KCNQ3 genes. Dorsal root ganglia (DRG) neurons cultured in the presence of a REST-expressing adenovirus showed 7.39 ± 0.11 fold ($p \leq 0.05$) increased REST protein, which led to a concomitant 2.20 ± 0.09 fold ($p \leq 0.05$) decrease in KCNQ2 protein and a corresponding 7.65 ± 0.49 fold ($p \leq 0.01$) reduction in M-current in DRG neurons, compared to vehicle control. We further show that REST protein expression was increased 3.65 ± 0.80 fold in cultured DRG neurons in response to inflammatory stimulation (1 μM bradykinin, 1 μM histamine, 1 μM ATP, 10 μM PAR2-AP and 1 μM substance P for 48 hrs). Increases in REST correlated with a 1.76 ± 0.38 ($p \leq 0.05$) fold decrease in KCNQ2 immunoreactivity. Similarly we observed a significant increase in REST mRNA (2.11 ± 0.01 fold) and protein levels and a reciprocal downregulation of KCNQ2 (1.75 ± 0.07 fold) and KCNQ3 (1.43 ± 0.01 fold) transcripts in DRGs from animals with neuropathic nerve injury (partial sciatic nerve lesion, PSNL). We propose that transcriptional regulation of KCNQ channels by REST will have profound effects on neuronal excitability and may contribute to the mechanisms of peripheral sensitisation in chronic pain.

Voltage-gated K Channels - Permeation

909-Pos Board B788

Permeation And Conformational Changes Of The Pore Domain Of The Kv1.2 Potassium Channel

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We have computationally determined the conductance of the pore-domain of the Kv1.2 potassium channel under physiologically relevant conditions of driving force and ion concentration using microsecond-timescale all-atom molecular dynamics simulations. The conductance, found to be limited in part by the rate of dehydration of the ion, is close to the experimental value for intact Kv1.2. We find that water and potassium ions are co-transported in a stoichiometric ratio close to one, as previously hypothesized, yet water and potassium favor different positions within the selectivity filter of the pore. On a microsecond timescale, the open conducting pore domain is found to undergo substantial conformational changes causing current attenuation, likely related to channel inactivation from the extracellular side. Finally, we observe reproducible closure events of the pore domain that involve pronounced conformational changes of the S6 and S4-S5 linker helices and of cavity-lining residues, whose net effect is to reduce hydration of the cavity and thus prevent its occupation by potassium ions.

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Mapping the Binding Site of the Alkoxypsoralen PAP-1 in the Voltage-Gated K+ Channel Kv1.3

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Kv1.3 is widely regarded as an attractive drug target for the treatment of effector memory T cell-mediated autoimmune diseases such as multiple sclerosis, type-1 diabetes and psoriasis. Schmitz et al. (2005) identified 5-(4-phenoxybutoxy)psoralen (PAP-1) as a potent and selective small molecule Kv1.3 blocker. Unlike the classical Kv1 blocker tetraethylammonium, the nucleophilic PAP-1 blocks Kv1.3 with a 2:1 stoichiometry. Following a hypothesis that nucleophilic ligands can coordinate a metal ion in the channel pore, we used Monte Carlo-energy minimizations to search for possible complexes of two PAP-1 ligands with a K^+ ion in the Kv1.2-based model of Kv1.3. In a predicted complex, the furocoumarin moieties of two ligands chelate a K^+ ion at the focus of the P-helices in the central cavity, while the 4-phenoxybutoxy arms extend into the intrasubunit S5/S6 interfaces and reach the S4-S5 linkers. The model predicted ligand-sensing residues in the S4-S5 linker, S5, P-loop, and S6. We next tested the model by introducing single amino acid substitutions into Kv1.3 and exploring the biophysical properties of the mutants and their sensitivity to PAP-1 in whole-cell patch-clamp experiments. So far we have confirmed L335 in the S4-S5 linker, L353 in S5, and V417 and T419 in S6 as PAP-1 sensing residues. Among the mutants, V417L exhibited the largest change in IC_{50}, 400 nM versus 2 nM for the wild-type channel. Interestingly, V417L and T419A also exhibit more of an open-channel type block rather than a C-type inactivated state block. The proposed model explains the actions of various nucleophilic ligands that block cationic channels with a Hill coefficient greater than 1, opening a new direction for structure-based design of ion channel drugs.
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Modeling of Binding of the Anti-Arrhythmic Compound Vernakalant to Kv1.5

