previously identified for temperature sensing in heat sensitive vanilloid receptors. Upon exchange of the region, the heat activation of the channel becomes reversible and the temperature dependence becomes considerably reduced as the wild type channels after sensitization. Interesting much of the hysteresis effect can be attributed to a single residue near the TRP box. The position of the residue suggests a mechanism of temperature-dependent gating of thermal TRP channels involving an intracellular region assembled around the TRP domain.

628-Pos  Board B408
The Spider Toxin GsMTx-4 Blocks TRPV4 Cation Channels Expressed in HEK-293 Cells
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The transient receptor potential channel TRPV4 is a calcium conducting, osmosensitive cation channel and expressed in motoneurons and skeletal muscles. In mouse muscle fibers TRPV4 forms or contributes to a mechanosensitive cation channel. The molecular architecture of this channel is still unclear. Since mechanosensitive cation channels can be blocked by the peptide toxin GsMTx-4, we studied whether TRPV4 itself is sensitive to GsMTx-4. To this end a TRPV4-YFP construct was functionally expressed in HEK-293 cells. Intracellular Ca2+ concentrations were monitored with the fluorescent Ca2+ indicator Fura-2 and the data presented as Fura-2 fluorescence ratios after alternate excitation at 340 and 380 nm. Non-transfected cells (n = 47) had an average resting ratio of 0.39 (range 0.38 and 0.42), while TRPV4 transfected cells (n = 524) showed on average an increased fluorescence ratio of 1.11 ranging from 0.44 to 2.71. Application of 4a-PDD (5 μM), a known TRPV4 activator, increased the fluorescence ratios in the HEK cells to 1.72, 1.73 and 1.77 with a peak or plateau at 1-3 min (n=13-19 cells, each experiment). The effect of 4a-PDD could be reversed within about 10-30 min by application of the TRPV4 blocker HC-067047 (1 μM) and GsMTx-4 (5 μM). The final resting ratios were 0.77 for HC-067047 and 1.16 for GsMTx-4. At a concentration of 1 μM the toxin was almost ineffective. The results indicate that TRPV4 activity can be inhibited within about 10-30 min by application of the TRPV4 blocker HC-067047 and 1.16 for GsMTx-4. We have recently shown that unexpectedly cholesterol enrichment up-regulates GIRK activity in atrial myocytes. In accordance, we also observed elevated GIRK currents in cholesterol-enriched Xenopus oocytes expressing the GIRK1/GIRK4 heteromers, the two pore-forming subunits expressed in the heart. Interestingly, whereas similarly to the heteromer GIRK1/GIRK4, the highly active homomeric pore mutant GIRK4* (GIRK4_S143T) was also enhanced by cholesterol, GIRK4* (GIRK1_F137S) was suppressed by cholesterol. Thus, in this study, we focused on identifying what determines whether a channel would be enhanced or suppressed by cholesterol.

In excitable cells, ion channel responses to patterned stimuli shape cellular behavior by regulating the duration, shape, and frequency of action potentials. We have investigated the behavior of voltage-gated potassium (Kv) channels subjected to repetitive stimuli, with a particular focus on the delayed rectifier Kv1.2. Genetic deletion of this subunit results in incomplete mortality within two weeks of birth in mice, highlighting a critical role for Kv1.2. To this end, we have examined a unique property of Kv1.2, previously described as "prepulse potentiation". In this study, we demonstrate that this property enables Kv1.2 channels to exhibit use-dependent activation during trains of brief depolarizations, causing dramatic increases in elicited current. Importantly, Kv subunits may assemble into heteromeric channels in the central nervous system, generating diversity of function and sensitivity to signaling mechanisms. We demonstrate that other Kv1.2 channels do not exhibit use-dependent activation, but this property is conferred when they coassemble with Kv1.2. These results are apparent in transfected mammalian cell lines as well as in tityustoxin-sensitive currents recorded from primary cultures of dissociated hippocampal neurons. Importantly, Kv1.2 assembly with different subunits can generate diverse current phenotypes with variable use-dependent activation or rundown. These findings illustrate that use-dependent activation is a unique property of Kv1.2 which persists in heteromeric channels and may influence physiological function of hippocampal neurons.

630-Pos  Board B410
The Impact of Cholesterol on GIRK Channels depends on a Transmembrane Region of the Channels
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In recent years, cholesterol emerged as a major regulator of ion channel function. The most common effect of cholesterol on ion channels is a decrease in channel activity. Here we focus on G-protein gated inwardly rectifying potassium (GIRK or Kir3) channels that play an important role in regulating membrane excitability in neurones, cardiac and epithelial cells.

