density and water distribution within the bilayer largely overlap, suggesting a high degree of protein hydration within the membrane. Solid-state NMR magnetization transfer data are consistent with deep penetration of water molecules into bilayer-embedded VSDs. We hypothesize that VSDs have water filled cavities, which may be essential for VSD function.

2496-Pos Board B466
Voltage and proton gradient sensing in H_v1 proton channels
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H_v1 voltage-gated proton channels appear to conduct H^+ through a voltage sensor domain (VSD) that is homologous to that found in voltage-dependent cation channels and phosphatases. A conserved S4 transmembrane helix that contains a series of at least three Arg residues is integral to the voltage sensing function of all VSD proteins. In contrast to other VSD-containing proteins, voltage-gated proton channels possess an additional unique biophysical property: coupling of the transmembrane pH gradient to voltage dependent activation. For both native voltage-gated H^+ currents and expressed H_v1 channels, the apparent voltage threshold for H^+ current activation (V_{thr}) shifts linearly ~40 mV per log([H^+]) over at least five pH units. The molecular mechanism of coupling between voltage and the pH gradients represents one of the central mysteries of proton channel function. What constitutes the pH sensor in proton channels and how does it interact with the voltage sensor?
DeCoursey and colleagues previously proposed a model for H^+ channel gating wherein protonation of discrete sites that are alternatively exposed to either the extra- or intra-cellular milieu regulates the voltage-dependence of channel opening (Cherny et al., 1996); the required first step in this model is deprotonation of an extracellular H^+ binding site. In order to identify residues that mediate pH-dependent regulation of voltage sensitivity in H_v1, we performed site-directed mutagenesis to convert each of the candidate H^+ acceptors in the H_v1 VSD to either neutral (alanine or asparagine), basic (arginine) or H^+-titratable (histidine) amino acids. Mutant channels were expressed in HEK-293 cells and V_{thr} was determined under a variety of imposed pH gradients using whole-cell voltage clamp. Surprisingly, charge-neutralizing mutations failed to abrogate pH gradient sensing in H_v1. Our findings are interpreted in the context of the Cherny and DeCoursey model for proton channel gating.

2497-Pos Board B467
Voltage-Dependent Conformational Changes of the Voltage Sensor of KVAP Measured with LRET
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The opening and closing of voltage-dependent ions channels depend upon conformational changes initiated in the voltage-sensors. The nature and extent of this rearrangement has been widely investigated in K^+ channels, using various techniques, but so far, there is no agreement on this fundamental mechanism in K^+-channel gating.
We investigated the voltage-sensor operation using a LRET technique employing Tb^{3+} ions bound to several Lanthanide-Binding-Tags (LBT) genetically encoded at the top of the S3 and S4 segments of the KvAP channel. A fluorescently-labeled pore-blocking toxin, the Agitoxin-2, was conveniently used as an acceptor placed at a non-mobile position near the pore-axis. Three mutations, S179G, K181D and P176E, were systematically introduced in the pore region to increase toxin binding. Various fluorophores were covalently attached to cysteines individually inserted at positions N5, Q13 and D20 of the agitoxin. After purification, mutant channels were reconstituted into proteoliposomes and submitted to a Nernst-clamp procedure combining the use of valinomycin and a K^+ chelator. This method allowed us to stably clamp the liposomal electrical potential at negative and positive potentials. Thus, this allowed us to stabilize the voltage-sensor in its closed state and in its open-inactivated state during the data acquisition period. Channels were then blocked with the fluorescent toxins and LRET measurements were recorded. Sensitized emissions of the acceptors were fitted with a square-base pyramidal multi-exponential model (Posson and Selvin, 2008) allowing the extraction of the four distances separating the position of the acceptor near the pore and the position of the donors located further away in the four subunits. The voltage-dependent coordinates of the LBTs indicate a rotational movement of the top of S4, with little participation of the top of the S3 segment.
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2498-Pos Board B468
Down-State Model of the KvAP Voltage-Sensing Domain
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Voltage-gated potassium (Kv) channels play a crucial role in the generation and propagation of electrical impulses in excitable cells by controlling the flow of K+ ions into the cell membrane in response to changes in transmembrane potential. The voltage-sensing domains (VSD) of these channels have at least two distinct conformations, the so-called up- and down-states, corresponding to the open and resting/closed states of the channel. To date, structures of three different Kv channels have revealed the up-state of the VSD; in the down-state, there is a great deal of functional data, but no crystal structures have been reported. We report here a model of the KvAP VSD in a down-state. The model was generated by molecular dynamics simulations (MD) of the VSD in a lipid bilayer in excess water using harmonic constraints to steer an equilibrated up-state sensor into a configuration that is consistent with the biotin-tethered avidin accessibility measurements of Ruta et al. (2005, Cell 123: 463). We have run a MD simulation of the system for 30 ns in the absence of constraints. The system is stable in the new configuration, and is consistent with newly reported avidin accessibility measurements (Banerjee and MacKinnon, 2008, JMB 381: 569) that were not used to generate the model. In addition, we have estimated the gating charge transfer between the up- and down-state configurations. Compared to the Kv1.2 model by Pathak et al. (2007, Neuron 56: 124), we see slightly larger transmembrane displacements for the S3 and S4 helices with significant differences in their orientation.

