2753-Pos Board B723
Aggregation of polyene antibiotics in aqueous solution. An MD study
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It has been proposed that the distinct action of polyene antibiotics on ergosterol or cholesterol containing membranes can be due to the insertion of the drug in the monomeric or aggregated form in the ergosterol containing membrane whereas in the cholesterol containing membrane only aggregates of the drug can insert into the membrane. Aggregation can occur in the aqueous solution previous to absorption onto the membrane. It is therefore interesting to study the aggregation of polyenes in aqueous solution. In this work a molecular dynamics study of the aggregation of Amphotericin B (AmB), and several derivatives having greater and smaller selectivity for ergosterol vs cholesterol membranes, where performed. The molecular mechanism for aggregation were studied. That is, if aggregation is driven by hydrophobic forces, hydrogen bond formation or dipole-dipole interactions. Validation of the potential constructed for the polyenes were tested against the known aggregation thresholds of AmB in water and dimethyl sulfoxide (DMSO), as well as a comparison with nuclear magnetic resonance (NMR) available for aggregation in DMSO.

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An Approach to Characterizing Single-Subunit Mutations in the Anthrax Protective Antigen Prepore and Pore
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Heptameric pores formed by the Protective Antigen (PA) moiety of anthrax toxin translocate the intracellular effector moieties of the toxin across the endosomal membrane to the cytosol. To elucidate the effects of selected mutations in PA, we devised a protocol for characterizing heptameric pore precursors (prepores) and pores containing an inhibitory mutation in only one subunit. We prepared monomeric PA containing two mutations: the test mutation and an innocuous Cys-replacement mutation (K563C) at an external site on the prepore. The introduced Cys was derivatized with a biotinylating reagent, and the mutated protein was allowed to cooligomerize with a 20-fold excess of wild-type PA. Finally, the biotinylated prepores were purified by avidin affinity chromatography and characterized in various assays. We used this protocol to examine mutations at D425 and F427, two residues where mutations are known to have strong inhibitory effects. The D425A mutation caused an inhibition by >104 of pore formation and a corresponding abrogation of transport activity. The F427A mutation caused ~100-fold inhibition of translocation across planar bilayers and smaller effects on pore formation and ligand affinity. These results show definitively that the protein transport activity of PA may be abrogated by altering a single residue in one subunit of the heptameric prepore, explaining the dominant-negative phenotype. The protocol described may be applied to the detailed characterization of various mutations in PA and other homooligomeric systems.

