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.
Supported by NSERC, CIHR, NIH, and HHMI.

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.
This work was supported by a grant from the Deutsche Forschugsgemeinschaft (Gr848/14-1).

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.