1326-Pos Identifying the Lipid-Protein Interface of the 5-HT_{3A}R: Hydrophobic Photolabeling Studies with Affinity-Purified 5-HT_{3A}Rs

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Board B302

The 5-HT_3R is a member of the Cys-loop superfamily of ligand-gated ion channels (LGICs) and mediates excitatory fast synaptic transmission in the CNS. Despite the clear physiological importance of the 5-HT_3R, only a small number of published studies have directly examined the structure of the receptor. The principal objective of this work is to express and affinity-purify 5-HT_3Rs and initiate structural studies. A mouse 5-HT_{3A}R containing a C-terminal α-bungarotoxin (αBgTx) pharmatope tag (1) was constructed and stably transfected into HEK 293 cells. To obtain sufficient quantities of receptor protein for affinity-purification, αBgTx-5-HT_{3A}R-HEK cells were cultured either in 140 mm dishes (∼1000 dishes) or in a 5 L spinner flask containing microcarrier glass beads (Cytodex-3). Typically, cells were treated with 100 μM serotonin 24 h prior to harvesting resulting in a ∼2.5 fold increase in receptor expression (upregulation). αBgTx-5-HT_{3A}Rs ([^{125}I]αBgTx Kd ∼10 nM) were affinity-purified (αBgTx-derivatized Sepharose 4B affinity column) from detergent (1% CHAPS) solubilized membranes. In this first study, the lipid-protein interface of the 5-HT_{3A}R was examined by hydrophobic photolabeling with [^{125}I]TID. Our data demonstrate that [^{125}I]TID photoincorporates into the 5-HT_{3A}R and the labeling maps to two proteolytic fragments, designated V8-17K and V8-8K. N-terminal sequencing of each rpHPLC purified fragment revealed that V8-17K starts at Val^{195} and based on its apparent molecular weight extends through the M1–M3 transmembrane segments. V8-8K starts at Val^{424} and contains the M4 segment. Approximately 60% of the total subunit labeling is localized to V8-8K suggesting that the M4 segment has the greatest exposure to lipid. Additional experiments are in progress to further identify lipid-exposed segments/residues in the 5-HT_{3A}R and the results will be compared with those previously determined for the *Torpedo* nAChR.

References

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1327-Pos Acid-Sensing Ionic Channels in Rat Anterior Pituitary Cells

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Board B303

Acid-sensing ionic channels (ASICs) are members of ENaC/degenerin family of channels. To date, four different isoforms have been cloned. Three of them, termed ASIC1-3, form functional homo- and/or heteromeric channels transiently opening in response to a drop in extracellular pH. The ligand for ASIC4 and functional role of this isoform remains elusive. They are widely distributed in central and peripheral nervous system and they have been implicated in neuronal ischemia, mechanoreception, and nociception. At present time, little is known about the expression and role of these channels and the pH sensitivity of anterior pituitary cells in general. Here we show that mRNA transcripts for ASIC1, 2 and 4 are expressed in anterior pituitary cells. In agreement with this data, drop in extracellular pH below 7.0 evoked transient inward current in one third of cells tested. The current was characterized by fast desensitization and diminution in response to repeated low pH application. ASIC-like current was inhibited by amiloride (50 μM) but was not affected by the TPRV1 antagonist ruthenium red (10 μM). Channels underlying ASIC-like current were permeable to sodium and potassium with no permeability for calcium. These data suggest expression of ASIC channels in pituitary cells, with possibility for their heteromeric composition. Further experiments are needed to clarify their exact structure and roles in pituitary cells, including their possible modulation by peptidergic hormones.

Voltage-gated K Channels - III

1328-Pos Differential Effects of Amidoarone and Dronedarone on hERG and KCNQ1/KCNE1 Channels

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Board B304

Purpose. To address the question whether dronedarone, a new noniodinated methanesulfonanilide derivative of amiodarone, may differ from amiodarone, we comparatively studied the effects of DR and AM on HERG and KCNQ1/KCNE1 channels and determined the binding sites of both drugs within HERG channel.

Methods. HERG (wild and mutant) and KCNQ1/KCNE1 (wild) channels were expressed in Xenopus Oocytes and currents were measured using 2-electrodes voltage clamp technique. To determine binding sites, ala-scanning mutagenesis of S6 (L646-Y667) and pore helix (L622-V625) in HERG channels were performed.

Results.

(i) DR potently blocked both HERG channel ($IC_{50\%}$: 1.7±0.3 μM, n=5) and KCNQ1/KCNE1 channels ($IC_{50\%}$: 13.4±3.3 μM, n=5), whereas AM preferentially blocked HERG channel ($IC_{50\%}$: 5.6±1.4 μM, n=5) rather than KCNQ1/KCNE1 channels ($IC_{50\%}$: >100 μM, n=5).

(ii) DR rapidly binds (time constant (tau), 264±47 ms, n=5, 40mV) to and unbinds (tau=360±24 ms, n=5, −80mV) from

HERG channel. On the contrary, AM 5–50 fold slowly binds (tau=1.2±1.1 s, n=3) to and unbinds (tau=33±18 s, n=3).

(iii) Both DR and AM have the common binding amino acid residues on HERG channel; three form the pore helix (T623A, S624A, and V625A) and two from S6 domain (Y652A and F656A), G648A HERG was not blocked by both drugs suggesting different binding sites from methanesulfonanilide.

I647 A current was more blocked by dronedarone rather than amiodarone.

Conclusions.

1. DR may inhibit both I_{Kr} and I_{Ks} which is more like a long-term treatment rather than a short-term treatment of AM.
2. The removal of iodine and the addition of methanesulfonanilide structure from AM to DR may result in rapid binding and unbinding to HERG channels which helps to exert antiarrhythmic action, but did not affect binding sites except I647.
3. KCNQ1/KCNE1 block by DR might attribute to the methanesulfoanilide structure.

