functional responses to changes in membrane voltage. We then used a family of tarantula toxins that interact with voltage-sensing domains and alanine scanning mutagenesis to explore the structural integrity of these modular motifs and their disposition with respect to the lipid membrane. Our results indicate that the voltage-sensor paddle, a motif composed of S3b and S4 helices, can drive channel opening with membrane depolarization when transplanted from KvAP, Hv1 or Ci-VSP into eukaryotic Kv channels. Tarantula toxins that partition into membranes can interact with these paddle motifs at the protein-lipid interface and similarly perturb voltage sensor activation in both ion channels and voltage-sensing domain proteins. Our results show that paddle motifs are modular, that their functions are conserved in voltage sensors, and that they move in the relatively unconstrained environment of the lipid membrane. The widespread targeting of voltage-sensor paddles by toxins also demonstrates that this modular structural motif is an important pharmacological target.

1357-Pos Kinetic of Activation of Heterotetrameric *Shaker* K^+ Channels With Neutralized Gating Charges

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It is a consensus that the activation of voltage-gated K^+ channels upon depolarization requires the movement of the four voltage sensors that contain gating charges. However, the operation of each individual voltage sensor has been difficult to establish. To assess the role of each voltage sensor in the activation of the *Shaker*, we first mutated the four arginines (the gating charges) in the S4 segment of the *Shaker* zH4 Δ(6–46) (R362Q, R365Q, R368N and R371Q). This quadruple mutant was expressed in *Xenopus* oocytes and studied using cut-open oocyte voltage clamp but no ionic current could be detected. We then constructed cDNA concatemers using this mutant voltage sensor construct (mut) and the wild type (wt) voltage sensor with stoichiometries of 4wt, 2wt+2mut and 1wt+3mut. The tetramer 4wt has the same biophysical properties of the *Shaker* injected as monomers. The tetramers 2wt+2mut and 1wt+3mut also conduct K^+ ions, giving currents slightly lower than the 4wt when the same amount of mRNA was injected. However, their GV curves are shifted toward more negative potentials compared to the 4wt: the GV of 2wt+2mut being intermediate between 4wt and 1wt+3mut. A large fraction of the delay in the opening of K+ channels reflects the contribution of the individual subunits and can be determined by the Cole-Moore shift. The delay measured at 8 °C was ~2.5 ms for the 4wt, ~1.75 ms for the 2wt+2mut and < 1 ms for the 1wt+3mut. These results suggest that the subunits with neutralized voltage sensors are in the open state and allow the study the kinetics of one voltage sensor at a time.

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Voltage-gated K Channels - IV

1358-Pos Membrane integration of the voltage sensor of the Shaker channel

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Board B334

Knowledge of the topogenesis of the voltage sensor in voltage-dependent K^+ (Kv) channels is important for understanding the voltage gating mechanism. Membrane-embedded voltage-sensor domains in voltage-dependent potassium channels (Kv channels) contain an impressive number of charged residues. How can such highly charged protein domains be efficiently inserted into biological membranes? In the plant K_v channel KAT1, the S2, S3, and S4 transmembrane helices insert co-operatively, as neither the S3, S4 or S3-S4 segments have any membrane insertion ability by themselves [. Here, we show that in the *Drosophila* Shaker Kv channel, which has a more hydrophobic S3 helix than KAT1, S3 can both insert into the membrane by itself and mediate the insertion of the S3–S4 segment in the absence of S2. An engineered KAT1 S3–S4 segment in which the hydrophobicity of S3 was increased or where S3 was replaced by Shaker S3 behaves as Shaker S3–S4. Electrostatic interactions between charged residues in S2, S3, and S4, including the salt bridges between E283 or E293 in S2 and R368 in S4, are required for fully efficient membrane insertion of the Shaker voltage-sensor domain. The salt bridges between E283 or E293 in S2 and R368 in S4 were strictly constrained by the side-chain length of the residues for the membrane insertion. These results suggest that cooperative insertion of the voltage-sensor transmembrane helices is a property common to Kv channels, and that the degree of co-operativity depends on a balance between electrostatic and hydrophobic forces.

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1359-Pos Neutron Diffraction Studies of Voltage Sensors in Voltage-Gated Potassium Channels

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Neuronal signal are propagated via action potentials that are generated by voltage-gated ion channels. In voltage activated potassium channel (Kv), the critical element in voltage sensing is the S4 segment which comprises a few positively charged Arginine residues. The available crystal structures of Kv1.2 (Long S.B. et al., Science *309*:903, 2005) and KvAP (Youxing J. et al., Nature *432*:33, 2003) place segment S4 at the periphery of the channel in contact with the lipid. The mechanism of voltage gating is still not well understood. In a current view, S3b–S4 segments form a 'paddle' which moves against the lipid membrane to open the pore. Lipid exposure of the paddle is also supported by experiments with tarantula toxin (VSTX1) that partitions into the bilayer and bind to the paddle motif. Neutron diffraction is proposed as an investigation technique to show the distribution and conformation of the S4 segment of KvAP and the inhibitor toxin VSTX1 in lipid bilayers. While our results indicate that the S4 segment is contained withing the lipid bilayer, the position and orientation of the peptides remains to be determined in future experiments by using specific deuterium labeling. However, within this context, we have shown that the toxin VSTX1 which is thought to inhibit the channel conduction by binding to the paddle, partitions into the bilayer and is located in the interfacial region. The neutron diffraction investigations can offer important clues over the conformation of the voltage sensor and the amplitude of its motion in the membrane.

