous beating rate, action potential (AP) triggered RyR Ca^{2+} releases, characteristics of spontaneous LCRs in single ES derived cardiocytes from wild type (WT) and the RyR mutant (E4872Q) mouse embryonic stem cells (ES) with intact sarcolemma and in permeabilized cells in the absence of AP's, and the expression of SR Ca^{2+} proteins in SAN lysates. We also measured the heart rate in vivo, which was 15% lower in mutant mice vs WT mice. Compared to WT cardiocytes, cells harboring the RyR mutation had a reduced spontaneous AP firing rate and reduced spontaneous RyR Ca^{2+} release. Expression of RyR protein was reduced, and calsequestrin and calreticulin were increased in mutant vs that of WT cells.
Conclusions: Numerous luminal SR Ca^{2+} sensing mechanisms linked to regulation of spontaneous RyR activation, regulator of the spontaneous AP firing rate of pacemaker cells. Alterations in spontaneous RyR activation in RyR mutant cells were a mechanism for a reduction of their AP firing rate and for reduced heart rate in mutant mice in vivo.

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Phosphorylation-Dependent Synchronization of Random Spontaneous Local Diastolic Ca^{2+} Releases Regulates Action Potential Firing Rhythmicity of Pacemaker Cells

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Substantial variability in heart beat intervals is detected within EKG time series analyses of numerous species (from mice to humans), and has often been linked to variation in autonomic neural impulses to the heart. Studies in single isolated sinoatrial nodal cells (SANC), however, indicate that mechanisms intrinsic to pacemaker cells not only regulate the average AP beating interval (APBI), but also determine APBI variability (APBIV). Furthermore, in permeablized SANC, which do not generate APs, phosphorylation of SR Ca^{2+} cycling proteins is one mechanism that regulates the spatiotemporal synchronization of spontaneous local RyR activation resulting in local Ca^{2+} release (LCRs) of shorter periods and reduced period variability. We tested the idea that the spatiotemporal synchronization of spontaneous local diastolic RyR activation linked to phosphorylation of Ca^{2+} cycling proteins is a determinant of both the average APBI and APBIV of intact SANC. Reduced SR Ca^{2+} cycling protein phosphorylation and increased APBI of cultured adult rabbit SANC (c-SANC) were accompanied by reduced kinetics and increased beat-to-beat variation of the SR refilling rate with Ca^{2+}, as reflected in the decay time of AP induced Ca^{2+}-transients; spatiotemporal de-synchronization of spontaneous, local RyR activation, was manifested by increased average LCR period and its variability, and by increased variability of surface membrane AP parameters. Increased protein phosphorylation effected by beta-AR stimulation in both c-SANC and freshly isolated SANC, accelerated the kinetics and reduced beat-to-beat variability of SR Ca^{2+} refilling, increased the spatiotemporal synchronization of LCR periods, reduced the variability of AP parameters and reduced both APBIV and average APBI. Thus, both the spontaneous AP firing rhythm and average firing rate of isolated SANC are linked to synchronization of random, local spontaneous RyR activation, modulated by SR Ca^{2+} cycling protein phosphorylation.

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Electron-Conformational Transformations in Nanoscopic RyR2 Channels Govern both the Heart's Contraction and Beating

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We argue that the gating of the ryanodine receptor (RyR) channels, key molecular determinants in the Ca^{2+} homeostasis, recognized as important novel therapeutic targets, is determined by electron-conformational transformations described by a simple electron-conformational model (ECM).
The model differs from conventional markovian models in several points, in particular, these are the RyR energy, inter-RyR coupling, conformational dynamics and unconventional quantum effects.The model describes the RyR gating under varying *cis* and *trans* [Ca^{2+}] with the same set of the parameters. We present an overview of computer modeling of the stochastic RyR2 gating in cardiomyocytes and sinoatrial node cells (SANC). The model does explain main features of the *in vitro* single RyR dynamics including modal gating and adaptation phenomena, effect of the *cis*[Ca^{2+}] and *cis*[Mg^{2+}], the temperature effects. Cooperative dynamics of the RyR clusters in Ca release units (CRU) and the Ca^{2+} spark features have been studied in a series of model simulations for 11x11 square RyR lattice incorporated into the cell calcium dynamics. The model does explain and describe the spontaneous oscillatory regime of the CRU both in SANCs (so-called Ca^{2+} clock) and in cardiomyocytes under Ca^{2+} sarcoplasmic reticulum overload. Puzzlingly, the intracellular clock obeys the Bowditch behavior without any membrane clock assistance. Given strong enough RyR-RyR coupling we observed novel effect of sudden inhibition of the oscillations with emergence of stable subclusters (2x2, 2x4,…) of opened channels and a steady-state Ca^{2+} leakage. The CRU oscillatory regime is restored by external membrane stimuli, so only working synergistically two types of clocks ensure robust and flexible pacemaker function. Despite the ECM is intentionally simplistic, it offers novel insight into the actual physical mechanisms involved in the gating behavior of the RyR channels with a sound framework for future studies.

540-Pos Board B320

MG56, a Member of the MBOAT Family of Proteins, Regulates Intracellular Calcium Signaling in Striated Muscle

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Calcium-induced-calcium-release (CICR) from the sarcoplasmic reticulum (SR) plays an integral role in excitation-contraction coupling - the driving mechanism behind synchronous cardiac muscle contractions. Alterations in CICR are commonly found in individuals suffering from cardiac arrhythmias, and closely associated with ventricular fibrillation, tachycardia, and sudden cardiac death. The ryanodine receptor 2 (RyR2) is a key mediator of CICR, functioning as a calcium channel along the SR. Elucidating the role of proteins that modulate RyR2-mediated CICR represents a major interest in cardiovascular research. We recently discovered a novel SR-resident membrane protein named mitsugumin 56 (MG56), which belongs to the membrane-bound o-acyltransferase (MBOAT) family of proteins. Knockout of MG56 produced a postnatal lethal phenotype. We have observed elevated Ca spark activity in MG56 null muscle fibers when compared with the wild type littermates. Using HEK293 cells with