1329-Pos hERG K+ Channel Drug Block Underlying Acquired Long-QT Syndrome Probed With Natural And Unnatural Amino Acid Mutagenesis In Mammalian Cells

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Board B305

Through systematic mutagenesis of critical residues in the hERG pore to other natural and/or unnatural amino acids (UAAs) we aim to guide the rational redesign of molecules to reduce their hERG block. We perform our studies in mammalian cells because a major limitation in *Xenopus* oocytes is that the test substance accumulates in the oocyte yolk, resulting in significant inconsistencies in potency estimates. To validate the methodology, we examined the effect of the known hERG blockers risperidone, amperozide and sparfloxacin on the conventional hERG mutants T623S, S624A, S624T and Y652F when expressed in CHO cells. The wild-type IC_{50} for each drug was compared to its IC_{50} for each mutant and used to calculate $\Delta\Delta G$, the difference between the Gibbs free energy of association between the drug and the wild-type channel versus the mutant channel. The $\Delta\Delta G$'s indicate the type of interaction between the drug and channel pore at each position. More precise probing of drug-channel interactions requires UAA mutagenesis of hERG. We identified a bacterial tyrosine aminoacyl-tRNA synthetase (bTyrRS)/tRNA (bTyr-$tRNA_{CUA}$) pair that rescue the expression of a hERG Y652TAG mutant. The voltage dependence and waveforms of rescued currents were identical to those of wild-type, within experimental error. In control experiments, if either the bTyrRS or the bTyr-$tRNA_{CUA}$ were omitted from the transfection, no hERG expression was detected. Directed evolution of the bTyrRS binding site has generated a mutant that specifically aminoacylates our bTyr-$tRNA_{CUA}$ with the UAA, cyclohexylalanine (CHA). Specific incorporation of CHA in Y652TAG and F656TAG hERG mutants expressed in mammalian cell lines is presently under investigation.

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1330-Pos Pharmacological Rescue of Syntaxin 1A-Dependent Trafficking Impairment of hERG Channels

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Board B306

The SNARE protein, syntaxin-1A (STX1A), is involved in cellular trafficking and regulation of ion channels. We have reported the expression of STX1A in cardiac myocytes, and its interaction with the cardiac K^+ channels $K_V2.1$, $K_V4.2$ and K_{ATP} channels. We examined the effects of STX1A on hERG (human ether-à-go-go-related gene) utilizing HEK 293 cells stably transfected with hERG and transiently transfected with STX1A for electrophysiological and biochemical interactions. STX1A reduced peak hERG currents (at +10 mV) from 69.1±5.8 pA/pF (hERG, n=16) to 28.2±4.1 pA/pF (hERG + STX1A, n=14) ($p<0.001$), induced a hyperpolarizing shift in the midpoint of the voltage dependence of steady-state inactivation, and accelerated the slow time constant of deactivation at negative potentials. Incubation with 5 μM E4031 for 24hrs, followed by a 1-hr washout period partially rescued hERG currents; peak current at +10 mV was 50.5±8.0 pA/pF (hERG + STX1A + E4031, n=10) which was not significantly different from control-treated cells 57.1±4.0 pA/pF (hERG + E4031, n=21). Western blot analysis demonstrated that E4031 rescued channel trafficking as shown by an increase in the 155 kDa mature hERG protein. To further probe the protein-protein interaction, we co-immunoprecipitated STX1A and HA-tagged C-terminal truncated hERG constructs and the FLAG-tagged hERG1b, which possesses a shorter 36 amino acid N-terminus compared with its 376 amino acid full-length hERG counterpart. STX1A co-immunoprecitated hERGΔ899, hERGΔ860, and hERGΔ814 strongly, while hERGΔ860–899 was weaker. hERG1b co-immunoprecipitated very weakly with STX1A suggesting that the N-terminus is a possible site of interaction. Our studies suggest that STX1A may be an important intrinsic modulator of hERG channel trafficking, current amplitude and gating through its interaction with the hERG N-terminus.

(Supported by HSFO)

1331-Pos Herg Channel And Propafenone Derivatives: Chemical Structure Effects On Drug Trapping

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Board B307

Inhibition of HERG channels prolongs the ventricular action potential, correspondingly the QT-interval with the risk of *torsade de pointes* arrhythmia that may result in sudden cardiac death. We have previously analyzed the mechanism of HERG inhibition by 8 inhibitors [1]. Four of them (domperidone, E-4031, terfenadine, bepridil) were found to be "trapped" at channel closure while the remaining four (amiodarone, cisapride, droperidol and haloperidol) were able to dissociate from the closed channel. No correlation between trapping and inhibitor properties (molecular weight, size, hydrophobicity etc) was observed. Here we systematically study the kinetics of HERG inhibition and recovery from block by new propafenone derivatives with conserved apparent pharmacophore structure but different side chains attached. HERG channels were expressed heterologously in *Xenopus* oocytes and currents were measured with the two microelectrodes voltage clamp technique. Frequency-dependent block was observed for the bulky derivative GPV-0576, GPV-0005 induced similar pulse-dependent block at all frequencies and propafenone induced "tonic" (pulse-independent) block. No substantial recovery from block was observed for propafenone and GPV-0005. HERG channels recover from block by GPV-0576 within $\approx$ 1 min with a voltage dependent rate. Our data suggest that propafenone and GPV-0005 (apparently "trapped" compounds) dissociate from the open channel state while GPV-0576 dissociates from open and resting states. Propafenone and its two derivatives were docked into homology models of the open and closed HERG channels. Binding scores from docking studies were compared with the experiment results to obtain insights into state dependent drug binding modes.

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References

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1332-Pos Does The Phosphoinositide PIP_2 Stabilize HERG Channel Open State?

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Board B308

Phosphatidylinositol-4,5-bisphosphate (PIP_2) is a plasma membrane phospholipid which has many functions such as cytoskeleton attachment or precursor of second messengers. Another important role is the modulation of many ion channels: in particular, it regulates several inward rectifier and voltage-gated K^+ channels. PIP_2 notably increases their maximum open probability. More precisely by a macropatch study of the PIP_2 regulation of Kv7.1 combined with a kinetic model of the channel, we suggested that PIP_2 acts on the transitions corresponding to the channel opening, rather than transitions corresponding to the voltage sensors movement. Here we investigate the generalization of this mechanism by studying the effects of PIP_2 on HERG, a Kv channel which is responsible for the cardiac potassium current I_{Kr}. Previous studies evidenced the up-regulation of HERG by PIP_2 giving details on the modifications of the biophysical parameters of the channel studied in the whole-cell configuration [1]. We have performed electrophysiological measurements of excised giant patch of COS-7 cells overexpressing HERG, in strictly the same conditions as KCNQ1 in our previous study. We monitored the channel activity in response to large variations of PIP_2 concentration due to depletion of endogenous PIP_2 (run-down) followed by addition of external PIP_2. Run-down entails

1. a decrease of the full-activated current, an acceleration of the activation and deactivation that are reversed by PIP_2, but not by the short chain analog diC8-PIP_2
2. a hyperpolarizing shift of the half-activation potential that is not restored.

The biophysical modifications of HERG by PIP_2 are close to those of Kv7.1 in the same conditions: we are thus testing in a kinetic model if the hypothesis that PIP_2 stabilizes the open state of Kv7.1 applies to other Kv channels as HERG.