Epithelial Channels & Physiology

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Small G protein-induced trafficking of the Epithelial Na^+ channel
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The long term control of blood pressure involves Na^+ homeostasis through the precise regulation of the Epithelial Na^+ Channel (ENaC) in the aldosterone-sensitive distal nephron. ENaC activity is regulated, in part, by trafficking to the plasma membrane. Membrane levels of ENaC reflect constitutive delivery and regulated retrieval. To explore the action of small G proteins on the activity and trafficking of ENaC we utilized electrophysiological recordings of ENaC, biochemistry and fluorescence microscopy. RhoA increases ENaC activity by promoting channel trafficking to the plasma membrane. Direct visualization of ENaC movement near the plasma membrane with TIRF-FRAP revealed that RhoA accelerates ENaC trafficking toward the membrane. RhoA-facilitated movement of the channel was sensitive to disrupting the endomembrane system. Moreover, facilitating retrieval decreased ENaC activity but not trafficking toward the membrane. Rab11a and Rab3a are well-established as a participant in the regulation of recycling trafficking. Co-expression of Rab11a and Rab3a with ENaC results in a significant increase in channel activity. Biochemical and imaging methods demonstrate that Rab11a colocalized with ENaC and increased ENaC activity by affecting the plasma membrane levels of this channel. Rab11a increases ENaC activity in an additive manner with dominant-negative dynamin, which is a GTPase responsible for endocytosis. Brefeldin A, an inhibitor of intracellular protein translocation, blocked the stimulatory action of Rab11a on ENaC activity. This is consistent with a mechanism of increased trafficking toward the plasma membrane. We conclude that RhoA, likely through effects on the cytoskeleton, promotes ENaC trafficking to the plasma membrane to increase channel membrane levels and activity. Moreover, we hypothesize that ENaC channels, present on the apical plasma membrane, are being exchanged with channels from the intracellular (recycling) endosomes in a Rab11-dependent manner.
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Quantitative Analysis Of DEG/ENaC Subunits Interaction
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Members of the DEG/ENaC protein family are voltage-independent, amiloride-sensitive cation channels. This family includes acid sensing ion channels (ASICs), which are involved in sensory function, modulation of learning and memory in the nervous system. Another branch of the DEG/ENaC family includes epithelial sodium channels (ENaCs) that are key players in sodium transport across epithelia. 'Classical' ENaCs are formed by three different subunits named α, β and γ. A forth subunit, δ, has been found in humans and other primates. It can form channels with ENaC β and γ but it is mainly expressed in non-epithelial tissues such as the nervous system. Several lines of evidence suggest that DEG/ENaC subunits may interact promiscuously to form heteromeric ion channels with diverse biophysical properties. Thus, δ could modulate αβγ channels or form channels with other DEG/ENaC subunits in tissues where α, β or γ are not expressed. ASICs have been proposed to interact with ENaC subunits to form heteromeric ion channels and also to directly modulate other ion channels such as BK. To assess the physiological relevance of these interactions, we need to: a) verify subunits co-expression in native tissues; b) quantify the efficiency of subunits interaction; c) biophysically characterize the different subunit assemblies. In this work we address some of these issues. We have quantified efficiency of subunits interaction by measuring membrane expression of fluorescently-tagged DEG/ENaC subunits in Xenopus oocytes. Our results show that δ is less efficient than α to form channels with β and γ subunits. Experiments to compare interaction efficiencies between other DEG/ENaC members and other ion channels are currently underway.

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Apical Methyl-β-cyclodextrin (mβCd) Treatment In A6 Renal Cells Does Not Affect Basolateral PGE_2-induced Cl^- Secretion , But Stimulates An Early Cl^- Peak Current, Activated By Hypotonic Shock
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We explore in this study whether cholesterol content of the apical membrane of distal kidney cells from *Xenopus laevis* (A6) affects the behavior of two types of anion channels, recently described by Bao et al. (AJP,2008,295,G234). Voltage clamp technique in an Ussing-type chamber, enabling the recording of short-circuit current, was applied on polarized monolayers of A6 cells, grown on permeable filter supports for 13 - 31 days. To create a gradient for Cl^- secretion, the apical solution was Cl^- and Na^+ free, whereas the basolateral solution contained normal NaCl. Twice basolateral addition of PGE_2 (1 μM) during 15 minutes with an interval of 60 minutes produced a first transient peak increase in I_{sc} of 7.25 ± 0.98 μAcm^{-2} (N=6), followed by a plateau of 2.77 ± 0.50 μAcm^{-2}. The second PGE_2 stimulation elicited a peak current of 6.63 ± 1.20 μAcm^{-2}. When the apical membrane was treated for at least 1h with 10 mM mβCD, PGE_2 evoked Cl^- secretion was not different from control. A hypotonic shock was induced twice with an interval of 60 minutes on the basolateral side by a sudden reduction of solution osmolality from 260 to 140 mOsm/kg H_2O. In control a transient increase in I_{sc} of 0.27 ±0.11 μAcm^{-2} (N=3) was observed during the second stimulation only. After 60 minutes treatment with 10 mM mβCD the hypotonic shock evoked an augmented transient I_{sc} of 1.82 ± 0.08 μAcm^{-2} (N=5). These results suggest that apical Cl^- channels in A6 cells, activated either by PGE2 or by cell swelling, are regulated differently by the membrane cholesterol content. This work was supported by the grants FWO Flanders G.0270.07 and BOF 03B0BF.

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Bile Acids Selectively Activate Iberiotoxin-sensitive Potassium Channels In Native Pancreatic Duct Cells
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Bile reflux into the pancreas is a common causes of acute pancreatitis. Our group has shown that luminal chenodeoxycholate (CDC) at low doses