recorded voltage ramps in the interval ±200 mV in the presence of 2 M internal sucrose. In these conditions, outward currents become voltage independent at voltages >150 mV, providing size estimates consistent with the size of the internal mouth derived from the crystallographic structure. The ion density profile obtained from molecular dynamics simulations using an applied voltage reveals a high density of K+ ions near P475D.
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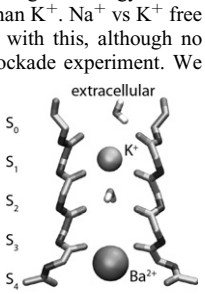
3064-Pos Board B169
Calculating Conductance and Size of the Entrance to the Inner Cavity of BK Channels with Side-Chain Replacement and a Two-Resistor Model
Yanyan Geng, Karl L. Magleby.
BK channels have the largest conductance (~250 pS) of all K+ selective channels. Previous studies suggest that residues E321/E324 in BK channels are located at the entrance to the inner cavity. We find that attachment of thiol reagents MPA and MTSET to E321C/E324C altered outward single-channel currents, suggesting that 321/324 face the ion conduction pathway. Therefore, substituting E321/E324 with different sized amino acids should change the size of the entrance to the inner cavity. We find that decreasing the size of the entrance decreases the conductance, whereas increasing the size of the entrance has little effect. Increasing [K+]i from 0.15 to 2.5 M negates differences in single-channel current associated with different side-chain volume. Plots of conductance vs. side-chain volume are approximated with a simple two-resistor model, where the ion conduction pathway is described by two resistors in series. R2 is a variable resistor, with resistance inversely proportional to the volume of the entrance to the inner cavity. R1 is a fixed resistor arising from the other parts of the conduction pathway including the selectivity filter. Fitting the data indicates that R1+R2 is ~5.4 gigaohm for glycine substitution, with an R1/R2 ratio of ~17, and effective radius and length of the entrance to the inner cavity of ~9.0 and 5.4 Å, respectively. (The volume of K+ and water are not taken into account.) The calculated size of the entrance to the inner cavity of BK channels is consistent with the crystal structure of large conductance bacterial MthK channels. These observations suggest that a large entrance to the inner cavity is required for the large conductance of BK channels, as decreasing the entrance size decreases the outward single-channel currents. Support: NIH-AR32805.

3065-Pos Board B170
Mechanism for Selectivity-Inactivation Coupling in KcsA Potassium Channels
Jason G. McCoy, Wayland Cheng, Ameer N. Thompson,
Crina M. Nimigean, Colin G. Nichols.
Potassium channels containing the GYG motif often diverge in their selectivity for monovalent cations, but the molecular basis is unknown. Using the prokaryotic potassium channel KcsA as our model, we have investigated the role of the interaction between glutamate 71 and aspartate 80, located behind the selectivity filter, in determining the selectivity of the channel as well as its influence on the conformation of the filter. In E71A KcsA channels, Na+ permeates at higher rates in both the presence and absence of K+, as seen with 86Rb+ and 22Na+ flux measurements. Single channel recordings indicate that Na+ "punches through" E71A KcsA channels at lower voltages than wild-type KcsA, and in the punchthrough regime the Na+-blocked current appears significantly larger. A crystal structure of E71A KcsA reveals that in contrast to what was seen for wild type KcsA, the selectivity filter does not collapse in the absence of K+, but instead assumes a "flipped" conformation. This flipped conformation is the same one observed in previous E71A KcsA structures in the presence of K+. The data reveal the importance of this E71-D80 interaction in both favoring inactivation and maintaining high K+ selectivity. We propose a molecular mechanism by which inactivation and K+ selectivity are linked, a mechanism that may also be at work in other channels containing the canonical GYG signature sequence.