References

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1333-Pos Inhibition of HERG human K^+ channels by *Lindera erythrocarpa*

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Board B309

Lindera erythrocarpa Makino (*L. erythrocarpa*) belongs to the large family Lauraceae and is used as a traditional medicine for analgesic, digestive, diuretic, antidote, and antibacterial purposes. We studied the effects of *L. erythrocarpa* on the human *ether-a-go-go*-related gene (*HERG*) channel expressed in *Xenopus* oocytes. Application of methanol (MeOH) extract of *L. erythrocarpa* showed a dose-dependent decrease in the amplitudes of the outward currents measured at the end of the pulse (I_{HERG}) and the tail currents of HERG (I_{tail}). When the n-butanol (BuOH) fraction and H_2O fraction of *L. erythrocarpa* were added to the perfusate, both I_{HERG} and I_{tail} were suppressed, while the hexane fraction, chloroform ($CHCl_3$) fraction, and ethyl acetate (EtOAc) fraction did not inhibit either I_{HERG} or I_{tail}. The shifts in the potential required for half-maximal activation ($V_{1/2}$) caused by EtOAc fraction, BuOH fraction, and

H_2O fraction were significant, while the hexane fraction and $CHCl_3$ fraction did not change the $V_{1/2}$. The BuOH fraction and H_2O fraction (100 μg/ml) decreased g_{max} by 59.6 and 52.9%, respectively. The H_2O fraction- and BuOH fraction-induced blockades of I_{tail} progressively decreased with increasing depolarization, showing that the H_2O fraction and BuOH fraction of *L. erythrocarpa* block I_{tail} voltage-dependently. Our findings suggest that *L. erythrocarpa*, a traditional medicine, blocks HERG channel and rapidly-activating delayed rectifier K^+ current, which could make heart prone to arrhythmia.

1334-Pos Multiple Splicing Defects Caused by a Splice Site Mutation in hERG

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Board B310

Mutations in the human ether-a-go-go-related gene (hERG) cause type 2 long QT syndrome (LQT2). We analyzed the LQT2 mutation 2592+1G>A, a splice site mutation in the 5′ splice donor site of intron 10. The functional consequence of this mutation was studied using a minigene containing the hERG genomic sequence spanning from exon 8 to exon 12. In these experiments, HEK293 cells were transfected with wild-type or 2592+1G>A mutant minigene. RT-PCR analysis revealed that the 2592+1G>A mutation disrupted the normal splicing and led to three aberrantly spliced transcripts. Sequence analysis showed that the major transcript was due to the use of a cryptic 5′ splice donor site in exon 10 located 72 nt upstream of the normal site. In the two minor transcripts, one was due to the utilization of an intron 10 cryptic 5′ splice donor site located 118 nt downstream of the normal site, and the other resulted from complete intron 10 retention. The use of the cryptic site in exon 10 resulted in a large deletion of 72 nt in hERG mRNA and led to an in-frame deletion of 24 amino acids in the distal portion of the cyclic nucleotide binding domain. The deletion of 24 amino acids resulted in defective trafficking of mutant channels. Translation of the two minor transcripts would result in a read-through into intron 10 and generate 5 additional amino acids before reaching a premature termination codon. These results suggest that the 2592+1G>A mutation disrupts normal splicing and leads to multiple splicing defects due to the activation of two cryptic sites and complete intron 10 retention. Our findings underscore the importance of studying LQT2 splice site mutations at the mRNA level.

1335-Pos The Co-chaperone Fkbp38 Promotes Herg Trafficking

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Board B311

The KCNH2 or human ether-a-go-go related gene (HERG) encodes the Kv11.1 α-subunit of the HERG potassium channel that underlies the rapidly activating delayed rectifier current I_{Kr}. In the heart, mutations in KCNH2 cause a reduction in I_{Kr} resulting in the proarrhythmic type 2 long-QT syndrome (LQT2). While multiple factors can cause the loss of the functional phenotype, the dominant mechanism has been reported to be a trafficking deficiency that results in endoplasmic reticulum retention (ER). It has been shown that trafficking-deficient HERG mutants can be rescued by a number of factors including a putative ER-resident HERG chaperone FKBP38 that we recently reported immunoprecipitates and co-localizes with HERG. In the present study we demonstrate *in vitro* that translated HERG C-terminus and cyclic nucleotide binding domain (CNBD) co-precipitate with added purified FKBP38, re-constituting the HERG-FKBP38 interaction observed in cells. Functionally, while siRNA knockdown of FKBP38 reduces wild type (WT) HERG trafficking, overexpression of FKBP38 is able to partially rescue several LQT2 trafficking mutants including F805C, S818L and R823W. Preliminary results show that FKBP38 lacking the TPR domain (ΔTPR), CaM binding site (ΔCaM) and TM domain (ΔTM) all co-precipitate with HERG from HEK cells; however, FKBP38 mutants lacking the PPIase domain (ΔPPIase), or containing the PPIase domain alone, do not. These data are consistent with an interaction through the PPIase domain of FKBP38 and at least one other site. Using these same constructs, we show that deletion of any domain (ΔTPR, ΔTM, ΔCaM, ΔPPIase) results in the loss of rescue of HERG trafficking mutants. We propose that the membrane-anchored co-chaperone FKBP38 provides a direct link between the cytosolic Hsp70/Hsp90 chaperone system and ER retention or export mechanisms of HERG.

1336-Pos Visualization of binding sites for Ergtoxin-1 on the Voltage Sensing Domain of hERG K+ Channel by AFM Recognition Imaging

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Board B312

Human ether-à-go-go-related (hERG) potassium (K+) channels are essential for normal electrical activity in the heart. The inhibition of hERG channels is the major cause of long QT syndromes inducing fatal cardiac arrhythmias. Ergtoxin 1 (ErgTx1) belongs to the scorpion toxins, known to inhibit hERG K+ channels with high specificity and low nanomolar affinity. A characteristic feature of the action of ErgTx1 on hERG is incomplete block of macroscopic current even at very high concentrations (∼ 1 microM, Kd ∼ 10 nM). Such phenomenon is supposed to be consistent with highly dynamic conformation of the outer pore domain of hERG. In this study simultaneous topography and recognition imaging (TREC) on hERG HEK 293 cells was used to visualize binding sites on extracellular part of hERG channel (on S1-S2 region) for Anti-Kv11.1 (hERG-extracellular-antibody). The recognition maps of hERG channels contained "dark" spots, haphazardly distributed and organized in clusters. Recognition images after the addition of

ErgTx1 at high concentrations (~ 1 microM) revealed subsequent partial disappearance of clusters indicating that ErgTx1 was bound onto S1–S2 region. These results were supported by AFM force spectroscopy data. Collectively, our results show for the first time that voltage sensing domain (S1–S4) of hERG K+ channel might be one of the multiple binding sites of ErgTx1.

