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,

0.75 octanol, 90 1,6-hexanediol, 30 halothane, 50 chloroform, and 700 isoflurane. In the presence of n-alkanols, macroscopic KvAP activation shifted towards negative voltages, while at +100 mV currents activated faster and inactivated slower (time constants ~30ms and ~1s, respectively). Ethanol and cholesterol, which have opposing influences on lateral pressure, had antagonistic effects on unitary conductance. The potency of n-alkanol action on KvAP conductance and gating depended monotonically on chain length. These observations are in qualitative accord with Traube's rule if the perturbation of conductance results from n-alkanol-induced decrease in surface tension and/or other associated changes in the LPP. Thus, the susceptibility of KvAP to SAA modulation may make it possible to compare LPPs for a membrane protein under basal conditions and in the presence of SAAs.

1384-Pos Voltage Range For Tuning Of Phosphatase Of Ci-vsp As Measured By Two PIP2-sensors

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VSPs, voltage-sensing phosphatases, have both voltage sensor and phosphatase that are coupled with each other. Moved charge of voltage sensor in ascidian VSP, Ci-VSP, does not saturate until up to 120 mV. Previous our study indicates that phosphatase activity increases over 50 mV (Murata & Okamura, J. Physiol., 2007). However, it remains unclear whether phosphatase activity is increased over the range where the extent of voltage sensor movement still increases. To clarify this point, we took two approaches. First, kinetics of PI(4,5)P2 depletion was compared at distinct membrane potentials by monitoring cell surface translocation of GFP fused with a PH-domain (PHD-GFP) that is derived from PLC-delta subunit under the line-scanning mode of confocal microcope. Second, kinetics of outward current through engineered inward-rectifier potassium channel, IRK1(3mut), with three mutations at sites critical for inward-rectification (Fujiwara & Kubo, J. Physiol., 2002) were measured under the two-electrode voltage clamp in Xenopus oocyte. The change of translocation of PHD-GFP occurred at time constant of 22.8 ± 15.0 sec (n=5) at 50 mV and 7.7 ± 2.8 sec (n=5) at 100 mV. Decay of outward current through IRK1(3mut) also increased from 50 mV to 100 mV. These are consistent with the idea that phosphatase activity still increased at a range over 50 mV to 100 mV where voltage sensor movement still does not saturated. We are currently comparing kinetics at further positive level.

1385-Pos Pip_2 Regulates The Activation And Voltage Dependence Of Voltage-gated Potassium Channels

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Phosphatidylinositol 4,5-bisphosphate is a signaling phospholipid of the plasma membrane that has been shown to play a key role in the regulation of a wide range of ion channels. Voltage-gated potassium channels constitute a large family of K channels, which have six transmembrane domains and whose opening and closing involve the control of molecular conformation by the voltage across the membrane. A single study by Oliver et al. (2004, Science *304*:265–70) reported effects of PIP_2 on the N-type inactivation of Kv channels. Effects on the activation mechanism of Kv channels have not yet been reported. Here, we tested for effects of PIP_2 on Kv currents lacking N-type inactivation, using perfusion of inside-out macropatches under voltage clamp conditions. Kv1.2 and Kv2.1, which belong to different subfamilies of Kv channels with significantly different cytoplasmic domains, showed a common dual dependence of their activities on PIP_2. Channel activity was inhibited by the PIP_2 scavenger poly-lysine, while 10μM PIP_2 partially reactivated the channels. Additionally, polylysine caused a left shift in the voltage-dependence of steady-state activation, while a right shift was obtained in response to PIP_2. This dual regulation of PIP_2 on Kv channels is reminiscent of similar regulation of voltage-gated calcium channels (Wu et al., 2002 Nature *419*:947–52). Mutagenesis work has identified channel sites that greatly affect PIP_2 regulation of Kv channel activity. Our results suggest that coupling between PIP_2 and the voltage gating apparatus may account for the shifts of the voltage-dependent activation upon PIP_2 depletion and replenishment.

1386-Pos Molecular Modeling and Binding of PIP_2 to Kv7 K^+ Channels

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We sought to describe the structural determinants of Kv7 channels critical for PIP_2 binding using site-directed mutagenesis and patch clamp of heterologously-expressed CHO cells. Chimeric channels were constructed with combinations of C terminal domains from high PIP_2-affinity Kv7.3 and low PIP_2-affinity Kv7.4 subunits. The putative PIP_2-binding site was localized to a C-terminal region of 71 residues located between the 1st and 2nd conserved domains in the C-terminus of the channels. Substitution of conserved positively-charged residues identified K425, K452, R459, R461, R463 and R467 as important molecular determinants for PIP_2 apparent affinity of Kv7.2, 7.3 and 7.4. To gain insight into the specific interactions between PIP_2 and the channels, a model of this domain of Kv7.2 was constructed using the solved crystal structure of the C-terminus of IRK1 (Kir2.1) (Pegan et al., *Nat Neurosci.* ***8*****:** 279–87, 2005) as a template using SWISS-MODEL (Schwede et al., *Nucleic Acids Research* ***31***: 3381–3385, 2003). Superposition of the C terminal structure of IRK1 and the predicted structure of Kv7.2 showed marked structural similarity with a CαRMS of 2.38 Å. Point mutations were introduced to the model and the resulting structural re-arrangements calculated. Docking of PIP_2 to the predicted Kv7.2

