PIP2 activation of a voltage-dependent potassium channel (Kv7.1). Kv7.1 channels have a canonical Kv structure with a central pore-gate domain (PGD) surrounded by four peripheral voltage-sensing domains (VSDs). Kv channel activation is thought to involve two general steps. First, membrane depolarization is sensed by gating charges in the VSDs resulting in conformational changes within the VSDs from the resting state to the activated state. After all four VSDs have been activated, the second general step in Kv activation involves a concerted motion during which the PGD opens to allow ion permeation. In this study we ask if PIP2 regulates activation of Kv7.1 by potentiating the early VSD conformational changes, the concerted opening of the PGD, or both. Using the voltage-clamp fluorometry (VCF) technique to assay local conformation changes in the VSDs and the PGD simultaneously, we are able to show that although depletion of PIP2 eliminates the ionic current, the early conformational changes in the VSD do not require PIP2. This result indicates that the later conformational changes that couple VSD activation to the opening of the PGD are eliminated by PIP2 depletion. We continue this work by dissecting the molecular details of why PIP2 is required for opening of the PGD in response to VSD activation. These results may provide insights on the common principle of how Kv channels are modulated by PIP2.

1684-Pos Board B454
Activation of Voltage Sensing Domains during KCNQ1 Channel Opening: Concerted or Independent?
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In the heart, Iks current is a major contributor in limiting the duration of the action potential. The Iks channel consists of four α-subunits (KCNQ1) which assemble with β subunits (KCNE1). Mutations in either KCNQ1 or KCNE1 cause multiple cardiac arrhythmia syndromes such as LQT syndrome, short QT syndrome and familial atrial fibrillation. Iks channels, characterized mainly by its slow activation and deactivation kinetics and opening at depolarized voltages, differ from those of functional tetrameric voltage-gated KCNQ1 channels expressed alone: fast activating and deactivating kinetic and opening at less depolarized voltages. Understanding the channel structure-function relationship represents a valuable tool in predicting not only cardiac arrhythmia risks, but most importantly possible therapeutic solutions. We aim to unveil the molecular mechanism underlying channel opening in wild type (wt) KCNQ1 channel and its interaction with KCNE1, as well as the molecular mechanism underlying arrhythmia-inducing mutations. To date, two models have been proposed for KCNQ1 channel activation: 1) a cooperative S4 movement, in which the channel opening occurs after a coordinated S4 voltage-sensing domain movement and 2) S4 moves independently to each other concomitantly rendering a step-wise current activation. To test the validity of these models, we characterize the electrophysiological properties of natural occurring LQT syndrome mutations such as R231 in KCNQ1 channel using two electrode voltage clamp (TEVC) and voltage clamp fluorescence (VCF) techniques. R231A mutation has been shown to cause KCNQ1 channel constitutively activated, probably by keeping S4 voltage-sensing domain locked in the activated state. We performed TEVC and VCF measurements of tetrameric constructs of R231 combined with wt KCNQ1 channel to determine whether the S4 voltage-sensing domains in KCNQ1 move independently of each other. Our data will provide insight into the mechanism by which KCNQ1 channel operates.

1685-Pos Board B455
N and C Terminal Interactions Underlie Channel Gating of M Channels
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Neuronal M-type K channels composed of KCNQ2 and KCNQ3 subunits regulate firing properties of neurons. Presynapticlly, KCNQ2 subunits were also found to regulate neurotransmitter release by directly influencing presynaptic function. Previously, we have showed the existence of constitutive interactions between the cytosolic N and C termini of homomeric KCNQ2 or KCNQ3 channels in living cells, and demonstrated that a modulation of the N-C interactions of KCNQ2, but not of KCNQ3, by two regulatory proteins, syntaxin 1A and calmodulin, accompany a reduction in single-channel open probability, suggesting that closer N-C termini proximity underlies gating downregulation. Furthermore, in KCNQ3, identified N terminal and C terminal structural determinants, employ the preclusion of the regulatory proteins effects on the N-C rearrangement and gating regulation. This strongly suggested that N and C structural determinants confer the isoform-specific gating downregulation. Notably, the N-C interactions of both subunits were found to consist of a basal interaction, similar in both channels, and an additional interaction in KCNQ3 formed by an NT distal-end module. Here, we show that the basal N-C interaction is common to both KCNQ2 and KCNQ3 subunits and is essential for proper channel gating of both types of channel. We demonstrate, using optical, biochemical, electrophysiological and molecular biology analyses, that mutations and truncations at specific locations at the N or C termini of the channels abolish, partially or totally, the channels N-C interaction, as well as single-channel gating. Further, we look into the involvement of calmodulin in the basal N-C interactions.