1337-Pos Functional Properties of Heteromeric hERG 1a/1b Channels at Physiological Temperatures

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Board B313

Cardiac I_{Kr} is a repolarizing current whose perturbation by inherited mutations or drug block can cause life-threatening arrhythmias associated with long QT syndrome (LQTS). Recent evidence suggests that native I_{Kr} channels are composed of hERG 1a and 1b subunits, yet the functional contributions of the 1b subunit are poorly understood. We compared the biophysical properties of hERG 1a and 1a/1b channels expressed in HEK-293 cells at near-physiological temperatures using whole-cell patch clamp techniques. Outward currents normalized to peak inward tail current, assumed to be proportional to the number of channels in each cell, revealed that hERG 1a/1b channels passed more outward current than homomeric1a channels in response to step voltage clamp commands or those mimicking the ventricular action potential. We found no differences in the $V_{1/2}$ of steady-state activation plots but the activation and deactivation kinetics were approximately two-fold faster for heteromeric channels. The apparent rates of inactivation were unaltered whereas the recovery from inactivation was faster, by approximately two-fold, for heteromeric 1a/1b channels. Thus, the increase in hERG 1a/1b current amplitude reflects an attenuation of rectification attributable to faster activation and recovery from inactivation. Deactivation, although accelerated in hERG 1a/1b currents, reduced current amplitude only at negative voltages and thus contributed little to differences in current properties. Development of E-4031 block was slower for heteromeric channels and showed four-fold reduced sensitivity when compared to homomeric 1a channels. Our work predicts that mutations selectively targeting 1b subunit will reduce I_{Kr} and thus represent a novel potential mechanism for LQTS.

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1338-Pos The Trafficking of hERG Channels is Inhibited by Dominant Negative Rab11B Mutations

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Board B314

Many long QT syndrome missense mutations in the human Ether-a-go-go Related Gene (hERG) channel decrease its intracellular transport (trafficking) to the Golgi apparatus and plasma membrane. The trafficking of proteins is regulated in part by Rab GTPase-proteins. The purpose of this study was to identify Rab GTPase-proteins that regulate the trafficking of hERG channels. We transiently expressed WT hERG channels with different dominant negative Rab mutations in HEK293 cells. We found that the trafficking of WT hERG channels was inhibited by dominant negative mutations in Rab11B. Co-expression of N124I-Rab11B reduced hERG current (I_{hERG}) by 76% compared to control cells ($n=6-7$ cells per group, $p<0.05$). The export and trafficking of hERG channels from the Endoplasmic Reticulum to the Golgi can be visualized using Western blot analysis because the addition of sugars to hERG in the Golgi (Golgi processing) increases the MW of each subunit from 135 to 155kDa. Co-expression of N124I-Rab11B prevented Golgi processing of hERG channels. We developed a new 'live-cell' Western technique that quickly measures the cell surface membrane expression of ion channels by imaging living cells on cell culture plates. This technique employs an extracellular hERG antibody and a secondary antibody that is labeled with an infrared dye. We found that co-expression of N124I-Rab11B reduced the infrared labeling of cells expressing hERG channels by 80% compared to control cells ($n=3$). We found similar electrophysiological, Western blot, and infrared labeling results for a second dominant negative Rab11B mutation, S25N, tagged with green fluorescent protein (S25N-Rab11B-GFP). Confocal imaging showed that co-expression of S25N-Rab11B-GFP caused intracellular retention of hERG channels. Together, these data imply that Rab11B regulates the trafficking of hERG channels to the Golgi apparatus and cell surface membrane.

1339-Pos hERG Channel (Kv11.1) Expression and Deactivation is Regulated by Conserved Charged Residues in S2 and S4

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Board B315

Studies suggest that salt bridges between charged residues form in the voltage-sensor of voltage gated K^+ channels (Kv) and they are important for normal functional expression and gating. The human-Ether-a-go-go Related Gene (hERG) encodes Kv11.1, which underlies the rapidly activating delayed rectifier K^+ current in the heart. Using the HEK293 cell expression system, we found that engineering a mutation that reversed the charge of a conserved acidic residue in the 2nd transmembrane segment (S2) at amino acid position 466 (D466K) reduced the functional expression of Kv11.1. We engineered a secondary charge reversal mutation in a conserved basic residue at amino acid position 534 (R534D) in the 4th transmem-

brane (S4) segment to generate a D466K/R534D-Kv11.1. In contrast to D466K-Kv11.1, D466K/R534D-Kv11.1 expressed at the cell surface. Cells expressing WT-Kv11.1 current ($I_{Kv11.1}$) had a mid-point potential for half maximal activation ($V^1/_2$) of −17.9±1.7mV and a slope factor of 7±1mV/e-fold change in current (n=8). In contrast, cells expressing D466K/R534D-Kv11.1 had two distinct components of $I_{Kv11.1}$, a constitutively active component and a voltage-dependent component that activated with a $V^1/_2$ of −78.5±3.5mV and a slope factor of 17±3mV/e-fold change (n=4). The kinetics of WT $I_{Kv11.1}$ deactivation can be described as a double exponential process with a voltage-dependent fast and slow component. In contrast, the kinetics of D466K/R534D $I_{Kv11.1}$ deactivation could be described as a weakly voltage-dependent single exponential process with a voltage-independent offset to account for the constitutively active component of $I_{Kv11.1}$. The voltage-dependent inactivation rate of $I_{Kv11.1}$ was slightly faster than WT, but there was not a large difference in the reversal potential of WT or D466K/R534D $I_{Kv11.1}$. These data suggest that interactions between D466 and R534 are important for Kv11.1 expression and channel closing.

1340-Pos Site-specific Regulation of hERG Potassium Channels by Intracellular Cysteine Modification

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Board B316

Human ether à go-go related gene potassium channels (hERG1) play a crucial role in cardiac repolarization. Oxidative stress in cardiac tissues diminishes hERG-mediated I_{Kr} currents and could give rise to cardiac arrhythmias. Here we aimed at elucidating the molecular mechanisms by which hERG channels are affected by oxidative stress. For that purpose, hERG1 was expressed in HEK 293 cells and currents were recorded in the whole-cell patch-clamp configuration. While extracellular application of the thiol-reactive and therefore cysteine-specific agent MTSES was without noticeable effects on channel function, intracellular application of MTSES via the patch pipette resulted in a loss of channel function. MTSES (1 mM) progressively decreased hERG current with a time constant of about 60 s down to 30% of the initial magnitude 200 s after patch rupture. Searching for target residues, hERG without the cytosolic N-terminal domain (ΔN=Δ2–373) was less sensitive to MTSES (remaining current after 200 s: 63%). The results using a C-terminal deletion (Δ866–1159) and single-point mutants showed that C723S has the strongest impact on the action of MTSES (63% remaining current). Combination of ΔN and C723S rendered channels practically insensitive to intracellular MTSES: only 8% current reduction within 200 s MTSES application. Thus, the function of hERG channels is strongly affected by chemical modification of cysteine residues, both in the N- and the C-terminal domains of the channel protein.

