outer tryptophan-rich lip, as well as D400C, E758C, K1237C and A1529C of the DEKA locus) helped us to identify residues playing a key role in aminoquinoline binding. In full agreement with our computed results, tryptophan W756 is crucial for the reversible blocking effects of PQ. W756C abolished the blocking effect of PQ in voltage-clamp assays.

600-Pos

Defining the Voltage Sensor Properties and Pharmacology of Nav1.9

Frank Bosmans[1], Michelino Puopolo[2], Marie-France Martin-Eauclaire[3], Bruce P. Bean[2], Kenton J. Swartz[1].
[1]NIH, Bethesda, MD, USA, [2]Harvard Medical School, Boston, MA, USA, [3]CNRS, Marseille, France.

The voltage-activated sodium channel Nav1.9 is preferentially expressed in DRG neurons where it is believed to play an important role in pain perception. However, progress in revealing the gating characteristics and pharmacological sensitivities of Nav1.9 has been slow because attempts to express this channel in a heterologous expression system have been unsuccessful. Here we use a protein engineering approach to study the contributions of the four Nav1.9 voltage sensors to channel function. We define individual S3b-S4 paddle motifs within each voltage sensor and show that these structural motifs sense changes in membrane voltage and determine the kinetics of voltage sensor activation. Toxins from tarantula and scorpion venom interact with each of these four motifs and can be used as pharmacological tools to alter Nav1.9 currents in native DRG neurons. Our results provide answers to fundamental questions on the functional role of the four voltage sensors in Nav1.9 and may be useful in developing new strategies to combat pain.

Voltage-gated K Channels-Permeation

601-Pos

KcsA Barium Permeation Blocked by External Potassium

Kene N. Piasta, Christopher Miller.
Brandeis University, Waltham, MA, USA.

Block by Ba^{2+} is a distinctive property of K^+ channels, and in a few cases this block can be used as a tool to determine the affinity for various ions at specific sites in the selectivity filter. We measure the discrete block of single E71A KcsA channels, a non-inactivating mutant, with micromolar concentrations of internal Ba^{2+} and find at high concentrations of external K^+ the block time distribution is described by a double exponential. This suggests there are two Ba^{2+} sites in the selectivity filter, fitting well with the published Ba^{2+} containing structure of KcsA where a Ba^{2+} ion resides approximately in S2 and S4. Utilizing a kinetic analysis of the blocking events, we can determine the occupancy of K^+ and other cationic monovalent ions in an extracellular site, presumably S1, and thus determine selectivity for this particular site. Our kinetic data has shown KcsA has an unusually high selectivity for K^+ over Na^+ with a ddG^0 of -5.6 kcal mol^{-1}.

602-Pos

The Role Of Oligo-(R)-3-Hydroxybutyrates in the *Streptomyces lividans* KcsA Channel

Alexander Negoda, Elena Negoda, Rosetta N. Reusch.
Michigan State University, East Lansing, MI, USA.

The *Streptomyces lividans* potassium channel KcsA is a tetramer of four polypeptides, each covalently modified by oligo-(R)-3-hydroxybutyrate (cOHB), which envelop a core molecule of inorganic polyphosphate (polyP). It has been proposed that the polyanion, polyP, attracts, binds and conducts K^+ in response to an electrochemical stimulus whilst the polypeptides govern access to polyP and regulate its selectivity. The function of cOHB, however, has been undefined.Digestion of KcsA with CNBr yields a 6.7 kDa fragment that contains most of the ion pathway (residues 97-154). This fragment was shown to contain cOHB by Western blot and chemical assays. The conjugation sites for cOHB were determined to be on S102 and S129. The effects of the single mutations S102, S129 and the double mutation S102:S129 on channel activity were examined. Wild-type KcsA, incorporated into planar lipid bilayers of POPC:POPE:POPG (3:3:1) between aqueous solution of 200 mM KCl, 5 mM $MgCl_2$, 20 mM Hepes, pH 7.4 *cis* and 20 mM KCl, 5 mM $MgCl_2$, 20 mM Hepes, pH 7.4 *trans* forms well-structured channels of 147 pS conductance. Under the same conditions, S102G exhibits irregular conductance with a major conductance state of 75 pS and a minor conductance state of 103 pS, S129G exhibits frequent but unsuccessful attempts to insert into the bilayer, and S102:S129 exhibits no channel activity whatsoever. The results suggest that cOHB-modification of KcsA polypeptides is essential for normal channel function.