1360-Pos Multimeric Nature Of Voltage-Gated Proton Channels

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Voltage-gated potassium channels are comprised of four subunits - each with a pore domain and a voltage-sensing domain (VSD) - in which the four pore domains together form one single central pore and the four individual VSDs control the gate of the pore domain. Recently, a family of voltage-sensing proton channels, called H_V or VSOP channels, was discovered that contain a single VSD, but no pore domain. How a protein containing only a VSD and no pore domain can conduct ions is not known, but it has been assumed that VSOP channels are monomeric channels with a single VSD that functions both as the voltage sensing domain and the pore domain. Using fluorescence measurements and biochemical crosslinking, we show here that VSOP channels are functionally multimeric channels. Our data suggest that two subunits form a cooperatively gated dimer with the fourth transmembrane domain S4 from each subunit forming the dimer interface. The finding that VSOP is not a monomer also has important implications on the greater family of voltage-gated ion channels. The assumption that VSOP is monomeric has been used as an argument for the functional independence of the VSDs in other voltage-gated ion channels, but our findings indicate cooperativity among VSDs in VSOP.

1361-Pos Ci-VSP: Beyond Voltage Gated Ion Channels

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With the discovery of the Ciona intestinalis voltage sensor-containing phosphatase (Ci-VSP), the study of the voltage sensing domain (VSD) has expanded beyond the voltage gated ion channel. A combination of single molecule optical methods and voltage clamp fluorometry indicate that a functional Ci-VSP protein is monomeric, in contrast to the classic ion channel composition which is usually tetrameric. Interestingly, Ci-VSP still exhibits complex protein motions similar to those seen in multi-subunit channels. In ion channels, these motions have been attributed to the cooperative interactions found between the multiple subunits. Given the monomeric state of Ci-VSP, these motions may reflect multiple conformational changes from a single VSD suggesting that the VSD may undergo more complicated motions than previously thought. Here, we use voltage clamp fluorometry to investigate these voltage driven conformational changes in real time.

1362-Pos Charge movement of the Voltage Sensitive Fluorescent Protein

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The N-terminus of the Voltage Sensitive Phosphatase from *Ciona intestinalis* (Ci-VSP; Murata, *et al.*, 2005) is a voltage sensing domain (VSD), which controls the activity of a phosphatase domain (PD) on the C-terminus of the protein. By replacing the PD with a tandem of fluorescent proteins (TFP), CFP and YFP, a family of Fluorescence Resonance Energy Transfer based, genetically-encoded voltage sensing probes (VSFP) was created (Sakai et al., 2001; Dimitrov et al., 2007). Although fluorescent signals from VSFPs are clearly modulated by membrane potential, very little is known about how these optical signals are correlated to the charge movements of the VSD, or "sensing" currents. A new VSFP version, VSFP2.3, was successfully expressed in *Xenopus laevis* oocytes, allowing for simultaneous recording of fluorescence and "sensing" current. VSFP2.3 showed large changes in YFP emission upon changes in membrane potential with CFP excitation. The time course of the fluorescence had two components: The fast component was close to the time constant of the "sensing" current and the slow component was in all cases around one order of magnitude slower than the one from the "sensing" current. For instance, by depolarizing to +60 mV from a holding potential (HP) of −90 mV the current showed a time constant around 4 ms whereas the fluorescence had

components of 9 and 155 ms. This indicates that the TFP reported a secondary conformational transition of the VSD, not resolved in the "sensing" current recording. Furthermore, at a HP of 0 mV the voltage dependency of the fluorescence shifted toward negative potentials. These observations resemble the shift of the charge movement voltage-dependency in voltage-gated channels with positive HP and it is described in detail for the Ci-VSP by Villalba-Galea *et al.* (this meeting).

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1363-Pos eGFP Fluorescence as a Tool to Compare the Gating Charge per Voltage-dependent Ion-channel in *Xenopus laevis* Oocytes

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cDNA coding for single and multiple eGFP (up to 4 in sequence) was inserted into the C-terminal of Shaker-K-channel (non-conducting W434F mutant) and rat-brain Na-channel using a high expression vector. After 2 to 14 days of expression in Oocytes, gating-currents were measured (TEVC) after taking whole-cell images from which the total membrane fluorescence was estimated. Improvements on the previous report (Biophys. J., Suppl., 470a, Abstr., 2007) are:

1. The fluorescence from a defined membrane area along the equator (~14 % of the total sphere) was integrated and extrapolated onto the whole cell sphere (F_{tot}).
2. Voltage-clamp protocols were used to obtain the total gating charge Q_g.

KG1, i.e. K-channel with one eGFP in each of the four subunits showed the highest expression, up to 120 nC (about 3*10^10 channels) while KG2 and KG3 showed clearly a lower expression, most likely due to the many EGFPs (up to 12 per channel). However, all constructs showed normal gating. F_{tot} was plotted vs. Q_g for a wide range of expression levels and the relation was strictly linear for all constructs. The ratio of F_{tot} / Q_g was directly proportional to the number of eGFPs per channel. These results obtained for K-channels show that F_{tot} is a relative measure of the number of marked channels. Upper limits of the contribution of submembraneous channels are presented. Extending this method to other voltage-gated channels, an estimate of the relative size of the single channel gating charge is obtained.

