2625-Pos
Structure Function Studies of the Proton Coupled Folate Transporter
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Folate vitamins are essential for DNA replication and cellular proliferation. However, mammalian organisms are devoid of folate biosynthesis and rely on dietary sources to meet their metabolic requirement for folate cofactors. The proton coupled folate transporter (PCFT/SLC46A1) has been recently identified as the molecular entity responsible for intestinal folate uptake displaying optimal transport activity at acidic pH. PCFT is also involved in transport of chemotherapeutically used antifolates. Currently, there is limited experimentally derived information about the structure and transmembrane topology of PCFT. Hydropathy analysis suggests 10 to 12 transmembrane segments for PCFT. The aim of our study is to construct a reliable homology model and study the structure function interplay in more detail experimentally. We have made various DNA constructs with different N- and C-terminal tags for the heterologous expression and purification of PCFT: 1) for bacterial expression of the gene, 2) for expression in oocytes and mammalian cells, 3) for cell free expression using the Membrane Max cell free expression kit. We will screen the expression systems to determine which is optimal for generating suitable quantity / quality protein that will be used for structural studies. We will engineer individual Cysteines to study the topology with the Substituted Cysteine Accessibility Model (SCAM). Accessibility will be assessed by Western Blotting (PEGylation, biotinylation). The lipid-protein interface will be investigated by hydrophobic photoaffinity labeling. Both SCAM and photoaffinity labeling will be used to study substrate pathways. We will complement these studies with functional assays (uptake and two electrode voltage clamping). Results from our structure function studies of PCFT will be used in exploring therapeutic strategies for folate malabsorption and in optimizing antifolate drug therapies.

2626-Pos
Substrate Specificity of the Aminophospholipid Flippase and Other Phosphatidylserine Binding Proteins
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The generation and maintenance of the asymmetric distribution of phosphatidylserine across the cell plasma membrane is regulated by an ATP-dependent, substrate specific lipid flippase. Transport activity is strongly dependent on the structure of its preferred substrate, 1,2-sn-glycerophospho-L-serine. With the exception of methylation of the primary amine group, any modification to the structural elements comprising the polar portion of the molecule results in significantly reduced rates of substrate transport. Substrate lipids are stereospecifically recognized by the flippase. The 1,2-sn-glycerol isomer, but not the 2,3-sn-glycerol isomer of PS is transport competent, but the enzyme does not distinguish between the L- and D-serine isomers of PS. This substrate specificity defines the characteristics of the lipid binding site and also provides biochemical criteria for the identification of putative flippases. By searching for ATPases that are stereoselectively activated by PS, a candidate flippase has been purified from human erythrocytes. This enzyme is uniquely activated by PS in both detergent micelles and in phospholipid bilayers, and demonstrates a stereochemical specificity identical to that expressed by the flippase. A member of a new class of P-type ATPases, which have been associated with PS flippase activity, has been expressed in insect cells and purified. This enzyme (ATP8A1) is also activated exclusively by the 1,2-sn-glycerol isomer of PS, regardless of the stereochemistry of the serine headgroup. The substrate specificity expressed by the flippase and these two ATPases is unique among PS-binding proteins, including blood clotting factors, protein kinase C or the macrophage PS receptor, each of which interact only with the L-serine, and not the D-serine isomer of PS. These studies indicate that at least two distinct PS binding motifs have evolved to enable proteins to selectively interact with PS.

Auditory Systems

2627-Pos
Selective Activation of Vestibular Hair Cells by Infrared Light
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Optical stimulation using pulsed infrared light is novel method with potential for selectively stimulating a small group of neurons. In present study, we show that infrared light can selectively activate vestibular hair cells and may offer a novel method to investigate biophysical mechanisms underlying the response. In experiments conducted in toadfish, O. tau, horizontal semicircular canal afferents showed a mix of inhibitory and excitatory responses evoked by infrared radiation. In a subset of afferents the background discharge rate decreased, while in other afferents the background discharge rate increased with infrared radiation. Excitatory vs. inhibitory afferent responses correlated with the dynamic adaptive properties of afferent responses observed during mechanical stimuli. Primary semicircular canal afferents in the toadfish are known to receive convergent inhibitory (GABA) and excitatory (glutamate) synaptic input from hair cells that ultimately shape the afferent discharge response. The present data indicate that afferents known to synapse on glutamatergic hair cells increase their discharge rate with incident infrared light, consistent with depolarization of hair cells and increased tonic release of glutamate. Acceleration-coding afferents which synapse on combinations of Glutamatergic and GABAergic hair cells were observed to reduce their discharge rate with infrared light, again indicating depolarization of hair cells but, in this case the inhibition was consistent with increased release of GABA. Repeated optical radiation elicited depolarization/repolarization of hair cells. Sensitive afferents were observed to phase-lock their discharge with the pulsed light stimulation. Since the entire epithelium was irradiated, this argues against simple kT thermodynamic modulation of hair cell channel kinetics. The results compel the hypotheses that infrared light stimulation increases the open probability of IR-sensitive ion channels and depolarizes hair cells due to the influx of cations. [Supported by NIH R01 DC06685 (Rabbitt, RD) and NIH grant 1R41DC008515-02 (Richter, CP)].

2628-Pos
Organ of Corti Micromachine Enables Hair Bundles to Deform the Stiff Basilar Membrane
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The organ of Corti (OC) is a highly organized structure in the mammalian cochlea that houses the sensory hair cells. It is believed that the OC functions to optimize force transmission from the outer hair cell (OHC) to the basilar membrane and the inner hair cell. Recent studies reveal that the OC cannot be considered as a rigid body and has a complex mode of deformation. We developed a 3-D finite element model of the OC to dissect its mechanics. Geometric and mechanical information was taken from the gerbil cochlea at 2 and 10 mm from the stapes, positions encoding high and low frequencies respectively, and in each case several hundred microns longitudinal extent was simulated. The model included all structurally significant components: OHCs, pillar cells, Deiters cells and reticular lamina. The model was validated by reproducing experimental results on point stiffness and longitudinal space constant measured at the basilar membrane and response to current steps through the OC. Deformation of the OC by two different active OHC forces (the OHC somatic force and the stereociliary-based force) was then simulated. A surprising result was that despite smaller magnitude, the stereociliary-based force (0.1and 0.7 nN at apex and base) was nearly as effective as the somatic force (10 nN) in displacing the basilar membrane. The results also suggested that for the active forces to work efficiently the radial stiffness of the tectorial membrane must be comparable to or greater than the hair bundle stiffness. Funded by NIH RO1 DC01362.

2629-Pos
Friction and Adhesion in the Hair Bundle's Glycocalyx
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To enable us to hear high-frequency sounds, the mechanosensitive hair bundles of our inner ears must oscillate and amplify at frequencies up to 20 kHz, overcoming both viscous drag and friction within the hair bundles. How can such movements be accomplished for a lifetime without harming cellular structures and causing hearing loss?
The hair bundle is a cluster of linked, finger-like projections, the stereocilia, emerging from the hair cell's apical surface. Each stereocilium is composed of an actin core surrounded by membrane endowed with a glycocalyx, a layer of glycoproteins and glycolipids. The stereocilia stand apart from one another at their basal insertions, but lean toward one another at their tips, where they are connected along the axis of mechanosensitivity by fragile tip links. For efficient sensory transduction to occur, mechanotransduction channels must open in unison when the bundle is deflected, suggesting that the stereocilia exhibit some form of low-friction sliding adhesion. One possible mechanism by which such sliding adhesion might be implemented is through charged sugars such as sialic acid. If stereocilia are coated with negatively charged sugars, they