3066-Pos Board B171
Structural Characterization of the Voltage Sensor Domain of the KvAP Channel Vectorially-Oriented within a Phospholipid Bilayer Membrane
Sanju Gupta, J. Liu, A. Tronin, J. Strzalka, J. Krepkiy, K. Swartz,
J. Kent Blasie.
Voltage-gated cation (Na^+, K^+) channels are responsible for the generation and propagation of action potentials in neurological signal transmission. Kv-channels are transmembrane proteins consisting of a homo-tetramer of 4 subunits that assemble about a 4-fold axis normal to the membrane plane to form the K^+ ion-selective pore. Each of the four subunits is comprised of six transmembrane helices, the S1-S4 helices forming the voltage-sensor domain (VSD) and the S5-S6 helices contributing to form the pore domain (PD). Despite several advances in the field, a complete understanding of the mechanism of electromechanical coupling interconverting the closed-to-open states is yet to be achieved. Positively charged arginine residues predominately in the S4 helix of the VSD are responsible for voltage sensing and the VSD's are arranged around the periphery of the PD in extensive contact with the lipid bilayer. This prompted us to focus initially on the structure of VSD itself within a phospholipid bilayer environment for the present study. A hydrated, phospholipid bilayer membrane environment has been reconstituted for the VSD of KvAP, vectorially oriented on the surface of inorganic multilayer substrates. This has been established by X-ray and neutron reflectivity (enhanced by interferometry), the latter employing a specifically deuterated phospholipid and water contrast variation, for the reconstituted membrane at both the solid-vapor and solid-liquid interfaces. This accomplishment now allows an investigation of the profile structure of the VSD within the lipid bilayer as a function of the applied transmembrane electric potential via x-ray reflectivity with millisecond time-resolution, employing high energy x-rays (> 20KeV) & pixel array detectors, and neutron reflectivity, employing selectively deuterium-labeled VSD proteins achieved via semi-synthesis. The same approach can be extended to the intact KvAP channel.

3067-Pos Board B172
KcsA Ion Affinity at an External Site Probed by Barium Block
Kene N. Piasta, Christopher Miller.
Block by Ba^{2+} is a distinctive property of K^+ channels since Ba^{2+}, a doubly charged analog for K^+, is electrostatically stabilized in the permeation pathway. Ba^{2+} block was used in BK channels as a tool to determine the equilibrium binding affinity for various ions at specific sites in the selectivity filter. In this work, we applied this approach to discrete block of single E71A KcsA channels, a non-inactivating mutant, in order to determine a thermodynamic measure of selectivity in a channel with abundant high-resolution structural information. We find at high concentrations of external K^+ the block time distribution is described by two distinct populations of Ba^{2+} block events. This argues there are at least two Ba^{2+} sites in the selectivity filter, fitting well with the published Ba^{2+} containing structure of KcsA where a Ba^{2+} ion resides approximately in S2 and S4. Utilizing a kinetic analysis of the blocking events as a function of external K^+, we determined the equilibrium dissociation constant of K^+ and other monovalent cations in an extracellular site, presumably S1, to arrive at a selectivity sequence for this particular site: Rb^+ (1 M) > K^+ (19 M) >> Na^+ (>1M). This represents an unusually high selectivity for K^+ over Na^+ with a $\Delta\Delta G^0$ of at least −7 kcal mol^{-1}. We are currently determining affinities for Li^+, NH_4^+, and Cs^+ at this site. The results fit well with other kinetic measurements of selectivity as well as with the many structures in various ionic conditions.

3068-Pos Board B173
QM/MM Modeling of Ba^{2+} Blockades in Potassium ion Channels
Christopher N. Rowley, Bogdan Lev, Sergei Noskov, Benoit Roux.
The robust selectivity of potassium channels for K^+ over Na^+ ions is a major component of the regulation of intracellular K^+ concentrations. The selectivity for K^+ was quantified through experiments measuring the Na^+ and K^+ dependence on Ba^{2+}-blockades,(1) indicating that K^+ has greater permeability by at least 150 fold. In thermodynamic terms, the relative binding free energy of Na^+ to the pore must be at least 3 kcal/mol less favorable than K^+. Na^+ vs K^+ free energy perturbation (FEP) simulations are consistent with this, although no simulations to date have modeled the actual Ba^{2+} blockade experiment. We have used MD simulations to calculate the relative binding energies of Na^+ and K^+ in the KcsA ion channel when the S4 site is occupied by Ba^{2+}. As Ba^{2+} is a strongly polarizing ion, we have used QM/MM FEP calculations using CHARMM interfaced to the deMon DFT code (2), as well as the polarizable Drude force field to correctly model the Ba^{2+}-filter interactions, with the aim of better interpreting the original selectivity experiments.



Schematic of ion binding in the selectivity filter.