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Vernakalant (RSD1235) is an investigational drug recently shown to convert atrial fibrillation rapidly and safely in patients as an intravenous formulation (Roy et al., 2004) and to maintain sinus rhythm when taken orally (Savelieva and Camm, 2008). In the present study, the modeling software AutoDock4 was used to explore potential binding modes of vernakalant to the open state of the Kv1.2 model, which is 100% homologous in the binding region with Kv1.5. Docking simulations were run with a maximum number of evaluations of 25,000,000 and a maximum number of generations of 27,000. Point mutations were made in the channel model based on earlier patch-clamp studies (Eldstrom et al., 2007) and the docking simulations re-run to evaluate the ability of the docking software to predict changes in drug-channel interactions. Each AutoDock run predicted a binding conformation with an associated value for free energy of binding (FEB) in kcal/mol and an estimated inhibitory concentration (K_i). Increasing the number of evaluations and thus the time allowed for the program to look for an optimal binding site decreased average FEB and Ki values, and resulted in two front runner binding conformations. The most favored conformation had a FEB of -7.12 kcal/mol and a predicted K_i of 6.08 μM. This conformation makes contact with all four T480 residues and when examined from the side view and from above appears to be clearly positioned to block the channel as it directly occludes the pore.

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Partnership interactions target Kv1.5 to distinct membrane surface microdomains

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Surface expression of voltage-dependent K^+ channels (Kv) has a pivotal role in leukocyte physiology. Although little is known about the physiological role of lipid rafts, these microdomains concentrate signaling molecules and their ion channel substrates. Kv1.3 associates with Kv1.5 to form functional channels in macrophages. Different isoform stoichiometries lead to distinct heteromeric channels which may be further modulated by targeting the complex to different membrane surface microdomains. Kv1.3 targets to lipid rafts, whereas Kv1.5 localization is under debate. With this in mind, we wanted to study whether heterotetrameric Kv1.5-containing channels target to lipid rafts. While in transfected HEK-293 cells, homo- and heterotetrameric channels targeted to rafts, Kv1.5 did not target to rafts in macrophages. Therefore, Kv1.3/Kv1.5 hybrid channels are mostly concentrated in non-raft microdomains. However, LPS-induced activation, which increases the Kv1.3/Kv1.5 ratio and caveolin, targeted Kv1.5 back to lipid rafts. Moreover, Kv1.5 did not localize to low-buoyancy fractions in L6E9 skeletal myoblasts, which also coexpress both channels, heart membranes or cardiomyocyes. Coexpression of a $Cav3^{DGV}$-mutant confined Kv1.5 to $Cav3^{DGV}$-vesicles of HEK cells. Contrarily, coexpression of Kvβ2.1 impaired the Kv1.5 targeting to raft microdomains in HEK cells. Our results indicate that Kv1.5 partnership interactions are underlying mechanisms governing channel targeting to lipid rafts.
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A Novel Screening Tool for Voltage-Gated Ion Channels: Light Induced Voltage Clamp

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Ion channels are a key target class with a high therapeutic potential. Conventional screening techniques yield insufficient data quality particularly when assessing voltage-gated ion channels. Thus, the development of new reliable technologies is desirable to integrate ion channel screening into early lead generation of drug discovery.
Here we demonstrate a method that allows non-invasive, millisecond light-induced activation of voltage-gated ion channels and the concurrent imaging of membrane potential changes using fast voltage-sensitive dyes. This light-induced voltage clamp method (LIVC) uses photostimulation through channelrhodopsin-2 (ChR2), to activate voltage-gated ion channels. ChR2 allows blue light (~ 470 nm) to be immediately transduced into a depolarizing ionic current, which causes voltage-gated ion channels to open. We coexpressed ChR2 with the voltage-gated potassium channel hKv1.5 in HEK293 cells and in Xenopus oocytes. In electrophysiological experiments we show that the light-induced cell depolarization through ChR2 sufficed to open hKv1.5 channels; the light-induced membrane depolarization was greatly reduced with active hKv1.5 channels compared to the full ChR2 response during hKv1.5 inhibition.