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hourglass-shaped model for ion channels, we calculated the resulting changes in its conformational equilibrium due to a coupling to domain elasticity. In the absence of cholesterol, we found a significant shift toward open channel states, while changes were only modest for the cholesterol containing fluid domains. This indicates a protective role of cholesterol in cellular signaling during sphingomyelinase activity.

2227-Pos Board B246
Cholesterol Sensing in Membranes
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Cholesterol levels in cells are maintained within narrow limits by a network of proteins that continuously measure cholesterol concentrations and adjust rates of its synthesis and uptake. The key protein of this network that acts as the master cholesterol sensor is Scap, a membrane protein in the endoplasmic reticulum (ER). The cholesterol concentration in the ER membrane is set at ~5 mole%. The sensitivity of the Scap sensor is switch-like, allowing for complete shutdown of cholesterol synthesis and uptake in response to small increases in ER cholesterol concentration above the 5 mole% set-point.
Our recent studies have shown that the binding of Perfringolysin-O (PFO), a cholesterol-dependent bacterial toxin, to purified ER membranes also shows the same switch-like behavior - no binding is seen below 5 mole% cholesterol, and close to complete binding is seen above 7 mole% cholesterol. This remarkable correlation suggests that PFO and Scap may sense ER cholesterol through a common mechanism related to the chemical activity of cholesterol in the ER membrane. Unfortunately, both Scap and PFO oligomerize in the membrane which could account for all or part of the sharp threshold-like response. In our latest studies we have engineered mutant forms of PFO that do not oligomerize. The binding of these mutant PFO molecules to model liposomes and ER membranes shows the same threshold behavior as wild-type PFO. This suggests that the chemical activity of cholesterol in the ER membrane controls its accessibility to proteins. The chemical activity of ER cholesterol is controlled by the ER phospholipid composition. This control of cholesterol accessibility is critical in triggering the recruitment of cholesterol-binding proteins to the membrane. It also allows us to study the initial cholesterol sensing reaction in isolation from subsequent oligomerization events.

2228-Pos Board B247
Membrane Bilayer Environment Influences Thermodynamics of Rhodopsin Membrane Protein-Lipid Interactions
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The interaction between lipid bilayers and membrane proteins plays an important role in the functionality of GPCRs such as rhodopsin. During rhodopsin photoactivation, the photoreactive 11-*cis* retinylidene chromophore is isomerized to an all-*trans* state attaining an equilibrium between the MetaI and MetaII states. Lipid bilayer interaction with rhodopsin during its photoactivation process is explained using the flexible surface model (FSM). The FSM describes the elastic coupling of membrane lipids to integral membrane proteins through a balance of curvature and hydrophobic forces in lipid-protein interactions [1]. The FSM predicts a shift of the MetaI-MetaII equilibrium towards the activated MetaII state for unsaturated phosphatidylcholines with an increase in acyl chain length [1] or temperature. using UV-visible spectroscopy, we test the FSM by evaluating how the MetaI-MetaII equilibrium and thermodynamic parameters are influenced by a change in membrane environment. Absorption wavelength maxima at 485 nm and 380 nm correspond to the MetaI and MetaII states (inactive and active MetaII-H+), respectively. Accurate results are obtained by using the Rayleigh-Gans approximation to Mie scattering to compensate for light scattering in the UV-visible spectra. We determine effects of the type of head group, lipid to protein ratio, acyl chain length, and temperature on the MetaI-MetaII equilibrium by comparing pH titration curves for reconstituted rhodopsin in different lipid bilayers to those for native disk membranes [2,3]. The thermodynamic parameters are obtained for this equilibrium by fitting a modified phenomenological Henderson-Hasselbalch function [2] to the pH titration curves. These thermodynamic parameters illustrate how free energy drives the structural changes in rhodopsin upon photoactivation and can be used to model other GPCRs.
[1] A.V. Botelho *et al.* (2006) *BJ* **91**, 4464-4477.
[2] M. Mahalingam *et al.* (2008) *PNAS* **105**, 17795-17800.
[3] E. Zaitseva *et al*. (2010) *JACS* **132**, 4815-4821.