1341-Pos Glycosylation Impacts Gating Of The HERG1 Channel

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Arrhythmias are often caused by aberrant ion channel activity, resulting in remodeling of the cardiac action potential. Two K^+ currents, I_{Ks} and I_{Kr}, contribute to phase III repolarization of the human action potential. Human *ether-a-go-go*-related gene 1 (hERG1), a voltage-gated potassium channel, underlies I_{Kr}. Alterations in the repolarization phase of the action potential, and in particular I_{Kr}, can lead to arrhythmias, long QT syndrome, heart disease, and sudden cardiac death. Our lab previously determined that sialic acids, often the terminal residue of N- and O-linked carbohydrate structures, can alter gating of voltage-gated Na^+ channels and the *Drosophila* Shaker K^+ channel. hERG1 has two potential N-glycosylation sites located in the S5-S6 linker region. The aim of this study was to determine if and to what extent glycosylation modifies hERG1 channel function. Voltage-dependent gating and kinetics of hERG1 were evaluated under conditions of full glycosylation, no sialylation, and in the absence of complex glycans. Under conditions of reduced sialylation and also in the absence of complex glycans, the half-activation voltage (V_a) for hERG1 shifted to more depolarized potentials by ∼10 mV. Reduced sialylation and complex glycans impacted steady-state inactivation to a slightly greater extent than the observed glycan-dependent shift in V_a. The half-inactivation voltage (V_i) for hERG1 shifted to more depolarized potentials by ∼13 and ∼19 mV, respectively. Additionally, a reduction in the levels of sialic acids and in complex glycan caused a significant 38% and 76% increase in deactivation rates at −50 mV. Overall, these data indicate a functional role for glycosylation in the modulation of gating of the hERG1 channel, suggesting that N-linked sugars promote hERG1 activity.

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1342-Pos Investigating The Origin Of The Voltage-sensitivity Of Inactivation In hERG Potassium Channels

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Board B318

The human ether a-go-go related gene (hERG) encodes the pore forming subunit of the cardiac delayed rectifier potassium channel. Among voltage-gated potassium (Kv) channels hERG has unique gating properties: slow activation and fast, voltage dependent inactivation. While the protein domains involved in voltage-sensitivity of activation in Kv channels have been identified, the origin of voltage-sensitivity of inactivation in hERG is not clear. Some evidence suggests voltage-dependent inactivation is linked to the voltage-sensor for activation (1), while other research points to a separate inactivation voltage-sensing domain in hERG (2). To

answer this question we have used site-directed mutagenesis to create a 'voltage sensor null' hERG construct where the positive charges in the S4 activation voltage-sensor have been sequentially neutralized. Whole cell voltage-clamp electrophysiology was then used to characterise voltage-dependent activation and inactivation in these mutant channels. Neutralization of the first 3 charges in the hERG S4 transmembrane helix (K525Q/R528Q/R531Q) shifted the $V_{0.5}$ of activation to -102.3 ± 5.2 mV compared to -15.1 ± 1.6 mV for wt hERG. On the other hand, voltage-dependent inactivation was unaltered ($V_{0.5}$ -73.9 ± 3.9 mV and -70.4 ± 3.3 mV for wt and 525Q/528Q/531Q). Our data therefore suggest that the origin of voltage-sensitivity for activation and inactivation in hERG channels lies in separate protein domains.

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1343-Pos Quinidine And Terfenadine Block Of Herg Show Opposite Dependency On Extracellular Potassium

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Board B319

Block of HERG by quinidine and terfenadine was measured using two electrode voltage clamp of *Xenopus* oocytes in extracellular solutions of 0 mM and 20 mM potassium. Consistent with published data, HERG block by quinidine is reduced with elevated extracellular potassium (1). Increasing extracellular potassium from 0 mM K^+ (no added potassium) to 20 mM K^+ reduced drug block approximately 9 fold from an IC_{50} of 2 μM in 0 mM K^+ to an IC_{50} of 18 μM in 20 mM K^+. However, increasing extracellular potassium from 0 mM K^+ to 20 mM K^+ had the opposite effect on HERG block by terfenadine. Increasing extracellular potassium from 0 mM K^+ to 20 mM K^+ increased HERG block by terfenadine approximately 3 fold from an IC_{50} of 1 μM in 0 mM K^+ to an IC_{50} of 0.29 μM in 20 mM K^+.

The mechanisms behind this different extracellular potassium dependency on block of HERG are not clear. Previous data from this lab indicates that HERG block by quinidine shows a better correlation with the permeant ion species than with inactivation. Thus HERG block by quinidine is greatest in solutions where the permeant ion is lowest, independent of the inactivation rate. This appears not to be the case for terfenadine. One possibility is that HERG channel gating has a different influence on HERG block by terfenadine compared to HERG block by quinidine. Consistent with this, terfenadine has been shown to be trapped in the channel after channel closure whereas quinidine must dissociate from the channel before channel closure.

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1344-Pos Internalized Kv1.5 traffics via Rab-dependent pathways

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Board B320

We have investigated the roles of three Rab-GTPases in the trafficking of Kv1.5 channels newly internalized from the plasma membrane. In imaging experiments, we found that internalized Kv1.5 recycles rapidly to the plasma membrane and that Rab5, Rab4 and Rab11 all co-localized with the channels, although over different time frames. Surface-labeled Kv1.5 appeared within minutes in both Rab5- and Rab4-positive vesicles whereas the channel colocalized with Rab11 only after prolonged periods of cell incubation. As measured electrophysiologically in H9C2 myoblasts and HEK-293 cells, dominant negative Rab5 and Rab4 constructs both caused a 2 to 3-fold increase in Kv1.5 channel surface expression. In addition, overexpression of wild-type Rab4 caused a modest increase in Kv1.5 currents in the myoblast cell line. A Rab11 dominant negative construct had no effect on Kv1.5 currents within 24 hours of transfection but did have a modest effect after 48 hours. Inhibition of the dynein motor by p50/dynamitin overexpression was not additive with the effects of Rab dominant negatives, indicating that all of these treatments were affecting the same trafficking pathway. With the exception of Rab11, the effects of disruption of the microtubule cytoskeleton by nocodazole were similarly not additive with the Rab dominant negatives. These results indicate that Kv1.5 channels are internalized via a Rab5-dependent pathway and very probably recycle to the membrane through a rapid, Rab4-dependent process. Some recycling via the slower Rab11 pathway is also likely. These findings illustrate that Rab-GTPases are dynamic targets for the regulation of Kv1.5 channel expression.

