rafts and coalesce into large ceramide-rich platforms during cell stress. However, the clustering mechanisms and their impact on membrane protein stability and function remain uncertain. We reported previously that the cystic fibrosis transmembrane conductance regulator (CFTR), which is mutated in the hyperinflammatory pulmonary disease cystic fibrosis (CF), forms clusters that are cholesterol-dependent and become incorporated into large and long-lived ceramide-rich platforms during hormonal stimulation. Using a combination of optimized fluorescence confocal imaging and quantitative analyses such as image correlation spectroscopy (ICS) and the k-space ICS (kICS), we report here that CFTR clustering at the plasma membrane of primary human bronchial epithelial cells does not involve known tethering interactions of CFTR with PDZ domain-containing proteins, filamin A or the actin cytoskeleton. It also does not require CFTR palmitoylation but is critically dependent on membrane lipid order and is induced by detergents that increase the phase separation of membrane lipids. Clustering and integration of CFTR into ceramide-rich platforms are impaired by the CF disease-causing mutations F508del and S13F and rescued by the CFTR modulators elexacaftor plus tezacaftor. These results indicate that CF therapeutics that correct mutant protein folding restore both CFTR trafficking and normal lipid interactions in the plasma membrane which results in its stability at the plasma membrane and its functional restoration.

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Conformational rearrangements associated with Panx1 channel gating

Jacqueline J. Ehrlich[1], Erik K. Henze[1], Toshimitsu Kawate[2].
[1]Molecular Medicine, Cornell University, Ithaca, NY, USA, [2]Cornell University, Ithaca, NY, USA.

Pannexins (Panx1-3) are ATP permeable membrane channels that are widely expressed in the human body. It has been demonstrated that pannexins play central roles in ATP release from a wide range of cell types including airway epithelia, blood cells, glial cells, and neurons. Pannexin mediated ATP release contributes to blood pressure regulation, neurotransmission, and apoptotic cell clearance. Our group and others determined structures of Panx1 using cryo-EM, which uncovered that Panx1 assembles into a unique heptameric channel with a potential pathway for the ATP molecule. However, how Panx1 controls ATP migration through the pore remains unclear. In fact, it is still uncertain whether the currently available Panx1 structures represent open or closed conformations. We recently discovered a cell metabolite as a bona fide Panx1 agonist. We performed cryo-EM single particle reconstructions in the presence or absence of this agonist to shed light on the Panx1 channel gating mechanism. We found a major conformational rearrangement in a region surrounding the ATP permeation pathway. Cysteine accessibility studies using whole cell patch clamp electrophysiology support that the conformational rearrangement in this region is important for Panx1 channel gating. This is in contrast to the proposed-gating mechanism in apoptotic cells, in which caspase-dependent C-terminal cleavage unplugs the pore for permeant molecules. Based on the current study, we provide a novel mechanism underlying Panx1 channel gating in non-apoptotic cells.

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Structural determinant of the ivabradine-induced block of pacemaker HCN channels

Andrea Saponaro[1], Alessandro Porro[2], Jan H. Krumbach[3], Atiyeh Sadat Sharifzadeh[1], Antonio Chaves-Sanjuan[1], Dario DiFrancesco[1], Kay Hamacher[3], Gerhard Thiel[3], Anna Moroni[1].
[1]University of Milan, Milan, Italy, [2]Elements SRL, Milan, Italy, [3]Technische Universität Darmstadt, Darmstadt, Germany.

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are the molecular correlate of the I_h (or I_f) current, which plays a key role in controlling rhythmic activity in cardiac pacemaker cells and spontaneously firing neurons. We have recently obtained the structure of HCN4 with the pore in the open and closed conformation, thus advancing the understanding of permeation and conductance in HCN channels. Being able to purify HCN4 molecules with the pore in the open state, we are currently characterizing, at atomic details, the action of Ivabradine, an open channel blocker specific to HCN channels and currently approved for clinical use in heart failure. By testing purified HCN4 proteins for Ivabradine-induced shifts in a thermal denaturation assay we obtained the first biochemical demonstration of Ivabradine binding to the open pore of HCN4 and proceeded to solve the structure of the complex by single particle cryo-EM. We can thus describe the pattern of contacts that Ivabradine develops with the residues facing the vestibule of the HCN pore. Strikingly, molecular dynamics simulation experiments uncovered the key role of the tertiary ammine of Ivabradine in the mechanism of block, thus explaining the current dependency of the drug-induced block.