Calcium Fluxes, Sparks, & Waves I

2229-Pos Board B248
Frequency of Triggered Ca^{2+} Waves is Increased in Atrial Myocytes during Heart Failure
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Triggered Ca^{2+} waves develop during rapid pacing in atrial myocytes and are therefore likely to contribute to action potential (AP) duration, thus establishing repolarization gradients and causing reentry. The goal of this study was to investigate how Ca^{2+} wave properties are altered in heart failure (HF). Myocytes were isolated from the left atrium of dogs paced rapidly for 5-6 weeks and Ca^{2+} cycling was studied during rapid pacing using rhod-2AM and confocal microscopy (37°C). In control myocytes, the incidence of triggered Ca^{2+} waves increased with pacing rate and this frequency dependence was shifted to slower rates in HF myocytes. The increase in wave frequency was directly related to the increase in diastolic $[Ca^{2+}]$ with increasing rate, which was exaggerated in HF myocytes. Propagation velocity and distance (percent of cell length) also decreased in HF and there was a shift in the distribution of propagation distance to more short waves and fewer long waves. A low concentration of caffeine (0.5mM, to increase RyR Ca^{2+} sensitivity) abolished triggered waves and restored normal Ca^{2+} transients even at high pacing rates. We conclude that 1) triggered Ca^{2+} wave frequency is increased in HF despite decreased propagation distance and velocity, 2) wave incidence in HF may be related to a greater sensitivity to the rate-induced rise in diastolic $[Ca^{2+}]$, and 3) the fact that low [caffeine] abolished waves and restored normal Ca^{2+} transients, suggests that triggered waves occur as a result of a reduced sensitivity of SR Ca^{2+} release to trigger Ca^{2+} influx. This form of aberrant Ca^{2+} cycling during the AP may affect the beat-to-beat regulation of AP duration, which may contribute to establishing the substrate for reentrant arrhythmias by formation of repolarization gradients, especially in the setting of HF.

2230-Pos Board B249
TRPC3 Channels in Angiotensin II-Induced Calcium- Dependent Arrhythmias in Mouse and Human Cardiomyocytes
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Angiotensin II (ATII) is associated with cardiac remodeling, heart failure and arrhythmias. In cardiomyocytes (CM), ATII signaling involves IP_3- and DAG-dependent pathways and results in increased diastolic Ca release from the sarcoplasmic reticulum (SR). Recent evidence suggests that TRPC3 channels and NCX may also be regulated by ATII contributing to arrhythmogenity. We investigated the mechanisms of Ca-mediated cellular arrhythmias induced by ATII. In CM from murine or human non-failing left ventricle, [Ca] transients (Fluo4-AM) during 1 Hz, as well as SR Ca load (caffeine), SR Ca leak (Ca spark frequency,SparkF) and arrhythmic action potentials (arrAP, as non-stimulated synchronized Ca release) were visualized confocally. Action potentials (AP) were recorded in a subset of cells. ATII (100 nM,20 min), IP3-inhibitor 2-ABP, TRPC3-inhibitor Pyr3, or Na/K-pump inhibitor ouabain (100 nM) were used in parallel experiments. In mouse CM ATII increased the [Ca] transient amplitude (F/F0: 4.1 ± 0.3 vs. 2.7 ± 0.2 in CTRL). ATII also induced Ca sparks (SparkF (s-1*pL-1): 277 ± 28 vs. 48 ± 20) and arrhAP (0.77 ± 0.12 vs. 0.03 ± 0.01 $p<0.05$) despite unchanged SR [Ca]. Interestingly, ATII induced significantly more arrhAP than ouabain at comparable SparkF. ATII increased AP duration from 53 ± 6 to 92 ± 34 ms (APD90; P=0.1). 2-APB significantly reduced ATII -induced Ca sparks and arrhAP. In CM matched for SparkF, ATII-induced arrAP were significantly reduced with Pyr3.ATII induced redistribution of TRPC3 to the sarcolemmal membrane. In human non-failing CM, Pyr3 also reduced ATII-induced arrhythmogeneity.-Conclusion: ATII facilitates Ca-dependent arrhythmias by mechanisms beyond an increase in SR Ca leak in mouse and human CM. Sarcolemmal TRPC3-channels may modulate the arrhythmogenity of diastolic SR Ca release in the presence of ATII.

2231-Pos Board B250
Properties of Calcium Transients in Rabbit Atrial Myocytes
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