1364-Pos Fluorimetric Analysis of the Kv1.2 S3–S4 Linker Shows Differences From Shaker Potassium Channels

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Voltage-clamp fluorimetry has been previously used in order to observe changes in channel conformation due to gating. Here we characterize the voltage-dependent conformational changes in the voltage-gated potassium channel Kv1.2 as shown by tetramethylrhodamine maleimide (TMRM) fluorescence after attachment to different sites in the S3–S4 linker. Each residue reports a different fluorescence profile, though common to each site is the contribution of fast and slow quenching components to the overall signal. Unlike in Shaker A359C, where only 15% of the fluorescence signal is contributed by a slow quenching component, Kv1.2 labelled at residue A291C (the equivalent residue to A359C) shows an initial rapid quenching phase, followed by a large slow quenching phase that composes 67.5 ± 2.3 % of the overall deflection after a 100 ms pulse. Given that this slow phase has been previously attributed to pore collapse associated with inactivation in Shaker, we tested whether the slow fluorescence movement in Kv1.2 also represents movement(s) associated with inactivation. We looked at fluorescence changes in response to high external potassium, which has been shown to inhibit inactivation in potassium channels (Lopez-Barneo et al. 1993). In the labelled A291C mutant, increasing the external potassium concentration from 3 mM to 99 mM significantly reduced the slow fluorescence phase contribution to 43.9 ± 3.4 % of the overall fluorescence signal, suggesting that this phase may be associated with inactivation. Based upon observation of ionic currents, Kv1.2 channels are not thought to display significant inactivation; however, these data suggest that a fluorophore attached within the S3–S4 linker reports changes within the Kv1.2 channel that are associated with an inactivation process that occurs with a similar time course to channel activation.

1365-Pos The Nature Of The Energy Barrier In Voltage Sensor Movement

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Recently we showed that the replacement of hydrophobic residues (I241 and I287) in S1 and S2 of the voltage sensor of the *Shaker* K^+ channel by histidine creates a connection between the external and internal solutions when the sensor is in its deactivated position. The spontaneous formation of disulfide bridges between these residues and the first charge in S4 (R362) at hyperpolarized potentials shows that they are close to each other when the channel is in the closed state, focusing the electric field in a hydrophobic plug formed, at least in part, by these two residues (Campos et al, 2007). This plug constitutes the lining of the pore where at least the first voltage sensing arginine crosses from the deactivated to the activated state of the sensor. This plug is expected to be the main energy barrier for arginine translocation that otherwise are in a hydrated state in the closed and open positions. Therefore we predict that by making the plug residues less hydrophobic the barrier should decrease and the sensor should translocate faster. The test was done by replacing I241 and I287 for several smaller and hydrophilic residues and probed their effect in the kinetics of the gating currents. We found that the

replacement of isoleucine in 287 for serine and threonine speeds up the gating current time course at all potentials but the effect is especially dramatic in the kinetics of deactivation of the voltage sensor. In I287T channels, the deactivation of the K^+ ionic current is several times faster when compared to wild type channels. These results support the notion that the energy barrier for the movement of the voltage sensor resides in the hydrophobic plug and that I287 rate limits channel closing.

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1366-Pos The V388C mutant of the human voltage gated Potassium Channel Kv1.3 promotes an ω-like current carried by sodium-ions

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Some residues in the inner vestibule of the *h*Kv1.3 channel are involved in its characteristic C-type inactivation or provide important binding sites for small molecule inhibitors. To obtain more information about the role of single amino acids in this region we introduced and investigated several cysteine mutations in the pore-helix with the whole-cell recording mode using a ramp protocol from −200 mV to +80 mV and KF as intracellular solution. We found that one mutation in the pore, at position V388 (*Shaker* position 438), generated a different current behaviour compared to the *h*Kv1.3 wild type channel. In extracellular Na-solution the V388C mutant channels promoted an influx of Na^+ at potentials more negative than -120 mV and this current could also be carried by NH_4^+, Cs^+ and Li^+. The observed inward current looked similar to the -current described by Tombola *et al.* 2005 (*Neuron* ***45***:379–388). In extracellular high K^+-solution (164 mM) the inward current disappeared and we observed a current similar to that in the wild type *h*Kv1.3 channel. The sodium inward current of the mutant channel was not affected by KTX and AgTX and could be blocked by verapamil. We therefore hypothesize that the *h*Kv1.3_V388C mutation in the P-region generates a channel with two ion pathways. One, the α-pore allowing K^+-flux in the presence of K^+ and the second pathway, similar but not identical to the -pathway, allowing several different ions to move along a special pathway beside the pore and is blocked by verapamil.

