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Adrenergic Regulation of the Human Ether-A-Go-Go-Related Gene Channel Protein Abundance Occurs at the Surface of the Endoplasmic Reticulum

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Long QT syndrome (LQTS) is a disorder of ion channels that can lead to the potentially lethal ventricular arrhythmias. Human *ether-a-go-go*-related gene product (HERG), a potassium channel responsible for the rapidly inactivating delayed rectifier current, is a genetic target in hereditary LQTS (specifically, LQTS2). Furthermore, pharmacological compounds of multiple classes can result in abnormal synthesis and/or function of HERG. Because arrhythmic events are often triggered in patients with LQTS2 following various stressors, it has been postulated that adrenergic regulation plays a key role in acute HERG regulation and LQTS pathogenesis. Recently, we have shown that chronic cAMP treatment (a surrogate for β-adrenergic signaling) significantly augments HERG protein abundance in HEK293 cells. Here, using velocity gradient centrifugations, we show that upon elevation of cAMP, the channel accumulates in the endoplasmic reticulum (ER) disproportionately more than at the plasma membrane. We also show that localized inhibition of protein kinase A (PKA) signaling at the surface of the ER by a targeted PKA inhibitory peptide (PKI) completely abolishes the effect; channels on the plasma membrane are unaffected by this inhibitor, as shown by patch-clamp experiments. Next, we targeted specific cAMP and PKA activity FRET-based biosensors (ICUE2 and AKAR3, respectively) to the ER surface; cell-permeable cAMP as well as the β-agonist isoproterenol elicited major FRET signal in the case of either biosensor, indicating that χΑΜΠ/ΠΚΑ σιγ ναλινγ ισ ινταχτ ιν τηισ χομπαρτμεντ. Τηε χΑΜΠ-δεπενδεντ αυγμεν τατιον ρεμαινσ πρεσεντ εππεν αφτερ τηε ΗΕΡΓ χοδινγ σενενχε ισ χοδον-οπτιμιζεδ (38% διφφερεντ φρομ τηε οριγιναλ ατ τηε πριμαρψ σενενχε λεππελ), ινδιχατινγ τηατ χΑΜΠ-δεπενδεντ αυγμεντατιον ισ νοτ μΡΝΑ δεπενδεντ. Τηερεφορε, ωε χονχλυδε τηατ β-adrenergic regulation of HERG occurs during the early stages of protein synthesis or folding at the level of the ER.

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Identification of the Escherichia Coli SecA Solution State Dimer Orientation using Forster Resonance Energy Transfer

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Over the past 8 years four different SecA dimer crystal structures have been solved. While the subunits of the dimer remain essentially the same, the orientation of the subunits is drastically different for each of the crystal structures. Some data has been published (Ding, H., Hunt, J.F., Mukerji, I., Oliver, D.B. 2003 *Biochemistry* 42, 8729-38) that suggest one of the anti-parallel dimers (Hunt, J.F., Weinkauf, S., Henry, L., Fak, J.J., McNicholas, P., Oliver, D.B., Deisenhofer, J. 2002 *Science* 297, 2018-26) is the solution state orientation, while other data suggests that it is the parallel dimer (Vassylyev, D.G., Mori, H., Vassylyeva, M.N., Tsukazaki, T., Kimura, Y., Tahirov, T.H., Ito, K. 2006 *J Mol Biol* 364, 248-58). Thus, the solution state orientation of the SecA dimer is currently unknown. The aim of the present study is to identify the dominant dimer orientation of *E. coli* SecA in solution. Förster Resonance Energy Transfer (FRET) was used to measure distances between dye-labeled monocysteine residues on SecA dimer subunits. Three fluorescent dye pairs with R_0 values of 34 Å, 62 Å, and 82 Å were used to measure distances from ~11 Å to 140 Å, which corresponds to the possible distances expected within any of the 4 proposed dimer orientations. The FRET measured distances were then compared to those measured in the SecA dimer crystal structures to determine if one of the structures represents the dominant solution state dimer orientation or if a mixed population of dimer states appears likely.