1. J. Neyton, C. Miller, *J. Gen. Physiol.* 92: 549-567.
2. B. Lev et al., *J. Comp. Chem.*, 31: 1015-1023.

3069-Pos Board B174
Human *ETHER-A-Go-Go*-Related Gene (HERG) K^+ Channel Inhibition by the Antidepressant Paroxetine
Hee-Kyung Hong, **Su-Hyun Jo**.
Paroxetine is a selective serotonin reuptake inhibitor (SSRI) for psychiatric disorders that can induce QT prolongation, which may lead to *torsades de*

pointes. We studied the effects of paroxetine on human *ether-a-go-go*-related gene (hERG) channels expressed in *Xenopus* oocytes and on action potential in guinea pig ventricular myocytes. The hERG encodes the pore-forming subunits of the rapidly-activating delayed rectifier K^+ channel (I_{kr}) in the heart. Mutations in hERG reduce I_{kr} and cause type 2 long QT syndrome (LQT2), a disorder that predisposes individuals to life-threatening arrhythmias. Paroxetine induced concentration-dependent decreases in the current amplitude at the end of the voltage steps and hERG tail currents. The inhibition was concentration-dependent and time-dependent, but voltage-independent during each voltage pulse. The S6 domain mutation Y652A did not affect the drug-induced hERG current block. In guinea-pig ventricular myocytes held at 36°C, treatment with 0.4 μM paroxetine for 5 min decreased the action potential duration at 90% of repolarization (APD_{90}) by 4.3%. Our results suggest that paroxetine is a blocker of the hERG channels, providing a molecular mechanism for the arrhythmogenic side effects during the clinical administration of paroxetine.

3070-Pos Board B175

Quinidine Block of Shab K Channels: Irreversible Collapse of the K^+ conductance, and Characterization of an External Selectivity Filter K^+ binding Site

Froylan Gomez-Lagunas, Elisa Carrillo.

Quinidine is a commonly used antiarrhythmic agent and a useful tool to study ion channels. We will show that: (1) quinidine (Qd) equilibrates within seconds across the plasma membrane of Sf9 insect cells, blocking the open pore of Shab K channels from the intracellular side of the membrane in a voltage-dependent manner with 1:1 stoichiometry. (2) On binding to the channels Qd interacts with pore K^+ ions in a mutually destabilizing manner. As a result, (3) when the channels are blocked by Qd with the cell bathed in an external medium lacking K^+, the Shab conductance G_K collapses irreversibly, despite the presence of a physiological [K^+] in the intracellular solution. (4) The Qd-promoted collapse of Shab G_K resembles the collapse of Shaker G_K observed with 0 K^+ solutions on both sides of the membrane: thus the extent of G_K drop depends on the number of activating pulses applied in the presence of Qd, but it is independent of the pulse duration. Taken together the observations indicate that, as in Shaker, the Qd-promoted collapse of Shab G_K occurs during deactivation of the channels, at the end of each activating pulse, with a probability of 0.1 per pulse at −80 mV. (5) Finally, we will compare the Ki (inhibition constant) with which different external cations destabilize the binding of Qd against the potency with which the same cations inhibit the collapse of G_K, in an attempt to characterize both the selectivity of the external K^+ binding sites (s1/s2) and their role in the stability of G_K.

3071-Pos Board B176

Effect of MTS Reagents on Wildtype and *h*Kv1.3_V417C Mutant Channels and its Implications for C-Type Inactivation

Sonja I. Schmid, Stephan Grissmer.

The voltage-gated *h*Kv1.3 channel, member of the *Shaker*-related potassium channels, is involved in T-cell activation and is characterized by its typical C-type inactivation. To characterize the three-dimensional structure of the C-type inactivated state a cysteine was introduced at position 417 (*Shaker* position 467) in the *h*Kv1.3 channel and a putative involvement in C-type inactivation was determined using MTS-reagents. MTSEA application led, in contrast to wildtype channels, to a fast and irreversible current reduction through *h*Kv1.3_V417C channels in the open or inactivated state indicating that a modification of both states was possible. This modification could be prevented by verapamil. In contrast, the closed state of this mutant channel could not be modified by MTSEA. Furthermore a current reduction was observed only when the positively charged MTSET was applied intracellularly and not when applied extracellulary to *h*Kv1.3_V417C channels. These experiments indicated that the binding site for MTS-reagents is intracellular and that a modification of the cysteine at position 417 in the *h*Kv1.3_V417C mutant channel was possible in the open and also inactivated state of the channel. In addition, the fact that the inactivated state of the *h*Kv1.3_V417C mutant channel could be modified by MTSEA indicated also that the activation gate must be open during inactivation, the side chain of the cysteine at position 417 does not move during inactivation in a way that it is not available for modification any more and the channel is, using the model by Cuello et al. (2010, *Nature* **466**:203), in the open-inactivated state.