1345-Pos In Xenopus oocytes Glycosylation of Shaker K+ Channels Alters Its Expression In The Plasma Membrane and Activation Kinetics

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Board B321

The Shaker K+ channel is particularly well suited for studies of ion channel biogenesis and protein quality control because it expresses well in a variety of systems, including Xenopus laevis oocytes. In this preparation, wild-type Shaker K+ channel protein folds and assembles into tetramers in the endoplasmic reticulum where the N-linked glycosylation is initiated. This membrane protein is glycosylated on two asparragine residues, N259 and N263, located in the first extracellular loop (Santacruz-Toloza et al, 1994). We examined the effect of removing both glycosylation sites on the expression and biophysical properties of a modified Shaker K-channel in which the

N-type inactivation domain was replaced by an HA-epitope. In Western blot analysis from total oocyte membrane wild-type (wt) channel, migrated as two populations (∼ 73 and ∼113 kDa), consistent with mannose-rich and full glycosylated species, respectively. Meanwhile, the double mutant (N259Q - N263Q) migrated as a single population of ∼68 kDa, consistent with a single unglycolsylated form. In harmony with their respective differences in the band intensities, injection of similar amounts cRNA, yielded ionic current densities for the mutant that were ∼5% of that for wt channels. Conductance vs. Voltage curve in the mutant was not significantly different from that of wt. However, the activation kinetics, during an 80 ms depolarizing pulse between −20 and +60 mV, were ∼3-fold slower in the mutant, while the inactivation kinetics were 2-fold slower. These results indicate that the lack of glycosylation decreases the expression and modify the kinetic of Shaker K-channels.

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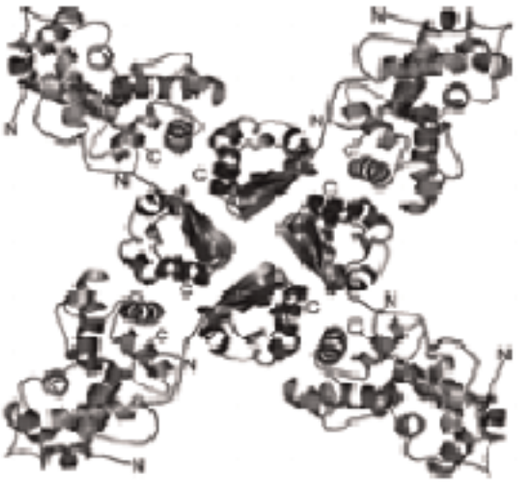

1346-Pos Effects of Tetrameric Clamping by KChIPs on Kv4 Trafficking

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Board B322

Cytoplasmic KChIPs that co-assemble with pore-forming Kv4 subunits to form a native complex regulate expression and gating properties of Kv4 K^+ channels. Our recent co-crystal structure consisting of Kv4.3 N-terminus and KChIP1 reveals a unique clamping mode of the complex in which a single KChIP1 molecule as a monomer laterally clamps two neighboring Kv4.3 N-termini in a 4:4 manner, thus generating two interfaces of the interaction. In the first interface, the proximal N-terminal peptide of Kv4.3 is sequestered by its binding to an elongated groove on the surface of KChIP1, indispensable to the modulation of Kv4.3 gating by KChIP1, whereas the same KChIP1 molecule binds to an adjacent T1 domain to stabilize tetrameric Kv4.3 channels, constituting a second interface of the interaction. A question arose as to whether the second interface is responsible for Kv4 trafficking. Using the structure-based mutagenesis, we generated mutants aimed at disrupting interactions within the second interface, and tested effects of those mutants expressed in mammalian cells on Kv4 trafficking using immunostaining. Preliminary results show that some key residues critical for KChIP1 clamping on tetrameric assembly of Kv4 within the second interface can affect Kv4 protein trafficking.

1347-Pos Mechanism Of The Blockade Of K_V Channels By Ketamine In Rat Mesenteric Artery Myocytes

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Board B323

Clinical doses of ketamine typically increase blood pressure, heart rate, and cardiac output. However, the precise mechanism by which ketamine produces these cardiovascular effects remains unclear. The voltage-gated K^+ (K_V) channel is the major regulator of resting membrane potential (E_m) and vascular tone in many arteries. Therefore, we sought to evaluate the effects of ketamine on K_V currents using the standard whole-cell patch clamp recordings in single myocytes, enzymatically dispersed from rat mesenteric arteries. Ketamine ((±)-racemic mixture) inhibited K_V currents reversibly and concentration-dependently with a K_d of 566.7 ± 32.3 μM and Hill coefficient of 0.75 ± 0.03. The inhibition of K_V currents by ketamine was voltage-independent, and the time courses of channel activation and inactivation were little affected. The effects of ketamine on steady-state activation and inactivation curves were also minimal. Use-dependent inhibition was not observed, either. *S*(+)-ketamine inhibited K_V currents with similar potency and efficacy as the racemic mixture. The average resting E_m in rat mesenteric artery myocytes was −44.1 ± 4.2 mV, and both racemic and *S*(+)-ketamine induced depolarization of E_m (15.8 ± 3.6 mV and 24.3 ± 5.0 mV at 100 μM, respectively). To verify the hypothesis that ketamine-induced E_m depolarization is attributed to K^+ channel inhibition, we measured the membrane conductance (G_m) by repetitively injecting brief hyperpolarizing current pulses. The ketamine-induced E_m depoarlization was accompanied by G_m decrease, indicating that the ketamine-induced E_m depolarization is associated mainly with the inhibition of K^+ conductance, not with the activation of some depolarizing conductance.

We conclude that ketamine induces E_m depolarization in vascular myocytes by blocking K_V channels in a state-independent manner, which may contribute to the increased vascular tone and blood pressure produced by this drug under a clinical setting.

1348-Pos The Molecular Basis Of High-affinity Binding Of Vernakalant, A Novel Atrial Antiarrhythmic Compound, To Kv1.5 Channels

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Board B324

Vernakalant (RSD1235) is an investigational drug recently shown to convert atrial fibrillation rapidly and safely in patients (1), and has been shown to target potassium currents important in atrial repolarization, including IKur. The present study describes the molecular mechanisms of interaction of vernakalant with the inner pore of the α-subunit of Kv1.5 (which is thought to underlie IKur), in comparison with the Class IC agent flecainide. Initial experiments showed that vernakalant blocks activated channels and vacates the inner vestibule as the channels close. Drawing on studies of other Kv1.5-selective blocking agents, site-directed mutagenesis targeting residues at the base of the selectivity filter and in S6 was used to explore drug binding. Block by vernakalant or flecainide of Kv1.5 wild-type (WT) and mutants was assessed by whole cell patch clamp experiments in transiently transfected HEK293 cells. The mutational scan identified several highly conserved amino acids, T479, T480, I502, V505 and V508, as important residues for affecting block by both compounds. In general, mutations in S6 increased the IC50 for block by vernakalant, with I502A causing an extremely local 25-fold decrease in potency. Specific changes in the voltage-dependence of block with I502A supported the crucial role of this position. A homology model of the pore region of Kv1.5 and molecular docking simulations predicted that, of these residues, only T479, T480, V505 and V508 are potentially accessible for direct interaction, and that mutation at additional sites studied may therefore affect block through allosteric mechanisms. For some of the mutations, the direction of changes in IC50 were opposite for vernakalant and flecainide, suggesting differences in the forces that drive drug-channel interactions.

