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.

910-Pos Board B789

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.

912-Pos Board B791

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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913-Pos Board B792

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.

We were further able to optically monitor the light-induced membrane depolarizations on a millisecond timescale with the voltage-sensitive RH421. The fluorescence readout reflected the concentration-inhibition relationship of the hKv1.5 inhibitor DPO-1.
LIVC represents a solely optical technology with remote activation of the target voltage-gated ion channels simply by the delivery of a flash of blue light and simultaneous detection of their activity employing voltage-sensitive dyes. It combines the high-throughput of optical methods with the high-content of patch clamp concerning and possible repetitive stimulation. Proof of concept and results from assay development for voltage-gated sodium and calcium channels as well as for the hERG channel underline the potential for LIVC to evolve into a high-throughput, high reliability assay for voltage-gated ion channels in general.

914-Pos Board B793
Kv1.7 - Interactions with Protons and a blocking Conotoxin
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We have examined the interactions of protons and an inhibitory, poly-cationic conotoxin with the human voltage-gated potassium channel, hKv1.7. This channel differs from some members of the Kv1 sub family by having a titratable histidine residue near the N-terminal end of the putative pore-supporting P-helix, suggesting that intrinsic channel functions and pharmacology may depend on the pH of the external solution. Channels were expressed in HEK-293 cells and studied by whole-cell patch clamp. The voltage dependence of channel activation was evaluated using a tail-current protocol in which the voltage was stepped to -40 mV following a variable activating pre-pulse. Lowering of the pH of the external solution from 7.4 to 5.0 produced a positive shift in the half-activation of 36 ± 3 mV (n=4). The lowering of pH also dramatically decreased the ability of the conotoxin to inhibit currents through the channels. Thus, following the largest depolarization, at pH5.0 very little inhibition was observed at a toxin concentration near IC50 for pH7.4. The tail current in the presence of the conotoxin was near the value seen in the absence of the toxin for external pH of 7.4. Our observations are consistent with a two-fold action of the conotoxin. First, they suggest that the positively charged toxin binds close enough to the S4 segment of the voltage sensor to inhibit activation following a depolarizing voltage step. Second, the observed decrease in maximal conductance at pH 7.4 following addition of the toxin is consistent with a block of current through the open channels. Toxin binding appears to be inhibited by protonation of a residue on the external surface of the channel, perhaps the histidine residue near the N-terminal end of the pore helix.

915-Pos Board B794
Pore Block of KCNQ1 Channels by Zn^{2+} is Modulated by Ancillary Subunits
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The basolateral membrane K^+ channel, KCNQ1 (KvLQT1, Kv7.1), plays a critical role in anion secretion by gastrointestinal tissues because it establishes an electrical driving force for anion exit. In secretory tissues, KCNQ1 associates with the KCNE3 ancillary subunit to form a voltage-insensitive K^+ channel, whereas, in the heart, it associates with KCNE1 (minK) to form a voltage-gated channel. To demonstrate the role of KCNQ1 in anion secretion, we used forskolin to activate secretory short circuit current (Isc) across T84 human colonic cell monolayers. We demonstrate that addition of Zn^{2+} (5 mM), an inhibitor of intestinal secretion, to the serosal bath reduced Isc by 34%. Subsequent addition of the K^+ channel blocker, Ba^{2+} (5 mM), reduced the current to near zero. To determine the mechanisms of Zn^{2+} block, we expressed KCNQ1 in *Xenopus* oocytes and determined the effects of extracellular Zn^{2+} on current-voltage relationships. When KCNQ1 was co-expressed with KCNE1, K^+ currents were very slowly voltage-activated and Zn^{2+} had a small inhibitory effect (23% at Vm = +40 mV). Co-expression of KCNQ1 with KCNE3 resulted in K^+ currents that were constitutively active and voltage-insensitive, however, Zn^{2+} caused these currents to become slowly-activating and dramatically reduced (64%). The dose-inhibition curve for Zn^{2+} on KCNQ1 expressed with KCNE1 revealed a single binding site (EC_{50} = 2 μM). The curve for KCNQ1 with KCNE3 also revealed a single binding component, but with a much greater affinity (EC_{50} = 0.1 μM) than we previously determined for Ba^{2+} (EC_{50} = 100 μM). These results suggest that the site of regulation of the open or activated state of KCNQ1 by KCNE1 or KCNE3 involves the extracellular pore region. In addition, the ancillary subunits cause state-dependent differences in the block of KCNQ1 by Zn^{2+}.

