indicating primarily elasticity changes, whereas amitriptyline and imipramine had equal effects on the different length gA, indicating primarily curvature changes.

2486-Pos

Thiazolidinediones Alter Lipid Bilayer Properties and Native Voltage-Gated Sodium Channel Function

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Thiazolidinediones (TZD) are selective peroxizome-proliferator receptor gamma (PPARγ) agonists that are used to treat hyperglycemia in type 2 diabetes. In addition to their hypoglycemic actions they have anti-inflammatory, anti-atherosclerotic and cardiovascular effects, but PPARγ activation does not account for all their actions. Three TZDs - troglitazone (Resulin), rosiglitazone (Avandia), and pioglitazone (Actos) - have been marketed; troglitazone was subsequently withdrawn due to hepatotoxicity and .a precursor TZD - ciglitazone- was discontinued after phase II trials. TZDs, with troglitazone being the most potent, modulate L-type calcium and delayed-rectifier potassium channels by a seemingly PPARγ-independent mechanism. This could result from the adsorption of the amphiphilic TZDs to the membrane/solution interface, which can alter bilayer properties such as thickness, intrinsic curvature and the elastic moduli, and thus membrane protein function. We therefore examined whether TZDs alter lipid bilayer properties. We exploited the sensitivity of gramicidin channels to changes in bilayer properties to test for TZD-induced bilayer effects. TZDs alter gramicidin channel function and shift the monomer-dimer equilibrium toward the conducting dimers. Using gramicidin channels of different lengths we find that the TZD effects do not vary with changes in hydrophobic mismatch. Increasing bilayer stiffness with cholesterol amplifies the TZD-mediated changes in gramicidin channel function. Based on the concentrations at which we observe changes in gramicidin lifetime and appearance frequency, the potency is troglitazone>rosiglitazone> ciglitazone>pioglitazone, consistent with their effects on native membrane proteins. We examined the TZDs effects in native membranes using neuronal voltage-gated sodium channels (Na_V) using whole-cell recordings. All TZDs caused a negative shift in the voltage-dependence of inactivation at concentrations similar to those that alter gramicidin channel function. Our results show that TZDs affect bulk membrane properties at concentrations that modulate native ion channels.

2487-Pos

NSAIDs Alter Bilayer Properties by a Common Mechanism

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Non-Steroidal Anti-inflammatory Drugs (NSAIDs) exert their primary action through inhibition of the Cyclo-oxygenase enzymes (COX-1 and COX-2). In addition to these COX-dependent effects, NSAIDs alter the function of a number of membrane proteins, by seemingly COX-independent mechanisms. Given that NSAIDs are amphiphiles, and that they modulate the function of different, structurally unrelated membrane proteins, we tested whether NSAIDs could alter lipid bilayer material properties at the same concentrations where they alter membrane protein function. To measure such changes in bilayer material properties, we used gramicidin A (gA) channels as molecular force transducers. We found that Aspirin, Salicylate, Sulindac, Sulindac Sulfide, Ibuprofen, Diclofenac and Flurbiprofen alter bilayer mechanical properties. At pH 7, NSAIDs increase both the lifetime and appearance rate of channels formed by both short (13-residue) and long (15-residue) gramicidin analogues, meaning that they shift the gA monomer<->dimer equilibrium toward the conducting dimers. The changes in gA channel function depend on the channel-bilayer hydrophobic mismatch, as we observe the larger effects on the shorter channels - the channels with the larger hydrophobic mismatch. We also found that, when comparing the effect of each NSAID, the relative changes in the lifetimes of the shorter and the longer channels could be described by the same linear relationship. We therefore conclude that NSAIDs decrease lipid bilayer stiffness by a common mechanism, through an increase in bilayer elasticity, and that specific channel-NSAID interactions are not involved. These effects were achieved at the high end of clinically relevant concentrations, and raise the possibility that in both the clinical and research setting, NSAIDs may have effects that arise from modulation of lipid bilayer mechanical properties.

2488-Pos

Supported Lipid Bilayers on Skeletonized Zirconium Phosphonate Surfaces For the Study of Transmembrane Proteins

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Supported lipid bilayers that can fully represent biological cell membranes are attractive biomimetic models for biophysical and biomedical applications. In previous work, we developed a new approach to engineer stable supported lipid membranes. This system uses the zirconium phosphonate substrate as a reactive surface that tethers the lipid membrane via a highly covalent bond between surface zirconium ions and divalent phosphate groups in the lipid assembly. An advantage of this approach is that the zirconium phosphonate can be applied to any surfaces (gold, glass, silicon) allowing different analytical techniques to be used on the same system. However, the covalent interaction between the zirconium phosphonate film and the inner lipid monolayer that is responsible for the bilayer stability restricts membrane components such as transmembrane proteins from penetrating into the membrane and retaining their mobility. In contrast to other approaches that incorporate pillars to support the bilayers, our strategy involves the creation of reservoirs beneath the supported lipid bilayers to accomodate transmembrane proteins. Skeletonized zirconium phosphonate surfaces have been designed using the Langmuir-Blodgett (LB) technique. Sizes of the nanometer-holes were varied by controlling the amount of octadecylphosphonic acid in mixed LB layers and characterized by atomic force microscopy. The skeletonization of the film was optimized so the hollow spaces were large enough to incorporate the proteins but small enough for the membrane to bridge the spaces. The efficiency of this system as a support for transmembrane proteins has been characterized by surface plasmon resonance enhanced ellipsometry (SPREE) using the proteins Annexin V and the maxi-K ion channel to demonstrate the utility of the system as a functional cell membrane. This concept is innovative in the area of bilayer platforms and can be used with membrane and transmembrane proteins.

2489-Pos

Critical Dependence of the Biophysical Activity of Pulmonary Surfactant Films on Temperature

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Pulmonary surfactant is a lipid-protein complex that stabilizes the respiratory surface of lungs. Once secreted into the alveolar spaces, surfactant adsorbs rapidly at the air-liquid interface reducing surface tension upon compression to near 0 mN/m. Within a given animal species, surfactant composition is influenced by development, disease, respiratory rate, and/or body temperature. In principle, surfactant collected from animals functions optimally at the body temperature 3of the animal at the time of sample collection.

Changes in temperature can alter the physical state and the molecular packing of surfactant membranes and films, potentially altering their biophysical performance. We have analyzed the effect of temperature on the structure of native surfactant, by differential scanning calorimetry (DSC) and fluorescence spectroscopy with the fluorescent probes DPH (Diphenylhexatriene) and Laurdan (6-Lauroyl-2-(N,N-dimethylamino)naphthalene). The spectral properties of these probes have been used to assess lipid packing and fluidity in surfactant as a function of temperature and compression state. The effect of temperature on the interfacial performance of surfactant has been evaluated by analyzing spreading capabilities in a surface balance and compression-expansion dynamics in a Captive Bubble Surfactometer. Native surfactant from porcine lungs showed optimal adsorption at temperatures around 37 oC, reaching minimal surface tensions below 2 mN/m upon quasi-static or dynamic compression-expansion cycling. Critical structural transitions at temperatures above 39oC led to reduced interfacial adsorption and impossibility of compressed films to reduce surface tensions below 20 mN/m, suggesting that surfactant composition has been optimized to work at a narrow interval of temperatures and that regulatory factors may be involved in adaptation of surfactant structures to changes in body temperature.

2490-Pos

The Effect of Membrane Spanning Peptides on Laurdan and Di-4-ANEPPDHQ Emission Spectra

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