different types of diseases. These channels are nonselective and permeable to Na+, Ca++ and other cations and they are modulated by changes in the extracellular and intracellular concentrations of these cations. Our studies also show that the translocation of these proteins to the cell membranes is stimulated by defined chemical chaperones, proteasome inhibitors and modulators of ion homeostasis. They play a role in membrane trafficking and their translocation between different subcellular compartments is modulated by hormones involved in the homeostasis of Na+ and other ions. Cells expressing mutated TRPP and related proteins show abnormal trafficking of subcellular membrane vesicles. Such abnormalities may explain the symptomatology and the pathogenesis of the disorders caused by the mutations of the genes encoding these proteins.

904-Pos Board B783

Binding of Syntaxin 1A to the C-terminus of hERG Channels Affects Channel Trafficking and Inactivation

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The SNARE protein, syntaxin 1A (STX1A), functionally regulates cardiac ion channels, including the human ether-à-go-go related gene (hERG) which encodes the pore-forming voltage-gated K^+ channel underlying I_{Kr} in the heart. The primary mode for STX1A-dependent inhibition of hERG channel function is trafficking impairment which can be rescued by reduced temperature or the high-affinity channel blocker, E4031. A secondary mode is achieved by the production of a hyperpolarizing shift in the voltage dependence of steady-state inactivation. Here we report the STX1A binding region on hERG channels. GST pulldown and coimmunoprecipitation demonstrates that the cytosolic SNARE motif-containing STX1A-H3 domain preferentially binds to hERG. This cytosolic domain is attached to the TM region by a short inflexible linker and is 60 residues in length. Use of N- (hERG-Δ2-16 and hERG-Δ2-354) and C-terminal truncation mutations (hERG-Δ1120, hERG-Δ1045, hERG-Δ1000, hERG-Δ960, hERG-Δ899, hERG-Δ860-899, hERG-Δ860, and hERG-Δ814) demonstrates that STX1A binds to all truncation mutations tested. The hERG C-terminus begins at approximately residue 670 immediately adjacent to the cytosolic portion of the S6 helices. Therefore, we deduce that the STX1A-H3 domain interacts with the C-terminus of hERG channels between residues 670 and 814. Functional analysis of C-terminal truncation mutations demonstrates that STX1A inhibits the trafficking of truncations up to hERG-Δ1000, but has no effect on hERG-Δ960 while enhancing trafficking and function of hERG-Δ899. We infer that STX1A-interaction disrupts normal protein folding thereby inhibiting channel trafficking, and may alter the movement of S6 helices affecting the hERG channel inactivation gate, shifting the voltage-sensitivity of channel inactivation. SNARE protein-mediated regulation of cardiac ion channels represents a novel biological mechanism that may have universally intrinsic implications for normal and diseased heart function.
(Supported by HSFO)

905-Pos Board B784

Rapidly Inducible Protein Modification Using Rapamycin-mediated Complementation Of Tobacco Etch Virus (TEV) Protease

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Traditional modalities of protein knock down rely on transcriptional or translation suppression and are thus hampered by slow endogenous protein turnover rates and compensatory mechanisms. Directly targeting proteins, rather than synthetic pathways, facilitates more rapid depletion of the targeted protein. Tobacco etch virus protease (TEVp), a potyvirus nuclear inclusion protein, cleaves at a canonical sequence, ENLYFQ/G, with sufficient specificity to be useful for targeting specific proteins. Starting with a TEVp mutant (S219V) deficient in autocatalysis (Kapust et al., *Protein Eng.* 14:993, 2001), we have humanized the codon bias and introduced mutations (van den Berg et al., *J. Biotechnology* 121:291, 2006) to increase solubility. Expression of the modified TEVp was well tolerated by Hela cells and resulted in efficient cleavage, as determined by Förster resonance energy transfer, of a co-expressed reporter consisting of fluorescent proteins (Cerulean and Venus) fused with a linker containing a TEVp cleavage sequence. Splitting the TEVp at residue 119 (Wehr et al., *Nat. Methods* 3:985, 2006) and fusing the fragments to FKBP12 and the c-terminus of FRB resulted in inducible enzyme activity following addition of rapamycin to living cells. Cleavage at 37°C proceeded exponentially with a time constant of approximately 100 minutes. In an effort to accelerate cleavage, the two halves of the complementation system were fused to form circular permutated unitary constructs. However, these constructs demonstrated constitutive activity without rapamycin. Fusing one half of TEVp to the reporter protein (cleavage in *cis*) also resulted in non-regulated activity. Current efforts are aimed at targeting one half of TEVp to the plasma membrane and recruiting the other half from the cytoplasm upon addition of rapamycin. Targeted protein cleavage was monitored by measuring $K_V3.4$ inactivation of a reporter construct consisting of EGFP-TEVP site-$K_V3.4$.