1686-Pos Board B456
Allosteric Properties of KCNQ1 (Kv7.1) Channel Gating Detected by Voltage Clamp Fluorometry
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KCNQ1 (Kv7.1) is a unique member of the superfamily of voltage gated K+ channels in that it displays a remarkable range of gating behaviors tuned by co-assembly with different β subunits of the KCNE family of proteins. Homomeric KCNQ1 channels activate quickly over a negative range of voltages; KCNQ1/KCNE1 channels activate very slowly over a depolarized range of voltages; and KCNQ1/KCNE3 channels are constitutively open. To better understand the basis for the biophysical diversity of co-assembled channels, we here investigate the basis of KCNQ1 gating in the absence of β subunits using voltage clamp fluorometry (VCF). Based on our previous work, the kinetics and voltage dependence of voltage sensor movements are very similar to those of the channel gate, suggesting a one-to-one relationship. Here, we have tested alterative hypotheses to explain KCNQ1 gating: 1) KCNQ1 voltage sensors undergo a single concerted movement that leads to channel opening, or 2) independent voltage sensor movements lead to channel opening before all voltage sensors have moved. We find that KCNQ1 voltage sensors move independently, but that the channel can conduct before all voltage sensors move. In some mutant KCNQ1 channels, transition to the open state occurs even in the absence of voltage sensor movement. In these mutants, voltage sensors display more depolarized voltage dependence than the channel gate, implying that voltage sensors move after the channel has opened. To interpret these results, we propose an allosteric gating scheme wherein KCNQ1 is able to transition to the open state after 0-4 voltage sensor movements, with each successive voltage sensor movement strengthening the opening transition. This model allows for widely varying gating behavior depending on the relative strength of the opening transition, which physiologically is controlled by co-assembly with different KCNE family members.

Channel Regulation & Modulation II

1687-Pos Board B457
Characterization of the Role of PKC-Theta in the Modulation of ClC-1 Chloride Channel Function and Calcium Homeostasis in Fast- and Slow-Twitch Skeletal Muscle by using PKC-Theta Null Mice
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In skeletal muscle the resting chloride conductance (gCl), sustained by the ClC-1 chloride channel, controls membrane electrical stability as its absence causes myotonia. The gCl is typically higher in fast-twitch than in slow-twitch muscle and is negatively modulated by Protein Kinase C (PKC). Different PKC isoforms are expressed in skeletal muscle, including the PKC-theta. In PKC-theta-null mice (Sun et al., *Nature,* 2000) we found a significant increase in gCl of slow-twitch soleus muscle with respect to wild-type, being 1876 ± 53 $\mu S/cm^2$ (n=41) and 1356 ± 37 $\mu S/cm^2$ (n=19), respectively. A minor 13% increase of gCl was found in the fast extensor digitorum longus (EDL) muscle. Muscle excitability was reduced accordingly. Chelerythrine, a non-specific PKC inhibitor, further increases gCl by 25% in transgenic soleus muscle showing that other PKC isoforms are involved in the control of gCl. Preliminary experiments suggest indeed an up-regulation of the PKC-alpha isoform in these mice. Minor effect of chelerythrine was found in EDL of PKC-theta null mice. Fluvastatin, known to activate PKC (Pierno et al., *Br J Pharmacol,* 2009) reduced gCl in EDL muscle more than in soleus muscle of transgenic mice confirming that other PKC isoforms contribute to ClC-1 modulation. No modification of ClC-1 expression was found in soleus and EDL muscle of PKC-theta-null mice compared to wild-type. In these mice we also found

a significant reduction of resting calcium concentration in both muscle types due to decreased sarcolemmal permeability at rest. Our results indicate that PKC-theta contributes to the regulation of ClC-1 channel differently in the muscle types and that this isoform can also modulate calcium homeostasis likely by interacting with sarcolemma channels. (ASI-OSMA)

