Title

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essential membrane components and are vital for the organization and functioning of membranes of many organisms. However, different organisms produce different types and number of sterols, and the biological significance of such diversity is still poorly understood. For example, membranes of bacterial cells are highly permeable to weak acids and glycerol, while yeasts can maintain large concentration gradients of these substances. In this work, we studied the effects of various sterols on the stability of membrane structures by electroporation.

1777-Pos

Investigating the effects of alcohols and cholesterol manipulations on bovine aortic endothelial cell membrane properties using phasor analysis of laurdan fluorescence lifetime microscopy and spectral imaging

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Endothelial cell membranes are made up of a heterogenous mixture of lipids and proteins and include liquid ordered (L_o) domains called lipid rafts and caveolae. These compartments have higher concentrations of cholesterol and sphingolipids and are concentrated in signaling molecules. Perturbations in membrane fluidity, including changes in cholesterol content can lead to changes in the structure of the plasma membrane and impair signaling mechanisms. In order to study how changing membrane fluidity can lead to changes in cellular signaling, we must be able to characterize changes in fluidity. Here we utilize two different methods using the fluorescent dye Laurdan, which exhibits a red spectral shift in emission when in a more liquid disordered (L_d) domain, to examine the effects of alcohols and cholesterol manipulations on membrane fluidity and cholesterol content in bovine aortic endothelial cells (BAEC). Laurdan-labeled BAECs were treated with dodecanol and benzyl alcohol to fluidize the membrane, methyl-β-cyclodextrin (MβCD) to remove cholesterol leading to an increase in membrane fluidity and MβCD complexed with cholesterol to enrich cells with cholesterol and decrease membrane fluidity. The emission spectra and the fluorescent lifetime images (FLIM) in two channels were collected. Analysis of the resulting spectral phasors and FLIM channel 1 phasors offer insight into changes in the polarity of the environment of the Laurdan molecule within the membrane, while FLIM channel 2 provides details on changes in water dipolar relaxations and cholesterol content.

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Polyunsaturated fatty acids differentially modulate membrane elasticity in membranes containing high cholesterol

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Membrane composition is important for the function of membrane proteins including ion channels and membrane-embedded receptors. One class of proteins that are especially sensitive to membrane composition are mechanosensitive channels, which activate in response to changes in membrane tension. Recently, polyunsaturated fatty acids (PUFAs) were demonstrated to alter mechanosensitive channel behavior, but the mechanism for these observations remains unknown. Mechanosensitive channel behavior is expected to be dependent on membrane mechanical properties such as the membrane area expansion modulus (K_a), which measures the extent to which membrane tension exerts a change in bilayer area. Despite these observations, the impact of non-esterified PUFAs on membrane K_a has never been measured. Using micropipette aspiration techniques, we measured the K_{app} (apparent K_a) of phospholipid model membranes containing various non-esterified polyunsaturated fatty acids. We found that polyunsaturated fatty acids with three or more unsaturated bonds decrease membrane K_{app}. We then determined how select ω-3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) affect the K_{app} of membranes containing cholesterol. Our results demonstrated that in the presence of high amounts of cholesterol, EPA and DHA had distinct effects on membrane $K_{app,}$ but this difference could not be observed when the cholesterol concentration was low. We hypothesize this result may be attributed to the propensity of membranes to phase-separate in the presence of high amounts of cholesterol. We also measured the effect of ω-3 PUFAs on other related membrane properties such as fluidity and bending rigidity to determine the relationship between the effect on K_{app} and other membrane properties. Our results point to the importance of the distribution of membrane amphiphiles and demonstrates that lipid raft formation may alter the bulk mechanical properties of the bilayer that are important for membrane protein behavior.

1779-Pos

Effect of procaine hydrochoride on lipid bilayers

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We are interested in the influence of natural osmolytes, *e.g.* Urea and TMAO (Trimethylamine N-oxide) on the fluidity and stability of lipid model membranes. Here we extend our interest to Procaine Hydrochoride. We used a combination of simultaneous SAXS/WAXS/DSC measurements, aiming to identify the structures, their dimensions and the phase transition temperatures. Thermal scans show morphologies similar to aqueous solution of the pure lipids, although with different dimensions and transition temperatures. Surprisingly in POPC, we observe a phase transition and upon temperature cycling, a variation of the lattice parameters of these phases. Fully hydrated POPC prepared in 400 mM Procaine Hydrochoride surprisingly produces a large structure at low temperatures ($15<T<27^oC$) that transforms into another with a smaller lattice parameter. The patterns on the scan show only one diffraction peak, not enough for a structure characterization, however we assume it to be lamellar. Moreover such diffraction peaks are of low intensity. Interestingly, these structures initially decrease slightly their lattices upon concentration of Procaine Hydrochoride, reaching a 'stable value' that minimally decreases further upon increasing the amount of additive. Upon the scan temperature range the SAXS scan signal indicates an organized structure, believed to be a stack of bilayers. From the observations above we assume a strong interaction of Procaine Hydrochoride with the surface of the lipid bilayer that can be overcome by heating, leading to a phase transition. Because this transition leads to a smaller lattice upon heating we propose that the Procaine Hydrochoride solubilizes in water and shifts to the excess water environment.

1780-Pos

The effect of dopamine on model lipid membrane structure

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Dopamine is a neurotransmitter released by neurons to send signals to other nerve cells. Previous work on the interaction of dopamine with model lipid bilayers made using solution-state NMR spectroscopy has shown that dopamine interacts with phosphatidylcholine (PC) and phosphatidylserine (PS) lipid headgroups primarily through the aromatic side opposite to the hydroxyl groups [1]. Here we use low-angle x-ray scattering (LAXS), solid-state deuterium NMR, as well as electron paramagnetic resonance (EPR) to determine the effect of dopamine on the physical properties of lipid membranes. X-ray measurements on oriented samples show that dopamine increases the interlamellar spacing (D) at full hydration. Preliminary analysis of scattering data also indicates a decrease of both membrane bending rigidity (K_C) and of the compression modulus (B) which is consistent with the increased D-spacing. However, the order parameters measured by solid-state deuterium NMR decrease only marginally in the presence of dopamine which indicates that dopamine neither enters the hydrocarbon region nor changes the thickness of the membrane. The fact that dopamine's effect is primarily on the headgroup region is further supported by EPR measurements of rotational correlation times of N-tempoyl palmitamide (N-TEMPO). This spin probe resides at the lipid-water interface and its correlation times are increased by dopamine in both PC and PS lipid membranes. These results can be relevant to a more complete understanding of signaling processes involving dopamine.

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1781-Pos

Amantadine preferential binding and disordering of phase separated membranes

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