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Activation of the alkaline-activated K_{2P} K^+ channel talk-2 requires opening of two functionally coupled gates

Lea C. Neelsen[1], Elena B. Riel[1,2], Susanne Rinné[3], Björn C. Jürs[1,4], Aytug K. Kiper[3], Bisher Eymsh[1], Mauricio Bedoya[5,6], Jan Langer[1], Niels Decher[3], Thomas Baukrowitz[1], Marcus Schewe[1].
[1]Institute of Physiology, Christian-Albrechts University of Kiel, Kiel, Germany, [2]Department of Anesthesiology, Weill Cornell Medical College, New York, NY, USA, [3]Institute of Physiology and Pathophysiology, Philipps University of Marburg, Marburg, Germany, [4]Medical School Hamburg, University of Applied Sciences and Medical University, Hamburg, Germany, [5]Ingeniería en Bioinformática, Universidad de Talca, Talca, Chile, [6]Millennium Nucleus of Ion Channels-Associated Disease, Universidad de Talca, Talca, Chile.

Two-pore domain K^+ (K_{2P}) channel activity was thought to be primarily regulated by a selectivity filter (SF) gate, but recent structures have also revealed a lower gate at the cytoplasmic entrance in the K_{2P} channels TASK-1 and TASK-2. Here we report a lower gate in the cardiac two-pore domain K^+ channel K_{2P}17.1 (TALK-2, TASK-4) that produced state-dependent pore access of intracellular applied blockers and cysteine modifying probes similar to the helix-bundle crossing gate in voltage-gated K_v channels. Moreover, we report small molecule modulators (i.e. 2-APB), native ligands (i.e. oleoyl-CoA) and mutations (introduced at positions corresponding to the X-gate in TASK-1) that open this lower gate. TALK-2 channels show also prominent SF gating induced by permeating ions such as Rb^+, membrane depolarization and extracellular alkalization. Remarkably, these SF stimuli also induce opening of the lower gate suggesting positive gate coupling. In agreement, activation of the lower gate (either by ligand binding or mutation) reduced the voltage required to open the SF gate as evident by large shifts in the G-V curves. These shifts represent the mechanical load for opening the lower gate via the voltage dependent ion binding to the SF. In summary, ion conduction in TALK-2 channels requires the opening of two gates located at both ends of the pore (SF gate and lower gate). The lower gate resembles the X-gate in TASK-1 and we report stimuli to open the gate. Further, we established assays to monitor the activity of each gate separately that revealed strong positive gate coupling which represents a unique feature in K^+ channels.

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Acute PKA modulation of the cardiac sodium current is mediated by 14-3-3

Yang Zheng[1,2], Angelina Ramirez-Navarro[1], Haiyan Liu[1], Snizhana Chorna[1], Xiaoping Wan[1], Isabelle Deschenes[3].
[1]Physiology and Cell Biology, The Ohio State University, Columbus, OH, USA, [2]Biomedical Engineering, Case Western Reserve University, Cleveland, OH, USA, [3]The Ohio State University, Cleveland, OH, USA.

Nav1.5 is an essential component of the cardiac action potential. Dysregulation of Nav1.5 contributes to cardiac arrhythmias and Nav1.5 mutations have been linked to lethal congenital cardiac diseases. However, myriad aspects of Nav1.5 function and regulation, mainly as it relates to associated regulatory proteins, remain to be elucidated. PKA or β-adrenergic stimulation is a regulator of cardiac function, and could modulate Nav1.5 function under both healthy and diseased conditions. 14-3-3 is a Nav1.5 interacting protein. Mass spectrometry studies revealed proximity between Nav1.5 PKA phosphorylation sites and the 14-3-3 binding site. Importantly, the 14-3-3 binding site on Nav1.5 was suggested to be phosphorylated by PKA. This led us to hypothesize that PKA could acutely modulate Nav1.5 through 14-3-3. We studied acute modulation by PKA of Nav1.5 using high-throughput automated patch clamping. Our data showed that acute PKA modifications could significantly change the sodium current. Interestingly, inhibition of 14-3-3 abolished the acute PKA effects. Serine 460 is part of the putative 14-3-3 binding motif and mutating Serine 460 to alanine (S460A) behaves like 14-3-3 inhibition and uncouples sodium currents. We therefore tested the effect of PKA modulation on Nav1.5-S460A and found that similarly to 14-3-3 inhibition, PKA had no effect on Nav1.5-S460A currents. To confirm the specific involvement of S460 and 14-3-3 in PKA modulation of Nav1.5, we mutated other phosphorylation sites found in the DI-DII linker. We found that except for S460A, all the other phospho-inhibiting mutations responded to PKA modulation. Our study demonstrated that PKA or β-adrenergic stimulation can acutely modulate Nav1.5 through protein 14-3-3 and Nav1.5-S460 appears to be central to this modulation. We conclude that 14-3-3 does not only regulate the Nav1.5 function and coupling, but also plays a key role in the PKA modulation.