obtained by knocking down PKCα with small interfering RNA duplexes. Taken together, these data demonstrate that PtdIns(4,5)P2 itself targets PKCα to the plasma membrane through the polybasic cluster located in the C2 domain, with this interaction being critical in the signaling network involved in neural differentiation.

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Probing Phosphoinositide Kinetics With A Voltage-sensitive Phosphatase

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Voltage-sensitive phosphatases (VSPs) have a voltage sensor linked to a phosphoinositide (PI) 5-phosphatase, which hydrolyzes plasma membrane PI-(4,5)-bisphosphate (PIP2) to PI(4)P (Iwasaki, PNAS 105, 7970). We used PIP2 hydrolysis by VSP from Ciona intestinalis (ci-VSP) and zebrafish (dr-VSP) to better understand PIP2 binding and resynthesis. PIP2 was monitored using the PIP2-sensitive M-current (KCNQ2/3) and FRET between a pair of PIP2-binding probes (PH-PLCδ1-CFP & PH-PLCδ1-YFP). Depolarizations to +100 mV lasting >50 ms reduced M-current and PH-probe FRET. PIP2 depletion was saturated by depolarizations lasting 500-1000 ms. Evidently PH-probe FRET and M-current respond quickly to changes in plasma membrane PIP2.

After repolarization, PH-probe FRET and M-current relaxed to baseline values with time constants of ~10 s in a wortmannin-insensitive manner. This reflects endogenous PIP 5-kinase converting PI(4)P back to PI(4,5)P2. Overexpression of PIP 5-kinase increased the length of depolarization required to deplete PIP2, and speeded PIP2 recovery after repolarization.

Recovery of PIP2 after VSP activation is ~10x faster than after PLC activation. However, it only requires PIP 5-kinase, whereas recovery after PLC activation requires PI 4-kinase and PIP 5-kinase in series. Thus PI 4-kinase must be the slower enzyme. To estimate the rate of PI 4-kinase, we compared translocation of a fluorescent probe that reports plasma membrane PI(4)P (PH-OSH2, T.Balla) to translocation of the PIP2-binding probe in confocal time-lapse imaging. Upon PLC activation through M1 receptors, the plasma membrane PI(4)P signal decreased 20 s later than the PIP2 signal. Both probes recovered with similar time courses. FRET photometry between either PIP2-probes or PI(4)P-probes showed comparable results. The simultaneous recovery of both probes is consistent with the hypothesis that recovery of PIP2 is governed by rate-limiting synthesis of PI(4)P by the PI 4-kinase, followed by rapid conversion of PI(4)P into PI(4,5)P2 by the PIP 5-kinase.

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Interaction Of $PTEN_{1-21}$ Peptide With Phosphatidylinositol-4,5-Bisphosphate: A ^{31}P NMR Relaxation Study.

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Phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) is by far the most abundant of all phosphoinositides (about 1% of all membrane phospholipids) and is found primarily at the cytoplasmic leaflet of the plasma membrane. $PI(4,5)P_2$ can be converted by class I PI 3-kinases to $PI(3,4,5)P_3$, which is a second messenger molecule affecting processes like cell survival and proliferation. PTEN is an important tumor suppressor protein that converts $PI(3,4,5)P_3$ back to $PI(4,5)P_2$, thereby keeping the basal levels of $PI(3,4,5)P_3$ low. We have demonstrated recently that PTEN is allosterically activated by $PI(4,5)P_2$, which interacts with the N-terminal end of PTEN. Here we study the interaction of the $PI(4,5)P_2$ binding domain of PTEN (PTEN1-21) with model membranes containing $PI(4,5)P_2$ using ^{31}P-NMR. Using both magic angle spinning (MAS) and static solid state NMR we probe the interaction of PTEN1-21 with lipid phosphates. We determined the intrinsic T_1 and T_2 relaxation times of the phosphomonoester groups of $PI(4,5)P_2$ and the phosphodiester group of dioleoylphosphatidylcholine, which form the lipid matrix into which $PI(4,5)P_2$ was dispersed. PTEN1-21 is highly basic, containing several Lys and Arg residues which are thought to give rise to a largely electrostatic PTEN/$PI(4,5)P_2$ interaction. We show here that the binding of PTEN1-21 to $PI(4,5)P_2$ bilayers dramatically affects the membrane structure, indicating that the PTEN1-21/$PI(4,5)P_2$ interaction is likely more than a simple electrostatic interaction. This is in accordance with our recent findings that PTEN/phosphoinositide interaction is specific for $PI(4,5)P_2$. Lys13 is crucial for this specific interaction and this study explores the interaction of the Lys13 of PTEN with the phosphomonoester groups of $PI(4,5)P_2$.