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1367-Pos Distinct Pore Properties Of Leak And Voltage-activated K^+ Channels Underlie Their Unique Roles In Electrical Signaling

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Voltage-activated (Kv) and leak (K_{2P}) potassium channels play key yet distinct roles in electrical signaling in the nervous system. Here, we examined how differences in the operation of the activation and slow inactivation pore gates of Kv and K_{2P} channels underlie their unique roles in electrical signaling. *Shaker* Kv and KCNK0 leak channels demonstrate opposite closed and open pore conformational stability phenotypes, respectively. Replacement of the pore domain of the *Shaker* channel with that of KCNK0 resulted in a substantial stabilization of the open pore conformation. Systematic replacement of activation gate hydrophobic residues of the *Shaker* channel to the corresponding glycine residues of KCNK0 resulted in dramatic open-state stabilization effects. Complementary experiments revealed dramatic closed state stabilization effects. By using macroscopic and single channel recordings we were able to monitor the conformational states (whether closed or open) of both the activation and inactivation gates of KCNK0. Our results revealed that

1. leak potassium channels also possess a lower activation gate,
2. that the activation gate is an important determinant controlling the intrinsic conformational stability of the K^+ channel pore,
3. the lower activation and upper slow inactivation gates of leak channels crosstalk and
4. in contrast to Kv channels, where the two pore gates are negatively coupled, i.e. the opening of the lower activation gate stimulates the closure of the upper slow inactivation gate, the two gates are positively coupled in K_{2P} channels.

Our results demonstrate how basic thermodynamic properties of the K^+ channel pore, particularly, intrinsic conformational stability and coupling between the pore gates, underlie the specialized roles of Kv and K_{2P} channel families in electrical signaling.

1368-Pos The KvAP Channel in a Lipid Bilayer: Structure and Dynamics

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Board B344

The native fold of voltage-dependent potassium (Kv) channels appears to be highly constrained by the lipid bilayer environment (Lee et al. 2005, PNAS *102*: 15441). To date, no single crystallographic study of Kv channels has been able to provide a model-free structure that features fully resolved, to the level of residue side chains, voltage-sensor (VS) domains in their native orientation. We present results of an atomistic simulation of the KvAP tetramer in a POPC bilayer in excess water. Our initial configuration is the KvAP model of Lee et al. The four VS domains exhibit a pattern of backbone thermal fluctuations as a function of sequence that is atypical for a transmembrane (TM) α-helix bundle structure, while the pore domain appears rigid in comparison. Each VS domain develops a different pattern of structural drift from the initial model, consistent with a diversity of solvation environments for the highly

conserved charged residues associated with the Kv gating charge. The Arg residues in the S4 TM domain are in a polar environment composed of water molecules, lipid phosphate groups, and acidic side chains. Characterization of VS motions is crucial to the understanding of voltage-dependent gating function, yet different kinds of experimental measurements appear to yield conflicting results. We compare our simulation results to the biotin accessibility measurements of Ruta et al. (2005, Cell *123*: 463) and the LRET experiments of Richardson et al. (PNAS *103*: 15865) in order to assess how different experimental techniques sample the dynamics of the KvAP channel and to examine the intrinsic uncertainty associated with their respective reference frames.

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1369-Pos Addition of a Charge to the *Shaker* Potassium Channel Voltage Sensor

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Board B345

Voltage-gated potassium channels contain a voltage sensor domain in each of its four subunits that confers its exquisite sensibility to membrane potential. The fourth α-helical transmembrane segment (S4) of each subunit has seven highly conserved basic amino acids, periodically spaced by two hydrophobic residues. The four outermost arginine residues in S4 travel most of the membrane electrical field upon depolarization. This charge movement (12–13 e_0 per channel) promotes a conformational change leading the opening of the channel conduction pathway. We carried out punctual substitutions of V363 (located between the two outermost arginines R362 and R365) for either an arginine or an aspartate. Surprisingly, both additional charge mutations decreased the charge movement coupled to the opening (6-8e_0 per channel) measured by the limiting slope method. To test whether the reduced effective valence introduced by the additional charge was caused by a disruption of the electric field sensed by the S4, we assessed the accessibility of methanethiosulfonate derivates for the double-mutants: R362C / V363R or R362C /V363D. We speculate that in the mutant channels with an additional charge at position 363, Arg 362 could reside outside the electric field.

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1370-Pos New insights into *Shaker* K+ channel voltage sensing using LRET

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Voltage-gated potassium channels open in response to membrane depolarization. The voltage-sensor (S1–S4) moves S4 arginines (gating charges) across the transmembrane electric field, coupling voltage changes to open probability. Understanding the structure and movement of this voltage-sensor is of fundamental importance. Luminescence Resonance Energy Transfer (LRET), a form of FRET, was shown to be a suitable technique for measuring distances and distance changes (between probes) on the *Shaker* channel (Cha et al., *Nature*, *402*, 1999). Recently, we used LRET to demonstrate that the vertical movement of S3–S4 is rather small (estimated 2 Å, Posson et al., *Nature*, *436*, 2005). Here we present further measurements that expand our previous studies to the S1 and S2 segments as well as a more complete scan near S4. We report a new geometric analysis of our data that allows a determination of 4 simultaneous distances between each of the voltage-sensors and an asymmetrically placed acceptor labeled toxin (charybdotoxin). We are now in the position to visualize estimated probe locations relative to known K+ channel structures. We show very little movement of S1 and S2. For S4, the data suggest that upon depolarization S4 moves outward at an angle tangential to the channel axis with a vertical movement greater than previously estimated, 5 ± 2 Å.

