110-Plat
Functional Consequences of Lipid-Mediated Interaction Between Rhodopsin Molecules
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In the retina, rhodopsin is densely packed in a rod outer segment disk membrane rich in phospholipids with PC and PE headgroups. Increasing rhodopsin packing density in model PC membranes has been shown to alter metarhodopsin-II (MII) formation. This observation is deemed to be the consequence of rhodopsin association promoted by non-specific properties of the membrane. Here, we studied the effect of rhodopsin packing density on MII formation in membranes characterized by different intrinsic curvature and different interfacial hydrogen bonding propensity. Rhodopsin was reconstituted into a series of POPC bilayers doped with DOPC, di-and mono-methylated DOPE or DOPE at rhodopsin/lipid ratios ranging from 1:250 to 1:70. The level of rhodopsin activation and rate of MII formation were determined by steady-state and time-resolved UV/vis spectroscopy. In PC membranes, lower rhodopsin concentrations shift the MI/MII equilibrium towards MII and result in a faster rate of MII formation, in agreement with previous findings. On the contrary, in membranes rich in lipids with PE headgroups, the MII concentration is independent of rhodopsin packing density and rates of MII formation, while reduced, show only a slight dependence on rhodopsin crowding. In addition, at low or high protein density, the amount of MII formed depends to a larger extent on the ability of the annular PE headgroups to establish hydrogen bonds with the MII state than on changes in membrane curvature elasticity. These results show clearly that MII formation and interaction between rhodopsin molecules depend strongly on interactions between annular lipids and rhodopsin, highlighting the fundamental role of the first layer of lipids surrounding the protein.

111-Plat
Hydrophobic Mismatch Modulates the Kinetics of G Protein Binding and Receptor Conformation Change
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A recent study demonstrated that rhodopsin in 14:0,14:1 PC (hydrophobic thickness = 21.4 angstroms) has 8% less helical content than rhodopsin in 18:0,18:1 PC (hydrophobic thickness = 29.2 angstroms). We investigated the effects of hydrophobic thickness on rates of MII and transducin (G_t) binding and phospholipid dynamics and packing order. Purified rhodopsin was reconstituted in liposomes consisting of 14:0,14:1 PC and 18:0,18:1 PC at a lipid:protein ratio of 200. Kinetics of MII formation and G_t binding where measured with flash photolysis, and membrane properties were assessed via time-resolved fluorescence anisotropy decay measurements of diphenylhexatriene (DPH). MII formation was analyzed in terms of the square model. Analysis of the DPH anisotropy decay data in terms of the P2-P4 model showed that lipid dynamics and fractional free volume (f_v) were higher in the 14:0,14:1 PC membrane. Previous studies demonstrate that an increase in these two bilayer properties is associated with enhanced MII formation, but in this case equilibrium concentration of MII and the rate of MII formation was higher in 18:0,18:1 PC at all temperatures. Analysis of the temperature dependence of the kinetics in terms of reaction rate theory showed this was chiefly due to increased activation enthalpy for two of the forward rates; Lumi to MI-380 and MI-480 to MII. At 30 ^{o}C in 18:0,18:1 PC, MII formed with a time constant of 0.69 ms and the MII-G_t complex formed in 0.79 ms. This near-immediate formation of MII-G_t following MII is similar to what is observed for rhodopsin in the native membrane. In 14:0,14:1 PC MII formed in 5.43 ms and MII-G complex formed in 37.6 ms. This long lag between appearance of MII and G_t binding demonstrates that hydrophobic mismatch has deleterious consequences for G protein-coupled signaling.