491-Pos Board B370

Plasma Membrane Order In T Cell Signalling

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Plasma membrane nanodomains, referred to as lipid rafts, more ordered than the bulk membrane play an important role in T cell signalling by forming signalling platforms in activated T cells. However, the existence of lipid rafts in resting T cells is contentious. Using laurdan, a membrane probe whose peak emission wavelength depends on the lipid environment, evidence is presented for the existence of ordered nanodomains in resting T cells.

T cell signalling can be initiated by stimulating the T cell receptor (TCR), crosslinking the lipid raft markers GM1 (sphingolipid) or glycosylphosphatidylinositol (GPI) anchored proteins. The aggregation of lipid raft components induces the same response in Jurkat T cells as the ligation of an antigen to the TCR. Changes in membrane order linked with reorganization of the plasma membrane upon Jurkat T cell activation were followed at 37°C. Fluorescent images were analyzed for generalised polarisation values - a measure of the relative abundance of liquid ordered and liquid disordered domains. TCR patching does not increase the overall membrane order suggesting that membrane domains of high order are brought together in the patches. This supports the existence of small ordered membrane domains in resting T cells that aggregate upon activation. Patching of GM1, the GPI-anchored protein CD59 and the non lipid raft marker CD45 significantly increases the overall membrane order. So does general crosslinking of membrane components with Concanavalin A. Remodelling of the actin cytoskeleton is an integral part of TCR signaling and T cell activation. Disrupting actin polymerization using latrunculin B decreases membrane order and stabilizing actin filaments with jasplakinolide increases membrane order. An increase in membrane order appears to be a general effect of plasma membrane component patching and is likely due to a global induction of actin polymerization at the plasma membrane.

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Adsorption Of Bar-domain Proteins To Charged Lipid Membranes Causes Deformations And Lipid Demixing

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Many proteins participating in cellular processes contain BAR domains that have been implicated in membrane shaping and deformation. These BAR domains can either induce significant membrane curvatures or sense high-curvature regions on cell membranes, but the mechanism for this action is still not well understood. One suggestion is that BAR domains work collectively and achieve significant bilayer deformations only through a suitable organization at membrane interfaces. In contrast, evidence from some atomistic simulations suggests that a single BAR can substantially deform a lipid membrane locally. Here we present results from a self-consistent mean-field model of BAR domain association on membranes, suggesting that a single Amphyphisin BAR is capable of producing a steady state, where the initially near-planar membrane curves significantly. However, using our approach we predict that such deformation will occur only for membrane patches that have the propensity to attain high spontaneous curvature, and that such favorable preconditioning may be the result of either local lipid demixing, or of a preceding insertion of the BAR domain's amphyphatic N-helix. Both events have been predicted to bring about asymmetry in the two membrane monolayers. To contrast, our simulations also show that local segregation of charged lipids under the influence of the adsorbing BAR domain alone cannot produce high enough asymmetry between bilayer leaflets, and that in the absence of additional energetic sources that favor membrane asymmetry, the membrane will remain near-flat within fluctuations upon BAR adsorption. Thus, we conclude that N-helix insertions may have a critical mechanistic role in the function of the BAR domain, and that the electrostatic interactions between BAR and membrane are essential for sensing and stabilization of bilayer curvature.

