paired with the native short A359. In consequence, we found that the paired arginine to serine mutations at the subsequent positions 362,365 and 365,368 also produce omega current. This was taken as evidence that S4 slides in 3 steps through a pore towards the activated state. Each pair of 2 short serines in between the thread of long arginines appears as a gap which allows a leak when in the omega pore.

Here, we present further studies of the above mutants over a wider voltage range, in addition with the alpha pore open (434W) to detect the open state with alpha current. We found that S4 in all 3 omega constructs can reach the final open state where omega current has disappeared and alpha current appears. This provides clear evidence that all 3 S4 constructs - despite the arginine-to-serine gap - still proceed to the open state, although at different voltages. We also analyzed in detail the voltage range over which the omega pore remains open, i.e. is occupied by the gap. In the cases where the pore could be closed, the voltage dependence for the closing process was studied. While all constructs close in outward direction, a clear inward directed closing of the omega-pore could so far only be demonstrated for the RR/365,368/SS construct.

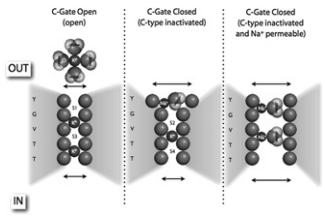
2699-Pos

Pore Dilation in C-Type Inactivation of Potassium Channels

Toshinori Hoshi[1], Wonpil Im[2], **Clay M. Armstrong**[1].
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]University of Kansas, Lawrence, KS, USA.

It is commonly thought that C-type inactivation is the result of constriction or "collapse" of the outer mouth of a voltage-gated K^+ channel. It is well established that conduction requires that the selectivity filter be a good fit for a dehydrated K^+ ion. In theory, failure to conduct could thus result from dilation of the filter, making it too large to effectively complex a K^+ ion. Our modeling and simulations as well as a review of the literature give support to this idea. Lowering or removing K^+ from the external (and/or the internal) solution is known to promote C-type inactivation. Our simulations show that external K^+ removal causes dilation of the outermost site in the selectivity filter, an expected consequence of the mutual repulsion between the oriented dipoles of the filter.

Dilation makes the outer site capable of accepting a partially hydrated Na^+ ion, but inactivates the channel because the Na^+ cannot move into Sites 2-4, which are K^+ selective. Removing internal K^+ causes the inner sites to dilate, making the pore Na^+ selective. The chain of events that follow channel activation and allow pore dilation are under investigation.



2700-Pos

Time and Voltage-Dependent Slowing in the Off Gating Currents from Kv3.1

Ivan Kopljar, Alain J. Labro, Tessa de Block, Dirk J. Snyders.
University of Antwerp, Antwerp, Belgium.

Kv3.1 channels are characterized by fast activation and deactivation kinetics with a quite positive threshold of activation. In the central nervous system they are involved in the high-frequency firing pattern of neurons. Given some controversy on Kv3.1 channel gating, we performed an in-depth analysis of WT Kv3.1 gating currents elicited in mammalian Ltk^- cells. The Q/V curves determined from integrating either Q_{ON} or Q_{OFF} were comparable and were best fitted with a double Boltzmann distribution. The Q/V curves were shifted by approximately 15 mV towards more hyperpolarized potentials compared to the ionic G/V curve, indicating that a substantial charge was moved during closed-state transitions. The double component in the Q/V curve and a bi-exponential decay of I_{gON} indicated that the charge was carried by at least two distinct transition steps, contrary to earlier reports for Kv3.2b, but more consistent with *Shaker*, Kv1.5 and Kv2.1 channels. Since the integrals of Q_{ON} and Q_{OFF} were identical, there was no charge immobilization within a 125 ms depolarization. The time course of I_{gOFF} was fast after short depolarizations, but displayed *Shaker*-like slowing with increasing step duration for depolarizations positive to the threshold for ionic current activation. Since 4-AP blocks the concerted opening step in *Shaker*, we used it to separate the two components of I_{gOFF}. In the presence of 3 mM 4-AP only the fast I_{gOFF} remained, indicating that the slowing is linked to a final concerted step in channel opening which appears to be a general gating feature of Kv channels.

2701-Pos

Molecular Determinants of the Slow Off-Gating Component in Shaker K^+ Channels

Zarah Batulan, **Georges A. Haddad**, Rikard Blunck.
Université de Montréal, Montréal, QC, Canada.