2499-Pos Board B469
Structural models of NaChBac: Does the secondary structure of S4 change during gating?
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NaChBac is a prokaryotic 6TM tetrameric voltage-gated sodium channel with a locus point in homology space connecting channels from all major voltage gated channels superfamilies. The voltage-sensing domain of NaChBac exhibits the familiar RxxRxxR motif of S4 and conserved negative residues on S2 and S3. Thus, the voltage sensing mechanism of NaChBac is probably shared with other voltage gated channels. We have used the crystal structure of the Kv1.2/2.1 chimera to model NaChBac's open conformation and that of the MlotiK channel to model its closed conformation. In the closed MlotiK structure the first part of S4 forms a 3_{10} helix and the last part forms an α helix, whereas in the open Kv1.2/2.1 structure the first part is an α helix while the rest is a 3_{10} helix. This elastic type of transition between secondary structures during gating can explain some apparent discrepancies regarding the magnitude of S4 motion reported for several potassium channels. However, this type of transition alone is not sufficient to explain the large gating charge movement reported for NaChBac and other channels. To account for this, we have incorporated the α-3_{10} transition into the "helical screw model" in which the α-helix part of S4 moves in a screw-like fashion while the 3_{10} part of S4 moves in a simple axial translation. In our models four positively charged residues of S4 moves outwardly during activation across a transition barrier formed by highly conserved hydrophobic residues on S1, S2, and S3. S4 movement is coupled to opening of the activation gate formed by S6 through interactions with the segment linking S4 to S5. Consistencies of our models with experimental studies of the NaChBac and Kv channels will be discussed.

2500-Pos Board B470
Modulation of HCN Channel Deactivation Kinetics by CAMP and Depolarization Can Be Amplified by Mode Shift
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HCN ion channels belong to the voltage-gated K-channel superfamily, but their voltage-sensing (S4) helices produce an unusual hyperpolarization-activation mechanism. This hyperpolarization-activation can be enhanced by (a) cyclic AMP (cAMP) binding to a cytoplasmic C-terminal region, and (b) a voltage-independent "mode-shift" after gate-opening that forms a secondary open state ("Mode II"). We previously [*Biophys. J.* **94**, 1400-Pos.] found that a mutation (K381E) within S4 produced a cAMP-dependent ''ultra-sustained activation'' phenotype without disrupting voltage-activation. Cyclic AMP applied to K381E channels in excised inside-out patches greatly slows deactivation kinetics, increasing decay time constants beyond 6 s at −40mV. In this study, we activated K381E channels with hyperpolarizing pulses too short for significant mode shift to occur, thus isolating open channels predominantly in the Mode I state. Deactivation transients were well-described with a sum of three exponential components, consistent with three subpopulations of open states. The three time constants (τ_{fast}, τ_{medium}, and τ_{slow}) were widely separated in the absence of cAMP, enabling the rapidly deactivating Mode I kinetics (τ_{fast} ~50 ms) to be clearly delineated from those of the more stable Mode II states (τ_{medium} ~300

ms and τ_{slow} ~900 ms). Slowing of deactivation by cAMP was slight for Mode I (~2-fold increase in τ_{fast}) in contrast with the ultra-sustained activation properties of Mode II (~9-fold increase in τ_{slow} to ~8 s). The voltage-dependence of Mode I versus Mode II deactivation was also markedly different (accelerating <1.5-fold versus ~4-fold respectively for 20-mV depolarization) near −40mV where ultra-sustained activation is most prominent. Thus, it is not Mode I states but specifically Mode II states whose kinetic stability shows the strong dual dependence on voltage and cAMP responsible for ultra-sustained activation.