We have recently shown that unexpectedly cholesterol enrichment up-regulates GIRK activity in atrial myocytes. In accordance, we also observed elevated GIRK currents in cholesterol-enriched Xenopus oocytes expressing the GIRK1/GIRK4 heteromers, the two pore-forming subunits expressed in the heart. Interestingly, whereas similarly to the heteromer GIRK1/GIRK4, the highly active homomeric pore mutant GIRK4* (GIRK4_S143T) was also enhanced by cholesterol, GIRK4* (GIRK1_F137S) was suppressed by cholesterol. Thus, in this study, we focused on identifying what determines whether a channel would be enhanced or suppressed by cholesterol. Although the major difference between these channels is in the cytosolic domain, our data showed that the impact of cholesterol did not depend on the extended C-terminus of GIRK1 as compared with GIRK4. Rather, our results show that a transmembrane region that includes residues from both the inner and outer alpha helices of the channel determines the impact of cholesterol on the channel.

631-Pos  Board B411
The Significance and Mechanisms of Clustering by SLC4 Cotransporters in the Plasma Membrane
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The Solute Carrier 4 (SLC4) family are integral membrane cotransporters that reabsorb or secrete anions across the basolateral membranes throughout the body, especially the kidney, eye, and red blood cells. Defects of SLC4A4 (NBCe1-A) lead to type-II acidosis and myriad ocular abnormalities; those of SLC4A1 (AE1) causes type-I acidosis and spermatogenic. We demonstrate that SLC4s form patches in the membrane of proximal tubule cells, corneal endothelial cells, and Xenopus oocytes. This new discovery of homophilic interactions or self-associations was initially suggested from our biophysical studies of the N-terminal domain of NBCe1-A (Nt) in solution, and by others studying the transmembrane domain (TMD) of AE1. The role and mechanisms of self-associations are further investigated, with insight into the role of the devastating R298S hereditary mutation found in type-II RTA afflicted individuals. We use confocal-microscopy imaging of fluorescently-tagged NBCe1-A and NBCe1-A-R298S molecules expressed in the aforementioned cells types and from in-depth biophysical studies of their Nts, including our Nt crystal structure at 2.4 Å resolution, melting-temperature, and homodimer dissociation constant (Kd) analyses by composition-gradient multi-angle light scattering (CG-MALS). The structure reveals that the Nt dimerizes by two interlocking arms and contains two variable sequence regions, one at the extreme N terminus (V1) and another in the middle (V2). The V1 domains are flexible segments that gate substrate entry into the Nt, and that the V2 are large solvent accessible loops that extend ~20 Å from the core Nt structure. Truncation mutants were rationally generated, and surface plasmon resonance (SPR) was used to investigate their roles in self-association. Based on these findings, we discuss the role of clustering in HCO3- sensing and transport in health and provide insight to the pathogenic processes observed in patients with point mutations.

632-Pos  Board B412
The Role of ROS in Tethering CFTR within Ceramic Platforms at the Plasma Membrane
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The cystic fibrosis transmembrane conductance regulator (CFTR) is an anion channel which is expressed at the apical surface of many secretory epithelia. CFTR and reactive oxygen species (ROS) are both involved in innate immune responses against bacterial pathogens, however the link between them during host defense is not well understood. Here we study the surface expression and dynamics of CFTR in epithelial cells during protein kinase C (PKC) stimulation using fluorescence microscopy and k-space image correlation spectroscopy (kICS). kICS analysis was used to measure the confined...
dynamics of CFTR under control conditions and during PKC stimulation while simultaneously monitoring CFTR expression at the cell surface as total fluorescence intensity. During PKC stimulation, CFTR became aggregated into large (1–2 μm diameter) structures on the plasma membrane called platforms. PKC induced a significant (1.5-fold) increase in CFTR surface expression and increased the CFTR confinement within platforms, as indicated by a decline in its confined diffusion coefficient from 0.011 ± 0.001 μm²/s to 0.0033 ± 0.0002 μm²/s. Pharmacological inhibition of ROS or acidic sphingomyelinase (ASM) prevented the PKC-induced aggregation and tethering of CFTR. This suggests a scheme in which PKC stimulates the production of ROS, which activates ASM and ceramide synthesis, leading to the formation of platforms in the outer leaflet of the plasma membrane. Ceramide platforms may help stabilize CFTR in the membrane so that its surface expression and channel function are increased during secretagogue stimulation.

