Voltage-gated potassium (Kv) channels are tetramers of four z-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 boxes, which display a pattern of conservation within and between the Kv subfamilies assumed to determine subfamily specific tetrameric 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 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 Kv7 channels encoding the M-current, a non-inactivating current that controls neuronal excitability. Stimulation of M1 muscarinic receptors suppresses M-currents concomitantly decreasing the FRET efficiencies and the lack of modulation by the muscarinic agonist oxotremorine-M (oxo-M) in a concentration-dependent manner with a maximal inhibition of about 80% and an EC\textsubscript{50} of 0.5 ± 0.1 μM, similar to modulation of native M current. However, o xo-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\textsubscript{50} = 1.0 ± 0.3 μM). We hypothesize that channels with high PIP\textsubscript{2} apparent affinity, like Kv7.3, are more saturated at tonic levels of membrane PIP\textsubscript{2}, and less-sensitive to inhibition by M1 receptor-induced membrane PIP\textsubscript{2} depletion than channels with low PIP\textsubscript{2} apparent affinity, like Kv7.2/7.3 heteromers, that are not saturated by tonic levels of PIP\textsubscript{2} in the membrane and are thus more sensitive to M1 receptor-induced PIP\textsubscript{2} depletions.

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Two Distinct Molecular Mechanisms Underlie pH Sensitivity of the Human Potassium Leak Channel KCa2.1

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The mammalian KCa2.1 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 KCa2.1 channel in two distinct time scales. We have identified two histidine residues (i.e., H87 and H141), located in the first extracellular 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\textsubscript{q} pathway. We demonstrate that three residues within the C-terminus of KCa2.1 mediate the channel’s response to the observed GPCRs activation by acidic pH.

Taken together, our results highlight the physiological importance of human KCa2.1 channels as sensors of extracellular pH in the central nervous system.