The electromechanical coupling occurs between the ion conducting pore and the peripheral voltage sensors in voltage-gated ion channels. It has been established that the voltage sensor undergoes a complex conformational change upon depolarization of the membrane potential, which eventually leads to opening of the helical bundle crossing. The S4-S5 linker, the covalent link between voltage sensor and pore, has been shown to anneal to the C-terminus of the S6. This interaction is essential for electromechanical coupling. Still the molecular mechanism of the electromechanical coupling remains unclear. Here, we used voltage clamp fluorometry in a cut-open oocyte configuration in order to further elucidate the residues responsible for the energy transfer from the sensor to the pore. We identified an intersubunit interaction, coupling the S4-S5 linker to the neighboring S6. This interaction is responsible for the slow component of the gating current upon return to negative membrane potential. The slow off gating component has been described early on but its molecular determinants were not identified previously. Alteration of any of the three interaction partners involved variably leads to shift of the QV, uncoupling of pore from voltage sensor movement and abolishment / slowing (increase of binding energy) of the slow off gating component. We also demonstrated that the interaction does not require pore opening, although it is increased in the open pore state. The data suggest that this interaction stabilizes the activated state and thereby contributes to electromechanical coupling.

(This work is financially supported by CIHR MOP-81351 & CRC 202965.)

2702-Pos

Biochemical and Functional Conversion of KChIP4a into KChIP1 for Modulation of Kv4 Inactivation

Ping Liang, Hao Chen, **KeWei Wang**.
Peking University, Beijing, China.

Inactivation in Kv channels plays a key role in dynamic regulation of neuronal excitability. Multifunctional auxiliary subunit KChIPs1-4 co-assemble with pore-forming rapidly inactivating Kv4 α subunits to form a native complex that encodes somatodentritic A-type K^+ current in neurons. KChIP1 increases Kv4 surface expression, speeds up steady-state inactivation with a moderate effect on fast inactivation. In contrast, KChIP4 variant KChIP4a functions as a suppressor of inactivation to eliminate the fast inactivation of Kv4 channels, but neither promotes surface expression nor has any effect on recovery time constant. We have recently determined the crystal structure of KChIP4a that reveals a distinct hydrophobic N-terminus as well as a core structure showing overlap with known KChIP1. In this study, we transfected HEK 293 cells that were solubilized andFast Protein Liquid Chromatography (FPLC) analysis was performed with Size Exclusion Chromatography (SEC) using a Superdex 200 column. The SEC-FPLC assay reveals that KChIP4a proteins self-associate to form multimers with three peaks, compared with a single peak of KChIP1 dominated by monomer. Mutations in N-terminal key residues of KChIP4a can turn multimeric proteins into monomers with a single peak on SEC. Two-electrode voltage clamp in oocytes confirms that disruption of multimeric state of KChIP4a proteins can convert its non-inactivating function into fast inactivating like KChIP1. Taken together, our findings of key residues in N-terminal KChIP4a critical for conversion between fast and slow inactivation may be a site of action for chemical compounds that can alter channel inactivation for a potential therapy of membrane excitability-related disorders.

2703-Pos

Structural Determinants of Closed-State Inactivation Studied with Channel Chimeras

Jan Barghaan, Jessica Wollberg, **Robert Bähring**.
Zentrum für Experimentelle Medizin, Hamburg, Germany.

Shal-gene-related voltage-gated potassium (Kv4) channels exhibit a prominent low-voltage-induced closed-state inactivation. Recent experimental results show that the S4 voltage sensor drives closed-state inactivation (Dougherty et al., J Gen Physiol 131: 257-273, 2008), and that the S4S5 linker and the main S6 activation gate are instrumental in the installment of closed-state inactivation (Barghaan and Bähring, J Gen Physiol 133: 205-224, 2009). In particular, an inactivated voltage sensor conformation correlates with a temporary

uncoupling between the S4S5 linker and the S6 gate. We used two different chimeric approaches to further study the structural determinants of Kv4 channel closed-state inactivation: First, chimeric swapping of S4S5 linker and distal S6 sequences between N-terminally truncated Kv4.2*delta*2-40 A-type channels and non-inactivating *Shaker*IR channels; Second, chimeric insertion of the Kv4.3 cytoplasmic C-terminus or the Kv4.3 T1S1 linker in Kv4.1. The first approach was pursued to possibly prevent inactivation in Kv4.2*delta*2-40 or introduce inactivation in *Shaker*IR. The second approach was pursued to possibly transfer slow Kv4.3 inactivation kinetics to Kv4.1. By two-electrode voltage-clamp on cRNA-injected *Xenopus* oocytes and kinetic analysis of the recorded currents we found that *Shaker* sequences slowed Kv4.2*delta*2-40 inactivation, and that Kv4.2 sequences introduced a novel form of inactivation in *Shaker*IR. Furthermore, we found that, rather than Kv4.3 C-terminal sequences, chimeric introduction of Kv4.3 T1S1 linker sequences made Kv4.1 channels inactivate slower. Our data confirm a model of temporary uncoubling between S4S5 and S6 as a mechanism involved in closed-state inactivation. Furthermore, our data suggest that the T1S1 linker region plays a role in closed-state inactivation.

