We therefore investigated the kinetics of endophilin N-BAR domain binding onto single large unilamellar vesicles (LUVs), chosen to provide a large range of membrane curvatures and accessibility to time-resolved analysis. By tracking the time dependence of labeled protein fluorescence intensity on each vesicle, we found that the association kinetics is curvature dependent: smaller vesicles showed faster association kinetics. This finding likely implies that small vesicles bear more binding (insertion) sites, which is in line with hydrophobic insertion mechanisms for curvature sensing. The observed slow dissociation process and irreversible binding fraction after extensive rinsing suggested protein oligomerization and/or conformational changes. Effects of ionic strength and lipid compositions on membrane binding kinetics were also examined.

494-Pos Board B280

Shear Modulus and Viscosity of Deionized Suspensions of Charged Liposomes

Joel A. Cohen[1], Steven M.T. Wei[2], H. Daniel Ou-Yang[2].
[1]University of the Pacific, San Francisco, CA, USA, [2]Lehigh University, Bethlehem, PA, USA.

The frequency-dependent shear modulus and viscosity of suspensions of charged liposomes were measured by use of an oscillating optical trap. Deionized suspensions of extruded 100 nm unilamellar liposomes composed of PG:PC mixtures form electrostatically-stabilized gels. A 1.5 μm polystyrene probe particle is suspended in the gel, trapped by optical tweezers, and the trap is oscillated over a frequency range ω = 1-1000 Hz. The relative displacement and phase of the probe in the trap yield the complex response function $G(\omega)$, whose real and imaginary parts $G'(\omega)$ and $G''(\omega)$ are the storage and loss moduli, respectively, which are related to the shear modulus and viscosity of the gel. The dependence of shear modulus on liposome charge can thus be measured. For PG:PC 1:5 (mol:mol) liposomes at 12% volume fraction, we find $G'(\omega) \sim 1000$ dyne/cm^2 over the entire frequency range. This result is consistent with earlier measurements on similar liposome suspensions by an oscillating bobbin technique and is comparable to the shear moduli of colloidal crystals composed of polystyrene spheres of similar size at similar volume fractions. Given the polydispersity of the liposomes ($\Delta r/r \sim 0.3$) compared to that of the polystyrene spheres ($\Delta r/r \sim 0.02$), the liposome suspension is surprisingly rigid. We use the analysis of Joanny (1979) with Poisson-Boltzmann potentials to relate the shear modulus of the liposome gel to the average liposome charge. Such measurements offer a new way to determine the charge of liposomes, biological vesicles, and other nanoparticles.

495-Pos Board B281

Experimental Studies of the Fusion of Vesicles Containing Nystatin and Cholesterol with Phospholipid Bilayers

Carl S. Helrich, Ruth M. Wiens, Sarah M. Lerch.
Goshen College, Goshen, IN, USA.

Nystatin (nys) is an antifungal agent that preferentially forms ion channels in membranes containing ergosterol (erg) or cholesterol (chol). When vesicles containing nys and erg fuse with a sterol-free bilayer, characteristic spike changes in membrane conductance are observed, which result from nys/erg channels transported to the bilayer by the fusion of vesicles with the bilayer. We previously studied the decay of these conductance spikes when the mol fraction of erg in the vesicles was chosen to produce specific superlattices in vesicle membranes. Here we report the results of similar experiments in which vesicles containing nys and chol were made to fuse with phospholipid bilayers. We observe conductance spikes, which decay spontaneously after long periods of sustaining constant currents. Fusion experiments with erg rather than chol, but otherwise identical conditions continue to produce decaying spikes.

496-Pos Board B282

A Systematic Investigation of the Phase Behavior of Langmuir Monolayers Containing Polyunsaturated Fatty Acids

Ben Stottrup[1], Nathan Ly[1], Jacob Anderson[1], Saame Raza Shaikh[2].
[1]Augsburg College, Minneapolis, MN, USA, [2]East Carolina University, Greenville, NC, USA.

Langmuir monolayers present an ideal model system to investigate the phase behavior of raft forming lipid compositions. Here, we present the results of a systematic study of Langmuir monolayer phase behavior for ternary mixtures containing polyunsaturated acids (PUFAs). PUFAs have been implicated in modifying membrane organization. In this study, four different mixed acyl phospholipid species with varying degrees of unsaturation in the acyl chain (1, 2, 4, 6) were mixed with sphingomyelin and cholesterol. The investigated compositions include ratios of 1:2, 1:1, and 2:1 PUFA to sphingomyelin mixtures with cholesterol composition varying from 10 to 40%. Fluorescence microscopy images provide domain size analysis, area fraction and miscibility transition pressures. We observe an increase in miscibility transition pressure with increased unsaturation or decreased cholesterol composition. Finally, traditional Langmuir film balance techniques measured pressure-area isotherms as well as area condensation.