603-Pos

Potassium Channel Block by a Tripartite Complex of Neutral Ligands with a Potassium Ion

Pavel I. Zimin[1], Bojan Garic[2], Heike Wulff[1], Boris S. Zhorov[2].
[1]UC Davis, Davis, CA, USA, [2]McMaster University, Hamilton, ON, Canada.

Potassium channels are targets for medically important drugs of large chemical diversity. While classical hydrophilic cations like tetraethylammonium block Kv channels with a stoichiometry of 1:1, many uncharged lipophilic (neutral) compounds exhibit Hill coefficients of 2. An example is the alkoxypsoralen PAP-1, which blocks Kv1.3 channels in lymphocytes with an IC50 of 2 nM and constitutes a potential drug for autoimmune disease such as type-1 diabetes, multiple sclerosis, rheumatoid arthritis, and psoriasis. The atomic mechanism of Kv channel block by ligands like PAP-1 is unknown. We first studied structure-activity relationships of PAP-1 derivatives and found that the carbonyl group in PAP-1's coumarin ring is indispensable, but does not accept an H-bond from the channel. We next demonstrated that block by PAP-1 is voltage-dependent, a feature expected for cationic but not neutral ligands. We then employed molecular modeling to predict the PAP-1 receptor and arrived at a model in which the carbonyl groups of two PAP-1 molecules coordinate a potassium ion in the permeation pathway, while the hydrophobic phenoxyalkoxy side-chains extend into the intrasubunit interfaces between helices S5 and S6 and reach the L45 linker. We tested the model by generating 58 point mutants involving residues in and around the predicted receptor, and then determined their biophysical properties and sensitivity to block by PAP-1. We found excellent agreement between the atomistic model and the results of experimental studies. Besides the known drug-binding locus in the inner pore, which is rather conserved between different Kv channels, the PAP-1 receptor involves loci where sequence homology is low. These loci constitute attractive targets for the design of subtype-specific potassium channel drugs and offer new directions for structure-based drug design.
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604-Pos

Effect of Peptide Toxins on C-Type Inactivation of a Mutant Human Voltge-Gated Potassium Channel (*hKv1.3)*

Azadeh Nikouee Ghadikolaei, Stephan Grissmer.
Institute of Applied Physiology, Ulm, Germany.

Current through wt *h*Kv1.3channels is characterized by the typical C-type inactivation and the high affinity block by scorpion peptide toxins. These toxins belong to a short peptide toxin family with high similarities in 3D shape and sequence and are thought to block current through *h*Kv1.3 channels by interacting with the outer vestibule of the channel thereby physically occluding the channel pore. In this study we measured current through *h*Kv1.3_V388C mutant channels, which inactivated faster compared to the wt channels and recovery from inactivation was also slower. In our experiments we examined the effect of CTX, NTX, KTX, AgTX2 and MgTX, each at a concentration of 100 nM, on current through mutant channels. KTX and AgTX2 did not or hardly block peak current through the mutant channel at 100 nM, respectively, indicating a loss of affinity by a factor of >100 due to the mutation in the channel. In addition, KTX and AgTX2 did not change the inactivation time course of the current through the mutant channels. In contrast, MgTX, even at a concentration of 10 nM, almost completely blocked peak current, indicating similar affinities of MgTX to mutant and wt channels. Interestingly, CTX and NTX did not block peak current through the mutant channels, however, almost completely abolished inactivation. This is different from the effect of TEA that is known to prevent inactivation while blocking current through the channel. We conclude that CTX and NTX bind to the external vestibule of the channel thereby preventing structural rearrangements of the outer vestibule that normally occur when channels inactivate.
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605-Pos

Alprenolol Inhibits Herg Potassium Channels

Seung Ho Lee, Hyang Mi Lee, Bok Hee Choi.
Chonbuk National University Medical School, Jeonju, Republic of Korea.

The action of alprenolol, a non-selective beta blocker as well as 5-HT receptor antagonist, on the cloned cardiac human ether-a-go-go-related gene (HERG) channels was investigated using the whole-cell patch-clamp technique. Alprenolol reduced HERG whole-cell currents in a reversible concentration-dependent manner, with an IC_{50} value and a Hill coefficient of $12.3 \pm 0.8 \in 1/4M$ and 0.98 ± 0.06, respectively. Alprenolol affected the channels in the activated and inactivated states but not in the closed states. The alprenolol-induced blockade of HERG was found to be use-dependent, exhibiting a more rapid onset and a greater steady-state block at the higher frequencies of activation.