2501-Pos Board B471

Electrophysiologic Characterization of a Complex hERG Channel Activator

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Novel and specific activators of the human ether-a-go-go-related gene (hERG) K^+ channel have been reported recently to enhance hERG current amplitude (4 synthetic small molecules and one naturally occurring substance). Here, we characterize the effects of a novel compound (Abbott-x) on atrial and ventricular action potentials (using microelectrode techniques) and cloned hERG channels stably expressed in HEK-293 cells (using whole cell patch clamp techniques). Abbott-x shortened cardiac action potentials and enhanced the amplitude of the hERG current in a concentration- and voltage-dependent manner. The fully activated current-voltage relationship revealed that this compound (60 uM) increased both outward and inward K^+ current. The slope conductance of the linear portion of the fully activated I-V relation was increased in the presence of the compound. Abbott-x significantly reduced the time constants (τ) of hERG channel activation at two example voltages tested (-10 mV: $\tau = 100 \pm 17$ vs 164 ± 24 ms, $n = 6$, $P < 0.01$; +30 mV: $\tau = 16.7 \pm 1.8$ vs 18.9 ± 1.8, $n = 5$, $P < 0.05$) and shifted the voltage-dependence for hERG activation in the hyperpolarizing direction by 9 mV ($n = 7$, $P < 0.01$). The time course of hERG channel deactivation was significantly slowed at multiple potentials tested (−120 to −70 mV). Abbott-x also significantly reduced the rate of inactivation and shifted the voltage dependence of inactivation in the depolarizing direction by 15 mV ($n = 5$, $P < 0.05$). Recovery of hERG channel from inactivation was not significantly affected by Abbott-x. In conclusion, Abbott-x enhances hERG current in a complex manner by facilitation of activation, reduction of inactivation, and slowing of deactivation, and abbreviates atrial and ventricular repolarization.

2502-Pos Board B472

Membrane Localization of S4 Transmembrane Segment of Voltage-Gated Ion Channels

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Voltage sensor domain (VSD) plays a key role in the channel conductance of the voltage-gated ion channels. Out of four transmembrane segments in VSD, cationic fourth transmembrane segment (S4) is the principal voltage sensor. Various models based on X-ray crystallography and site directed mutagenesis studies have shown that S4 segment, in response to the membrane potential, undergoes a conformational rotatory motion in the hydrophobic core of the lipid bilayer. Functional studies using whole-cell/ patch clamp techniques have detailed the conductance properties of the channel. However, the exact mechanism of membrane interaction and orientation of the cationic S4 segment in the non-polar lipid bilayer is yet to be understood. Direct experimental evidence using native S4 segment and liposomes, without any other energetic cofactors, would allow a direct test of the underlying mechanism of localization of S4 in the lipid bilayer. We used various fluorescence techniques to study the interaction and penetrative depth of the native S4 peptide in the lipid bilayer. Detail information concerning autonomous partition of S4 peptides in lipids will be discussed.