domain structure was performed using the molecular docking algorithm MolDock of Molegro Virtual Docker (Thomsen and Christensen, *J. Med. Chem.,* ***49***: 3315–3321, 2006). In preliminary calculations, energy minimization of the docking was consistent with our experimental data and predicts PIP_2 to bind with very favorable hydrogen bonding (HBond) energy to wt Kv7.2, and reduced, or greater, HBond energies to Kv7.2 mutants with lower, or higher, PIP_2 apparent affinities, respectively, as measured using excised patches. We conclude that this domain serves as a critical determinant of PIP_2 apparent affinity and may be a site of PIP_2 binding.

1387-Pos Missense Mutations In The Mink Transmembrane Segment Impair Cardiac I_{Ks} Potasisum Channels

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Cardiac I_{Ks} potassium channels are formed with four pore-forming KCNQ1 α-subunits and two accessory MinK β-subunits with single transmembrane span. Missense mutations in KCNQ1 or MinK are associated with cardiac disorders. Three mutations (L51H, G52R, and T58P·L59P) in the MinK transmembrane segment are reported to cause human cardiac arrhythmias. Our previous perturbation analysis classified that MinK L51 and L59 residues face to protein and G52 faces to lipid. We investigated further how these three disease-related mutants defect structure and function of I_{Ks} potassium channels. We first tested a hypothesis that MinK G52R disrupts channel structure and function by introducing a non-conserved charge into a lipid environment. MinK G52R and other three charged substitutions significantly changed biophysical properties and isochronal free energy in I_{Ks} potassium channels, while non-charged substitutions with different side chains had no effect, suggesting that MinK residue 52 is intolerant to a charge and located in a lipid-exposuring environment. Second, we re-examined whether MinK residues 51, 58 and 59 interact with KCNQ1 by studying effects of alanine substitutions. MinK L51A and T58A significantly altered biophysical properties and isochronal free energy of I_{Ks} potassium channels, while L59A had sub-threshold effects. Previous reports implied that MinK residues 58 and 59 may interact with KCNQ residues 338 and 340. Application of membrane-permeable reagent Methyl methanethiosulfonate had no effects on I_{Ks} potassium channels formed with KCNQ1 S338C (or F340C) and MinK L59C (or T58C), indicating that no cysteine-cysteine disulfide bridge forms between KCNQ and MinK. In fact, MinK T58P·L59P changed activation kinetics and reduced current amplitude. Therefore, MinK residues 58 and 59 interact with KCNQ1 by mainly influencing gating kinetics. MinK L51H disrupted trafficking of I_{Ks} potassium channels possibly due to altering folding of I_{Ks} channel complexes.

1388-Pos Activation of Protease-activated Receptor-2 (PAR-2) Inhibits M-current in Rat Sensory Neurons Through a PLC-dependent Mechanism

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PAR-2 receptors are functionally expressed in sensory neurons and are implicated in the pathophysiology of inflammation and pain. Activation of PAR-2 is coupled to the $G_{q/11}$ class of G proteins and results in PIP_2 hydrolysis and Ca^{2+} release from ER stores through an IP_3-PLC pathway. One of the major contributors to the control of sensory neuronal excitability is the M-current, conducted by KCNQ (Kv7) channels, which are sensitive to changes in membrane PIP_2 and cytosolic Ca^{2+}. We therefore sought evidence for M-channel modulation by PAR-2 in rat dorsal root ganglion (DRG) sensory neurons.

Neurons were enzymatically dissociated from DRGs of 7 day old rats, and cultured for 2-7 days. Whole cell M-currents were recorded by perforated patch. For measurements of intracellular calcium, DRG were loaded with Fluo-4 (2μM)/pluronic acid (0.02%) for 30–60mins.

Acute addition of a specific PAR-2 agonist peptide 2f-LIGRLO-amide (10μM) strongly reduced the current density at -30mV from 19.3±2 to 11.2±2 pA/pF (n=11) and depolarized the resting membrane potential. The M-current blocker XE-991 inhibited a similar fraction of the current to 2f-LIGRLO-amide, however, when XE-991 was applied after 2f-LIGRLO-amide, no further inhibition was observed. M-current inhibition by 2f-LIGRLO-amide was attenuated by pre-exposure to the PLC inhibitor edelphosine (10μM) indicating that PAR-2 acts through a PLC-dependent mechanism. Calcium imaging revealed robust rises in $[Ca^{2+}]_i$ induced by 2f-LIGRLO-amide in a subset of DRG neurons, by Ca^{2+} release from intracellular stores. M-channels can be inhibited by both, PIP_2 depletion and Ca^{2+}, therefore we are now exploring which mechanism underlies the uncovered modulatory pathway.