493-Pos Board B372

Inducing and Reversing Anesthesia with Temperature Variation - Experiments on an Excised Frog Sciatic Nerve

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The Meyer-Overton Rule and other more recent experimental observations suggest that the fluidity of the lipid membrane is involved in nerve propagation and in mechanisms behind anesthesia. In other words, Hodgkin-Huxley may not be

telling the whole story. Under physiological conditions a cell membrane operates very close to the melting transition, i.e., to the point where the fluid membrane becomes a solid gel. Recent theoretical work indicates that signal propagation in a nerve cell may also involve a thermo-acoustic pulse of partial gellification. Natural selection should have led to optimal propagation under physiological conditions.
When apolar molecules are dissolved in the apolar membrane of the nerve cell, the freezing temperature of the membrane is lowered. This would interfere with pulse propagation and thus lead to anesthesia. However, if the theory is right, the effect should be reversed if we let the propagation take place at lower temperature. This is because the lower temperature would bring us closer again to the freezing transition.
We experimentally test this idea on the sciatic nerve of frogs. We follow the propagation of a signal with different concentrations of Argon in the medium and at different temperatures. Argon is an anesthetic that is chemically inert and it is expected to have its anesthetic effect just through interfering with the fluidity of the membrane. As a control we also perform the same experiments with Lidocain as the involved anesthetic. Lidocain is an anesthetic that is well known to work through interfering with voltage gated sodium channels.

494-Pos Board B373
Ci-VSP Is A Depolarization-Activated PI(4,5)P2 And PI(3,4,5)P3 5' Phosphatase
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Phosphoinositides are membrane-delimited regulators of protein function and control many different cellular targets. The differentially phosphorylated isoforms have distinct concentrations in various subcellular membranes, which can change dynamically in response to cellular signaling events. Maintenance and dynamics of phosphoinositide levels involve a complex set of enzymes, among them phospholipases and lipid kinases and phosphatases. Recently, a novel type of phosphoinositide-converting protein, termed Ci-VSP, was isolated, which contains a voltage sensor domain. It was already shown that Ci-VSP can alter phosphoinositide levels in a voltage-dependent manner. However, the exact enzymatic reaction catalyzed by Ci-VSP is not known. We used fluorescent phosphoinositide-binding probes and total internal reflection microscopy together with patch-clamp measurements from living cells to delineate substrates and products of Ci-VSP. Upon activation of Ci-VSP by membrane depolarization, membrane association of $PI(4,5)P_2$ and $PI(3,4,5)P_3$-specific binding domains decreased, revealing consumption of these phosphoinositides by Ci-VSP. Depletion of $PI(4,5)P_2$ was coincident with an increase in membrane PI(4)P. Similarly, $PI(3,4)P_2$ was generated during depletion of $PI(3,4,5)P_3$. These results suggest that Ci-VSP acts as a 5'-phosphatase of $PI(4,5)P_2$ and $PI(3,4,5)P_3$.

IP3 Receptors

495-Pos Board B374
Toward A Computational Model Of IP3R1-associated Ataxia
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Individuals with ataxia suffer impaired imbalance and incoordination of motor functions. Approximately 150,000 Americans are afflicted with ataxia, as are thousands of individuals worldwide. Among these are families with reduced levels of IP3R1 protein, the primary receptor for IP3 in cerebellar Purkinje neurons. Mice with reduced levels of IP3R1 are also ataxic; cerebellar microsomes from IP3R1 knockout mice exhibit little calcium release when probed with IP3. This suggests that altered calcium response to IP3 may mediate the pathophysiology of cerebellar ataxia associated with reduced IP3R1. Currently, there are no direct therapeutics for hereditary ataxias. We hypothesized that adjusting IP3R1 sensitivity to IP3 in the context of reduced IP3R1 could restore normal calcium response. To investigate our hypothesis, we adapted a computational compartmental model of a cerebellar Purkinje neuron previously published by our laboratory, using optimal parameters for calcium release. These parameters were dependent on the shape of the IP3 signal produced from PIP2 hydrolysis, determined in a recent study published by our group. In our optimized model, we reduced the value of *Jmax,* the variable representing IP3R1 abundance in Purkinje spines, to 50%, 40%, 30%, 20%, and 10% of the normal level of IP3R1 found in mouse cerebellum. Next, we adjusted the sensitivity of IP3R1 to IP3 in a similar cumulative fashion to see whether increasing sensitivity could rescue low abundance. We did this by varying values for d_{IP3}, the dissociation constant for IP3 from the receptor. We found that correspondent increases in IP3R1 sensitivity to IP3 restored normal calcium response when IP3R1 abundance was reduced to as low as 30% of its normal value. This promises significant therapeutic benefit for individuals with 'IP3R1-associated ataxia', as the phosphorylation status of IP3R1 can be regulated experimentally to adjust its sensitivity. (Supported by NIH RR013186)

