The phosphorylation levels of RyR2 at Ser^{2809} and phospholamban at Ser^{16} are substantially lower in c-SANC, suggesting that PKA signaling in c-SANC is down-regulated. Additionally, the level of RGS2 protein is substantially lower in c-SANC (149.9 ± 4.0, n=100) than in f-SANC (201.9 ± 6.0, n=88, $p<0.001$). β-AR stimulation with 1μM isoproterenol for 10min increases phospholamban and RyR2 phosphorylation, accelerates AP and Ca^{2+}-transient kinetics, reduces the LCR period, and accelerates the AP firing rate to a similar maximum in c-SANC (3.34 ± 0.05Hz, n=150) and f-SANC (3.55 ± 0.06Hz, n=126). A 2 hour incubation of 1μM isoproterenol enhances RGS2 staining density. A specific PKA inhibitor, PKI, completely inhibits all isoproterenol effects. Overexpression of RGS2 (Ad-RGS2), or overnight pertussis toxin treatment to disable Gi signaling, partially (66% and 85%, respectively) rescues the reduced spontaneous beating rate. These results indicate that a reduction in PKA-dependent Ca^{2+}-cycling protein phosphorylation that is Gi-dependent is involved in the reduced spontaneous beating rate of c-SANC, and that this deficit can be reversed by pharmacologic or genetic manipulation.

522-Pos

Spontaneous Beating of Rabbit Sinoatrial Node Cells Requires Basal Protein Kinase C Activity

Tatiana M. Vinogradova, Edward G. Lakatta.
NIA, NIH, Baltimore, MD, USA.

Cardiac rhythm is governed by the spontaneous rhythmic excitations of rabbit sinoatrial node cells (SANC), a crucial component of which is cAMP-mediated, PKA-dependent local subsarcolemmal Ca^{2+} releases (LCRs) from ryanodine receptors (RyR). LCRs activate inward Na^{+}-Ca^{2+} exchange current during late diastolic depolarization (DD) leading to the increase in the DD rate and spontaneous SANC beating rate. Here we show that in addition to PKA-dependent phosphorylation, spontaneous SANC beating requires basal PKC activity. In freshly isolated SANC the PKC inhibitor GF109203X, 10 μmol/L, suppressed spontaneous SANC beating rate, recorded using perforated patch by ~2.5-fold (from 154 ± 7 to 60 ± 14 beat/min, n=6). To study whether IP3 receptor-mediated Ca^{2+} release is involved in the PKC-dependent modulation of the spontaneous SANC beating, IP3 receptors were inhibited by 2 μmol/L 2-APB. However, 2-APB produced no significant changes in the spontaneous SANC beating rate suggesting no direct involvement of IP3-mediated Ca^{2+} release in the spontaneous SANC beating. Confocal imaging of isolated SANC loaded with the Ca^{2+}indicator, Fluo-3, demonstrated that PKC inhibition markedly decreased LCR size from (from 8.6 ± 0.5 to 4.0 ± 0.4 μm, n=3, $P<0.01$); LCR number per each spontaneous cycle (from 1.5 ± 0.3 to 0.6 ± 0.2, n=3, $P<0.05$), and prolonged the LCR period, the time from AP-induced Ca^{2+} transient to the time of LCR appearance (from 406 ± 7 to 836 ± 80 ms, n=3, $P<0.05$). The increase in the LCR period accompanied the increase in the spontaneous SANC cycle length, strongly suggesting that LCRs could be the major target of the basal PKC activity. Thus, our data show for the first time that the basal PKC-dependent activity regulates spontaneous SANC beating rate through modulation of LCRs from RyR, while IP3 receptors make no substantial contribution in this effect.

523-Pos

Mechanisms Underlying Spontaneous Beating in Human Embryonic Stem Cell-Derived Cardiac Myocytes

Frank Fabris[1], Ardo Illaste[2], Michelle Park[1], Eric D. Adler[1], Eric A. Sobie[1].
[1]Mount Sinai School of Medicine, New York, NY, USA, [2]Tallinn University of Technology, Tallinn, Estonia.

Cardiac myocytes derived from human embryonic stem cells (hESC-CMs) are increasingly considered a promising therapy for a variety of pathologies, but many questions persist regarding the physiology of these cells and how it changes during differentiation. We sought to determine the mechanisms underlying spontaneous beating in hESC-CMs derived from HES2 line. Enrichment for developing cardiomyocytes was achieved by exposing differentiating Embryoid Bodies (EBs) to the following growth factors: BMP4, DKK and VEGF, and DKK, VEGF and bFGF. By day 20, >85% of EBs were spontaneously beating and roughly 70% of all cells were expressing troponin T. To monitor intracellular calcium (Ca^{2+}), EBs from various time points were plated on cover slips and imaged with a laser scanning confocal microscope. Recordings of Ca^{2+} transients in hESC-CMs at different stages of differentiation revealed the following features: 1) rate of spontaneous beating increased, and Ca^{2+} transients decayed more quickly, in older (> 45 day) compared with younger (< 45 day) hESC-CMs; 2) coordinated spontaneous beating was abolished in 100% of EBs when external Na^{+} was replaced with Li^{+}; 3) reducing extracellular [Ca^{2+}] to 0.5 mM caused slower spontaneous beating; 4) spontaneous beating was abolished in most (>75%) EBs when 20 mM caffeine depleted sarcoplasmic reticulum (SR) Ca^{2+}; 5) field-stimulation induced Ca^{2+} transients recorded in 20 mM caffeine decayed more slowly than control Ca^{2+} transients, and the difference in decay rate was more pronounced in older hESC-CMs. Together these results are consistent with a model in which spontaneous SR Ca^{2+} release induces inward Na^{+}-Ca^{2+} exchange current, and the membrane depolarization provided by this current initiates action potentials. Differentiation time increases the importance of SR Ca^{2+} cycling in the function of these cells, suggesting a more mature phenotype in older compared with younger hESC-CMs.