2704-Pos

Differential Integration of DPLP Protein Variants Regulates Inactivation Kinetics of Neuronal A-Type Current

Henry H. Jerng, Paul J. Pfaffinger.
Baylor College of Medicine, Houston, TX, USA.

Voltage-sensitive potassium channel complexes consisting of Kv4 pore-forming subunits and modulatory subunits mediate the neuronal subthreshold A-type current (I_{SA}), a regulator of membrane excitability and action potential firing patterns. Both the KChIP (Kv channel-interacting proteins) and DPLP (dipeptidyl peptidase-like protein) modulatory subunits are expressed as N-terminal variants, and two DPLP variants (DPP10a, DPP6a) possess the unusual property of inducing similar fast inactivation kinetics of Kv4 channels in reconstitution studies. To investigate whether their effects are similar on native I_{SA}, we characterized I_{SA} from DPP10a-expressing cortical layer II/III pyramidal neurons (CtxPN) and DPP6a-expressing cerebellar granule neurons (CbGN) in rat brain slices. Surprisingly, CtxPN I_{SA} and CbGN I_{SA} differ significantly in their inactivation kinetics. CtxPN I_{SA} undergo mono-exponential decay (τ = ~8 ms); CbGN I_{SA}, bi-exponential decay (at +36 mV: τ-1 = ~11 ms, τ-2 = ~120 ms). While CtxPN I_{SA} resembles heterologously expressed Kv4.2+KChIP3a+DPP10a current, CbGN I_{SA} is not recapitulated by the Kv4.2+KChIP3a+DPP6a current. Since CbGN reportedly also express robust levels of other DPP6 variants competing for Kv4 channels, we quantitated the levels of different DPP6 isoforms by qRT-PCR and determined their percentage contributions: DPP6a (DPP6-E) = ~32%, DPP6b (DPP6-K) = ~42%, DPP6c (DPP6-L) = ~8%, and DPP6d (DPP6-S) = ~18%. Since DPP6a and DPP6b variants together constitute the majority of the CbGN DPP6 species, we performed coexpression studies to investigate whether CbGN I_{SA} is mediated by ternary complex channels with these DPP6 variants. We propose that relative ratios of DPLP auxiliary subunit isoforms likely contribute to the variability of I_{SA} inactivation kinetics between neuronal populations.

2705-Pos

Pyridine Nucleotide Dependence of Kv Beta - Induced Kv Inactivation: Role of Kv Alpha C-Terminus

Oleg A. Barski, Xiao-Ping Li, Aruni Bhatnagar, Srinivas M. Tipparaju.
University of Louisville, Louisville, KY, USA.

Binding of ancillary β-subunits (Kvβ) to the N-terminal T1 domain of Kv1 and Kv4 regulates channel function and localization. The β subunits of Kv channels belong to the aldo-keto reductase superfamily (AKR6). These proteins bind NAD(P)(H) with high affinity, but the mechanisms by which nucleotides regulate channel gating are unclear. Herein we report that when coexpressed with Kv1.5 in COS-7 cells, Kvβ3 shifts the half-activation potential and imparts inactivation to slowly inactivating Kv1.5 current. Addition of NAD(P)H to the patch pipette increased rate and extent of inactivation, whereas $NAD(P)^+$ reduced inactivation. These results conform to a model assuming that NAD(P)(H) binding regulates rate and extent of inactivation synergistically by altering the number of Kvβ monomers involved in inactivation. Deletion of 56 C-terminal amino acids of Kv1.5 (KvΔC56) did not significantly affect Kv association with Kvβ or Kvβ-mediated inactivation. KvΔC56 did not, however, respond to changes in intracellular pyridine nucleotide concentration when co-expressed with Kvβ3 and neither NAPDH nor $NADP^+$ altered rate or extent of inactivation. Glutathione-S-transferase (GST) fusion protein containing peptides from the last 38 (Ile565-Leu602) and 60 (Arg543-Leu602), but not 19 (Asp584-Leu602), amino acids of Kv1.5 C-terminus precipitated Kvβ2 and Kvβ3 in pull-down assays from lysates of transformed bacteria. The C-terminal peptide (GST-C60) also precipitated Kvβ1 and Kvβ2 from mouse brain extracts. The GST-C60 construct did not bind to apoKvβ2, and it displayed higher affinity for Kvβ2:NADPH than for the Kvβ2:$NADP^+$ binary complex. These results suggest that nucleotide binding provides an efficient mechanism to adjust potassium flux in response to metabolic changes. The C-terminal domain of Kv1.5 from Arg543-Asp584 interacts with Kvβ and this interaction may be involved in sensing different conformational states of Kvβ bound to either reduced or oxidized pyridine nucleotides.

