3600-Pos

PH Domain Mutation Alters Membrane Targeting Specificity of GRP1 and AKT

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Protein kinase B (AKT1) and General Receptor for Phosphoinositides 1 (GRP1) regulate multiple cell signaling pathways essential for cell function and survival. AKT1 possesses an N-terminal PH domain (AKT1-PH) that binds important target phosphatidylinositol phospholipids (PIPs) such as PI(3,4)P3 and especially PI(3,4,5)P3. During a signaling event, PI(4,5)P2 gets converted to PI(3,4,5)P3, resulting in targeting of AKT1 to the cell plasma membrane by its PH domain. Our recent published work (Landgraf (2008) Biochemistry) revealed that the E17K charge reversal mutation adjacent to the PIP binding site in AKT1-PH alters the PIP specificity of the site, yielding high affinity binding to PI(4,5)P2 and constitutive binding to plasma membrane. The resulting hyper-activation of AKT1 inhibits apoptosis and explains the known oncogenic character of the E17K mutation, which is linked to multiple human cancers. Here we test the hypothesis that glutamate residues often found adjacent to the PIP binding sites of PI(3,4,5)P3 -specific PH domains are essential to PIP lipid specificity. Using a combination of biochemical, structural, and cell biology approaches, we compare the PIP lipid specificities and intracellular targeting of GRP1-PH and E345K-GRP1-PH. The latter mutant possesses a charge reversal mutation at a glutamate position adjacent to the PIP binding site. The preliminary findings, which will be presented at the meeting, indicate that the mutant exhibits high affinity binding to PI(4,5)P2 and constitutive binding to plasma membrane. These findings support the hypothesis that the adjacent glutamate residue plays an essential role in PI(3,4,5)P3 -specificity and PI(3,4,5)P3 -regulated plasma membrane targeting in cells. Finally, we are investigating the structural basis for the specificity change by attempting to solve the crystal structures of the E17K-GRP1-PH and E345K-GRP1-PH mutants bound to PI(4,5)P2.

3601-Pos

Engineered Nanolipoproteins as Biosynthetic Decoys for Pathogen-Binding

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The ability to exogenously present cell-surface receptors in a synthetic system offers an opportunity to provide host cells with protection from pathogenic toxins. Practical implementations suffer from serious limitations, primarily because of difficulties in mimicking the role of the membrane micro-environment during the complex and dynamic pathogen-receptor interaction. To this end, we have developed reconstituted lipoprotein - nanometer-sized discoidal lipid bilayers of arbitrary composition bounded by apolipoprotein - to serve as a versatile, biocompatible and stable platform to house pathogen-binding receptors in a membrane-like environment. Our approach exploits the notion that a control of biophysical properties of the membrane micro-environment allows to modulate interactions between membrane-embedded receptors and their pathogenic (e.g., bacterial toxin) targets. We demonstrate here, using a Foerster Resonance Energy Transfer (FRET) based assay, that ganglioside GM1 receptors incorporated at controlled concentrations in reconstituted lipoprotein bind cholera toxin with greater affinity than liposome-based systems. Furthermore, fluorescence microscopy investigations of cholera toxin presented to populations of mammalian cells show that GM1-laden lipoprotein can function as decoys without harming healthy cells.

3602-Pos

Crystallization of Calcium Carbonate Vaterite Associated with Liquid Crystal in Embryonic Yolk Sacs

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Calcium carbonate is often used as an efficient antacid that absorbs and neutralizes stomach acid while providing calcium for healthy bones. Taking advantage of the lack of adverse side effects of calcium, new drug delivery systems consisting of drug-supported spherical microparticles are being developed. We have reported in our previous studies that a natural process producing calcium carbonate microparticles can be found during avian development. These natural systems provide inspiration for designing more efficient microparticle facilitated drug-delivery systems. In this study, the formation and re-absorption of calcium carbonate crystals were tracked during Gallina *N. meleagris* embryogenesis and early postnatal development. The study demonstrated that the formation of calcium carbonate microparticles, as calcium is transferred from the eggshell into the egg sac, is a process of calcium preservation. X-ray diffraction showed that calcium carbonate crystal is mainly preserved in the vaterite isoform. Calcium incorporated into the yolk sac during this process can be easily assimilated as necessary during postnatal development. Eons of evolution have yielded a calcium preservation process that produces an iso-form of crystalline calcium most readily absorbed by the organism. Our previous results indicate that this biological system is likely a lyotropic process, the method that is currently being used for the production of microparticle drug delivery systems. In this work, our data suggests that calcium carbonate crystal can also initiate its crystallization from the center of liquid crystal, recognizable by a chimeric thermal phase transition. Our work provides valuable information for designing more efficient microparticle for drug-delivery.