These results suggest that alprenolol prolongs the QT interval by direct inhibition of activated HERG channels.
Keywords: alprenolol, HERG channel

606-Pos
Mutations at the Intron 9 Donor Splice Site in hERG Lead to Cryptic Splicing in LQT2
Matthew R. Stump, Qiuming Gong, Zhengfeng Zhou.
OHSU, Portland, OR, USA.
Long QT syndrome type 2 (LQT2) is caused by mutations in the human ether-a-go-go-related gene (hERG). More than 30% of LQT2 mutations are nonsense, frameshift, or splice site mutations that may affect mRNA stability and splicing. To date, relatively few studies have focused on the pathogenesis of hERG splice site mutations. We characterized three LQT2 mutations in the 5′ donor splice site of intron 9: 2398G>T, 2398+3A>T, and 2398+5G>T. G2398 is the last nucleotide of exon 9 and 2398G>T has been previously classified as a missense mutation (G800W). The functional consequences of these mutations were studied by RT-PCR analysis of RNA collected from HEK293 cells transfected with minigenes containing the wild-type or mutant genomic sequence spanning exon 8 to exon 11 of hERG. All three splice site mutants disrupt normal splicing and produce an aberrantly spliced transcript. Sequence analysis showed that this transcript results from the use of a cryptic 5′ donor splice site in intron 9 located 54 nt downstream of the normal site. Translation of this transcript would result in an in-frame insertion of 18 amino acids in the cyclic nucleotide binding domain. A full length hERG cDNA construct including the 2398G>T mutation and the additional 54 nt from intron 9 was expressed in HEK293 cells. Patch clamp studies revealed that the splice mutant channels did not produce hERG current. Western blot analysis showed that the mutant expressed the immature form of the hERG protein indicating defective channel trafficking. These studies underscore the importance of RNA analysis in describing the pathogenesis of LQT2. The intron 9 donor splice site appears to be a localized hot-spot for LQT2 mutations.

607-Pos
Lysine Versus Arginine: RNA Editing In The Eag Potassium Channel
Mary Y. Ryan[1], Rachel Maloney[2], Jeffrey Fineberg[1], Robert A. Reenan[2], Richard Horn[1].
[1]Thomas Jefferson University, Philadelphia, PA, USA, [2]Brown University, Providence, RI, USA.
Four RNA editing sites in *eag*, a *Drosophila* voltage-gated potassium channel, result in point mutations. One of these mutations, K467R, involves a highly conserved basic residue at the top of the S6 segment. We characterized wild-type and mutant channels using two-microelectrode voltage clamp and patch clamp in *Xenopus* oocytes. The homologous mutation is lethal in *Shaker* and hERG. Position 467 plays an important role in inactivation; the K467R mutation causes a 54% decrease in the fraction of inactivated current at +80 mV. The fraction of inactivated current is reduced at higher (10 mM) extracellular Mg^{+2} concentrations; constructs with a lysine at 467 are more sensitive to changes in extracellular Mg^{+2} than those with an arginine. Mutating position 467 to alanine, glutamine or cysteine resulted in intermediate inactivation phenotypes and a leftward shift of the peak current-voltage relationship, normalized at +80 mV. Using instantaneous IV measurements from cell-attached oocyte patches, we constructed normalized P_o curves for 467Q, 467R and 467K. The P_o-V curves for these mutations are superimposable, suggesting little effect on activation gating. However, 467Q and 467R produce inward rectification in instantaneous IV measurements, suggesting a change in ion permeation. Single channel current amplitudes at +40 mV, estimated from non-stationary noise analysis, are comparable for these mutants, which affect instantaneous rectification at more depolarized potentials. Preliminary experiments show no change in rectification between cell-attached and inside-out patches suggesting the permeation change is not due to block by cytoplasmic cations. Intracellular TBA (tetrabutylammonium) blocks 467R significantly better than 467K. Block by intracellular, but not extracellular, TEA (tetraethylammonium) interferes with inactivation. These results show that even a minor residue change can have a dramatic impact on channel biophysics.