524-Pos

Competitive NFAT Bottleneck For Transcriptional Activation of Endogenous Ca^{2+} ATPASE (SERCA2) in Adrenergic Hypertrophy

Anand M. Prasad, Giuseppe Inesi.
California Pacific Medical Center Research Institute, San Francisco, CA, USA.

A prominent feature of cardiac hypertrophy and failure is reduced SERCA2 expression and deficient Ca^{2+} signaling. To uncover a causal relationship between hypertrophy and downregulation of SERCA2, we use neonatal rat cardiac myocytes where SERCA2 transcription can be increased by exposure to 10 nM thapsigargin (TG), which allows cytosolic Ca^{2+} rise and calmodulin activation of calcineurin (CN). This SERCA2 rise is markedly reduced by cyclosporine (CsA), which inhibits CN phosphatase, and increased by KN-93, a calmodulin activated kinase (CAMKII) inhibitor which relieves CAMKII dependent phosphorylation and inhibition of CN. These CsA and KN-93 effects are also produced on TG enhanced luciferase expression, under the control of an NFAT (Nuclear factor of activated T Cells) dependent promoter. We conclude that NFAT dephosphorylation is a limiting factor for SERCA2 transcription. Exposure of myocytes to PE yields adrenergic hypertrophy, with rise of Atrial Natriuretic Factor (ANF) transcript, protein incorporation of ^{14}C-phenylalanine and fluorescent staining of actin, while SERCA2 is downregulated. The adrenergic response can be reproduced by direct stimulation of protein kinase C (PKC) with phorbol 12-myristate 13-acetate, indicating that inhibition of glycogen synthase kinase (GSK3β) by PKC and consequent reduced NFAT phosphorylation, as well as histone deacetylase (HDAC) phosphorylation by PKC activated MAPK, are involved in the mechanism of adrenergic hypertrophy. The hypertrophy response is markedly reduced by CN inhibition by CsA, indicating that CN dephosphorylation and nuclear import of NFAT play an important role in the development of hypertrophy, and SERCA2 downregulation is produced by competitive utilization of NFAT by the hypertrophy program. Interestingly, CAMKII inhibition limits the development of hypertrophy, emphasizing a rate limiting role of CAMKII dependent phosphorylation and nuclear export of HDAC in the extensive transcriptional hypertrophic program (Supported by 5 R01 HL069830-08).

525-Pos

Differential Regulation of β-Adrenergic Signaling Via Phosphoinositide-3-Kinase-γ

Gerrie P. Farman, Peter B. Helli, Peter H. Backx.
University of Toronto, Toronto, ON, Canada.

We have recently shown that phosphoinositide-3-kinase-γ (PI3Kγ) modulates cardiomyocyte contractility by locally regulating sarcoplasmic reticulum (SR) Ca^{2+} load via the type 4 phosphodiesterase (PDE4) (Kerfant Circ. Res. 2007). In addition, we have also shown that SR Ca^{2+} load and contractility are enhanced in PI3Kγ knockout (KO) mice both at baseline in the context of non-selective β-adrenergic receptor (β-AR) stimulation (Kerfant Circ. Res. 2004). Since PI3Kγ is activated by the βγ subunit of G-proteins which has also been shown to affect β-AR signaling in cardiomyocytes, we asked whether PI3Kγ differently affects β_1-AR versus β_2-AR signaling and whether PDE4 underlies these differences. We used L-type Ca^{2+} currents (I_{CaL}) and Ca^{2+} transients to assess the effects of β-AR stimulation. In isolated murine cardiomyocytes, specific β_1-AR stimulation increased I_{CaL}, and Ca^{2+} transient amplitudes in both wild-type (WT) and KO cells, although the peak transient response was more pronounced in the latter. Concomitant inhibition of PDE4 with 10μM rolipram had no effect on Ca^{2+} transient amplitudes in KO cells while in WT cells, transient amplitudes were further increased to levels observed in KO's. While specific β_2-AR stimulation increased Ca^{2+} transient amplitudes in both WT and KO cells (not to the same extent as β_1), this increase was not accompanied by any changes in I_{CaL}. Furthermore, while transients were larger in KO cells, rolipram reduced the parity between these groups. In summary, specific activation of the β_1 pathway enhances cardiac Ca^{2+} handling in a PI3Kγ/PDE4-dependent manner. Conversely, β_2-AR activation has little impact on I_{CaL}, plays a smaller role in Ca^{2+} handling, but also appears to be partially regulated by PDE4. Taken together, these results suggest that PI3Kγ plays an important role in both β_1, and β_2-AR signaling.