Voltage-gated Ca Channels II

3603-Pos

Stim1 Binds to and Inhibits CaV1.2 Voltage Gated Calcium Channels

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CaV1.2 and other L-type voltage gated calcium channels play a key role in regulating cardiac contraction, synaptic plasticity, insulin secretion and a variety of other cellular events. Phospho-inositide linked receptors like the muscarinic acetylcholine receptor, inhibit L-type calcium channels and this inhibition is important for parasympathetic regulation of heart contraction as well as for learning and memory in the brain. The mechanisms by which PLC coupled receptors inhibit L-type channels are still controversial though several hypotheses including reduction of cAMP and depletion of PIP2 from the cell membrane have been proposed. We report a new and unexpected mechanism by which PLC-coupled receptors inhibit L-type calcium channels in cells. We have found that depletion of ER calcium stores either down stream of muscarinic receptors or following application of the ER calcium ATPase inhibitor, thapsigargin, inhibits CaV1.2 channels. CaV1.2 inhibition depends on binding to Stim1, an ER calcium sensor protein that activates the Orai family of store operated calcium channels. In cells expressing CaV1.2, Stim1 translocates to ER-plasma membrane junctions and co-localizes with clusters of CaV1.2. In vitro and in vivo studies indicate that the CAD domain of Stim1 binds to a coiled coil in the II-III loop of CaV1.2. Stim1 lacking the CAD domain is unable to bind to CaV1.2 and fails to inhibit CaV1.2 currents following depletion of ER calcium stores. These studies support a new mechanism by which phosphoinositide-linked receptors inhibit L-type calcium channels and suggest that Stim1 dynamically regulates the relative contributions of Orai and CaV1.2 channels to calcium influx in excitable cells.

3604-Pos

Oxygen-Sensing of L-type Calcium Channels in Rat Cardiomyocytes: The Possible Role of Hemoxygenase

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Hemoxygenases (HO)-1 and −2 are enzymes that metabolize the heme group, generating CO, biliverdin and Fe^{+2}. HO-2 is believed to be an O_2 sensor in the carotid body controlling the release of dopamine as it needs O_2 for its function. HO-2 is necessary for the anoxic activation of nociceptive neuron and binds to calmodulin in neurons, and the complex is modulated by Ca^{2+}. Previously, we have found that L-type Ca^{2+} channel is suppressed by 22-43% with acute anoxia prior to metabolic impairment or run down of Ica . This property appeared to require the calmodulin-binding IQ motif on the cytoplasmic carboxy-tail of the channel. Here we probe whether HO mediates this rapid Ica-suppressant effect of anoxia thus serving as the O_2 sensor of the heart. Freshly isolated rat cardiomyocites were maintained in Tyrode containing (in mM): 135NaCl, 5.4KCl, 10Hepes, 10glucose, 1$MgCl_2$ and 2$CaCl_2$). Internal solution used was (in mM): 15NaCl, 118CsCl, 5.5Glucose, 14EGTA, 3.92$CaCl_2$, 10Hepes, 3MgATP, 0.5$MgCl_2$. Solutions bubbled with 100% O_2 or 100% N_2 were rapidly (50ms) applied. I_{Ba} was measured replacing $CaCl_2$ for $BaCl_2$. HO inhibitors ZnPP-IX 100nM or SnPP-IX 100nM were added to either 100% O_2 or 100% N_2 solutions. Both the O_2 removal and the inhibition of HO blocked

about 30% of the I_{Ca} and I_{Ba}. Administration of anoxic solution did not further inhibit I_{Ca} in cells previously exposed to ZnPP-IX or SnPP-IX. We conclude that both anoxia and HO suppress I_{Ca} and I_{Ba} with the same intensity and kinetics and O_2 sensing effect becomes negligible in cells exposed to HO inhibitors.