Since PAR-2 receptors are often activated upon inflammation, this new pathway of M-channel suppression may contribute to the development of 'peripheral sensitization', a hyperexcitability of sensory neurons often seen upon inflammation.

1389-Pos Cortisone potentiates Kv1 channel activities through Kvβ subunit

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The *Shaker* type voltage-dependent K^+ channels (Kv1) are expressed in a wide variety of cells and essential to regulating membrane potential and cellular excitability. Kv1 channels are

macromolecule complexes consist of the pore-forming Kvα subunits and cytoplasmic Kvβ subunits. We have found that Kvβ is a functional aldo-keto reductase that utilizes NADPH as cofactor and that the oxidation of the cofactor alters channel activities. These properties of Kvβ present a novel way of regulating Kv1 channels by targeting Kvβ. We have found that cortisone binds to Kvβ protein, and we were able to monitor the interactions between the two by following changes in NADPH fluorescence and in enzymatic activity of Kvβ. We have also tested cortisone on inside-out patches expressing both Kv1.1 and Kvβ1 and we found that cortisone significantly increased channel activities at micromolar concentrations. To further understand the modulation mechanism, cortisone was co-crystallized with the conserved aldo-keto reductase core of Kvβ2 and a high resolution structure (1.8 Angstrom) of the complex was obtained. The structure showed that each Kvβ binds to more than one cortisone molecule, and this has naturally led to the question of which binding site(s) is(are) responsible for channel modulation. Structural and functional studies are now being carried out on Kvβ mutations, and in parallel, on small-molecule compounds that bind to only one of the sites.

1390-Pos Modulation Of Neuronal K^+ Channel Current Activity By Alzheimer's Disease Related Protein BACE-1

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Increased β-secretase (BACE-1) activity results in the elevation and accumulation of the Alzheimer's disease associated peptide, amyloid β protein (Aβ). More recently it has been reported that endogenous BACE-1 co-associates with presenilin (PS1), possibly playing a role in regulating physiological levels of Aβ. Our previous studies reported that inhibiting endogenous Aβ with commercially available β-secretase inhibitors resulted in a 50% decrease in K^+ channel current. This was subsequently reversed by the exogenous application of Aβ. The exact mechanism behind the modulation of K^+ channel current by Aβ is still not fully understood. Here we used shRNAi lentiviral technology to investigate the effects of BACE-1 knockdown on K^+ channel current in human neuroblastoma SHSY5Y cells overexpressing PS1 (PS1wt).

PS1wt cells were transduced with lentiviral vectors expressing shRNAi targeting BACE-1 (shRNAi-BACE1) and the empty vector shRNAi pLentilox 3.7 (shRNAi-PLL) as control. Whole-cell patch clamp measurements of K^+ channel currents were carried out using quasi-physiological intra- and extracellular solutions. Statistical differences were assessed using repeated measures ANOVA with Tukey's post-hoc test or unpaired Student's *t*-test as appropriate.

The mean current-voltage (I-V) relationship for cells transduced with shRNAi-BACE1 was significantly decreased by 75%. At a test potential of +50mV following a prepulse of -140mV current was decreased from 0.12±0.02 nA/pF to 0.03±0.01 nA/pF (n=12, $p<0.001$). This decrease was reversed upon the exogenous application of 10 nM $A\beta_{1-40}$ which significantly increased the mean peak current density by 233%. At +50mV the values of mean peak current density increased from 0.03±0.01 nA/pF to 0.10±0.02 nA/pF (n=12, $p<0.001$).

These data further support the role of BACE-1 and Aβ as physiological modulators for K^+ channel function. Lentiviral shRNAi-BACE1 technology also proves to be more effective in reducing the K^+ current than pharmacological β-secretase inhibition.

1391-Pos Intracellular Mg2+ Is A Voltage Dependent Pore Blocker Of HCN Channels

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are activated by membrane hyperpolarization that creates time-dependent, inward-rectifying currents, gated by the movement of the intrinsic voltage sensor S4. However, inward rectification of the HCN currents is not only observed in the time-dependent HCN currents, but also in the instantaneous HCN tail currents. Inward rectification can also be seen in mutant HCN channels that have mainly time-independent currents (Chen et al., 2001). Here, we show that intracellular Mg2+ functions as a voltage-dependent blocker of HCN channels, acting to reduce the outward currents. The affinity of HCN channels for Mg2+ is in the physiological range, with Mg2+ binding with an IC50 of 0.53 mM at +50 mV in HCN2 channels. The effective electrical distance for the Mg2+ binding site was found to be 0.19. Removing a cysteine in the selectivity filter reduced the affinity for Mg2+ suggesting that this residue forms part of the binding site deep within the pore. Our results show that Mg2+ acts a voltage-dependent pore blocker and, therefore, reduces outwards currents through HCN channels. The pore blocking action of Mg2+ may play an important physiological role, especially for the slowly gating HCN2 and HCN4 channels. Mg2+ could potentially block outward hyperpolarizing HCN currents at the plateau of action potentials, thus preventing a premature termination of the action potential.