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1349-Pos External Barium Block of Kv1.5 channels and the Effects of Low pH_o

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Board B325

Under low pH_o conditions, Kv1.5 channels exhibit accelerated P/C-type inactivation and decreased peak macroscopic conductance and channel availability. As these effects are relieved by elevated $[K^+]_o$ and/or the R487V mutation, manoeuvres that also attenuate P/C-type inactivation, the two phenomena may share a similar mechanism. Here, external Ba^{2+} was used to determine whether low pH_o is associated with outer pore constriction, a presumed mechanism of P/C-type inactivation. Following a 2 min application of Ba^{2+} at pH_o7.4, the current time course was slowed, an effect that was sensitive to changes in $[K^+]_o$ and test potential. These results are similar to observations in N-type-inactivation removed *Shaker* (*Sh*IR) channels and are consistent with a blocking site in the pore that is accessible at rest and becomes unblocked during channel activation. Contrary to findings in *Sh*IR, block onset was more rapid and followed a single exponential time course with no evident "fast" component, while current recovery was slower, with a closed-channel off-rate more than two magnitudes lower than that observed in *Sh*IR. Qualitatively, however, the relationship between the blocking rate and $[Ba^{2+}]_o$ can be predicted by a two-site binding model previously described for *Sh*IR. When Ba^{2+} was applied after switching to pH_o5.5, which reduced Kv1.5 currents by over 90%, binding was largely prevented and there was little slowing of current activation upon return to pH_o7.4. Conversely, pulses applied at pH_o5.5 following Ba^{2+} loading (at pH_o7.4) were unable to cause Ba^{2+} unblock. These results are consistent with a low pH_o-induced conformational change in the outer pore of Kv1.5 that prevents Ba^{2+} binding at rest or Ba^{2+} unbinding during depolarization. If a pore constriction similar to that proposed to occur during P/C-type inactivation is involved, it would imply that closed-state inactivation occurs under acidic conditions.

1350-Pos 4-Phenoxybutoxy-substituted anellated heterocycles, a new class of Kv1.3 Blockers

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Board B326

The voltage-gated potassium channel Kv1.3 provides a promising target for the selective suppression of effector memory T cells in T-cell mediated autoimmune diseases, such as multiple sclerosis. In earlier studies, potent and selective Kv1.3 blockers have been synthesized starting from 5-methoxypsoralen isolated from Ruta graveolens. The best results in terms of potency, selectivity and drug-like properties were obtained by attaching a 4-phenoxybutoxy side chain in 5-position of the psoralen system as in PAP-1 (IC50 = 2 nM). In contrast, the unsubstituted 5-methoxypsoralen, which does not have the above-mentioned side chain, inhibits Kv1.3 with more than 1000-fold lower potency. We therefore investigated, whether attaching of the 4-phenoxybutoxy side chain to other heterocyclic systems, different from the psoralen scaffold, would also produce potent Kv1.3 blockers. A high percentage of our newly synthesized

compounds, such as 4-phenoxybutoxy substituted quinolinones, furoquinolins, coumarins or furochromons blocked Kv1.3 with IC50s in the range of 150 nM to 10 μM in whole-cell patch-clamp experiments. Our most potent new compound is the 5-(4-phenoxybutoxy)coumarin, which resembles the psoralen scaffold except for the missing furan ring (IC50 = 150 nM). Quinolins, quinazolins and phenanthren system substituted with the 4-phenoxybutoxy side chain in contrast had no effect on Kv1.3 at all. Taken together, our results demonstrate that attachment of a 4-phenoxybutoxy alone is not sufficient to render any aromatic ring system a potent Kv1.3 blocker.

1351-Pos HpTx2 Binding and Gating Modification of Kv4 Channels are Dependent on Distinct Amino Acids in S3b

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Board B327

Kv4 channels are responsible for many of the fast-inactivating K+ currents found in the cardiovascular and nervous systems. HpTx2 is an inhibitor cysteine knot (ICK) gating modifier toxin that selectively inhibits Kv4 channels, making it an excellent probe for understanding Kv4 structure and function. Previous experiments in our lab suggested that the S3-S4 region of Kv4.3 contains the toxin-binding region. To characterize the molecular determinants of interaction, alanine scanning of the S3b-S4 linker region was performed. L275A and V276A significantly decreased rHpTx2 inhibition of Kv4.3; the double mutant LV275AA nearly eliminated inhibition. The analogous amino acids on Kv4.1 also eliminated inhibition. These amino acids are located in the "hot spot" for gating modifier toxin binding, previously identified. However, unlike some other ICK toxins, HpTx2 does not require a charged amino acid for channel interaction. These results show that despite the specificity of HpTx2, its binding determinants are shared with other ICK toxins. Even though their binding sites are identical, HpTx2 gating modification of Kv4.3 and Kv4.1 is distinct with respect to voltage dependence of inhibition, and voltage dependence of both steady-state activation and inactivation. To understand these differences, we modeled Kv4 kinetics and interaction with HpTx2. This model is consistent with our experimental data that suggests HpTx2 affects voltage-dependent transitions between closed states (C0–C4) in Kv4.3, while in Kv4.1, it modifies the voltage-independent transition between the pre-open closed to open state (C4-O). The molecular basis for this difference was due to four non-conserved amino acids in S3b, adjacent to the HpTx2 binding site. Swapping this region between the two channels resulted in a phenotype switch with respect to toxin interaction, suggesting that S3b is important in the voltage-dependence of Kv4 gating.

