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Negative charges in the loop between the A and B box of the T1 domain are involved in Kv2.1 and Kv2.1/Kv6.3 channel tetramerization
Elke Bocksteins, Alain J. Labro, Evy Mayeur, Dirk J. Snyders.
University of Antwerp, Antwerp, Belgium.
Voltage-gated potassium (Kv) channels are tetramers of four α-subunits. Formation of homo- or heterotetramers is determined by their subfamily specific N-terminal T1 domain. This domain contains two regions designated A and B box, which display a pattern of conservation within and between the Kv subfamilies assumed to determine subfamily specific channel assembly. However, the linker between both boxes is not conserved and its function is uncertain. Here we report that negatively charged residues in this linker are involved in Kv2.1 channel assembly. Mutating these negative charges to arginines caused at least a 15-fold down regulation in current density. To investigate if this was due to a trafficking or a tetramerization deficiency we used FRET analysis to determine the amount of unpaired channel subunits in the plasma membrane versus the ER. Using N-terminal CFP and YFP constructs we observed a 2.5 to >10-fold decline in the FRET efficiency in the ER for these charge reversal substitutions, indicating that there were more unpaired CFP-labeled subunits and that the reduction in current is most likely due to deficient tetramerization. These negative charges are also present in Kv6.3, an electrically silent subunit that fails to form functional homotetramers but selectively co-assembles with the Kv2.x subunits and generates heterotetrameric channels in which Kv6.3 modulates the Kv2.x currents. Mutating this charge cluster in Kv6.3 resulted likewise in a tetramerization defect with Kv2.1 as shown by the reduced FRET efficiencies and the lack of modulating Kv6.3 effects. These results indicate that besides the T1 domain's A and B boxes, the A-B linker is involved in channel tetramerization and could be one of the determinants for the selective co-assembly of Kv6.3 with Kv2.x channels.

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Structural Insights into KChIP4a Modulation of Kv4.3 Inactivation
Ping Liang[1], Huayi Wang[2], Hao Chen[1], Yuanyuan Cui[1], Jijie Chai[2], KeWei Wang[1].
[1]Peking University, Beijing, China, [2]National Institute of Biological Sciences, Beijing, China.
Dynamic inactivation in Kv4 A-type K^+ current is critical in regulating neuronal excitability by shaping action potential waveform and duration. Multifunctional auxiliary KChIPs1-4 subunits that share a high homology in the C-terminal core regions exhibit distinctive modulation on inactivation and surface expression of pore-forming Kv4 subunits. How structural differences of KChIPs in determining their functional diversity remain unknown.
Here, we describe a crystal structure of KChIP4a resolved at 3.0 Å resolution. The KChIP4a structure shows the distinct N-terminal α-helices that differentiate it from other KChIPs. The N-terminal residues of KChIP4a form a long α-helix followed by a short rigid coil that binds to a well-defined hydrophobic pocket formed by the conserved structural components. Structural comparison indicates that the hydrophobic pocket of KChIP4a is similar to that of KChIP1. This hydrophobic pocket has recently been shown to be the same type of binding groove that is recognized by the Kv4.3 N-terminus in KChIP1(ref. 1-2). The core KChIP4a (without its N-terminal α-helices) reveals that the hydrophobic groove can sequester the N-terminus of Kv4.3. Structural observations suggest that N-termini of both Kv4.3 and KChIP4a can competitively interact with the same hydrophobic pocket of KChIP4a. Biochemical experiments showed that competitive binding of Kv4.3 N-terminal peptide to the hydrophobic groove of core KChIP4a causes the release of its N-terminus that suppresses the inactivation of Kv4.3 channels. Electrophysiology confirmed that the N-terminal first α-helix peptide of KChIP4a either by itself or fused to N-terminal truncated Kv4.3 can confer the slow inactivation. We propose that N-terminal binding of Kv4.3 to the core KChIP4a mobilizes the KChIP4a N-terminus which may serve as a slow inactivation gate.
References:
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Divergent PIP_2 Sensitivity Confers Differential Muscarinic Agonist Efficacy For Suppression Of Kv7 K^+ Channels
Ciria C. Hernandez, Mark S. Shapiro.
UT Health Science Center, San Antonio, TX, USA.
Kv7 channels encode the M-current, a non-inactivating current that controls neuronal excitability. Stimulation of M_1 muscarinic receptors suppresses M-current via depletion of membrane phosphatidylinositol 4, 5-bisphosphate (PIP_2). We have suggested Kv7 channels to be directly regulated by PIP_2 binding at a carboxy-terminal inter-helix domain and divergent PIP_2 apparent affinity to underlie the differences in gating of these channels (Hernandez et al., *J Gen Physiol.* 132: 361-81, 2008). Taking advantage of the differences in PIP_2 affinities for different Kv7 subunits and the biophysical differences in current amplitude and unitary conductance displayed by the Kv7.3 (A315T) pore mutant suggested to "unlock" the channels into a conductive conformation (Zaika et al., *Biophys J.* Sep 12, 2008), we sought to determine the relationship between receptor-induced modulation and PIP_2 apparent affinity. Using perforated patch clamp, we studied CHO cells in which cloned Kv7 channels were co-expressed with M_1 muscarinic receptors. Heteromeric Kv7.2/7.3 currents were depressed by the muscarinic agonist oxotremorine-M (oxo-M) in a concentration-dependent manner with a maximal inhibition of about 80% and an EC_{50} of 0.5 ± 0.1 μM, similar to modulation of native M current. However, oxo-M induced a much weaker suppression of homomeric Kv7.3 (A315T) currents, manifested by a significantly reduced maximal inhibition (~40%) and a small shift of the dose response curve to the right (EC_{50} = 1.0 ± 0.3 μM). We hypothesize that channels with high PIP_2 apparent affinity, like Kv7.3, are more saturated at tonic levels of membrane PIP_2, and less-sensitive to inhibition by M_1 receptor-induced membrane PIP_2 depletion than channels with low PIP_2 apparent affinity, like Kv7.2/7.3 heteromers, that are not saturated by tonic levels of PIP_2 in the membrane and are thus more sensitive to M_1 receptor-induced PIP_2 depletions.