1392-Pos N-linked Sialic Acids Are Responsible For The Entire Effect Of Sugars On $K_V1.5$ Gating, While Other Sialic Acids Modulate $K_V2.1$ Gating

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$K_v1.5$ and $K_v2.1$ produce a slowly inactivating current, which is responsible for a portion of cardiac repolarization. K_v channels are heavily and differentially glycosylated, with glycosylation sites varying in number, location, and type of sugar linkages. Previously,

our lab showed that the loss of the terminal sugar residue, sialic acid, modulates gating of the *Drosophila* Shaker K^+ channel, resulting in a depolarizing shift in the voltage of half-activation (V_a). Here, we investigated whether alterations in glycosylation, including sialic acids, can modulate voltage-gated potassium channel function. Voltage-dependent gating of both $K_v1.5$ and $K_v2.1$ was evaluated under conditions of full and effectively no sialylation. Under states of reduced sialylation, the V_a for $K_v1.5$ and $K_v2.1$ were significantly shifted to more depolarized potentials by 21 mV and 8 mV, respectively. The asparagine residues of the N-glycosylation consensus site for $K_v1.5$, located on the S1–S2 linker, and $K_v2.1$, located on the S3–S4 linker, were mutated to prevent the addition of N-glycosylation. For $K_v1.5$, the data suggest that N-linked sialic acids account for the entire effect of sialic acids on channel gating. However, $K_v2.1$ mutagenesis had no effect on the impact of sialic acids on channel gating, indicating that, if $K_v2.1$ is N-glycosylated, these sugars do not exert any measured functional effect. These data suggest that sialic acids other than N-linked sialic acids modulate $K_v2.1$ gating. Furthermore, similar sialic acid-dependent shifts were observed for the activation time constants and V_a for $K_v1.5$ and $K_v2.1$. Overall, the data indicate a functional and isoform-specific role for sialic acids in the modulation of K_v channel gating.

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1393-Pos Tetrameric Structure of Kv Channels and Kvα C-terminus Allow Regulation of Kvβ3-Imparted Rate of Inactivation by Pyridine Nucleotides

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Board B369

Voltage-gated potassium (Kv) channels are a tetrameric assembly of Kv (α) proteins and ancillary β-subunits that regulate channel kinetics and localization. When co-expressed with Kvβ3 in COS-7 cells, Kvα1.5 generated rapidly inactivating outward currents. Inclusion of reduced pyridine nucleotides NAD(P)H in the patch-pipette solution increased the overall extent of inactivation and accelerated the fast inactivation rate constant to a maximal value $\tau_{fast} = 19 \pm 1.7$ ms. Inclusion of $NAD(P)^+$ dose-dependently increased the value of τ_{fast} to 64±16 ms before totally abolishing inactivation. Mathematical analysis showed that simultaneous changes in the extent and rate of inactivation cannot be explained by simple ligand competition at the single Kvβ active site but is a function of tetrameric structure of Kv channels. Theoretical curves were generated from empiric data with the following assumptions:

1. Each conformation with different number of NADPH molecules per channel $Kv_4(NADPH)_x$ has its inactivation rate constant;
2. overall degree of inactivation is proportional to the number of channels bound to at least one molecule of reduced nucleotide per tetramer.

Sharply contrasting inactivation behavior was observed when myocardial normoxic (no inactivation) or hypoxic (fast inactivation) mixtures of 4 pyridine nucleotides were included in the patch pipette. Fitting these data using the model equations predicts the ratio of affinities for NADPH/NAD^+ of 2.5-fold. Thus, Kvβ3 does not appear to discriminate between nucleotides and its ability to impart inactivation to Kv currents depends upon the total metabolic state of the cell. Furthermore the observation that deletion of 58 C-terminal amino acids abolishes the nucleotide sensitivity of Kvα1.5 co-expressed with Kvβ3 suggests that the C-terminal domain of Kv1.5 provides a mechanistic link translating different conformational states of Kvβ into Kv inactivation rates.