1352-Pos Interaction Of Tarantula Toxins With Voltage-activated Proton Channels

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Board B328

Voltage-activated proton channels (VSOP; HVCN1) were recently cloned and shown to be similar to S1–S4 domains in voltage-activated potassium (Kv) channels (Sasaki et al., 2006; Ramsey, et al., 2006). However, few inhibitors, other than divalent cations, exist to study their physiology and biophysical mechanisms. We recently showed that the voltage-sensor paddle motif (S3b-S4) can drive opening with membrane depolarization when transplanted from Hv1 into classical Kv channels, suggesting common mechanism of voltage sensing (Alabi et al., 2006). We examined whether tarantula toxins that interact with paddle motifs in Kv channels can also interact with similar regions in HVCN1. When applied to the external solution of HEK cells expressing HVCN1, hanatoxin (HaTx) and to a lesser extent VSTx1 inhibit voltage-activated proton currents. When examined over a range of membrane voltages, both toxins shift activation to more depolarized voltages. To examine whether these toxins interact with the paddle motif of HVCN1, we performed alanine scanning mutagenesis within the paddle motif and find that mutations within the paddle region alter sensitivity to HaTx. These results suggest that tarantula toxins can interact with the paddle region of proton channels and will be useful tools for studying these interesting channels. We are currently studying the mechanism of toxin inhibition to determine whether proton channel can activate with tarantula toxins bound.

1353-Pos Identification and Purification of a Novel Tarantula Toxin Targeting the VSD protein, H_v1

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Board B329

Voltage sensing domain (VSD) proteins consist of a S1-S4 voltage sensing module and are capable of responding to changes in membrane potential, thereby coupling these changes to ion permeation or enzymatic activity (Sasaki et al., Science, v. *312*, 589–92; Ramsey et al., Nature, v. *440*, 1213–16;Murata et al., Nature, v. *435*, 1239–43). The recently described VSD protein, H_v1, functions as a voltage-gated H^+ channel and is primarily expressed in human immune tissues. A recognizable pore region does not exist in H_v1 and the voltage sensing mechanism is not clearly established. In the past, toxins from various venomous animals have been effectively used to investigate structural elements involved in both voltage sensing and ion permeation (MacKinnon et al., Neuron, v. *5*, 767–771;Swartz & MacKinnon, Neuron, *18*, 665–682). Tarantula toxins, in particular, have been shown to interact with a variety of voltage sensors to modify gating. The well-characterized toxin, HaTx, inhibits $K_v2.1$ by stabilizing the resting state and produces a similar effect on H_v1

but with modest affinity (μM). We are currently screening tarantula venom for high affinity activity against H_v1 and have found that a 1:2000 dilution of venom from the spider, *Grammostola spatulata*, causes robust inhibition and a positive shift in the voltage-dependence of activation of H_v1 channels stably expressed in HEK cells. HPLC fractionation of the venom demonstrates that potent inhibitory activity (85% inhibition with slow recovery) of H_v1 associates with peaks that are distinct from other known gating modifier toxins. We are currently working to purify these new toxins to homogeneity and characterize their effects on H_v1.

1354-Pos Molecular Basis Of Toxin-paddle Interactions At Protein-lipid Interfaces

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Board B330

Tarantula toxins partition into membranes and inhibit Kv channels by interacting with the voltage-sensor paddle, a structural motif composed of S3b and S4 helices (Lee, Nature. 2004; Swartz, Toxicon. 2007; Milescu, J. Gen. Physiol. 2007). In the latter of these studies, we showed that the toxin-channel complex is stabilized by the presence of specific lipids (i.e. sphingomyelin), suggesting that key residues on the voltage-sensor paddle are lipid exposed and raising the possibility that specific lipids interact with the channel in the vicinity of where the toxin binds. To further understand these interactions at the protein-lipid interface, we studied the effects of sphingomyelinase D (SMaseD), an enzyme that converts sphingomyelin to the anionic ceramide-1-phosphate, on the activation of Kv channels (Ramu, Nature. 2006) and their sensitivity to tarantula toxins. SMaseD typically shifts activation of Kv channels to negative voltages, an effect that can be accentuated by mutations in S4, but diminished by mutations in the S3b helix. In addition, SMaseD also typically increases tarantula toxin affinity, and this effect can be diminished by mutations in the S3b helix. These results support the idea that sphingomyelin interacts with voltage-sensor paddle motifs and that the toxins and paddles form a ternary complex with lipids.

1355-Pos Peptide toxins show surprising selectivity for heteromeric Kv1 channels

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Board B331

The majority of Kv1 channels expressed in mammalian cerebral cortex are heteromeric combinations of α subunits from subfamily members. A pharmacopeia of drugs that can distinguish between heteromeric Kv1 channels would provide invaluable research tools with therapeutic potential. To examine drug action against stoichiometrically-controlled hetero-tetrameric channels, concatenated genes encoding Kv1.1, 1.2, 1.3, 1.4 and/or 1.6 subunits were recombinantly expressed in mammalian cells. The pharmacological profiles of the resultant channels were investigated with snake (αdendrotoxin, dendrotoxin-k), scorpion (hongotoxin-1, tityustoxin Kα, kaliotoxin) and anemone (ShK) toxins, by patch-clamp, rubidium efflux, and displacement of radiolabelled toxin. These peptides are known to discriminate between Kv1 channels by binding to variable residues external to the pore. As the footprint of a single toxin spans multiple subunits, affinities for Kv1 heteromers are not readily predictable from their parental monomers. For example, Kv1.2 homomers were insensitive to kaliotoxin, but including a single copy of Kv1.1 or 1.3 imbues hetero-tetrameric channels with sensitivity to this toxin. Kv1.4 homomers are not blocked by any known peptide toxin, while Kv1.2 homomers bind several toxins, including αdendrotoxin, hongotoxin and ShK. However, heteromers containing Kv1.4 and Kv1.2 are blocked by αdendrotoxin while having greatly reduced sensitivity to hongotoxin and ShK. As the potential tetrameric subunit combinations are manifold, emphasis is placed on the limited number of subunit combinations predominant in mammalian brain, with the aim of deciphering general rules for toxin susceptibility of heteromeric channels. Findings indicate that individual Kv1 subunits can be classified as necessary for, permissive towards or repulsive against binding of each toxin. Delineation of principles that define the toxin vulnerability of heteromeric Kv1 channels will markedly aid researchers seeking to identify native K+ channel subtypes.

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1356-Pos Portability of Paddle Motif Function and Pharmacology in Voltage Sensors

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Board B332

Voltage-sensing domains enable membrane proteins to perform critical signaling functions in response to changes in membrane voltage. The repertoire of these functions has recently expanded with the identification of a voltage-gated proton channel (Hv1) and a voltage-dependent phosphatase (Ci-VSP), which both contain identifiable S1–S4 voltage-sensors. The mechanism underlying voltage sensing has been the subject of intense study, with much of the work focusing on eukaryotic ion channels. Recent studies on the archaebacterial voltage-gated potassium (Kv) channel, KvAP, and the voltage-sensing domain proteins (Ci-VSP and Hv1), raise important questions regarding the degree of conservation that exists between the voltage sensing mechanism in these distinct proteins. Using an extensive chimera approach, we examined whether specific structural elements within voltage-sensors could be transferred between Kv channels and voltage-sensing domain proteins while preserving

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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Board B333

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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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.

References

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

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