3605-Pos

A New Paradigm for Gem Regulation of Voltage-Gated Ca^{2+} Channels

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The RGK (Rem, Rem2, Rad, Gem/Kir) family of Ras-related monomeric small GTP-binding proteins has emerged as potent inhibitors of high-voltage activated (HVA) Ca^{2+} channels. All RGK proteins bind all four subfamilies of HVA Ca^{2+} channel β subunits (Ca_vβs), and Ca_vβ is required for RGK-induced inhibition. Two modes of RGK action have been reported: (1) RGKs interfere with channel trafficking to the plasma membrane and hence reduce the number of surface channels; (2) RGKs inhibit channels already on the plasma membrane. It is generally believed that both forms of inhibition absolutely rely on the RGK/Ca_vβ interaction. However, this central hypothesis has not been tested directly. We investigated the molecular mechanism of Gem inhibition of P/Q-type Ca^{2+} channels expressed in *Xenopus* oocytes and HEK 293T cells. Gem inhibited P/Q channels without affecting their surface expression. Application of a purified Gem protein domain in inside-out membrane patches acutely inhibited P/Q channels. This acute inhibition was completely abolished when Ca_vβ was removed from surface P/Q channels, but it was fully restored after the channels regain Ca_vβ. These results unequivocally demonstrate that Ca_vβ is indispensable for Gem inhibition of surface P/Q channels. Surprisingly, however, complete disruption of the Gem/Ca_vβ interaction, as shown biochemically, did not affect Gem inhibition. On the other hand, we discovered that Gem associated with Ca_v2.1 in a Ca_vβ-independent manner. Finally, we identified a 12-amino acid region in the C-terminus of Gem that was sufficient to produce inhibition in inside-out patches and another site in the core region of Gem that was also involved in Gem inhibition. Based on these findings, we propose that Gem directly binds and inhibits Ca_Vβ-primed HVA Ca^{2+} channels on the plasma membrane.

3606-Pos

The β Subunit of Voltage-Gated Ca^{2+} Channels Acts as a Transcriptional Regulator

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Ca^{2+} channel β subunits (Ca_vβs) are essential for the surface expression and proper gating of high-voltage activated (HVA) Ca^{2+} channels. In yeast two-hybrid screens aimed at discovering novel Ca_vβ-interacting proteins, we identified a new splicing isoform of Pax6, a transcription factor crucial for the development of a variety of organs and tissues, especially the eye. Pax6 contains two DNA-binding domains (paired domain and homeodomain), a glycine-rich linker connecting these two domains, and a carboxyl (C)-terminal proline, serine and threonine (PST)-rich transactivation domain. The newly isolated isoforms, named Pax6(S), retains the paired domain, linker and homeodomain of Pax6, but its C-terminus is composed of a truncated classic PST domain and a unique S tail. In contrast to Pax6, which is 100% conserved from rodent to human and is expressed in both embryo and adult, Pax6(S) is completely conserved only in human and chimpanzee, and it is expressed only at early stages of development, suggesting that Pax6(S) has a noncanonical function. Pax6(S) retained strong transcriptional activity, although its C-terminus showed less transactivity compared with the canonical PST domain. The interaction between Pax6(S) and Ca_vβ was mainly endowed by the S tail of Pax6(S). Co-expression of Pax6(S) with a HVA Ca^{2+} channel complex containing the β_3 subunit in *Xenopus* oocytes did not affect channel properties. However, the transcriptional activity of Pax6(S) was markedly suppressed by β_3. Furthermore, in the presence of Pax6(S), β_3 was translocated from the cytoplasm to the nucleus. These results suggest that full length Ca_vβs may function as transcription regulators, independent of their role in regulating Ca^{2+} channel activity.

3607-Pos

Increased Intracellular Magnesium Attenuates β-Adrenergic Stimulation of the Cardiac Cav1.2 Channel