608-Pos
Overlapping LQT1 and LQT2 Phenotype in a Patient with Long QT Syndrome Associated with Loss-of-Function Variations in KCNQ1 and KCNH2
Jonathan M. Cordeiro[1], Guillermo J. Perez[1], Ryan Pfeiffer[1], Elena Burashnikov[1], Martin Borggrefe[2], Christian Wolpert[2], Rainer Schimpf[2], Charles Antzelevitch[1].
[1]Masonic Medical Research Laboratory, Utica, NY, USA, [2]University of Mannheim, Mannheim, Germany.
Background: Long QT Syndrome (LQTS) is an inherited disorder characterized by prolonged QT intervals and potentially life-threatening arrhythmias. Mutations in several ion channel genes are responsible for LQTS. Here we describe a patient with LQTS who has a mutation in KCNQ1 as well as a polymorphism in KCNH2. **Methods and Results:** The proband (MMRL0362), a 32 yo female, exhibited multiple ventricular extrasystoles and episodes of syncope. Her ECG (QTc=518ms) showed an LQT2 morphology in leads V4-V6 and LQT1 morphology in leads V1-V2. Genomic DNA was isolated from lymphocytes. All exons and intron borders of 7 LQTS susceptibility genes were amplified and sequenced. Variations were detected predicting a novel missense mutation (V110I) in *KCNQ1* as well as a common polymorphism in KCNH2 (K897T). We expressed WT or V110I *KCNQ1* channels in CHO-K1 cells co-transfected with *KCNE1* and performed patch clamp experiments. In addition, WT or K897T *KCNH2* were studied by patch clamp. Current-voltage (I-V) relations for V110I showed a significant reduction in both developing and tail current densities compared to WT at potentials >+20 mV ($p<0.05$), suggesting a reduction in I_{Ks} currents. K897T-HERG channels displayed a significantly reduced tail current density compared to WT-HERG at potentials >+10 mV. Interestingly, channel availability assessed using a triple-pulse protocol was slightly greater for K897T compared to WT ($V_{0.5}=-53.1\pm1.13$ mV and -60.7 ± 1.15 mV for K897T and WT, respectively, $p<0.05$). Comparison of the fully activated I-V revealed no difference in the rectification properties between WT and K897T channels. **Conclusions:** We report a patient with a loss-of-function mutation in KCNQ1 and a loss-of-function polymorphism in KCNH2. Our results suggest that a reduction of both I_{Kr} and I_{Ks} underlies the combined LQT1 and LQT2 phenotype in this patient.

609-Pos
Divergent Effects of AF- or LQTS-Associated HERG Mutations on Endogenous I_{Kr}
Jianguo Han, Franck Potet, Wen Shuai, Dan M. Roden, Dawood Darbar, **Sabina Kupershmidt**.
Vanderbilt University, Nashville, TN, USA.
Mutations in HERG not only reduce I_{Kr} to cause QT syndrome (LQTS) but have also been associated with atrial fibrillation (AF). The mechanisms in AF are unknown. To identify genetic defects conferring AF susceptibility, we screened HERG in 375 patients with typical and lone AF, and identified three probands with rare, non-synonymous HERG variants absent in control populations (284). The first was a C-terminal HERG variant (R1047L), previously reported in LQTS, in 2 probands. One proband was part of a kindred that included 2 other family members with AF or palpitations, and all 3 were mutation carriers; no family was available in the 2nd proband. A second variant (R954C) located only six residues from a previously identified LQTS variant (S960N) was also identified in a lone AF proband. In mutation carriers, QT intervals during sinus rhythm were normal. These variants are particularly interesting because AF and LQTS mutations are likely to be located in close structural proximity. We compared the functional effects of these mutations and WT in two heterologous cell systems: HEK cells stably expressing endogenous HERG (HERG-HEK) or 'empty' HEK cells. R1047L caused a 1.4 fold *increase* in current amplitude in HERG-HEK. In empty HEK cells, there was no difference between R1047L and WT. R954C generated currents that were similar to WT in both HERG-HEK and empty HEK cells, although the nearby S960N variant *reduced* current 1.6 fold in HERG-HEK and 2 fold in empty HEK cells. These results suggest that relative expression levels of normal and mutant alleles determine net effect on ionic current and action potential controls. Variability in these mechanisms, across or within chambers, may contribute to phenotypes that manifest in only one chamber.

610-Pos
K^+ Occupancy of the Pore Critically Determines the Selectivity-Stability of K^+ Channels. A Study with Shab Channels
Imilla I. Arias-Olguín[1], Manuel Soriano-García[2], Froylan Gomez-Lagunas[1].
[1]School of Medicine, UNAM, Mexico City, Mexico, [2]Institute of Chemistry, UNAM, Mexico City, Mexico.
Potassium channels are characterized by their ability to select K^+ excluding the smaller Na^+ ions. Based on crystallographic images of the pore this selectivity is commonly explained in terms of protein structural elements alone. On the other hand, it is well known that some pore properties such as the stability of the K^+ conductance itself critically depend on the K^+ occupancy of the pore. Here it will be shown functional data demonstrating that (a) both the stability and the selectivity of the pore of Shab K^+ channels change in