1394-Pos Single Channel Analysis Of Kv2.1 Indicates That Channels In Cell Surface Clusters Are Not Active K+ Channels

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Voltage-gated K+ channels regulate action potential duration and spike frequency. The delayed rectifier Kv2.1 is frequently found localized in high- density clusters on the cell surface. The channel is constitutively phosphorylated and changes in phosphorylation state are associated with dispersion from these clusters as well as a hyperpolarizing shift in the voltage-dependence of activation. Recently we demonstrated that a population of Kv2.1 channels resides outside these cluster domains, even under basal conditions. Since these clusters can be recapitulated in heterologous expression systems, we expressed GFP-Kv2.1 in HEK293 cells and used cell-attached patch clamp in physiological K+ concentrations to investigate the functional difference between Kv2.1 inside and outside these clusters. Using confocal microscopy, we were able to position the patch pipette directly on GFP-Kv2.1 clusters. Under these conditions, patches on clusters typically contained 1 – 3 channels, with a slope conductance of 7.6 pS, consistent with previous estimates of Kv2.1 single channel conductance. The average open probability at -35 mV was 0.1. In contrast, patches outside the cluster domain yielded large (>200 pA) delayed rectifier currents consistent with those derived from Kv2.1. It was not possible to measure single channel activity in these patches. These results are consistent with a model in which the channels within the cluster domain are silent, whereas channels outside the cluster function as K+ channels. We hypothesize that the channels within the cluster may be a reserve pool of surface channel to be recruited during periods of hyperexcitability, while the active channels outside the cluster are responsible for the whole-cell current under basal conditions. Additionally, though they appear to minimally flux K+, these channels may still act as voltage sensors, coupling changes in membrane excitability to intracellular signaling pathways.

1395-Pos Lipoelectric Modification of Ion Channel Voltage Gating by Polyunsaturated Fatty Acids

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Polyunsaturated fatty acids (PUFAs) have beneficial effects on epileptic seizures and cardiac arrhythmia. However, their exact molecular mechanism is not known. We have studied the effect of different fatty acids on the voltage-gated Shaker K channel expressed in Xenopus oocytes using the two-electrode voltage-clamp technique. We show that -3 and -6 all-cis-PUFAs affect the voltage dependence of the Shaker K channel by shifting the conductance versus voltage (G(V)) curve in negative direction along the voltage axis. In contrast, uncharged methyl esters of the PUFAs do not affect the voltage dependence and the positively charged GsMTx-4 toxin shifts the G(V) curve in positive direction along the voltage axis. pH and charge mutations on the channel surface affect the size of the PUFA-induced shifts. These results suggest an electrostatic effect on the channel's voltage-sensors. PUFAs also shift the gating charge versus voltage curve in negative direction along the voltage axis, however this shift is smaller than the G(V) shift. This indicates that PUFAs electrostatically affect the concerted opening step more than the early independent voltage-sensor movements. Monounsaturated and saturated fatty acids, as well as trans-PUFAs do not affect the voltage dependence. Therefore, fatty acid tails with two or more cis double bonds near the methyl end may be needed for placing the negative carboxylate charge of the fatty acids in a position to affect the channel's voltage dependence. We propose that charged lipophilic compounds could play a role in regulating neuronal and cardiac excitability by electrostatically affecting the channel's voltage sensor. Lipoelectric modification of the channels voltage dependence could be a future new approach for pharmacological treatment, that is voltage sensor pharmacology.

1396-Pos Fast-inactivation Mechanism of Kv Channels Revealed by RNA Editing

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In hKv1.1 mRNAs, genomic recoding by adenosine-to-inosine editing results in an Ile to Val conversion at residue 400 that is located in the intracellular pore cavity. Although this substitution is conservative, it leads to a substantial increase in the off rate from fast-inactivation, a process that results from an interaction between the N-terminus of an auxiliary beta-subunit and the permeation pathway of the alfa-subunit. We hypothesized that the small change in hydrophobicity due to RNA editing underlies the rapid unbinding kinetics. To study this interaction in greater detail we used the *Shaker* K^+ channel because its inactivation particle is part of the alfa-subunit and it does not exhibiting highly regulated gating modes like hKv1.1. We substituted I470, equivalent to 400 in hKv1.1, to cysteine as target for chemical modification with MTS-reagents. Two hydrophobic reagents, methyl and propyl-MTS, significantly slowed the recovery from fast-inactivation by 30 and 90 fold, respectively. In contrast, polar MTS-reagents of similar size changed recovery by two fold. Although these results supported our hypothesis, MTS reagents are relatively large, potentially changing the distance between the N-terminus and position 470. To identify the specific N-terminal residue that interacts with 470, we introduced pairs of cysteines: one at 470 and the other at position 2 through 8 of the N-terminus. After excising inside-out patches, we observed that only the 470C-2C pair showed an irreversible decrease in current resulting from the formation of a disulphide bond which can be recovered by reducing reagents, suggesting that the N-terminus can enter deep into the cavity in a rather extended conformation. We conclude that by changing an Ile to a Val, RNA editing perturbs the intimate interaction between the cavity and the second position of the N-terminus, thereby altering the fast-inactivation process.

