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Structural Basis for Alcohol Modulation of a Ligand-Gated Ion Channel Homolog

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The molecular mechanisms underlying direct effects of ethanol and other n-alcohols on the nervous system, including intoxication and anesthesia, are poorly understood. Although physiological and behavioral evidence implicates ligand-gated ion channels in the action of alcohols and other general anesthetics, a lack of high-resolution structural information for these receptors has limited structural characterization of alcohol modulation. Recent structural and functional studies of the prokaryotic ligand-gated ion channel homolog GLIC show it to be a valuable model for structure, function, and modulation of these receptors. We are investigating the effects of alcohols on GLIC, and identifying specific protein sites underlying alcohol modulation. Our results demonstrate that GLIC exhibits differential modulation by short- and long-chain n-alcohols, similar to homologous cys-loop receptors. Mutations at putative alcohol binding sites modify alcohol modulation, while labeling with MTS reagents mimics alcohol effects. Our work supports a role for specific residues in the GLIC transmembrane domain in mediating alcohol binding and modulation.

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Smase D Activates Voltage-Gated Cation Channels

Hyeon-Gyu Shin, Yanping Xu, Zhe Lu.

Voltage-gated ion channels generate electric impulses in nerve, muscle and endocrine cells. These channels open in response to membrane depolarization. Previously studies from our group show that extracellular application of sphingomyelinase (SMase) D shifts the G-V curve of voltage-gated K^+ channels in the hyperpolarized direction, thereby activating the channels in hyperpolarized potentials where they otherwise remain deactivated. SMase D hydrolyzes the phospholipid sphingomyelin which is primarily present in the outer leaflet of cell membranes. In doing so, it removes the positively charged choline group of sphingomyelin. Enzymatic removal of the positively charged choline groups makes it energetically easier for the positively charged voltage sensor to move outwardly, assuming an activated conformation. We have recently found that extracellular application of SMase D also shifts the conductance-voltage (G-V) relation of voltage-gated Na^+ and Ca^{2+} channels in the hyperpolarized direction. Thus, SMase D apparently activates all three major classes of voltage-gated cation channels at hyperpolarized potentials where they otherwise remain closed.

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PI(4,5)P2 Regulation of TRPV1 Reconstituted in Model Lipid Membranes

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TRPV1 channels are non-selective cation channels activated by capsaicin, protons, and heat. We have previously shown that TRPV1 activation is potentiated in vivo by PI(4,5)P2. We are now investigating the mechanism of PI(4,5)P2 potentiation using TRPV1 channels reconstituted in synthetic lipid vesicles studied with patch-clamp electrophysiology. We will present our results exploring the effects of natural PIP2 extracts and di-C16 PI(4,5)P2 incorporated into asolectin giant unilamellar vesicles (GUVs) and synthetic lipid GUVs. We will compare the effects of these non-soluble forms of PIP2 to the effects of the more water soluble di-C8 PIP2 forms used in many cellular electrophysiology experiments.

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Caspases Mediate Pannexin 1 Channel Activation in Apoptotic Cells

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Pannexins represent a recently discovered family of membrane proteins that form large conductance (400-500 pS) membrane channels permeable to ions and small intracellular signaling molecules. The mechanisms that regulate pannexin channel activity and their physiological roles remain incompletely understood. Here, we used whole cell voltage clamp recordings from Jurkat cells to demonstrate induction of a Pannexin 1 (Panx1)-dependent current only in apoptotic cells; the current was not observed in Jurkat cells that were not undergoing apoptosis, even when Panx1 was overexpressed. The apoptosis-induced currents were attributed to Panx1 through a combination of pharmacological profiling, over-expression, and siRNA knockdown experiments. We found that Panx1 is a target of apoptosis-induced effector caspases (caspases 3 and 7), and that a specific caspase-cleavage site within the channel C-terminus is required for activation of Panx1 currents during apoptosis. Furthermore, expression of a Panx1 construct that is truncated at the C-terminal caspase cleavage site resulted in constitutive Panx1 currents, even in non-apoptotic cells. In addition, uptake of Yo-Pro or To-Pro dyes characteristic of apoptotic cells was altered by these manipulations in direct correspondence with changes in membrane current, suggesting that dye uptake is mediated by Panx1 channels and provides a faithful surrogate for Panx1 currents. Likewise, ATP and UTP release from apoptotic cells was Panx1-dependent. Together, these data reveal a novel mechanism of Panx1 activation by caspase-dependent cleavage in apoptotic cells. Moreover, this work identifies a new physiological role for Panx1 channels in apoptotic cell clearance since Panx1 activation mediates release of ATP and UTP, two nucleotides that provide 'find-me' signals for recruitment of phagocytes to apoptotic cells.