906-Pos Board B785

Resolving the Structural-Functional Interaction Between HIV-1 Vpu and TASK Channels by FLIM/FRET

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An established membrane potential is fundamental to the survival of a cell. The majority of cellular and physiological processes foremost require orchestration of various ion channel activities and interactions in a spatially and temporally interdependent fashion. Previously we found that Vpu, a single transmembrane protein encoded by HIV-1, could interact with the assembly of endogenous K_{2P} channels that set resting potentials. This Vpu function reflects its viroporin structure, conferring the propensity to oligomerize into homomeric channels or promiscuously with homologous endogenous channel subunits. By interfering with normal assembly of background K^+ channels, Vpu suppresses the stabilizing K^+ conductance and dissipates the transmembrane voltage constraint on viral particle discharge. In this report, we measured membrane potential changes with respect to TASK-Vpu interaction in single cells by implementing a FRET pair with potential-tracking imaging. Our combined measurements from FLIM/FRET and confocal microscopy showed a structural-functional coupling between the two physical parameters (protein-protein interaction; membrane potential), and confirmed the previous experimental results obtained by independent means.

907-Pos Board B786

The Positive Effect Of STREX On BK Channels

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The large conductance voltage and calcium sensitive potassium channel (BK) is encoded by a single gene KCNMA1. The inclusion of a stress regulated exon (STREX) at a splice site (C2) in the intracellular carboxyl (C) terminus of BK confers differing properties to the channel. STREX insertion generates a putative polybasic region. In a number of other channels, polybasic regions are suggested to interact with negatively charged phospholipids, such as phosphoinositides, to control ion channel gating and membrane targeting. We hypothesised that the polybasic region including STREX may serve as a membrane targeting domain of the STREX BK channel C-terminus. To test this, a GFP-tagged carboxyl terminal construct spanning the S6 transmembrane domain to the COOH end region of the intracellular carboxyl terminus was constructed (S6-COOH-STX) and transiently transfected into HEK293cells. The construct localised at the plasma membrane and this was abolished when the STREX insert was deleted. To test whether the polybasic region is important for plasma membrane targeting, two approaches were taken: Firstly, site directed mutagenesis to change selected positive residues into neutral (alanine) residues, also abolished membrane targeting of S6-COOH-STX to the plasma membrane. Secondly, to discern whether the polybasic region may interact with negatively charged phosphoinositides at the plasma membrane, the S6-COOH-STX construct was co-transfected with a 5′phosphatase, IPP. Cells co-expressing IPP displayed significantly reduced plasma membrane targeting of S6-COOH-STX, however a phosphatase null mutant of IPP did not effect plasma membrane expression. These data suggest that the polybasic region generated by inclusion of the STREX insert is an important determinant of STREX domain interaction with the plasma membrane. The functional role of the polybasic region and the interaction of phospholipids on BK channel calcium and voltage sensitivity were elucidated using patch clamp electrophysiology and high throughput dyes.

908-Pos Board B787

Regulation Of Kcnq2/3 Channels By The Transcriptional Repressor REST In Nociception

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Understanding how excitability of sensory neurons is regulated is an important goal since this excitability underlies pain transmission and unfortunately almost everyone will suffer from inflammatory pain at some point in their life. Recent studies have identified expression of M-type K^+ channels (encoded by KCNQ genes) in damage-sensing (nociceptive) sensory neurons, where they are thought to control excitability. Accordingly, receptor-induced inhibition of M-current in these neurons has been shown to contribute to peripheral sensitisation and inflammatory pain. Here we describe a new mechanism for downregulation of KCNQ channel expression in nociceptors. We identified binding sites for the transcriptional repressor REST within both the KCNQ2 and

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.