natriuretic peptide (BNP) is regarded as an early compensatory response to hypotension and heart failure, although the use of a recombinant BNP agonist in clinical trials did not provide the expected benefit. Emerging data shows that BNP decreases cardiac sympathetic neurotransmission by attenuating activation of neuronal calcium signaling via a GMP-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 Ca2+ handling in cardiac sympathetic neurons from 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. [Ca2+]i transient was measured by ratiometric fluorescence imaging. BNP significantly reduced the magnitude of the Ca2+ 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 in producing [Ca2+]i in the SHR. In conclusion of PDE2A null 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 [Ca2+]i and the neuronal calcium current by BNP is impaired in the SHR, and this may be associated with apparent 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.

537-Pos Board B317 Reduced Heart Rate in Mice Harboring an SR Luminal Ca2+ Sensor Mutation (E487Q) is Linked to Abnormal Ca2+ Release and Pacemaker Function in Isolated Cardiocytes Derived from the Mutant RyR Clone Syveda Sirenko, Ihor Zanichyk, Yelena Tarasova, Daniel R. Riordon, Wenqian Chen, Wayne S.R. Chen, Edward G. Lakatta1,2

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Rationale: The coupled-clock theory of cardiac pacemaker normal automaticity integrates numerous facets of pacemaker cell Ca2+ cycling and electrophysiology. Details of intrinsic RyR molecular mechanisms that regulate spontaneous RyR activation to generate local Ca2+ releases (LCRs) of the “Ca2+ clock”, that drive normal automaticity, however, have not been elucidated.

Objective: We hypothesized that spontaneous RyR activation to generate LCRs of the “Ca2+ clock” will be attenuated in cells harboring mutant SR luminal Ca2+ sensor (E487Q).

Methods and Results: We measured the spontaneous beating rate, action potential (AP) triggered RyR Ca2+ releases, characteristics of spontaneous LCRs in single ES derived cardiocytes from wild type (WT) and the RyR mutant (E487Q) ES and embryonic stem cells (ESC) with intact sarcolemma and in permeabilized cells in the absence of APs’s, and the expression of SR Ca2+ 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 Ca2+ release. Expression of RyR protein was reduced, and calsequestrin were increased in mutant vs WT cells.

Conclusions: Numerous luminal SR Ca2+ 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.

538-Pos Board B318 Phosphorylation-Dependent Synchronization of Random Spontaneous Local Diastolic Ca2+ Releases Regulates Action Potential Firing Rhythmicity of Pacemaker Cells Dongmei Yang, Alexey E. Lyashkov, Yael Yaniv, Bruce D. Ziman, Edward G. Lakatta1,2

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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 (APBV). Furthermore, in permeabilized SANC, which do not generate APs, phosphorylation of SR Ca2+ cycling proteins is one mechanism that regulates the spatiotemporal synchronization of spontaneous local RyR activation resulting in local Ca2+ 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 Ca2+ cycling proteins is a determinant of both the average APBI and APBV of isolated SANC. Reduced SR Ca2+ 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 Ca2+, as reflected in the decay time of AP induced Ca2+-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 Ca2+ refilling, increased the spatiotemporal synchronization of LCR periods, reduced the variability of AP parameters and reduced both APBI and average APBV. 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 Ca2+ cycling protein phosphorylation.

539-Pos Board B319 Electron-Conformational Transformations in Nanoscopic RyR2 Channels Govern both the Heart’s Contraction and Beating Alexander Moskvkin1,2, Alexander Ryvklin1, Nikolaev Zorin1, Kirill Soulim1, Bogdan Yarapov1, Olga Solovyova1,2, Vladimir Markhasin1, Alexey E. Lyashkov, Yael Yaniv, Bruce D. Ziman1, Matthew Sermersheim2, Jordan W. Stocum2, Matthias B. Reuter1, Alexander Zakharenko1, Yu-Cheng Liu1, Alexey E. Lyashkov, Yael Yaniv, Bruce D. Ziman1, Matthew Sermersheim2

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We argue that the gating of the ryanodine receptor (RyR) channels, key molecular determinants in the Ca2+ 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 [Ca2+] with the same set of 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 nodal gating and adaptation phenomena, effect of the cis[Ca2+] and cis[Mg2+], the temperature effects. Cooperative dynamics of the RyR clusters in Ca release units (CRU) and the Ca2+ 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 Ca2+ clock) and in cardiomyocytes under Ca2+ sarcoplasmic reticulum overload. Puzzlingly, the intracellular clock obeys the Bowdich 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 Ca2+ leakage. The CRU oscillatory regime is restored by external membrane stimuli, so only working synergistically types of clocks ensure robust and reliable contraction 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 Mg26, a Member of the MBOAT Family of Proteins, Regulates Intracellular Calcium Signaling in Striated Muscle Matthew Sermersheim1, Xin Yu Zhou2, Ki Ho Park1, Pei-Hui Lin1, Jacob Yount1, Wayne Chen1, Miyuki Nishi3, Hiroshi Takeishima1, Jianjie Ma4

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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 premier interest in cardiovascular research. We recently discovered a novel SR-resident membrane protein named mitsugum 56 (Mg26), which belongs to the membrane-bond o-acetylsalicylate (MBOAT) family of proteins. Knockout of Mg26 produced a postnatal lethal phenotype. We have observed elevated Ca spark activity in Mg26 null muscle fibers when compared with the wild type littermates. Using HEK293 cells with