2706-Pos

Depenedence of Ependence 6β-Acetoxyl-7α-Hydroxyroyleanone Block of Kv1.2 Channels on C-Type Inactivation

Yuk M. Leung[1], Kar-Lok Leung[2], Chia-Huei Lina[1], Chia-Chia Chaoa[1], Chun-Hsiao Choua[1], Li-Yun Changc[3], Shao-Wei Chend[4], Tzu-Hurng Chengd[4], Yue-Hsiung Kuoe[5].
[1]Graduate Institute of Neural and Cognitive Sciences, China Medical University, Taichung, Taiwan, [2]Department of Anesthesia, China Medical University and Hospital, Taichung, Taiwan, [3]Graduate Institute of Molecular Systems Biomedicine, China Medical University, Taichung, Taiwan, [4]Department of Biological Science and Technology, China Medical University, Taichung, Taiwan, [5]Tsuzuki Institute for Traditional Medicine, China Medical University, Taichung, Taiwan.

Voltage-gated K^+ (Kv) channels repolarize excitable cells by providing a pathway for K^+ efflux. Kv channels activate when the membrane is depolarized, and subsequently exhibit slow inactivation (C-type inactivation) during continuous depolarization. A selective pharmacological agent targeting C-type inactivation is so far lacking. Here we reported that 6β-acetoxy-7α-hydroxyroyleanone (AHR), a diterpenoid compound isolated from *Taiwania cryptomerioides* Hayata, could selectively modify C-type inactivation of Kv1.2 channels. Extracellular, but not intracellular, AHR (50 μM) dramatically speeded up the slow decay of Kv currents and left-shifted the steady-state inactivation curve. AHR blocked steady-state Kv currents with an IC_{50} of 17.7 μM and the effects of AHR were completely reversible. AHR did not affect at all the kinetics and voltage-dependence of Kv1.2 channel activation. The degree of block of Kv currents by AHR was independent of the intracellular K^+ concentration. In addition, effect of AHR was much attenuated in a Kv1.2 V370G mutant defective in C-type inactivation. Furthermore, ATP-sensitive K^+ (K_{ATP}) channel, which does not display C-type inactivation, was not affected by AHR. Therefore, block of Kv1.2 channel by AHR did not appear to involve direct occlusion of the outer pore but may depend on the C-type inactivation gate. AHR could thus be a pharmacological tool targeting the C-type inactivation gate of Kv channels.

Ligand-gated Channels-Glutamate Receptors

2707-Pos

TARP Modulation of AMPA Receptor Pharmacology: Polyamine Block and Competitive Antagonism

David MacLean, Derek Bowie.
McGill, Montreal, QC, Canada.

External polyamines have been a valuable tool as pharmacological markers of Ca^{2+}-permeable AMPARs (CP-AMPARs). However, recent work in the developing retina has revealed a population of CP-AMPARs which are unexpectedly insensitive to external polyamines. Because TARPs attenuate internal polyamine block, we hypothesized that TARP association with CP-AMPARs would also diminish external polyamine block, and thus display the phenotype observed in the retina. Similarly, TARPs have also been reported to reduce the potency of another useful pharmacological tool: the competitive antagonist CNQX. We therefore examined the impact of TARPs on CP-AMPARs for both of these antagonists using excised patches. We found that TARPs did attenuate internal block, however external block was not substantially reduced. Indeed, the rate of onset of block was actually accelerated. Moreover, TARPs do not significantly reduce CNQX inhibition of peak responses. In the presence of TARPs, CNQX has been reported to