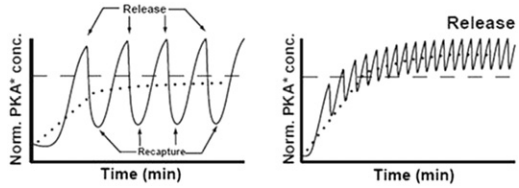
PLC-epsilon with a mutated Ras Association (RA) domain, or a H1640L PLC-epsilon that is catalytically dead, were both ineffective. Since 8-pCPT-2'-O-Me-cAMP-AM failed to facilitate CICR in WT beta cells transduced with a GTPase activating protein (RapGAP) that down-regulates Rap activity, the available evidence indicates that a signal transduction ''module'' comprised of Epac2, Rap, and PLC-epsilon exists in beta cells, and that the activities of Epac2 and PLC-epsilon are key determinants of CICR in this cell type.

2811-Plat

PKA is a Control Node in a Calcium-Dependent Oscillatory Circuit in Pancreatic Beta Cells

Ambhighainath Ganesan, Qiang Ni, Nwe-Nwe Aye-Han, Xinxin Gao, Michael D. Allen, Andre Levchenko, Jin Zhang.
Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Protein Kinases are often critical nodes in signaling cascades that encode diverse inputs and effect appropriate outcomes requiring complex spatiotemporal regulation of kinase activities. We show here using FRET-based biosensors that calcium, cAMP and PKA activity oscillate synchronously in insulin secreting MIN6 pancreatic beta cells, suggesting the presence of a tightly regulated oscillatory circuit involving PKA mediated feedback onto calcium signaling. We observed that PKA is essential for this circuit as well as capable of initiating oscillations. Further, mathematical modeling in conjunction with experimentsindicates that PKA activity could modulate the frequency of oscillations resulting in diverse spatiotemporal outcomes (simulations shown in figure).



Catalytic subunits of PKA could be periodically released and recaptured for ''local'' target phosphorylation at low frequency conditions (left panel). However, at high frequency conditions (right panel), the mean PKA activity (dotted line) may cross an arbitrary threshold (dashed line) leading to continued release of catalytic subunits resulting in phosphorylation of ''global'' targets. Our findings therefore suggest that temporal regulation of PKA activity could be used to control complex cellular functions and thus influence the functionality of the pancreatic beta cell.

2812-Plat

Basal Phospholipase C (PLC) Activation is Obligatory for Cardiac Pacemaker Activity

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

Spontaneous firing of sinoatrial node cells (SANC) is controlled by sarcoplasmic reticulum (SR) generated local subsarcolemmal Ca^{2+} releases (LCRs) which appear during diastolic depolarization and activate an inward Na^{+}-Ca^{2+}exchange current, regulating diastolic depolarization rate and SANC beating rate. Whether the ubiquitous enzyme, PLC that plays a key role in Ca^{2+} signaling of numerous cell types, controls spontaneous SANC firing is unknown. Here we show that PLC inhibitor U-73122 (6μmol/L, n=7), but not its inactive analog U-73343 (n=4), stops spontaneous firing (perforated patch-clamp recordings) of freshly isolated rabbit SANC, indicating important role of basal PLC activity for cardiac pacemaking. To determine whether signaling via the cAMP-sensor Epac elevates PLC activity in SANC and stimulates PLC-mediated IP_3-dependent Ca^{2+} release, we employed Epac activator cpTOME or IP_3 receptor inhibitor 2-APB, respectively. Neither cpTOME (10 μmol/L, n=3), nor 2-APB (2 μmol/L, n=4) altered the spontaneous SANC beating rate. PLC inhibition by U-73122, in a time-dependent manner, suppressed LCRs (confocal microscopy, Ca^{2+}indicator Fluo-3), i.e. decreased LCR's size, amplitude and prolonged the LCR period (the interval between AP-induced Ca^{2+} transient and subsequent LCR); afterwards eliminated LCRs and stopped SANC firing. The time-dependent increase in the LCR period predicted the concomitant increase in the spontaneous cycle length, suggesting that Ca^{2+} cycling could be a major target of PLC-dependent control of SANC firing. LCRs are critically dependent upon amount of Ca^{2+} in SR, supplied by L-type Ca^{2+}current ($I_{Ca,L}$). U-73122, but not U-73343, markedly suppressed $I_{Ca,L}$ amplitude (from -11.8 ± 4.8 to -5.9 ± 2.4 pA/pF, n=6, $P<0.001$). We conclude that PLC-dependent control of spontaneous SANC firing, independent of either Epac signaling or IP_3-dependent Ca^{2+} release, regulates Ca^{2+} influx through L-type Ca^{2+} channels and, thus, SR Ca^{2+} cycling and via these mechanisms, in part at least, is obligatory for SANC normal automaticity.

2813-Plat

Altered Nuclear Calcium Signaling in Tachycardia-Induced Remodeling in Rabbit Atria: A Mechanism of Altered Excitation-Transcription Coupling in Atrial Fibrillation?