1371-Pos *In Vivo* Measurements of Intramolecular Distances Using Genetically Encoded Reporters

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The lanthanide-binding tag (Nitz *et al.*, 2003) was encoded into two different membrane proteins expressed in *Xenopus laevis* oocytes: the *Shaker* potassium channel and the Voltage Sensitive Phosphatase from *Ciona intestinalis* (CiVSP). LBTs carrying bound terbium (Tb^{3+}) ions served as luminescent donor probes and were used in combination with different acceptor probes: with an encoded hexahistidine (6-His) tag or with fluorophores bound to introduced cysteine residues. 6-His tags bind transition metal ions like Ni^{2+} and Cu^{2+} and as acceptors yield relatively short R_o values with respect to Tb^{3+}: 12Å and 20Å, respectively. LBT/6-His pairs were used to measure intra- and trans-subunit distances in *Shaker* K^+ channels tagged as monomeric or dimeric constructs. *Shaker* channels tagged with the LBT in the S3–S4 loop and with the 6-His in external trans-segment loops gave reliably measurable transfer using Ni^{2+} and Cu^{2+} as acceptors. S4-S1 and S4–S5 distances obtained with Ni^{2+} were 14Å and 15Å, respectively. S4-S2 and S4-S3 distances obtained with Ni^{2+} and Cu^{2+} coincided and gave 16Å and 16.6Å, respectively. Analogous distances were also measured in CiVSP and yielded slightly different results: 15Å for S4-S2, 9Å for S4-S3 and 16Å for S4-S1. *Shaker* dimers with one LBT on one subunit and 6-His/Cu^{2+} on the adjacent subunit gave 25Å between S1-S1 and 27Å between S4-S1. The reported applications show that LRET based distance measurements utilizing genetically encoded probes is a useful technique to study membrane proteins in their natural environment: the cell membrane.

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1372-Pos Voltage-Dependent Conformational Changes of the Voltage Sensor of K_VAP Measured with LRET

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Board B348

Voltage-dependent potassium channels respond to changes in the membrane electrical field by opening, inactivating and closing ion-selective pores. Their structure is comprised of 4 subunits, each of 6 transmembrane segments, S1 through S6, with the S1–S4 region, mainly the S4, being the voltage sensor domain (VSD), responsible for the voltage-dependent control of the pore. One of the best studied VSD at a structural level belongs to K_VAP, a prokaryotic potassium channel, for which it has been proposed that a "paddle-like" S3/S4 structure undergoes large voltage-dependent conformational changes.

To test these predictions, we employed an LRET-based strategy to track voltage-dependent dynamic motions in the VSD of purified K_VAP reconstituted into liposomes. We used a genetically encoded tag, the Lanthanide Binding Tag (LBT), to encage a lanthanide donor in the linker between S3 and S4, and a fluorescein acceptor covalently bound to a cysteine of the pore-blocking scorpion toxin Agitoxin2-D20C. Toxin binding was ensured by modifying the pore with the S179G and K181D homologous mutations, which enhance Agitoxin2 blocking of *Shaker* channels. Specific donor-acceptor pair distances were determined at 0 mV for inactivated channels by monitoring the time constant of the sensitized emission of the acceptor. The combined use of an ionophore and a chelator for potassium ions was then employed to establish a transmembrane potential in the liposomes, allowing us to track changes in donor-acceptor distances upon steady-state voltage hyperpolarization, followed by depolarization. Thus, the technique allows distances estimations in the closed, the open and the open-inactivated states. Our data suggests that the distances of the LBT relative to the toxin are similar between the closed and open-inactivated states, but that the distance increases going to the open state.

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1373-Pos KCNE Proteins Stabilize Voltage-Sensing S4 Segment of KCNQ1 Channel

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Gating property of voltage-gated K+ channel can be modulated by the presence of auxiliary beta subunit. Gating property of KCNQ1, a causative gene for cardiac arrhythmia and deafness, is dramatically altered by the presence of KCNE protein family. KCNE1 significantly slows the activation kinetics of KCNQ1 channel, while KCNE3 makes KCNQ1 channel constitutively active. In spite of the apparent change of the voltage dependence, it has not been addressed how the voltage-sensing S4 segment behaves under the influence of KCNE proteins. To address this question, we introduced series of cysteine-scanning mutation from the S3–S4 linker to the middle of the S4 segment for applying SCAM (substituted cysteine accessibility method). A226C at the top of the S4 segment turned out to be an appropriate mutant to apply SCAM, because the site seemed to be exposed to the outside of the membrane only during depolarization. Application of cysteine-modifying reagent MTSES locked A226C mutant in the open state either in the absence or presence of KCNE1. We analyzed and compared the modification kinetics by MTSES in the absence and the presence of KCNE1. The modification kinetics by MTSES was substantially slower (13 times) in the presence of KCNE1. This result suggests that S4 segment of KCNQ1 is stabilized in the down state under the presence of KCNE1. On the other hand, in the presence of KCNE3, the modification kinetics was less voltage-dependent and A226C seemed to be accessible even at hyperpolarized potential. S4 segment seems to be stabilized in the up state under the presence of KCNE3. Although the modulatory effects of KCNE1 and KCNE3 on KCNQ1 are completely different, both KCNE proteins stabilize S4 segment either in the down state or the up state.