633-Pos Board B413
Refinement and Evaluation of a CFTR Homology Model and Identification of Residues Controlling Channel Gating
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Cystic Fibrosis, which affects nearly 1 in 2,500 births in the Caucasian population, is caused by various mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. CFTR is a member of the diverse ATP-binding-cassette (ABC) protein superfamily. While most ABC proteins are transporters, CFTR has been shown to function as an ion channel. Currently, no crystal structure of full length CFTR exists, but due to its biological relevance, many CFTR homology models have been proposed. Using a recently published and experimentally supported model of CFTR, we have carried out all-atom Molecular Dynamics (MD) in an attempt to further characterize the dynamics of this recalcitrant protein. First, we refined the model utilizing MD flexible fitting (MDF) and structural data from a 9-Å cryo-EM map of CFTR (Rosenberg et al., 2011). Next, equilibrium MD simulations of the model within a membrane were used to probe 3 physically relevant states: the apo state, the proposed semi-apo state (1 ATP bound), and the bound state (2 ATP bound). Initial results show a difference in dimerization in the nucleotide-binding domains (NBDs) between the three states. In other experiments, residues in CFTR’s NBDs indicating sequence divergence from the canonical ABC protein sequence (suggesting that they may be involved in slowing the ATP hydrolysis rate, which would likely support longer open durations) were returned to the canonical sequence. Burst durations (in ms) measured for single-channels expressed in oocytes were WT-CFTR (683 ± 59), S573E-CFTR (427 ± 83)*, G576S/Y577A-CFTR (431 ± 46)*, (*p = 0.05). These results suggest that CFTR’s NBDs evolved in a manner that would suppress the shift from transporter to channel function. (Support: 5R01-DK054618).

634-Pos Board B414
Solution NMR to Investigate Gating in the NaK Channel
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The NaK channel is a small bacterial non-selective cation channel that has proved to be an excellent model system to study basic biochemical and biophysical questions concerning ion channels. NaK has been crystallized in the closed state and as an open channel using a truncated construct (NaKa19) missing the M0 helix. NaK shares a homologous architecture to tradition K+ channel pores such as KcsA, but it is non selective. However, small mutation in the selectivity filter can change NaK into a K+-selective channel. Solution NMR spectra of NaK solubilized in isotropic bicelles show superior spectral resolution, allowing for detailed solution NMR studies of NaK structure and dynamics in bicelles. Regulation of NaK is currently unknown, however the location of the amphipathic M0 helix on the membrane surface packed against two adjacent monomers makes it a likely contributor to channel gating. Preliminary work suggests that the full length NaK channel exists in at least two states in slow exchange at 45°C. Mutations to the M0 helix shift the equilibrium to a single population, supporting a role for the M0 helix in determining the structural and functional state of the channel. NaK19 NMR spectra are also sensitive to addition of amino acids just before the M1 helix. This suggests that this region plays a role in gating. Mutations to the M1 helix? are impeding in determining NaK dynamics. Comparison of our results with functional studies highlights specific interactions between the M0 helix and the pore domain that determine the structural and functional state of the channel.

635-Pos Board B415
A New Class of Positive Gating Modulators of hKv3.2 Channels: Insights into the Mechanism of Action
Qiansheng Liang1, Giuseppe Alvaro2, Charles Large2, Manuel Covarrubias3, 1Thomas Jefferson University, Philadelphia, PA, USA, 2Aptifony Therapeutics Limited, London, United Kingdom, 3Neuronal Kv3 channels are implicated in neurological (tinnitus, hearing loss) and psychiatric (schizophrenia) disorders, and are potential therapeutic targets. Here, we investigated two new compounds (AUT3 and AUT5) developed by Aptifony Therapeutics, which may act as specific modulators of this K+ channel sub-family. Initial screening of heterologously expressed Kv3 channels in Xenopus oocytes demonstrated that AUT5 acts as a relatively selective positive modulator of hKv3.2. AUT3 (2 μM) induces a substantial parallel negative shift in the conductance-voltage relation (ΔD1/2 = –24.1 ± 2.0 mV, n = 9). However, 2 μM AUT3 has little to no effect on Kv1.2, Kv3.4, Δ28-Kv3.4 and Kv4.2. The positive modulatory effect on hKv3.2 is concentration-dependent with an EC50 = 2 – 4 μM. AUT3 also acts as a positive modulator of hKv3.2, albeit the effect is ± 10-fold weaker. Toward investigating the mechanism of action, we examined tail current kinetics and found a dramatic slowing of deactivation with 2 μM AUT3 (τa = 24.7 ± 6.2 ms vs 6.5 ± 1.1 ms of control at –50 mV, n = 6). We observed similar positive modulation by AUT3 and AUT5 in mammalian cell lines (HEK-293 and stably transfected cells). We hypothesize that a direct interaction between AUT compounds and the channel’s activation machinery favorably affects the energetics of hKv3.2 activation gating. To test this hypothesis, we are currently investigating possible effects on voltage sensor movements, extending the screening to additional Kv channels, and searching for putative binding sites in the Kv3.2 protein.