Benoit-Gilles Kerfant, Marion Kuiper, Arne Van Hunnik, Maura Greiser, Sander Verheule, Ulrich Schotten.
CARIM, Maastricht, Netherlands.

Nuclear calcium (Ca2+) signalling play a major role in cardiac excitation-transcription coupling. The origin of nuclear Ca2+ transient is still controversial. It has been suggested that Ca2+ diffusion from the cytosol through the nuclear pores or perinuclear and intranuclear Ca2+ release mechanisms, mostly via the inositol-3-phosphate receptors (IP3R), participate to nuclear Ca2+ transient. In rabbits subjected to rapid atrial pacing (RAP, 600bp, 5 days), a model for atrial fibrillation (AF), we recently observed that central-cellular, but not subsarcolemmal Ca2+ transient, was largely reduced. Since nuclei are often at the center of atrial myocytes, we hypothesized that nuclear Ca2+ transient is also decreased in RAP cells. Using confocal microscopy we recorded transverse line-scan images in field-stimulated sham and RAP atrial cells. Nuclear Ca2+ transient amplitude in relation to subsarcolemmal Ca2+ transient was decreased in RAP compared to sham cells (at 1Hz, DFnuclear/DFsubsarcolemmal was $0{,}38 \pm 0{,}03$ in 17 RAP cells, compared to $0{,}81 \pm 0{,}06$ in 16 sham cells, $p<0{,}01$). Isoproterenol restored central-cellular as well as nuclear Ca2+ transient amplitude in RAP cells. Interestingly, at 2,5Hz, in 9 out of 16 sham cells and in 4 out of 16 RAP cells, a nuclear Ca2+ transient did not follow the cytoplasmic Ca2+ transient suggesting that cytosolic Ca2+ diffusion is not the only mechanism eliciting atrial nuclear Ca2+ transient. Indeed, we observed that, in RAP, L-phenylephrine enhanced both nuclear Ca2+ transient and perinuclear Ca2+ release, via the IP3R, while it had no effect in sham cells. Taken together, our data indicate that both cytoplasmic Ca2+ transient and perinuclear Ca2+ release determine atrial nuclear Ca2+ transient. Furthermore, nuclear Ca2+ transient is reduced while IP3-mediated nuclear Ca2+ signalling is enhanced in RAP, possibly affecting Ca2+-mediated transcription pathways.

2814-Plat

Alterations of Membrane Currents, Contractility and Calcium Signaling in Gq/G11 Single and Double Ko Mice

Qinghai Tian, Sara Pahlavan, Sandra Ruppenthal, Anke Scholz, Kathrina Wiesen, Martin Oberhofer, Lars Kaestner, **Peter Lipp**.
Saarland University, Homburg/Saar, Germany.

In contrast to its role in pathophysiology very little is known about the role of the Gq/G11 signaling cassette in the physiology of cardiac myocytes. We have generated transgenic mice combining a G11-KO approach with the Cre-recombinase tool to knock-down (KD) Gq in a tissue-specific inducible manner. Western-blot analysis depict the rapid loss of Gq protein in less than 2 weeks while the use of a reporter mouse substantiated tissue specificity. We analyzed the effects of (i) Cre expression, (ii) vehicle/Tamoxifen injection, (iii) G11-KO and (iv) Gq-KD on resting potential and capacitance, action potentials (AP) and I(to) by patch-clamping ventricular myocytes. Calcium homeostasis was investigated by Fura2 video-imaging and contraction behavior by measuring sarcomere length. While basic cellular parameters were largely unchanged (cell capacitance, resting membrane potential) in all genotypes and treatments, AP, calcium handling and contractile behavior were altered by vehicle/Tamoxifen injection, G11-KO and Gq-KD. Vehicle injection alone caused upregulation of I(to) and shortening of APs similar to G11-KO. In contrast additional Gq-KD, restorated'' AP properties and I(to). Vehicle injection itself into wt mice caused increases in basal calcium that were absent after Tamoxifen injection, Cre expression had mild effects on calcium handling. Tamoxifen injection and Gq/G11-KO caused major changes in calcium handling and contractile behavior. In conclusion, despite the broad application of the Cre-recombinase system, care has to be taken for possible effects of Cre expression and vehicle/Tamoxifen injection. The Gq/G11 signaling system plays an important role in the physiological modulation of proteins involved in electrophysiology and calcium homeostasis most likely regulated by basal levels of neurohormonal stimulation.

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2815-Plat

Nuclear Inositol 1,4,5-Triphosphate is an Absolut Requirement for Cardiomyocyte Hypertrophy

Lilian M. Arantes[1], Carla J. Aguiar[1,2], M. Jimena Amaya[3], S. Guatimosim[1], M. Fátima Leite[1,4].
[1]UFMG, Belo Horizonte, Brazil, [2]Izabela Hendrix Institute, Belo Horizonte, Brazil, [3]Yale School of Medicine, New Haven, CT, USA, [4]Howard Hughes Medical Institute, Chevy Chase, MD, USA.

Ca^{2+} mediates a wide range of cellular responses and the release of this ion from inositol 1,4,5-triphosphate receptor ($InsP_3R$) is known to play a critical