1688-Pos Board B458

Interleukin-1β Decreases Aquaporin-3 Expression via Trans-Repression by CCAAT Enhancer Binding Protein

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Aquaporin-3 (AQP3), which is expressed in the basal layer of keratinocytes and airway epithelial cells, regulates not only water and glycerol permeability at plasma membrane, but also cell migration. We have previously shown that the expression of AQP3 is decreased by inflammatory cytokines, such as TNF-α and IL-1β. However, underlining mechanism for this decrease in AQP3 remains to be clear. In the present study, therefore, we examined the effect of IL-1β on AQP3 mRNA expression and promoter activity in A549 lung epithelial cells. IL-1β decreased both AQP3 protein and mRNA expression, in a concentration- (0.5-50 ng/ml) dependent manner. IL-1β also decreased promoter (−990/+88) activity, suggesting that IL-1β repressed AQP3 gene transcription. The IL-1β-induced decrease in AQP3 was relieved with PD98059, a MEK/ERK inhibitor. We then prepared a series of 5'-deletion constructs of AQP3 promoter. Among these, IL-1β did not decrease the activity of mutant promoters without −374/-272 region. The promoter, which has point mutation at CCAAT enhancer binding protein (C/EBP) binding element located at −276/-262, was also insensitive to IL-1β. Taken together, it was suggested that IL-1β decreases AQP3 expression via MEK/ERK- and C/EBP-dependent signaling.

1689-Pos Board B459

Inhibition of a Dimer Interface Mutant of CLC-0 by Intracellular Cadmium Ion

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The CLC chloride-transporting proteins play a variety of physiological roles: regulate the cellular excitability in skeletal muscle cells, facilitate the electrolytes transport in epithelial cells and control the pH in intracellular compartments. These membrane proteins are homodimers with each molecule containing two identical subunits. A double-point mutation (I201W/I422W) at the dimer interface in the bacterial CLC molecule (CLC-ec1) has recently been shown to destablize the dimer interaction so that the functional unit of the I201W/I422W mutant of CLC-ec1 is a monomer. We made the corresponding double mutant at the dimer interface of CLC-0, and the fluorescence resonance energy transfer (FRET) experiments indicated that the mutant channel still consists of two subunits. This dimer-interface mutant of CLC-0, however, shows very different functional properties in comparison to the wild-type CLC-0: the mutant channel is activated only at hyperpolarized voltages. Furthermore, the mutant channel significantly enhances the inhibition of the channel by the intracellularly-applied cadmium ion (Cd^{2+}). The Cd^{2+} inhibition of this dimer-interface mutant appears to be state-dependent with the closed-state channel being more sensitive to the Cd^{2+} inhibition than the open-state channel. We are currently mutating the endogenous cysteine and histidine residues of CLC-0 to identify the potential Cd^{2+}-binding site.