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Increases in intracellular Mg^{2+} (Mg_i^{2+}), as observed in transient cardiac ischemia, decrease L-type Ca^{2+} current of mammalian ventricular myocytes. Transient cardiac ischemia is also associated with an increase in sympathetic tone, which could stimulate the L-type Ca^{2+} current. Therefore, the effect of Mg_i^{2+} on L-type Ca^{2+} current in the context of increased sympathetic tone is unclear. We hypothesized that increased Mg_i^{2+} in ventricular myocytes might alter the β-adrenergic stimulation of L-type Ca^{2+} current. Using the whole-cell patch clamp method to study ventricular myocytes from C57BL6 mice, we tested the impact of increased Mg_i^{2+} on the stimulatory effect of the β-adrenergic receptor (β-AR) cascade activation on L-type Ca^{2+} current. We observed that exposure of myocytes to higher Mg_i^{2+} concentration decreased isoproterenol stimulation of the L-type Ca^{2+} current from 75 ± 13 % with 0.8 mM Mg_i^{2+} (n=11) to 20 ± 8 % with 2.4 mM Mg_i^{2+} (n=7) ($p < 0.01$). Because Mg_i^{2+} could act at multiple sites in the β-AR cascade, we activated this signaling cascade at different steps using pharmacological tools to determine the site(s) of Mg_i^{2+} action. We found that exposure of ventricular myocytes to increased Mg_i^{2+} attenuated the stimulation of L-type Ca^{2+} current mediated by isoproterenol (β-AR stimulation), forskolin (adenylate cyclase stimulation), and IBMX (phosphodiesterase inhibition). These experiments rule out significant effects of Mg_i^{2+} on the β-AR, Gs protein, adenylate cyclase, and phosphodiesterase (I-V). Taken together, our results suggest that, in transient ischemia, increased Mg_i^{2+} reduces the entry of Ca^{2+} via the L-type Ca^{2+} current by directly acting on the Ca_V1.2 channel in a cell-autonomous manner, effectively decreasing the metabolic stress imposed on ventricular myocytes until blood flow can be re-established.

3608-Pos

Localized Calcineurin in Calcium- Dependent Inactivation of L-type Calcium Channels

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The open probability of Ca_V1.2 L-type Ca^{2+} channels is enhanced by cAMP-dependent protein kinase (PKA), which is scaffolded to Ca_V1.2 channels by A-kinase anchoring proteins (AKAPs). Ca_V1.2 channels also undergo negative autoregulation via Ca^{2+}-dependent inactivation (CDI). CDI relies upon binding of Ca^{2+}/calmodulin (CaM) to an IQ motif in the carboxy tail of Ca_V1.2 L-type channels, a mechanism seemingly unrelated to phosphorylation-mediated channel enhancement. In neurons, AKAP79/150 anchors both PKA and the Ca^{2+}-activated phosphatase calcineurin (CaN) to Ca_V1.2 channels. Using transfected tsA201 cells or neurons, and tools such as the isolated calcineurin autoinhibitory peptide, over-expression of the catalytically-inactive CaN_{H151A} mutant, and RNAi suppression of AKAP79, we have found that channel-linked CaM serves as a Ca^{2+} sensor for CaN, and that Ca^{2+}/CaM-activated CaN participates in CDI by reversing channel enhancement by kinases such as PKA. We have also observed that I→E substitution in the IQ motif produces a mutant $Ca_V1.2_{I/EQ}$ channel that - when co-expressed with AKAP79 in tsA201 cells - unexpectedly exhibits ultra-fast inactivation. Ultra-fast inactivation is eliminated in Ca^{2+}-free Na^+ external solution, as well as by over-expression of the CaN_{H151A} mutant or stimulation of PKA with forskolin. One interpretation is that the intact IQ motif's affinity for Ca^{2+}/CaM limits the speed of CDI, and that reducing IQ affinity for Ca^{2+}/CaM via I→E substitution allows CDI to proceed at a greatly speeded rate. FRET results with the $Ca_V1.2_{I/EQ}$ mutant or with AKAP79 lacking the CaN anchoring motif suggest that, during periods of elevated channel activity, the IQ-CaM and AKAP79-CaN interactions are both necessary for CaN-mediated reversal of current enhancement by PKA. In sum, our work supports a synthetic view fusing previous ideas regarding CaM and phosphorylation signaling in CDI.

3609-Pos

Sumoylation of Voltage-Gated Alpha1a Calcium Channels

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The trinucleotide repeat disorder Spinocerebellar ataxia type 6 (SCA6) results form alterations in the *CACNA1A* gene coding for the α_{1A} subunit of the neuronal voltage-dependent P/Q-type calcium channel (Ca_V2.1). We have previously reported that the α_{1A} subunit is susceptible to proteasomal and lysosomal-mediated degradation, and that ubiquitin-mediated degradation is abnormal in SCA6 α_{1A}, consistent with a glutaminopathy component in SCA6. The Small Ubiquitin-related Modifier (SUMO) is only distantly related to ubiquitin (18% homology), but shares many similarities with it, including a similar protein size, tri-dimensional structure, a C-terminal glycine-glycine motif for substrate conjugation and many PEST motif-containing substrate targets. SUMO's primary functions involve nuclear events and also include