496-Pos Board B375
Electron Cryomicroscopy of IP_3R1 Calcium Release Channel
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The inositol 1, 4, 5 - trisphosphate receptor (IP_3R) is an intracellular Ca^{2+} release channel that mediates ligand-gated release of Ca^{2+} from the endoplasmic reticulum (ER) into the cytoplasm. IP_3R1 is the predominant type in the cerebellar ER membrane where it forms homotetramers with a M_r over 1.2 MDa. The gating of IP_3R1 channel is still poorly understood due to the lack of high-resolution structure of the channel complex. Although several low-resolution 3D structures of the IP_3R1 were reported, these 3D maps are broadly consistent in the overall size and shape. To achieve a reliable structure of IP_3R1 channel at higher resolution, substantial improvements were made to cryo-specimen preparations that allowed acquiring electron images of ice-embedded channel protein, which exhibit substantially improved contrast and image quality. The structure of IP_3R1 was analyzed under conditions favoring the closed channel conformation, i.e. in the absence of the two co-agonists, Ca^{2+} and IP_3. Ice-embedded IP_3R1 particles were imaged at 60,000X magnification on a JEOL 2010F electron cryomicroscope with a Gatan 4k x 4k CCD camera. Image processing and the reconstruction were performed using EMAN. The improved map clearly exhibits more structural detail in both the cytoplasmic and membrane-spanning regions of the channel, connected through the stalk-like region. Available x-ray structures of the IP_3-binding core region (pdb code: 1N4K) and the ligand binding suppressor domain (pdb code: 1XZZ) were docked into the cryo-EM density map to interpret visualized structural domains. Currently, structural analysis of IP_3R1 in other physiologically relevant functional states is being performed to reveal the gating mechanism of the IP_3R1 channel.
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497-Pos Board B376
The Amplification Of InsP3R Activity By NCS-1 Is Attenuated By Medications Used In The Treatment Of Bipolar Disorder
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Neuronal Calcium Sensor-1 (NCS-1) is a high-affinity, low-capacity calcium-binding protein abundantly expressed in neuronal and neuroendocrine cells. We previously showed that NCS-1 interacts with the inositol 1,4,5-trisphosphate receptor (InsP3R) and modulates calcium signaling by enhancing InsP3-dependent InsP3R channel activity and intracellular calcium transients. Furthermore, it is known that NCS-1 is overexpressed in the prefrontal cortex of bipolar disorders and schizophrenic patients. Because we had reported that addition of lithium, a compound used for treatment of bipolar disorders, attenuates the NCS-1/InsP3R association, we hypothesized that other medications used for these disorders also might target the interaction between NCS-1 and the InsP3R. After overexpressing NCS-1 in a human neuroblastoma cell line to simulate the situation in the prefrontal cortex of bipolar patients, and using calcium sensitive dyes, we assessed the effect of the three main categories of medications used in bipolar disease on InsP3R-dependent intracellular calcium transients. We found that long-term treatment (8h) of cells overexpressing NCS-1 with therapeutic concentrations of chlorpromazine (CPZ) or valproic acid (VPA) attenuate the amplification effect of NCS-1 on InsP3-mediated Ca^{2+} release. This finding is dependent on NCS-1 overexpression and was not observed in cells with reduced NCS-1 levels due to shRNA mediated NCS-1 knockdown. Furthermore, no alterations due to treatment were observed in either the calcium loading of the intracellular stores or in the expression level of NCS-1 or $InsP_3R$. Therefore, the treatment with all three main categories of bipolar medications - lithium, anticonvulsants like VPA and antipsychotics like CPZ - appear to target the interaction between NCS-1 and the $InsP_3R$. This study suggests a new approach to investigating and understanding the etiology and treatment of bipolar disorder.

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The Role of the Pore-forming Region in the Regulation of IP3 Receptor by Luminal Ca2+
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It is well known that submaximal concentrations of IP3 release only a portion of the intracellular Ca2+ store via the IP3 receptor (IP3R), a phenomenon known