1374-Pos Modulation Of KCND3 By The Transmembrane And Juxtamembrane Domains Of DPP6 And DPP10

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DPP6 and DPP10 are dipeptidase-like proteins (DPLs) homolog to DPP4 (CD26) but lacking catalytic activity. They are type-2 transmembrane proteins expressed predominantly in excitable cells and modulate the kinetics of the transient outward currents I_{to}, the corresponding potassium channel is encoded by KCND3 (Kv4.3). The long extracellular portion of DPLs proteins is dispensable for electrophysiological effects on I_{to}. Since the trans-membrane (TM) and juxta-membrane (JM) domains are highly conserved between DPLs and among species, we investigated whether the TM or JM+TM domains are sufficient to reproduce the modulatory effects of full-length DPLs (WT) on I_{to}. As an experimental model we used CHO cells stably expressing Kv4.3 and KChIP2a. Surface immunostaining showed that TM is sufficient for DPLs incorporating to the cell membrane. In comparison to WT DPP10 current density was not changed in cells transfected with JM+TM; in contrast, TM significantly reduced I_{to} amplitude. The fast time course of activation and inactivation obtained with WT DPP10 (τ_{act} 0.25±0.02ms; τ_{inact} 8±1ms) could not be reproduced with the truncated proteins. Time constants of activation and inactivation were increased with both TM (τ_{act} 0.69±0.1ms; τ_{inact} 68±4ms) and JM+TM (τ_{act}

0.77±0.2ms; τ_{inact} 59±4ms). Time course of recovery from inactivation was also slowed with the truncated DPLs compared to WT DPP10. The effect of DPLs on voltage dependence of activation and inactivation was diminished with TM and JM+TM. The half-maximum potentials $V_{0.5}$ of activation (TM: 3±2mV; JM+TM: 0±2mV) and inactivation (TM: −26±1mV; JM+TM: −36±2mV) were shifted to more positive potentials compared to DPP10 ($V_{0.5act}$: -12±2mV; $V_{0.5inact}$: −46±2mV). In conclusion, the electrophysiological properties of DPLs on Kv4.3/KChIP2a currents cannot be reproduced with the conserved TM and JM+TM domains of DPLs, the whole polymorphic intracellular portion of DPLs is necessary.

1375-Pos Interaction of Kv4.3 with Long QT associated mutations of KCNE2

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The human cardiac transient outward current I_{to}, encoded by the Kv4.3 gene, exhibits a characteristic "overshoot" in recovery from inactivation, i.e. I_{to} amplitude transiently increases above steady state in the course of recovery. Accessory β-subunits modulate channel expression and function. Thus KChIP2 is required for Kv4.3 integration into the plasma membrane. Although KCNE2 was initially associated with the K^+ channels HERG (I_{Kr}) and KvLQT1 (I_{Ks}), we have recently demonstrated that KCNE2 (WT) also interacts with Kv4.3 to induce the I_{to} "overshoot". Since mutations of KCNE2 in patients with LQT-6 syndrome impair I_{Kr} and I_{Ks}, we investigated whether 2 of these mutations, M54T and I57T, will also affect I_{to} currents in CHO cells co-expressing Kv4.3 and KChIP2. While M54T had no effect, I57T reduced I_{to} current density from 199±49 (WT, n=16) to 120±21 (I57T, n=14). The mutants did not influence voltage dependence of I_{to}. The activation kinetics of I_{to} were significantly slowed, τ_{act} increased from 0.45±0.04ms (WT, n=14) to 1.9±0.7ms (M54T, n=9) and 2.8±0.6ms (I57T, n=14). The time course of inactivation was not affected. Recovery from inactivation was different with the 2 mutants: M54T produced a similar "overshoot" and time course of recovery as KCNE2 WT, whereas I57T significantly reduced "overshoot" amplitude and slowed the time course of recovery. In conclusion, the mutations of KCNE2 significantly modulate I_{to}, with M54T slowing activation kinetics only, and I57T additionally reducing current density, impairing the "overshoot" and slowing recovery from inactivation. Hence I57T may contribute to the development of LQT syndrome.

1376-Pos Investigating the Arrangement of MinK and KCNQ1 in IKs Channels by a Variant of Lanthanide-Based Resonance Energy Transfer (LRET) Utilizing Genetically Encoded Tags

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MinK is a single transmembrane protein that assembles with the pore-forming subunit KCNQ1 to create the slowly-activating, voltage-dependent current I_{Ks} in heart, kidney and auditory system of mammals. Functional and biochemical analyses indicate that I_{Ks} channels are formed by four KCNQ1 subunits and just two MinK subunits. To investigate the physical arrangement of the six subunits, we have employed a recently reported variant of lanthanide-based resonance energy transfer (LRET)[1]. The technique utilizes small genetically encoded tags and has several advantages over other LRET applications; it does not require indiscriminant chemical labeling of thiols and the tags can be directed to specific locations within subunits. Here, LRET tags are inserted into sites in MinK and KCNQ1 and channels are formed by expression of the proteins in *Xenopus* oocytes for study. The technique has been successfully applied to measure distances between MinK and KCNQ1.