1397-Pos Regulated RNA Editing and Functional Epistasis in Shaker Potassium Channels

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RNA editing is a pretranslational mechanism that can transform the genetic code by enzymatic modification of mRNA. Simple hydrolytic deamination by adenosine deaminases acting on RNA (ADARs) at specific mRNA locations reassign codons, altering amino acids in the resultant protein. We have identified four such sites that, in effect, produce point mutations in conserved positions of the *Drosophila Shaker* potassium channel: I360M(1), I464V(2), T489A(3), and Q491R(4). A single mRNA molecule can potentially represent any of 2^4=16 permutations (editoforms) of these mutations. Thus, we generated editoform expression profiles to assess sexually dimorphic, spatial, and temporal differences. Unexpected and striking tissue-specific expression was seen for particular editoforms. Pairwise comparisons between these distributions were significantly different from one another, except for male/female comparisons. Moreover, distributions of editoforms showed evidence for coupling (linkage) of editing sites. For example, in male wing, sites 3 and 4 were both unedited in 70% of the clones, but there were no clones in which site 3 was unedited and site 4 was edited. This was not due simply to the paucity of editing at site 4. We characterized the biophysical properties in nine of the 16 possible editoforms, with and without the inactivation ball. One editoform (edited at site 3 only) distinguished itself from the others, inactivating ~3-fold more slowly. We show that while individual editing sites confer modest natural variability in biophysical properties, an

unprecedented feature was revealed in multiply edited forms, namely, functional epistasis. For example, the biophysical phenotype of the slowly inactivating editoform cannot be explained by consideration of the consequences of individual mutations at the four sites. This result unmasks an apparent allosteric communication across disparate regions of the channel protein and between evolved and regulated amino acid changes introduced by RNA editing.

1398-Pos Five RNA Editing Sites In *Shab* Responsible For Activation And Deactivation Kinetics

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During transcription, RNA editing revises the genetic code at precise locations, creating single base changes in mRNA. These changes can result in altered amino acid sequences and modifications of protein function. Sequence analysis of the *Shab* potassium channel of *Drosophila melanogaster* revealed five such RNA editing sites; four are $\geq$ 50% edited (I583V, T643A, Y660C and I681V), and one is edited more in larval than in adult flies (T671A). These sites are located in the S4, S5–S6 loop and S6 segments of the channel. We examined the biophysical consequences of editing these sites by examining five mutant constructs, each containing the genomic (unedited) base at one of the five sites in the background of a channel in which all other sites are edited. We characterized these channels using two-microelectrode voltage clamp in *Xenopus* oocytes. Each 'unediting' mutation slowed activation kinetics. Four of the five editing-site mutations are relatively conservative. The V $\rightarrow$ I substitution at position 681, for example, is a minimal side chain modification; however it exhibited the most striking change in deactivation kinetics. Deactivation was slowest in a 'genomic' construct in which all five sites were unedited. Three point mutants exhibited a significant hyperpolarized shift in their midpoints of activation. One of the editing sites, position 660, aligns with the *Shaker* 449 residue, which is known to be important in tetraethylammonium (TEA) block. The aromatic, genomic residue tyrosine at this position in *Shab* enhances TEA block 14-fold compared to the edited residue, cysteine. These results show that both position of the RNA editing site and identity of the substituted amino acid are important for channel function. A further challenge is understanding why these sites are so uniformly and highly edited in this potassium channel.

1399-Pos Editing Of A Second K_V1 Potassium Channel mRNA From The Squid Giant Axon

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Previous reports have shown that in squid, mRNAs encoding proteins involved in excitability are extensively edited. The biological significance of these changes is largely unknown. RNA editing of mRNAs for SqKv1.1A has been previously reported (*Neuron 34*: 743–757).We have taken advantage of the cloning of SqKv1.2A (*Biophys. J. 80*: 434D), a second K^+ channel expressed in the giant axon system, to compare how RNA editing by adenosine deamination affects two similar channels from the same cell. Based on 50 individual cDNA clones and the genomic DNA sequence, adenosine residues within SqKv1.2A mRNAs can be edited at 13 positions, 6 of which are silent. Editing efficiency greatly varies between sites. The editing pattern of SqKv1.2A shares some similarities with SqKv1.1A. Each has two sites in S1 and two in S3. Unlike SqKv1.1A, SqKv1.2A does not have a large cluster of sites in the T1 domain. Based on their positions in conserved domains, two sites are noteworthy: R359G is in the hinge between the S4–S5 linker and S5, and D396G is on the outer mouth of the pore. As with SqKv1.1A, the biophysical effects of editing sites in SqKv1.2A are subtle. Most notably, I171M accelerates deactivation kinetics ~4 fold (−60mV). The most pronounced effect of editing appears to be on functional expression. When expressed in *Xenopus* oocytes, the unedited channel produces robust currents with as little as 2.2 pg of injected cRNA. Other single editing site mutants require 10–50 times more cRNA to produce similar sized currents. The fully edited channel does not produce detectable currents, even when 22 ng of cRNA is injected. Our results indicate that the most significant effect of editing on K^+ channels is to regulate the available K^+ conductance.