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AKAP79/150 Signal Complexes in G-Protein Modulation of Neuronal Ion Channels

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Voltage-gated M-type (KCNQ) K^+ channels play a critical role in modulation of neuronal excitability and action potential firing. A-kinase-anchoring protein (AKAP)79/150 mediated PKC phosphorylation of M channels is involved in M current (I_M) suppression by muscarinic M_1, but not bradykinin B_2 receptors. In this study, we first explored if purinergic and angiotensin suppression of I_M in superior cervical ganglion (SCG) sympathetic neurons involves AKAP79/150. Transfection into rat SCG neurons of ΔA-AKAP79, which lacks the A-domain necessary for PKC binding, or the absence of AKAP150 in AKAP150-/- mice, did not affect I_M suppression by the purinergic agonist UTP, nor by bradykinin, but did reduce I_M suppression by muscarinic agonists and by angiotensin II. Transfection of AKAP79, but not ΔA-AKAP79 or AKAP15, ''rescued'' the muscarinic suppression of I_M in AKAP150-/- neurons. We also tested association of AKAP79 or KCNQ channels with M_1, B_2, $P2Y_6$ and AT_1 receptors via fluorescence resonance energy transfer (FRET) experiments on CHO cells under total internal refection fluorescence microscopy, which revealed substantial FRET between AKAP79 with M_1 and AT_1 receptors, but only weak FRET with $P2Y_6$ or B_2 receptors. Similarly, we observed strong FRET between KCNQ2 with M_1 and AT_1, but not $P2Y_6$ or B_2, receptors. The involvement of AKAP79/150 in the regulation of N- and L-type Ca^{2+} channels in SCG neurons by $G_{q/11}$-coupled muscarinic receptors and by cAMP/PKA was also studied. We found AKAP79/150 to not play a role in the former, but to be necessary for forskolin-induced up-regulation of the L-type current. Our data suggest that AKAP79/150 orchestrates signal complexes that include PKC, KCNQ subunits and M_1 or AT_1 receptors, which correlates with the PIP_2-depletion mode of neuronal M current suppression, but does not generalize to $G_{q/11}$-mediated inhibition of N- or L-type Ca^{2+} channels.

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Effect of Cytoskeleton on Cell Volume Regulation in Mdck Cells

Jason Rahimzadeh, Susan Z. Hua.

Volume regulation is critical for cell survival. A change in cell volume reflects the movement of water across the cell membrane. That in turn is a function of the solute content and mechanical stresses within the cytoskeleton. Previous studies on regulatory volume decrease (RVD) used a Coulter Counter applied to free floating cells. To evaluate the role of cytoskeletal stress we studied RVD in adherent cells using a microfabricated cell volume sensor and compared them with Coulter measurements. Suspended cells subjected to a hypotonic challenge exhibited a rapid RVD. Adherent cells, with intact cytoskeletal networks, recovered their volume slowly and to a lesser extent. Modulating actin filaments with cytochalasin-D and jasplakinolide affected RVD in adherent cells more than in suspended cells. Cytochalasin-D (10 μM) disrupted actin networks and caused rapid and large decreases in volume in response to a hypotonic challenge, whereas RVD of suspended cells was unaffected. Jasplakinolide (500 nM) that stabilizes actin filaments increased the density of actin filaments in attached cells and diminished RVD. These results suggest that as expected stress in the cytoskeleton affects osmotic responsiveness. Since attached cells are not spherical there are clearly forces normal to the membrane and they play a role in volume regulation.

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Phospholipids Regulate the Voltage-Dependence and Selectivity of Plant VDAC

Lamia Mlayeh, Marc Leonetti, Martine Prevost, **Fabrice Homble**.

Membrane lipids have a significant effect on both structure and function of membrane proteins. Several comprehensive studies on the lipid composition of cell membranes indicate a high degree of adaptability to both endogenous and environmental cues. It is of physiological importance notably for plants which are static organisms to avoid lethal membrane injuries. VDAC channel constitutes the major transmembrane protein of the mitochondrial outer membrane, and is a key element in the regulation of solute exchange between mitochondria and cytoplasm. We have recently shown that sterol-VDAC