Biophysics of Ion Permeation

1690-Pos Board B460

Recharging the Phylogenetic Analysis of Voltage Sensor Domains

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Sequence and phylogenetic analysis of voltage-gated ion channels has famously resulted in the discovery of novel ion channels in previously unexplored phyla, and has provided insight into the molecular underpinnings of ion channel function. Because of their crucial role in human health, most current evolutionary analysis has quite reasonably centered on 'traditional' pore-forming channels (e.g., Yu, et al, 2005, Pharmacol. Rev., 57:387-395, among many others). Earlier phylogenetic analyses of voltage sensor domain (VSD) modules (e.g., Komanovics et al, 2002, FASEB J., 16:1623-1629, among others) were undertaken before the discovery and characterization of the genes for voltage sensitive phosphatases (VSP) and voltage gated proton channels (HV1), which contain VSDs but do not contain traditional ion pores. We recently reported two new discoveries: in one line of inquiry we found and characterized a dinoflagellate HV1, supporting the prediction of its existence in bioluminescent dinoflagellates by Fogel and Hastings in 1972 (PNAS, 3:690-693); and in the other we uncovered the universal selectivity filter of HV1's. Both of these studies were informed by large-scale sequence and phylogenetic analyses that focused on homologs of VSDs themselves, separate from their N- or C-terminal appendages. Here we present a full sequence and phylogenetic analysis that updates earlier work and reveals that VSDs may have taken a different evolutionary path from associated ion channel pore domains, which has mechanistic and physiological implications.

1691-Pos Board B461

Role of Central Cavity in Ion Permeation through the Kv1.2 Channel

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In the ion permeation pathway of the K^+ channel, the narrow selectivity filter is a critical region where ions and water molecules permeate with a single-file fashion. Theoretical studies have been concentrated on the permeation process in the selectivity filter, whereas the role of the central cavity, a common architecture that all the known K^+ channels have, is not systematically examined yet. Here we investigated ion permeation through the Kv1.2 channel by the molecular dynamics simulation and analyzed the role of the central cavity. It is found that the central cavity attracts the K^+ ions when the ion concentration in the bulk is below the physiological range. Accordingly, the concentration in the central cavity is higher than that in the bulk, increasing the entry chance of ions into the selectivity filter. Thus, the central cavity plays a role to make ion permeation rapid. On the other hand, the ion concentration in the central cavity is found to be saturated at high concentration in the bulk, and the conductance is saturated. These results indicate that the concentration of ions in the central cavity generates the Michaelis-Menten-type behavior of the conductance-concentration curve in the Kv1.2 channel. In contrast, it is known that the saturation of the conductance is not observed in the KcsA channel. We found that the ion concentration in the central cavity increases progressively as the bulk concentration increased up to 2 M, which is in agreement with the apparent non-saturating concentration-conductance curve in the previous studies.

1692-Pos Board B462

Interpreting the Barium Blockades of Potassium Channels with the Multi-Ion Permeation Free Energy Surface

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Barium blockade experiments on the BK channel provided an early quantitative determination of the ion binding selectivity of a potassium channel (Neyton, Miller, *J. Gen. Physiol.* 1988, 569). Recently, these experiments were repeated for KcsA by Piasta and Miller, providing the first site selectivity electrophysiological measurements for a channel of known atomic structure (Piasta, Theobald, Miller, *J. Gen. Physiol.* 2011). Site selectivity can be determined from these blockade experiments because the binding of an external alkali cation in the so-called lock-in site impedes the translocation of Ba^{2+} toward the external side, thus increasing the length of blockade. As K^+ and Na^+ impede the rate of Ba^{2+} translocation to vastly different degrees, their relative binding affinities to the lock-in site can be determined quantitatively. Here, we have used molecular dynamics simulations of KcsA to model the permeation process of Ba^{2+} by computing the ion permeation potential of mean force (PMF) with umbrella sampling enhanced by Hamiltonian exchange. Although a Ba^{2+}-bound crystallographic structure (Lockless, Zhou, MacKinnon, *PLoS Biology*, 2007, 5, e121) showed Ba^{2+} binding in the S4 and S2 sites, we find that Ba^{2+} can bind in all five internal sites, with site binding affinities ranked in the order: S2 > S1 ≈ S3 > S0 ≈ S4. Permeation of Ba^{2+} in the absence of external K^+ proceeds through the familiar knock-on mechanism, where the Ba^{2+} moves in concert with an internal K^+ ion. The barriers for the transition between binding sites are high (>15 kcal/mol), consistent with the observation of long-lived channel blockades and slow permeation of Ba^{2+} relative to K^+. The K^+ lock-in effect is examined by a multi-ion PMF of Ba^{2+} permeation in the presence of an external K^+ ion that impedes the permeation of Ba^{2+}.