References

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1377-Pos Additive Augmentation Of Kcnq Channels By Multiple Synthetic Agonists

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Ion channels are gated by ligands and/or voltages. Despite the major difference in sources of energy that causes conformation changes, ligand-gated ion channels and voltage-gated ion channels share some features including sensitivity to both ligand binding and transmembrane voltage. KCNQs are voltage-gated potassium channels. They are important regulatory proteins for controlling electrical excitability. Mutations of KCNQ2 and KCNQ3 potassium channel genes result in reduction or loss of potassium channel activity and cause benign familial neonatal convulsions (BFNCs). Therefore, ligand-mediated augmentation of the channel activities would be of great interest both for investigation of channel gating and for development of potential therapeutics. Retigabine and zinc pyrithione are reported to augment KCNQ potassium channels. These two activators displayed distinguishable potentiation effects and isoform specificity on KCNQ channels. We sought to test the hypothesis that these agonists recognize two different sites and thus

confer compounded effects when applied together. Using wild type and specific mutant KCNQ channels with differential sensitivities to ZnPy and retigabine, we have examined the channel modulations and gating properties. Our study indicates that there are multiple accessible agonistic sites on KCNQ channels where activators bind and exert combined modulations.

1378-Pos Identification of a Small Molecule Activator of KCNQ1/KCNE1 Channel Complexes

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KCNQ1 (Q1) is a homotetrameric voltage-gated K^+ channel that co-assembles with the KCNE family of type I membrane spanning proteins. In the heart, KCNQ1 voltage-gated K^+ channels associate with KCNE1 (E1) to form I_{Ks} currents. Mutations in either Q1 or E1, which lead to a decrease in the overall channel conductance, are known to cause the inherited form of Long QT syndrome (LQTS). Although Q1/E1 channel activators could potentially treat LQTS, there are few known activators of the Q1 channel. Moreover, known activators are either non-specific or have a reduced effect on the Q1/E1 channel complex. Our lab has identified a small molecule that potentiates Q1/E1 channel complexes in a voltage dependent manner. We found that potentiation is due to a slower rate of deactivation and a left shift in the voltage sensitivity of the channel complex. Potentiation is specific for KCNQ channels; however, the magnitude of potentiation varies depending on which KCNE peptide is present. Furthermore, we show that this activator rescues a LQTS mutant, demonstrating its promising therapeutic value.

1379-Pos Determinants Within The Turret And Pore-loop Domains Of KCNQ3 Channels Governing Functional Activity

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KCNQ1 and KCNQ4 homomers, and KCNQ2/3 heteromers, yield large currents, whereas KCNQ3 homomers yield small currents. Since the unitary conductance of KCNQ3 is five-fold, and >20-fold, greater than KCNQ4 or KCNQ1, respectively, these differences are especially striking. To test for differential membrane expression, we performed biotinylation and TIRF imaging assays, which both revealed only small differences. Normalized to KCNQ4, biotinylated surface expression of KCNQ3 was 0.36 ± 0.04. Under TIRF, KCNQ3 expressed at 0.59 ± 0.14 that of KCNQ4, and in both assays, surface KCNQ3 expressed alone was not significantly different from co-expression with KCNQ2. We examined two regions suggested to govern current amplitudes: the C-terminal, and the turret domain (TD) near the pore. Current densities of KCNQ1, KCNQ3 and KCNQ4 were 24.1 ± 1.5, 10.2 ± 2.1 and 60.9 ± 7.1 pA/pF, respectively. A chimera of KCNQ3 containing the TD of KCNQ1 yielded current densities of 32.8 ± 4.6 pA/pF. The proximal half within the TD in KCNQ3 contains PEVDAQG, whereas KCNQ1 has only VN, and their swap yielded 53.2 ± 14.4 pA/pF. Subsitution of N289 in KCNQ1, whose glycosylation was suggested to drive high expression, by the E of KCNQ3 had no effect (33.8 ± 4.3 pA/pF). When the C-terminal of the KCNQ3(TD)KCNQ1 chimera was replaced by that of KCNQ4, current densitities were 125 ± 18 pA/pF, suggesting the TD and C-terminal influences are additive. Unexpectedly, the A315T swap in KCNQ3, which corresponds to T441 in *Shaker* important for internal TEA sensitivity, dramatically boosted current densities to 135 ± 28 pA/pF, and the inverse T217A swap in KCNQ1 strongly reduced it to 2.5 ± 1.3 pA/pF. Our data suggest structural arrangements within the turret and pore-loop regulate functional activity of robustly-expressing, but mostly-dormant KCNQ3 channels.

1380-Pos Gain-of-function Mutations In KCNQ2 Cause Non-benign Neonatal Seizures

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Benign familial neonatal convulsions (BFNC) is an autosomal dominant epilepsy syndrome of newborns characterized by a benign course of the disease and normal psychomotor development. Loss-of-function mutations in the voltage-gated potassium channels KCNQ2 and KCNQ3 are associated with BFNC. Furthermore, two loss-of-function mutations in KCNQ2 have been described in families with BFNC and severe neonatal seizures, but the relevance of KCNQ2 mutations to the severe phenotype remained controversial due to extreme clinical variability associated with these mutations. We analyzed 45 unrelated individuals with severe neonatal or early-infantile seizures. In one patient, we identified a *de novo* missense mutation (M546V) in the calmodulin-binding domain in the C-terminal part of KCNQ2. In another patient we identified another *de novo* missense mutation (R560W). Functional analysis of the mutated proteins in combination with KCNQ3 subunits revealed minor differences in channel function in the case of M546V. However, in the case of R560W the current amplitude was increased 2-fold and was associated with altered gating properties. In contrast, the functional properties of the mutation in channels composed of KCNQ2 subunits (without KCNQ3 subunits) revealed a 3-fold increase in current amplitude without changes in voltage-dependence or channel kinetics for M546V while no differences were observed for R560W. Both genetic and functional findings provide evidence that heterotetrameric channels exclusively composed of KCNQ2 and its M546V mutant lead to the neonatal seizures with a

non-benign outcome, most likely caused by a gain-of-function due to an increase in trafficking. In the case of R560W, the similar clinical outcome is caused by a combination of increased trafficking of heterotetrameric channels composed of KCNQ3, KCNQ2 and its mutant R560W and altered gating properties.