Calcium Fluxes, Sparks, and Waves I

497-Pos Board B283

βAr-Stimulation Causes EADs but not DADs in Pre-Failure CAMKIIδC Transgenic Myocytes

Andrew G. Edwards, Johan Hake, Joan Heller Brown, Andrew D. McCulloch.
UCSD, La Jolla, CA, USA.

Ca^{2+}-Calmodulin dependent protein kinase II (CaMKII) is a nodal regulator of Ca^{2+}-handling and electrophysiology in the ventricular myocyte, and transgenic mice expressing CaMKIIδc (CaMKIIδc TG) exhibit cellular afterdepolarizations and triggered arrhythmias. Here we studied myocytes isolated from these mice to determine: (1) how CaMKII-hyperactivity promotes electrophysiologic instability early in progression to HF, and (2) how βAR stimulation exacerbates this instability. METHODS: Myocytes were isolated, prior to overt HF, from cardiac-specific CaMKIIδc TG mice (TG, n = 18), and WT littermates (n = 16). Steady-state action potentials, Ca^{2+}-handling, and electrophysiologic instability were assessed in whole-cell current clamp (1 Hz pacing), with simultaneous Ca^{2+} epifluorescence, and with and without βAR stimulation (100 nM Isoproterenol, Iso). RESULTS: EADs, but not DADs, were observed with Iso, and transgenic cells (8/18 cells, 19% of cycles) were more susceptible to EADs than WT (1/16 cells, 2% cycles; $p < 0.05$). Prior to EAD appearance, TG cells that later exhibited EADs (TG-EAD) also exhibited greater Iso-induced AP prolongation than cells that remained stable (52.3 ± 15.6 ms vs. 21.5 ± 3.9 ms; $p < 0.05$). TG-EAD cells also exhibited blunted baseline Ca^{2+} transient amplitude (CaT; 42 ± 9 nM vs. 123 ± 12 nM; $p < 0.05$), but a larger relative change in CaT with Iso (4.4 ± 0.47 fold vs. 2.8 ± 0.46 fold; $p < 0.05$). EADs could be abolished by caffeine, indicating a requirement for SR calcium release. CONCLUSIONS: Prior to overt HF, superimposing βAR stimulation upon CaMKIIδc overexpression elicits EADs without DADs. This EAD etiology requires SR calcium release, and given the larger relative effect of Iso on CaT in TG-EAD cells, it is likely that βAR stimulation combines withCaMKII overexpression to exceed the range of calcium-handling permissive of stable electrophysiology.

498-Pos Board B284

Activation of Ca^{2+} Sparks during β-Adrenergic Stimulation in Resting Cardiomyocytes May Involve CAMKII and No, But Not ROS

Daniel Gutierrez, Jakob Ogrodnik, Ernst Niggli.
University of Bern, Department of Physiology, Bern, Switzerland.

During β-adrenergic stimulation of cardiac myocytes, phosphorylation of the sarcoplasmic reticulum (SR) Ca^{2+} release channels (ryanodine receptors, RyRs) by PKA and CaMKII has been linked to the observed diastolic Ca^{2+} leak (as Ca^{2+} sparks) from the SR. Using confocal Ca^{2+} imaging, we previously showed that β-adrenergic stimulation with 1 μM isoproterenol (ISO) increases the spark frequency in quiescent voltage-clamped guinea-pig cardiomyocytes, without altering the SR Ca^{2+} content. Protein kinase inhibitors (KN-93 and H89) indicated an involvement of CaMKII in the change of spark frequency, but not PKA. Additional experiments showed that the change in sensitivity of the RyRs upon β-adrenergic stimulation may involve reactive nitrogen intermediates (RNI), as incubation with the NOSs inhibitor L-NAME (500 μM) and specific nNOS inhibitor AAAN (100 μM) prevented the increase in spark frequency without changes in SR Ca^{2+} content. This observation was confirmed by using the NO scavenger PTIO (130 μM). Furthermore, recent evidence suggests that redox activation of CaMKII or redox modifications of RyRs may contribute to the SR Ca^{2+} leakage. Therefore, we examined the effect of reactive oxygen species (ROS) on the spark frequency upon ISO application. Incubation with the SOD mimetic (and peroxynitrite decomposition catalyst) Mn-TBAP (100 μM) did not prevent the increase in spark frequency in cells with constant SR Ca^{2+} content. Similarly, acute application of the ROS scavenger TIRON (200 μM) could not suppress the progressive increase in spark frequency upon ISO application. Our observations suggest that CaMKII and RNI, but not ROS, are involved in the upregulation of spark frequency during β-adrenergic stimulation.