1400-Pos A Mutation in the Voltage-Sensing Helix of HCN4 Channels Enhances cAMP-Dependent Stabilization of a Secondary Open State

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HCN ("pacemaker") channels are hyperpolarization-activated and their voltage-gated activity is enhanced by the binding of cyclic nucleotides such as cyclic AMP (cAMP), but whether cAMP-gating is directly coupled to voltage-gating remains unresolved. With sufficiently long hyperpolarizations, HCN channels form a secondary open state after the initial opening step; this briefly sustains the activation of open channels after a return to weakly depolarized holding voltages where voltage-dependent steps are rate limiting for deactivation. The effect of cAMP on formation and decay of the secondary open state is unclear. We studied mouse HCN4 channels as homomers in excised oocyte membrane patches, and found cAMP does slightly increase sustained activation. Since charged residues in the voltage-sensing S4 helix are a likely determinant of voltage-dependent steps, we studied a charge-reversing mutation in

S4 (K381E). K381 is predicted to lie in the membrane, but the K381E mutation causes surprisingly little change in voltage-dependence (G-V Boltzmann slope factors within 20% of wildtype HCN4). This mutation also causes general open state stabilization in the absence of cAMP (a depolarizing $V_{1/2}$ shift, faster activation and slower deactivation). Notably, K381E also dramatically increases (~five-fold) the cAMP-dependence of sustained activation: current previously activated by hyperpolarization in the presence of saturating cAMP requires >15 seconds to become fully deactivated. The cAMP-dependence of sustained activation decreases as the holding voltage is increasingly depolarized, showing voltage-dependent steps are affected. Thus, the secondary open state decays during deactivation through a step that is both voltage- and cAMP-dependent. K381E dramatically increases the stability of the cAMP-dependent secondary open state, rather than altering the voltage-sensing capacity of S4. This suggests direct coupling of and physical interactions between voltage-sensing S4 and cAMP-modulatory machinery in HCN channels.

1401-Pos A Photoswitchable Affinity Label (PAL) for optical regulation of endogenous K+ channels

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Controlling ion channels with light offers great spatio-temporal advantages over chemical and electrical stimulation. Because most proteins are not naturally photosensitive, different strategies have emerged to render them light-responsive. We previously developed a light-regulated Shaker K+ channel by covalently attaching a photoswitchable pore blocker onto an engineered cysteine, which afforded optical control of neural firing in neurons expressing these mutant K+ channels. Here we report a method that allows optical control of endogenous K+ channels that do not contain surface cysteine residues, eliminating the need for foreign gene expression. The strategy, which we call the PAL (Photoswitchable Affinity Label) approach, is based on affinity labels that contain a photoisomerizable azobenzene linker between a ligand and electrophilic moiety that covalently reacts with the protein surface. To target K+ channels, a quaternary ammonium (QA) pore blocker was used as the ligand. By design, after covalent attachment at an appropriate distance from the QA binding site, photoisomerization of the azobenzene linker would control QA binding, thereby regulating K+ conductance. Indeed, photoswitchable QA ligands displaying various electrophiles were found to react with wild-type Shaker channels expressed in Xenopus oocytes. Pre-incubation with an acrylamide analogue (AAQ) conferred light sensitivity to numerous wild-type neuronal K+ channels, including the Kv1, Kv2 and Kv4 families when expressed in HEK-293 cells, but not to cells expressing the Kv3.1 subtype. Nevertheless, AAQ was found to react with the Kv3.1 channel and impart light sensitivity if voltage pulses were used to repetitively depolarize the cell and open the channel during treatment. This observation suggested a conformation-dependent affinity labeling reaction, such that the attachment site is only accessible for a given state of the channel.

1402-Pos Cell cycle-dependent expression of Kv1.5 is involved in myoblast proliferation

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Voltage-dependent K^+ channels (Kv) are involved in the proliferation of many types of cell, but the mechanisms by which their activity is related to cell growth remain unclear. Kv antagonists inhibit the proliferation from activated quiescent to highly cycling tumour cells, which is of physiological relevance in skeletal muscle. Although myofibres are terminally differentiated, some resident myoblasts may re-enter the cell cycle and proliferate. Here we report that the expression of Kv1.5 is cell-cycle dependent during myoblast proliferation. In addition to Kv1.5 other Kv, such as Kv1.3, are also up-regulated. However, pharmacological evidence implicates Kv1.5, but not other *Shaker* subunits, in myoblast growth. Pharmacological blockage of Kv1.5 led to cell cycle arrest during the G_1-phase. The use of selective cell-cycle blockers showed that Kv1.5 was transiently accumulated during the early G_1-phase. Furthermore, while myoblasts treated with S0100176, a Kv1.5 antagonist expressed low levels of cyclin A and D_1, the expression of $p21^{cip-1}$ and $p27^{kip1}$, two cyclin-dependent kinase inhibitors, increased. Our results indicate that the cell cycle-dependent expression of Kv1.5 plays a pivotal role in skeletal muscle cell growth. In addition, we demonstrate that the induction of $p21^{cip-1}$ and $p27^{kip1}$, concomitant with a decrease of cyclin A and D_1, underlie signalling mechanisms.