916-Pos Board B795
Discovery of a Novel Activator of KCNQ1-KCNE1 K+ Channel Complexes
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KCNQ1 (Kv7.1) associates with the family of KCNE peptides to form complexes with diverse gating properties and pharmacological sensitivities. The varied gating properties of the different KCNQ1-KCNE complexes enables the same K^+ channel to function in both excitable and non excitable tissues. Small molecule activators would be valuable tools for examining the gating mechanisms of KCNQ1-KCNE complexes; however, there are very few known activators of KCNQ1 channels and most are ineffective on KCNQ1-KCNE complexes. Our lab has identified a simple boronic acid, phenylboronic acid (PBA), which potentiates KCNQ1-KCNE channel complexes in a voltage dependent manner. Activation by the boronic acid moiety has some specificity for the Kv7 family members (KCNQ1, KCNQ2/3, and KCNQ4) since PBA does not activate Shaker or hERG channels. We show potentiation of current is due to a slower rate of deactivation and a hyperpolarizing shift in the voltage sensitivity of the channel complex. Analysis of different-sized charge carriers revealed that PBA targets the permeation pathway of KCNQ1 channels. The discovery that PBA activates physiologically relevant KCNQ1-KCNE complexes makes it a useful and readily available tool to investigate the molecular mechanisms of KCNQ1-KCNE complex activation.

917-Pos Board B796
Using Inducible Expression Vector Technology To Create Stable Cell Lines Expressing KCNQ2/3, KCNQ4, And KCNQ3/5 Currents Suitable For Automated Electrophysiology Platforms
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The KCNQ (Kv7) family of voltage gated ion channels conduct a number of hyperpolarising currents in various tissue types, including the heteromultimeric KCNQ2/3 M-current found in sensory neurones. Cell lines constructed using constitutive expression vectors to stably transfect KCNQ2/3, KCNQ4, and KCNQ3/5 genes gave acceptable performance when using rubidium efflux methodology. However, expression levels within the cell population were found to be variable when assessed using conventional electrophysiology. Cell morphology changed during passage and the cell lines were unsuitable for automated electrophysiology recording. Using RheoSwitch™ inducible vector technology we have created new stable cell lines where the production of ion channel can be closely controlled by addition of an inducer agent. Putative clones were screened using IonWorks® Quattro™ recording in single hole PatchPlate™ mode. For each of the three cell lines, clones were identified displaying more than 60% of the cells having greater than 0.5nA of current. The performance of the clones in single hole mode was suitable for progression to Population Patch Clamp™ (PPC) mode recording. Each cell line displayed acceptable seal properties and current amplitudes, KCNQ2/3 26 ± 5 MΩ, 0.77 ± 0.19 nA (n=250); KCNQ4 112 ± 49 MΩ, 0.44 ± 0.07 nA (n=372) and KCNQ3/5 159 ± 44 MΩ, 0.84 ± 0.50 nA (n=124). In addition, each cell line each cell line displayed the appropriate pharmacology for regitabine, linopridine, XE991, TEA and bepridil. The cell lines are suitable for compound screening and selectivity profiling using automated and conventional electrophysiology.

918-Pos Board B797
The Dipeptidyl-peptidase-like-protein DPP6 Determines the Unitary Conductance of Neuronal Kv4.2 Channels
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The neuronal subthreshold-operating A-type K^+ current regulates electrical excitability, spike timing and synaptic integration and plasticity. The Kv4 channels underlying this current have been implicated in epilepsy, regulation of dopamine release, and pain plasticity. However, the unitary conductance (γ) of neuronal somatodendritic A-type K^+ channels composed of Kv4 pore-forming subunits is larger (~7.5 pS) than that of Kv4 channels expressed singly in heterologous cells (~4 pS). Here, we examined the putative novel contribution of the dipeptidyl-peptidase-like-protein-6 DPP6-S to the γ of native (cerebellar granule neuron, CGN) and reconstituted Kv4.2 channels. Co-expression of Kv4.2 proteins with DPP6-S was sufficient to match the γ of native CGN channels; and CGN Kv4 channels from *dpp6* knock-out mice yielded a γ indistinguishable from that of Kv4.2 channels expressed singly. Moreover, suggesting electrostatic interactions, charge neutralization mutations of two N-terminal