and single-channel gA assays to examine the effects of (diC8) phosphoinositides -PI, PI(4,5)P_2, PI(3,5)P_2, PI(3,4)P_2 PI(3,4,5)P_3 as well as long-chain PI(4,5)P_2 on the lipid bilayer. The diC8 phosphoinositides, except for PI(3,5)P_2, alter lipid bilayer properties with potency that depends on their logP with PI being the most potent and PIP3- the least. When comparing di-oleoyl-PI(4,5)P_2 to the naturally occurring 1-stearyl-2-arachidonyl-PI(4,5)P_2 the naturally occurring PIP_2 is the more potent bilayer modifier, being active at 10 μM nominal concentrations in the planar bilayer assay. Our results show that application of exogenous PIP_2 and its structural analogues (with changes in acyl chain length or phosphorylation state) alters lipid bilayer properties. We propose that these PIP_2 lipid bilayer effects may play be important for some of its many different effects on membrane protein function.

2715-Pos Board B701
Fenamates Alter Bilayer Properties
R. Lea Sanford, Subhi J. Al'Aref, Roger E. Koeppe II, Olaf S. Andersen.
Fenamates are a family of non-steroidal anti-inflammatory drugs (NSAIDs). They are widely prescribed to manage pain and inflammation and, like other NSAIDs, inhibit the cyclooxygenases; they have also been proposed to have anti-epileptic and neuroprotective effects. The fenamates modulate a variety of ion channels, with mechanism(s) of action that range from direct fenamate-protein interactions (binding) to non-specific membrane-mediated effects. We therefore examined whether fenamates alter bilayer properties at concentrations where they modify membrane protein function. To this end, we used a gramicidin-based fluorescence assay to investigate whether the fenamates could alter bilayer properties (as sensed by a bilayer-spanning channel), and to establish dose response curves for the fenamates bilayer-modifying effects. The fenamates we examined were flufenamic acid, meclofenamic acid, mefenamic acid, niflumic acid and tolfenamic acid. All of them increased the rate of fluorescence quenching, meaning that they shifted the gramicidin monomer-dimer equilibrium in favor of the monovalent cation permeable dimers. These results thus show that fenamates alter bilayer properties, most likely by softening the bilayer. Niflumic acid was the most potent modifier of bilayer properties and tolfenamic acid the least potent. All the fenamates have limited solubility, which limited the concentration range that could be studied to 300 μM (or less). To examine the alteration of bilayer properties in more detail, mefenamic acid was tested using single-channel electrophysiology in planar bilayers. The electrophysiological results support the data from the fluorescence assay. The fenamates alter bilayer properties at the concentrations where they have been reported to alter membrane protein function. This suggests that there may indeed be a membrane contribution to the fenamates' mechanism of action.

2716-Pos Board B702
Statins Modify Bilayer Mechanical Properties
Subhi J. Al'Aref, R. Lea Sanford, Roger E. Koeppe II, Olaf S. Andersen.
Statins exert their primary mechanism of action through inhibition of HMG-CoA reductase, thereby preventing cholesterol synthesis. Additionally, statins have many, so called ''pleotropic'', effects that are independent of HMG-CoA reductase inhibition. Because statins are amphiphiles that modulate the function of different, structurally unrelated membrane proteins, we explored whether statins could alter lipid bilayer mechanical properties at the concentrations where they alter membrane protein function. To this end, we used a gramicidin-based fluorescence quench method as well as single-channel electrophysiology. We found that atorvastatin, fluvastatin, lovastatin, mevastatin, pravastatin, and simvastatin increase the rate of fluorescence quenching, meaning that they shift the gA monomer ↔ dimer equilibrium toward the conducting dimers. Statins thus alter bilayer mechanical properties, with fluvastatin being the most active and rosuvastatin the least active. When examined electrophysiologically, simvastatin, pravastatin, and fluvastatin increase the lifetime and appearance rate of channels formed by both short (13-residue) and long (15-residue) gramicidin analogues, with fluvastatin being the most active and pravastain being the least active. 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 conclude that statins alter lipid bilayer properties by a common mechanism, through an increase in bilayer elasticity, and that specific channel-statin interactions are not involved.