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Two Distinct Molecular Mechanisms Underlie pH Sensitivity of the Human Potassium Leak Channel $K_{2P}2.1$
Asi Cohen, Yuval Ben-Abu, Noam Zilberberg.
Ben-Gurion University, Beer-Sheva, Israel.
The mammalian $K_{2P}2.1$ potassium channel (TREK-1, KCNK2) is highly expressed in excitable tissues, where it plays a key role in the cellular mechanisms of neuroprotection, anaesthesia, pain perception and depression. Here, we report that external acidification, within the physiological range, strongly inhibits the human $K_{2P}2.1$ channel in two distinct time scales. We have identified two histidine residues (*i.e.,* H87 and H141), located in the first external loop of the channel, which govern the fast response of the channel to external pH (in the time scale of seconds). We demonstrate that these residues are within physical proximity to glutamate 84, homologous to *Shaker* E418, KcsA E51 and KCNK0 E28 residues, all previously reported to stabilize the outer pore gate in the open conformation by forming hydrogen bonds with pore-adjacent residues. We thus propose a novel mechanism for pH sensing in which protonation of H141 and H87 generates a local positive charge that serves to draw E84 away from its natural interactions, facilitating the collapse of the selectivity filter region, a mechanism which resembles C-type gating of voltage dependent potassium channels. In accordance with this proposed mechanism, the proton-mediated effect was inhibited by external potassium ions, modified the channel's ion selectivity and was enhanced by a mutation, S164Y, known to accelerate C-type gating. In addition, we show that the slow regulatory effect (in the time scale of minutes) is mediated by proton-sensitive G-protein coupled receptors (GPCRs), which activated phospholipase C via the G_q pathway. We demonstrate that three residues within the C-terminal of $K_{2P}2.1$ mediate the channel's response to the observed GPCRs activation by acidic pH.
Taken together, our results highlight the physiological importance of human $K_{2P}2.1$ channels as sensors of extracellular pH in the central nervous system.

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How Do Mutations in the L0-linker of ABCC8 Produce Neonatal Diabetes?
Andrey P. Babenko.
Pacific Northwest Research Institute, Seattle, WA, USA.
Numerous mutations in ABCC8 (SUR1), the neuroendocrine-type regulatory subunit of K_{ATP} channels, cause Neonatal Diabetes (ND). Many of these mutations compromising insulin release cluster around the first ND mutation identified in the SUR1 L0-linker, L213R (NEJM 355:456). Here we show the ND-SUR1 increased the on-cell open channel probability, Po, not the density of Kir6.2-based channels. Single-channel kinetics analyses demonstrate that the increase in the Po is due to a ligand-independent stabilization of the active (burst) state. A similar diabetogenic effect was reproduced by deletions in the Kir6.2 N-terminus (BBRC 255:231; Cell 100:645) that partners with the L0-linker controlling the "slow" (inter-burst) gating transitions (JBC 278:41577). These findings illustrate the physiologic significance of our original model of SUR1/Kir6.2 coupling (JBC 278:41577) where the L0-linker plays a key role in controlling the maximal Po.

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Comparison between the Functions of TRPP and Other Structurally-related Channels and their Links to Membrane Trafficking
Peter M. Vassilev, Philipp V. Fuchs, Marie Kanazirska, Liping Chen.
Brigham & Women's Hospital and Harvard Medical School, Boston, MA, USA.
TRPP and other structurally-related proteins belong to the same subfamily of TRP channels. Despite the similarities in their membrane topology, their cellular distribution is different and mutations in the genes encoding them cause