1381-Pos Chasing The Fate Of KCNE1 N-linked Glycosylation

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KCNE peptides are a family of type I transmembrane glycoproteins that assemble with and modulate the function of voltage-gated K^+ channels, enhancing K^+ current diversity in a variety tissues. Most KCNE peptides are heavily glycosylated with multiple N-linked consensus glycosylation sites located in the N-terminus. The glycosylation site closest to the N-terminus is conserved in all KCNE peptides and mutations that prevent N-linked glycosylation at this site in KCNE1 and KCNE2 lead to inherited and acquired LQTS (Long QT Syndrome) respectively. In order to investigate the importance of the individual N-linked glycans in protein stability, assembly with K^+ channels and cell surface expression, we made a panel of null glycosylation mutants that prevent glycosylation at either or both consensus sites in KCNE1. Using pulse-chase and cell surface labeling experiments, we determined that the glycosylation site proximal to the KCNE1 N-terminus is critical for the formation of KCNE1 peptide, whereas removal of the second glycosylation site has no significant effect on KCNE1 protein expression or assembly with its channel partner, KCNQ1. To determine whether the two consensus sites in KCNE1 were glycosylated similarly, we used short radioactive pulses to chase the fate of the single glycosylation mutants. We found that the first N-linked glycan is added co-translationally whereas the second glycan is primarily post-translationally attached. These results demonstrate that an N-linked glycan adjacent to the N-terminus is critical for KCNE protein stability, suggesting that LQTS mutations that prevent glycosylation at this site reduce the number KCNE peptides available for assembly with K^+ channel subunits in endoplasmic reticulum.

1382-Pos C-terminal Interactions Between KCNE1 and KCNQ1

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KCNE1, which encodes the minK protein, associates with KCNQ1 to create the cardiac slowly activating delayed rectifier IKs. Mutations of both genes are linked to the hereditary cardiac arrhythmias in the Long QT syndrome (LQTS). LQTS mutations may occur throughout either gene and analyses have generally indicated a phenotype of reduced effective potassium current (from trafficking defects or altered channel function). Functional channel mutations have been primarily described as altered rates of channel activation and reduced current density. The LQTS mutation KCNE1 D76N (within the C-terminus) has been studied and though accelerated deactivation rates were noted, the majority of the effect was ascribed to altered current density and activation. KCNE1 exerts its regulation of KCNQ1 activation via interactions between membrane-spanning segments. Less attention has been focused on channel deactivation rates. In an analysis of KCNE1/KCNQ1 interactions we have observed significantly altered rates of channel deactivation with C-terminal mutations. Specific analysis of deactivation rates in subunits expressed in CHO cells shows that D76N markedly accelerates voltage-dependent deactivation with subtler effects on current density and activation rates. Analysis of purified C-termini of KCNE1 and KCNQ1 shows that they are capable of physically associating with each other and that the D76N mutation did not disrupt the association. When KCNQ1 was expressed with a KCNE1 lacking its entire C-terminus, a similar (though less pronounced) acceleration of deactivation occurred with only modest effects on current density or activation rates. Rate-dependent accumulation of K+ conductance with protocols mimicking action potential trains was defective for KCNE1 C-terminal mutations and may relate to the clinical phenotype of arrhythmias triggered by heart rate elevations during exercise. These data point to C-terminal interactions between KCNE1 and KCNQ1 as important regulators of cardiac repolarization by means of deactivation control.

Voltage-gated K Channels - V

1383-Pos Alkanol and Anesthetic Modulation of KvAP Potassium Channel Conductance and Gating: a Tool for Probing Bilayer Mechanical Effects versus Binding Models of Action

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Whether general anesthetics and alcohols act on channels via the lipid environment or binding sites is a matter of contention. Direct mechanical stress modulates the activity of voltage-gated channels and recent evidence suggests an inextricable interaction between the prototypical channel KvAP and bilayer lipids. Alkanols lower the bilayer surface tension following Traube's rule: each added CH2 reduces by ~3-fold the effective [alkanol]. Therefore, changing the bilayer's lateral pressure profile (LPP) with short chain n-alkanols and other surface active agents (SAAs) is expected to influence KvAP function. We measured unitary conductance and gating kinetics of KvAP channels in PE:PG bilayers (symmetric 150mM KCl) with and without the following SAAs: ethanol, propanol, butanol, pentanol, hexanol, octanol, 1,6-hexanediol, halothane, chloroform, isoflurane, and cholesterol. Effects of n-alkanols and general anesthetics were reversible and dose-dependent. KvAP unitary conductance (~150pS) was reduced ~25% by (in mM) 900 ethanol, 200 propanol; 20 butanol, 7 pentanol, 2 hexanol,