2717-Pos Board B703
Effects of Fluorinated Alcohols on Lipid Bilayers Properties
Mike Zhang, Helgi I. Ingólfsson, Olaf S. Andersen.
Fluorinated alcohols are unique alcohols that, while sharing a common overall structure with ''normal'' alcohols, have fluorines bonded to the carbons instead of the familiar hydrogens. The high electronegativity of fluorine shifts the charge distribution in the molecules, which changes their pK and together the larger bulk of fluorine relative to hydrogen alters their physico-chemical properties. Fluorinated alcohols are extensively used as organic solvents for solubilizing polymers and proteins especially in organic synthesis and NMR studies. In addition, the fluorinated form of 2-propanol (hexafluoroisopropanol (HFIP)) is used in Alzheimer's disease research to solubilize the amyloid-beta peptide. The fluorinated alcohols modify the function of various membrane proteins and are know to interact with lipid bilayers, altering bilayer properties, structure and stability. Additionally, HFIP has been shown to increase the conductance of planar lipid bilayers (Capone et al, NR 16:1, 2009). The question then becomes to what extent do the fluorinated alcohols alter bilayer properties and at what concentrations? We probed the membrane-modifying potential of the fluorinated alcohols: trifluoroethanol (TFE), HFIP, and nonafluoro-tert-butyl alcohol (PFTB) using a gramicidin-based fluorescence assay. All the fluorinated alcohols tested alter bilayer properties in the low (PFTB) to high (TFE) mM range. Not surprisingly, the largest alcohol, PFTB, is the most potent and the smallest, TFE, the least. In addition, above their bilayer modifying concentration PFTB and HFIP break down lipid bilayer structures and solubilize lipid vesicles.

2718-Pos Board B704
Bilayer-Modifying Potential of Limonene and its Metabolites
Will R. Fletcher, R. Lea Sanford, Ruchi Kapoor, Olaf S. Andersen.
Membrane protein function depends on lipid bilayer properties, which can be modified by small molecules. This may account for some of the undesired clinical effects of amphiphatic drugs that modify bilayer properties. In this study, we assessed whether D-limonene and its metabolites (perillyl alcohol, perillaldehyde, and perillic acid) alter lipid bilayer properties, as sensed by bilayer-spanning gramicidin (gA) channels, using a fluorescence assay and single-channel electrophysiology. D-limonene and its metabolites are terpenes that are found in a variety of foods, particularly citrus oils. They have been shown as potential cancer prevention and treatment agents and as antimicrobials. These terpenes are also used as solvents and flavor and fragrance agents. Given the wide range of biological functions of these hydrophobic/amphiphilic compounds, it becomes important to determine to what extent they alter bilayer properties. Using the fluorescence assay, we find that at micromolar concentrations (nominal concentrations): D-limonene decreases gA channel activity, perillyl alcohol and perillaldehyde increase activity, and perillic acid has no apparent effect. When examined using single-channel electrophysiology, each terpene increased gA channel lifetime and appearance rate. In the case of perillaldehyde and perillyl alcohol there is agreement between the two assays but not for D-limonene and perillic acid. We are exploring the basis for these differences. The changes in gA channel function (bilayer properties) were observed at low membrane concentrations (mole fraction ~0.02-0.03), indicating that these terpenes are potent bilayer modifiers.

2719-Pos Board B705
Antidepressants Modify Lipid Bilayer Properties
Ruchi Kapoor, Helgi I. Ingólfsson, Roger E. Koeppe, Olaf S. Andersen.
Antidepressants canonically inhibit neurotransmitter re-uptake at synapses. In addition, the two major classes of antidepressants - tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) - have off-target effects that include inhibition of membrane proteins other than neurotransmitter transporters. The biological significance of these effects remains unclear, but this polypharmacy may contribute to the desired changes in brain function - including altered neuronal circuitry and connectivity. It may also contribute to these compounds' off-target and side effects. A common feature of many proteins modulated by antidepressants is that they span lipid bilayers. Thus, it may be important that antidepressants are amphiphiles that adsorb at the membrane-solution interface. Membrane proteins are coupled to the bilayer through hydrophobic interactions - meaning that conformational changes underlying normal protein function may involve local reorganization of the surrounding lipids. Because bilayer deformations incur energetic costs, which vary with bilayer properties, membrane protein function may be sensitive to changes in bilayer mechanical properties caused by amphiphile adsorption. That is, amphiphiles may affect membrane protein function by altering the bilayer contribution to the free energy difference between protein conformations. Using gramicidin (gA) channels as probes, we examined whether 19 different TCAs and SSRIs alter lipid bilayer properties and thus may be able to alter membrane protein function through bilayer-mediated mechanisms. All of the examined antidepressants alter gA channel activity in a dose-dependant manner, with citalopram (Celexa, Lexapro) being the least, and fluoxetine (Prozac), paroxetine (Paxil) and sertraline (Zoloft) the most bilayer-modifying. These effects are not enantiomer-specific, and are observed with gramicidins of varying lengths and with different bilayer thicknesses, demonstrating that antidepressants increase bilayer elasticity. The calculated octanol-water partition coefficient, as a measure of drug hydrophobicity, is insufficient to predict the relative bilayer-modifying potential of different compounds.