Platform K: Calcium Signaling in Heart & Non-excitable Cells

112-Plat
Recruitment of Multiple Spontaneous Ca^{2+} Release Initiation Sites Promotes Ca^{2+} Waves in Myocytes of Intact Rat Heart Under Conditions of Ca^{2+} Overload
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Spontaneous Ca^{2+} release (SCR) in the form of Ca^{2+} waves is responsible for cardiac myocyte depolarization and delayed afterdepolarizations (DADs) that can produce triggered beats. Whether or not a cell reaches threshold is determined by the magnitude and rate of spread of the Ca^{2+} wave in the cell and the resultant activation of inward current via forward mode Na-Ca exchange. In this study, we combined experimental observations with computer simulations in order to investigate the mechanisms by which the characteristics of Ca^{2+} wave activation influences DAD magnitude. Ca^{2+} waves were measured in individual myocytes in the left ventricular subepicardium in rat hearts using confocal microscopy (fluo-4 Ca^{2+} fluorescence). Extracellular Ca^{2+}, $[Ca^{2+}]_e$, was raised to increase sarcoplasmic reticulum (SR) Ca^{2+} load and induce Ca^{2+} waves. With increasing $[Ca^{2+}]_e$, the number of SCR sites increased along with the incidence of Ca^{2+} waves within myocytes. Interestingly, Ca^{2+} wave velocity at higher $[Ca^{2+}]_e$ was considerably heterogeneous_both faster as well as nearly equivalent to that at normal $[Ca^{2+}]_e$, the average at higher $[Ca^{2+}]_e$ being only moderately faster. Computer simulations demonstrated that the recruitment of multiple SCR sites is a highly effective means of increasing the magnitude and rate of cytoplasmic Ca^{2+}. The rapid delivery of Ca^{2+} to the Na-Ca exchanger increases DAD magnitude and the probability of producing a triggered beat. Our results suggest that it is the recruitment of multiple SCR initiation sites that determines DAD magnitude and whether or not depolarization can reach threshold. This mechanism is likely to contribute to arrhythmogenesis under conditions of SR Ca^{2+} overload and in genetically-based disease states in which ryanodine receptor function is altered, such as in catecholaminergic polymorphic ventricular tachycardia.

113-Plat
Emergence of Local Ca Oscillators in Cardiac Pacemaker Cells: 2d Ca Dynamics Measurements, An Analytical Theory, and Complex Systems Numerical Modeling
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The sarcoplasmic reticulum (SR) ofsanoatrial node cells (SANC) is capable of generating roughly periodic spontaneous localized Ca^{2+}releases (LCRs), recently dubbed as "Ca^{2+}clock". The LCRs interact with sarcolemma electrogenic molecules and are critically important for cardiac impulse initiation and pacemaker rate regulation. Mechanisms of emergence of the local Ca^{2+} oscillators by stochastically gated release channels remain unknown. **Methods:** We explored the emergence of rhythmic LCRs in rabbit SANC using a fast 2-D camera, an analytical theory, and complex systems numerical modeling. **Results:** Spontaneously beating SANC exhibit action potential-induced Ca^{2+} transients that are shortly preceded by multiple wavelet-like LCRs throughout the cell. The LCRs persisted in KCl-depolarized SANC and were recorded for 30-60 seconds. Autocorrelation analysis and histograms of intervals between persisting releases show that Ca^{2+}dynamics are roughly periodic in each spontaneously active cell location. The histograms exhibit a gap followed by a skewed peak that was interpreted in terms of an analytical theory that considers Ca^{2+}releases to have a restitution time followed by a Poisson process. The experimentally measured Ca^{2+}releases were closely reproduced by a complex systems numerical 2D-model featuring an array of stochastic but diffusively coupled Ca^{2+}release units (CRUs) with fixed restitution times. As the amplitude of the CRU releases increase from 0.5 to 1.25 pA in the model, the CRUs strongly interact via diffusion and Ca^{2+}induced Ca^{2+}release (CICR), resulting in a larger Ca^{2+}release size (between sparks and global waves), and a higher rhythmicity of release occurrence. **Conclusions**: SANC SR generates roughly periodic LCRs. The emergence of the local Ca^{2+}oscillators is an inherent fundamental property of an ensemble of diffusively interacting, stochastic CRUs having time-dependent restitution. The rhythmicity of the releases increases as interactions of CRUs enhance.

114-Plat
Cytosolic Ca-Dependent Na/Ca Exchange Regulation in Intact Cardiomyocytes: Role of Cytosolic Na
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Cytosolic Na ($[Na]_i$) strongly controls cardiac Ca handling and contractility by establishing the thermodynamic Na gradient and hence the operating point for Ca transport via Na/Ca exchange (NCX); e.g. low-$[Na]_o$ or glycoside-induced inotropy. Here we investigate how $[Na]_i$ might control not only NCX transport but also cytosolic Ca ($[Ca]_i$) dependent activation. We assayed NCX activation in intact rabbit cardiomyocytes (physiological solutions; 37C; no voltage clamp) by applying short (5-sec) 0 Na solution switch steps, which transiently increase $[Ca]_i$ when NCX is active. In rested cells, NCX was refractory to