Ligand-gated Channels

2503-Pos Board B473

Accessibility of Ag^+ in the Pore of P2X Receptor Channels

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P2X receptor channels are extracellular ATP gated cation channels composed of three identical or related subunits. In mammals, seven subunits have been cloned ($P2X_1$-$P2X_7$). Each subunit has two transmembrane (TM) helices flanking a large extracellular domain that contains the ATP binding site, with the NH_2 and COOH termini on the intracellular side of the membrane. To investigate the mechanism underlying gating, we recently mutated one at a time each residue in the two TMs to cysteine, and measured the rate of modification by extracellular methanethiosulfonate (MTS) when channels are either closed or activated by ATP. Whereas only one residue in the extracellular end of TM1 is modified by MTSET, several residues are modified in TM2 in a state-dependent manner. These results suggest that TM2 makes substantial contribution to lining the pore and that the external region of TM2 forms a barrier for MTSET [1]. We now report on the accessibility of Ag. Several residues in both TMs are modified by Ag in the open state, although high modification rates were only seen at a number of positions in TM2. The modification pattern by Ag supports the results with MTSET that the pore is primarily formed by TM2 and that the external region of TM2 forms a gate for small ions. In addition, the results suggest that in the open state there is a crevice between TM1 and TM2 that is accessible to ions. The implications of these findings for the gating motion of the TMs will be discussed.

1. Li, M., Chang, T.-H., Silberberg, S.D. and Swartz, K.J. (2008) Gating the pore of P2X receptor channels. Nature Neuroscience 11, 883-887.

2504-Pos Board B474

Mutation Analyses Of The Critical Regions For The Voltage And [ATP] Dependent "Gating" Of $P2X_2$ Receptor Channel

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$P2X_2$ is an extracellular [ATP] gated cation channel with 2 transmembrane (TM) regions. In spite of lacking a voltage sensor domain, inward current through it in the steady state after ATP application shows gradual increase upon hyperpolarization, implying "voltage-dependent gating". We analyzed the tail current and activation time constant using Xenopus oocytes under two electrode voltage clamp and observed that increasing [ATP] shifted conductance (g)-V relationship towards depolarized potentials and accelerated activation kinetics. The results show that in steady state after ATP application, the gating was dependent on both voltage and [ATP]. We aimed at identifying the critical region for the gating and first introduced mutations to the ATP binding region with a hypothesis that a negatively charged ATP itself or its complex with the binding site could be an origin of voltage dependency and introduced K71A/R, K69A/R, R290A/K, K308A/R mutants. Analyzed mutants showed no clear [ATP] dependent acceleration of the activation upon hyperpolarization. Except for K69R, no remarkable [ATP] dependent shift of g-V relationship was observed. Secondly, we focused on the linker region between the ATP binding site and 2nd TM. In G311A and G320A, [ATP] dependent g-V shift to depolarized potentials was much more prominent than in WT. In contrast, G311P and G320P had no clear g-V shift by [ATP], indicating an essential role of the glycine residues in the linker region. Thirdly, introduced mutations in TM regions F44C, Y47C in TM1 and T339S in TM2 were devoid of activation phase and no more voltage dependent gating was observed at higher [ATP]. Taken together, these results suggest the critical involvement not only of the ATP binding region but also the linker and the TM regions in voltage and [ATP] dependent "gating".

2505-Pos Board B475

A Single Amino Acid Mutation Turns a P2X3 Antagonist into an Agonist

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Purinergic P2X3 receptors (P2X3Rs) are ATP-gated cation channels expressed mainly in sensory neurons. Suramin inhibits P2X3R currents of several species and provides a useful tool for *in vitro* studies of P2X antagonism. A rhesus homolog of P2X3R was cloned, stably expressed, and pharmacologically characterized. Suramin was a more potent inhibitor of rhesus P2X3R (IC50: 0.4 μM) than of human P2X3R (IC50: 8.6 μM). Surprisingly, suramin activated rhesus P2X3R at higher concentrations (EC50: 4.7 μM). In contrast, suramin did not activate human P2X3R at similar concentrations. Other than differences in suramin agonism, the kinetic and pharmacological profile of rhesus and human P2X3Rs were similar. To investigate the molecular basis of suramin agonism with P2X3R, we generated single-point mutations in the P2X3R. Only four amino acid differences exist between rhesus and human P2X3Rs (S67F, L127F, L144F, and T162M). Mutant receptors were transiently expressed in HEK293 cells and their currents measured using an automated patch clamp instrument (PatchXpress, MDS Analytical Technologies, USA). A single amino acid mutation of human P2X3R (S67F) allowed suramin to act as an agonist and increased the potency of suramin. A corresponding mutation (F67S) in the rhesus P2X3R resulted in a loss of suramin agonism and a decrease of its inhibitory activity. This suggests that position 67 is critical for suramin modulation of P2X3R activity and that inhibition and activation by suramin may be mechanistically related.