

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.

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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)P₂ and PI(3,4,5)P₃-specific binding domains decreased, revealing consumption of these phosphoinositides by Ci-VSP. Depletion of PI(4,5)P₂ was coincident with an increase in membrane PI(4)P. Similarly, PI(3,4)P₂ was generated during depletion of PI(3,4,5)P₃. These results suggest that Ci-VSP acts as a 5'-phosphatase of PI(4,5)P₂ and PI(3,4,5)P₃.

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 J_{max} , 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 IP₃R1 Calcium Release Channel

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The inositol 1,4,5-trisphosphate receptor (IP₃R) is an intracellular Ca²⁺ release channel that mediates ligand-gated release of Ca²⁺ from the endoplasmic reticulum (ER) into the cytoplasm. IP₃R1 is the predominant type in the cerebellar ER membrane where it forms homotetramers with a M_r over 1.2 MDa. The gating of IP₃R1 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 IP₃R1 were reported, these 3D maps are broadly consistent in the overall size and shape. To achieve a reliable structure of IP₃R1 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 IP₃R1 was analyzed under conditions favoring the closed channel conformation, i.e. in the absence of the two co-agonists, Ca²⁺ and IP₃. Ice-embedded IP₃R1 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 IP₃-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 IP₃R1 in other physiologically relevant functional states is being performed to reveal the gating mechanism of the IP₃R1 channel.

This research is supported by grants from NIH (R01GM072804, P41RR02250).

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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 Ca²⁺ 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 InsP3R. Therefore, the treatment with all three main categories of bipolar medications - lithium, anti-convulsants like VPA and antipsychotics like CPZ - appear to target the interaction between NCS-1 and the InsP3R. 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