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1403-Pos Kv1.3 Currents in Human Jurkat T-Cells Recorded with Automated Planar Patch-Clamp Instrumentation

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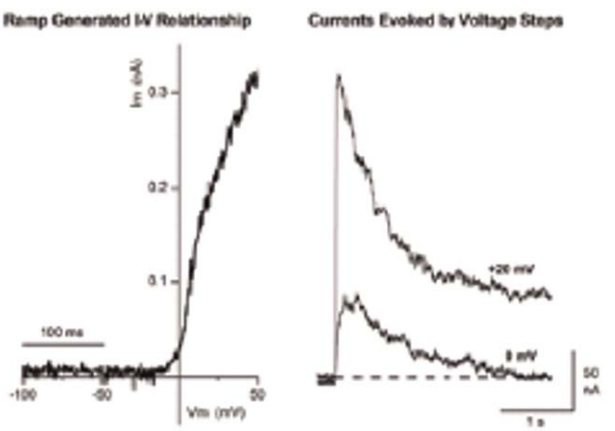
Board B379

Expanding the successful application of automated planar patch-clamp technology to an increasing diversity of ion channels, expressed in native and heterologous cell types, is now allowing the definite consolidation of ambitious screening programs of ion channels as targets for novel therapeutic purposes within the drug discovery industry. Voltage-gated Kv1.3 channels have been proposed as emerging prominent targets in different therapeutic fields

(i.e. immunological disorders, immuno-suppression, metabolic syndrome). Kv1.3 plays a critical role in the activation of human T-cells and are also normally expressed in Jurkat cells, a leukemic cell line well established as an experimental model. In this study perforated-patch whole-cell recordings from Jurkat cells were obtained with IonWorks™ HT and Quattro systems. Composition of the recording internal solution was (in mM): K-gluconate 100, $MgCl_2$ 1, EGTA 5, HEPES 5 (pH= 7.2 with KOH). The external solution was PBS (pH=7.4). Identification of Kv1.3 currents was accomplished by characterizing their kinetical, gating and pharmacological (Margatoxin blockade) properties. In all these respects the results obtained were entirely comparable to Giga-ϑ seal pipette recordings therefore validating the use of automated instrumentation for the electrophysiological study of this channel.



1404-Pos ICh-MASCOT- A Flexible And User Friendly Software For The Global Kinetic Modeling Of Ion Channel Gating

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Board B380

Ion channels in excitable cells play crucial roles in electrical signaling. To gain insight into the gating mechanisms of ion channels, Markov models are commonly used to describe their kinetics quantitatively. Here, we present the Windows-based software "ICh-MASCOT", which is a flexible and user-friendly program for global fitting of ion channel kinetics based on Markov models. The global fit uses experimental data obtained under different conditions to get a set of rate constants that describes the entire experimental data set simultaneously. ICh-MASCOT can use macroscopic data such as time dependence of the current in response to a voltage and/or ligand stimuli, the steady state properties and time constants. Ionic currents for several permanent ions and gating currents can also be included in the global fit. The program supports models for coupled gates described by a single kinetic scheme as well as models with independent gates described by several kinetic schemes. The models can assume arbitrary rate constants with certain voltage and ligand dependencies.

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Acetylcholine Receptors

1405-Pos The Mechanism Of Partial Agonism In The Nicotinic Receptor Superfamily

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Board B381

A partial agonist is a ligand which, at high enough concentrations to occupy all receptors, can elicit only a relatively small response. In the case of ligand-activated ion channels, it has been supposed ever since 1957 that the basis for the small response lies in the gating reaction, i.e. the change of conformation from an open channel to a shut channel. We investigated partial agonists for two members of the C-loop family, namely, tetramethylammonium for the nicotinic acetylcholine receptor and taurine for the glycine receptor. Single channel currents were recorded from HEK293 cells transfected with wild type acetylcholine receptor α, β, ε and δ or glycine receptor α_1 and β subunits in cell-attached configuration. Several mechanisms were fitted by maximising the likelihood of the entire sequence of open and shut time periods, with exact allowance for missed brief events (program HJCFIT[1]). Several records obtained at different agonist concentrations were fitted simultaneously. We found that the results can be well described by a 'flip' mechanism[2] in which after binding, the receptor moves through an intermediate shut conformation ('flip' state), before the channel opens. For both nicotinic and glycine receptors, full and partial agonists showed very similar gating reactions, so differences in gating were not responsible for partial agonism. Rather, the difference between full and partial agonists originated during the earlier conformation change (flipping) while the channel is still shut. This interpretation places the root of partial agonism earlier in the chain of events that follow binding than has been supposed up to now. That is something that might be detectable in structural measurements and could be exploited in rational drug design.

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1406-Pos Interplay between Cations, Anionic Lipids, and Lipid-Protein Interactions at the Nicotinic Acetylcholine Receptor

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The role of lipid ionization state in lipid-protein interactions at the nicotinic acetylcholine receptor (nAChR) has been investigated.