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Progesterone Impairs the Trafficking and Maturation of HERG Potassium Channels

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The prolongation of QT intervals in both mothers and fetuses during the later period of pregnancy implies that higher level of progesterone may have impact on the function of cardiac ion channels. In this study, we investigated the effect of progesterone on the expression, trafficking and function of human *ether-a-go-go-related* gene (HERG) potassium channel, a key ion channel responsible for controlling the length of QT intervals. We found that progesterone decreased fully-glycosylated form of HERG channels in both concentration- and time-dependent manners. Progesterone also significantly decreased HERG current density. Immunofluoresence microscopy showed that progesterone preferentially decreased HERG channel protein in the plasma membrane. Neither blockade of progesterone receptor with RU486 nor inhibition of protein synthesis with cycloheximide reversed the effect. The effect of progesterone was rescued by both lower temperature culture (27°C) and application of HERG channel blocker (E4031), but not by blockade of protein kinases including ERK1/2, JUNK, PI3K/Akt and PKA. Application of a sterol binding agent rescued the effect of progesterone. Moreover, disturbance of intracellular cholesterol homeostasis with simvastatin and imipramine mimicked the effect of progesterone. In conclusion, progesterone impairs HERG channel trafficking and maturation via disturbing intracellular cholesterol homeostasis. Our findings uncover the mechanism for the QT prolongation and high risk of arrhythmias during pregnancy.

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Overexpression and Biophysical Characterization of Human Interleukin-1 Alpha

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Interleukin-1 alpha (IL-1) regulates a wide range of important cellular processes. Structure- function data has been limited for IL-1α because of the difficulty in its overexpression in mammalian cells. In this study we propose the cloning, expression, biophysical and biological characterization of the human IL-1. Human IL-1 has been expressed and purified from *Escherichia coli* in high yields (~ 4 mg per liter of the bacterial culture). IL-1 was purified to homogeneity (~ 98% purity) using affinity chromatography and size exclusion chromatography. Results of the steady state fluorescence and differential scanning calorimetry experiments show that the recombinant IL-1 is in a folded conformation. Far-UV circular dichroism (CD) data suggest that IL-1 is an all -sheet protein with a -barrel architecture. IL-1 is a unique protein that affects nearly every cell type and for the many proteins that lack the N-terminal signal sequence, IL-1 is believed to be a model for understanding their endoplasmic reticulum- Golgi independent secretion. By characterizing and establishing the non-classical secretion route of IL-1, a more complete understanding can be accomplished for this special class of proteins. With the expected completion of the research outlined in this proposal, greater knowledge of other molecules with non-classical releases can be attained.

Exocytosis & Endocytosis I

2590-Pos

Elastic, Electrostatic and Electrokinetic Processes Contributing to Membrane Curvature - Membrane Shape as a Memory Element

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The curvature of cellular membranes is controlled by a variety of physical mechanisms, whose relative importance and interplay is still to be worked out. Moreover the notion that the membrane curvature controls a variety of biological processes, and that it also may be an important factor in regulating vesicular secretion, is gaining ground. In this study we evaluate what mechanical forces, such as may be generated by the cytoskeleton, are needed to generate required membrane deflection and deformation. We also consider the role the electrical forces play in the membrane deformation, and which may be present under physiological conditions. Membrane deformation is evaluated using a coupled system of linear elastic equations and electrostatic-electrokinetic (Poisson-Nernst-Planck) equations. If the fixed charges on the membrane are asymmetrical the electrostatic forces generated can produce significant bending of the membrane. Even when the distribution of fixed charges on both sides of the membrane is symmetrical the membrane bending occurs, if the intracellular and extracellular ion concentrations are different. Finally, upon removal of the forces (mechanical or electrical) that induce the membrane curvature the membrane relaxes toward the original configuration with a time course that depends on the membrane properties. The shape of the membrane can thus serve as a memory element regulating various biological processes including those of vesicular secretion.

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Molecular Mechanism of Membrane Constriction and Tubulation Mediated by the F-BAR Protein Pacsin

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