636-Pos Board B416
PKA Reduces the Rat and Human KCa3.1 Current, CaM Binding and Ca2+ Signaling, which Requires Ser332/334 in the CaM-Binding C Terminus
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The Ca2+–dependent K+ channel, KCa3.1 (KCNN4/Ki3/SK4), is widely expressed and contributes to cell functions that include volume regulation, migration, membrane potential and electrical excitability. KCa3.1 is now considered a therapeutic target for several diseases, including CNS disorders involving microglial activation; thus we need to understand how KCa3.1 function is regulated. KCa3.1 gating and trafficking require calmodulin binding to the two ends of the CaM-binding domain (CaMBD), which also contains three conserved sites for Ser/Thr kinases. While cAMP protein kinase (PKA) signaling is important in many cells that use KCa3.1, reports of channel regulation by PKA are inconsistent. Here, I first compared regulation by PKA of native rat KCa3.1 channels in microglia (and the microglia cell line, MLS-9) with human KCa3.1 channel expressed in HEK293 cells. In all three cells, PKA activation with Sp-8-Br-cAMPS decreased the current, and this was prevented by the PKA inhibitor, PKI1422. Inhibiting PKA with Rp-8-Br-cAMPS increased the current in microglia. Mutating the single PKA site (S334A) in human KCa3.1 abolished the PKA-dependent regulation. CaM-affinity chromatography showed that CaM binding to KCa3.1 was decreased by PKA-dependent phosphorylation of S334, and this was absent in the S334A mutant. Single-channel analysis showed that PKA decreased the open probability in wild-type but not S334A mutant channels. The same decrease in current for native and wild-type (but not S334A) expressed KCa3.1 channels occurred when PKA was activated through the adenosine A2a receptor. Finally, by decreasing the KCa3.1 current, PKA activation reduced Ca2+–release-activated Ca2+ (CRAC) entry following activation of metabotropic purinergic receptors.

637-Pos Board B417
Cardiac Sodium Channel: Activation by CaM Involves a NaV1.5-NaV1.5 Interaction
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Ionotropic Kv channels, and searching for putative binding sites in the Kv3.2 protein.

Cardiac Sodium Channel: Activation by CaM Involves a NaV1.5-NaV1.5 Interaction
Sandra B. Gabelli1, Agedi Boto1, Victoria Halpernin2, Mario A. Bianchet1, 1Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2Physiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA, 3Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA, 4National Synchrotron Light Source, Brookhaven National Laboratory, Upton, NY, USA.

The Ca2+–dependent K+ channel, KCa3.1 (KCNN4/Ki3/SK4), is widely expressed and contributes to cell functions that include volume regulation, migration, membrane potential and electrical excitability. KCa3.1 is now considered a therapeutic target for several diseases, including CNS disorders involving microglial activation; thus we need to understand how KCa3.1 function is regulated. KCa3.1 gating and trafficking require calmodulin binding to the two ends of the CaM-binding domain (CaMBD), which also contains three conserved sites for Ser/Thr kinases. While cAMP protein kinase (PKA) signaling is important in many cells that use KCa3.1, reports of channel regulation by PKA are inconsistent. Here, I first compared regulation by PKA of native rat KCa3.1 channels in microglia (and the microglia cell line, MLS-9) with human KCa3.1 channel expressed in HEK293 cells. In all three cells, PKA activation with Sp-8-Br-cAMPS decreased the current, and this was prevented by the PKA inhibitor, PKI1422. Inhibiting PKA with Rp-8-Br-cAMPS increased the current in microglia. Mutating the single PKA site (S334A) in human KCa3.1 abolished the PKA-dependent regulation. CaM-affinity chromatography showed that CaM binding to KCa3.1 was decreased by PKA-dependent phosphorylation of S334, and this was absent in the S334A mutant. Single-channel analysis showed that PKA decreased the open probability in wild-type but not S334A mutant channels. The same decrease in current for native and wild-type (but not S334A) expressed KCa3.1 channels occurred when PKA was activated through the adenosine A2a receptor. Finally, by decreasing the KCa3.1 current, PKA activation reduced Ca2+–release-activated Ca2+ (CRAC) entry following activation of metabotropic purinergic receptors.