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3072-Pos Board B177

Discrimination Among Heteromeric Potassium Channels by Pore-Blocking Conotoxins

Rocio K. Finol-Urdaneta, Stefan Becker, Baldomero M. Olivera, Heinrich Terlau, Robert J. French.

Screening of specificity of drugs affecting K channels commonly uses homotetrameric channels assembled following expression of a single monomer. However, in many tissues, voltage-gated K currents may reflect the properties of heteromeric channels. Recently, we described a cardioprotective action of the Kv1.2-blocking conopeptide κM-RIIIK, but concluded that this was unlikely to result from an interaction with homomeric Kv1.2 channels (Chen et al., 2010, J.Biol.Chem. 285:4882). Here, we examine target discrimination, among heteromers, of the related conotoxins κM-RIIIJ and κM-RIIIK by testing their activity on 12 different Kv1.2-containing channels, each formed after expression of a single dimeric construct. Expression of homodimeric Kv1.2 yielded channels with toxin sensitivity similar to homotetramers, suggesting that dimerization, *per se*, does not affect toxin sensitivity. κM-RIIIK was most potent against Kv1.2 homotetramers and 1.2/1.7 heteromeric channels, but did not discriminate based on the order of connectivity in the latter. κM-RIIIJ was most potent against 1.1/1.2 constructs, without regard for connectivity, but showed significant discrimination based on connectivity between the two constructs for both 1.5/1.2 and 1.6/1.2 heteromers. Preliminary data for two Kunitz family conopeptides Conkunitzin-S1 and Conkunitzin-S2 suggest that each of these peptides can also discriminate among targets based on their order of connectivity. In conclusion, peptide inhibitors are able to select among heteromeric K-channel targets based on both identity of the component monomers, and on their order of connectivity. Thus, the toxins may bind across monomeric boundaries. This may account for the wide variety of selectivity "fingerprints" observed for intact cells and tissues and maybe of major relevance for the physiological action of a given peptide.

3073-Pos Board B178

Scorpion Toxins Modify C-Type Inactivation in a Mutant Potassium Channel

Azadeh Nikouee Ghadikolaei, Stephan Grissmer.

The amino acid at position 399 in the outer vestibule of *h*Kv1.3 channels (in *Shaker* 449) critically determines the C-type inactivation time course. In the present study we generated an *h*Kv1.3_H399N mutant channel with asparagines in the outer vestibule. This mutant channel showed faster inactivation and recovery time courses compared to the wild-type channel. We investigated the effect of MgTX and CTX on C-type inactivation of the mutant channel in $NMDG^+$ (K^+:4.5 mM) solutions using the whole-cell patch-clamp technique. Our results showed that the inactivation time course of the mutant channel increased around 10-fold in the presence of MgTX and 3-fold in CTX. In both cases the toxin affinity to the mutant channel is much lower compared to the wild-type channel. Other peptide toxins (NTX, AgTX2 and KTX) did not show any remarkable effects on C-type inactivation. We think that MgTX and CTX can bind to the outer vestibule of the mutant channel thereby impeding the structural changes in the outer mouth of the channel that are involved in the inactivation process. Rearrangement of the outer vestibule during C-type inactivation has been proposed earlier (Grissmer et al., 1989, *BiophysJ* **55**:203; Choi et al., 1991, *PNAS* **88**:5092; Liu et al., 1996, *Neuron* **16**:859).We conclude that C-type inactivation in voltage-gated potassium channels induce structural changes in the outer vestibule and therefore differs from the C-type inactivation in *KcsA* channels (Cuello et al., 2010, *Nature* **466**:203), which shows little changes in the outer vestibule of the *KcsA* channel.

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3074-Pos Board B179

Quantification of Non-Conducting Kv2.1 Channels in Transfected HEK Cells and Cultured Hippocampal Neurons

Philip D. Fox, Robert Loftus, Emily Deutsch, Michael M. Tamkun.

Kv2.1 potassium channels retained within cell-surface clusters in transfected HEK cells are incapable of conducting potassium. Expression of GFP-tagged Kv2.1 reveals two distinct populations of channels, those retained within clusters and those freely diffusing throughout the membrane. We hypothesized that all whole-cell current is derived from non-clustered channel. The goals of our present work were to 1) determine how the number of freely diffusing Kv2.1 channels in transfected HEK cells relates to the number of channels conducting K+, and 2) compare levels of endogenous Kv2.1 and Kv current in cultured hippocampal neurons. To quantify GFP-tagged Kv2.1