Inhibitor-Induced Conformational Changes in ASIC1A
Lund, Camilla; Borg, Christian B.; Lynagh, Timothy Peter; Pless, Stephan

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Acid-sensing ion channels (ASICs) are involved in acid-induced neuronal injury during pathological conditions in the central nervous system, such as seizures and ischemic brain injury. These cation-permeable membrane proteins are also involved in several pain-related mechanisms in the peripheral nervous system. Understanding their function and pharmacology is therefore of great interest. ASICs are activated by protons and inhibited by both small molecule compounds, such as ibuprofen, and peptides like the tarantula toxin psalmotxin 1 (PcTx1). In this project, Voltage-Clamp Fluorometry (VCF) is used to track the conformational changes of mASIC1a in response to exposure to both ibuprofen and PcTx1. We show that ibuprofen induces global, concentration-dependent conformational changes in the extracellular domain of ASIC1a. These ibuprofen-induced conformational changes appear to be distinct from those induced by channel opening and/or desensitization and are therefore likely to be compound-specific conformational changes. Furthermore, mutation of a residue critical to functional ibuprofen inhibition, K422, rendered the channel less sensitive to ibuprofen, but did not affect the concentration-dependence of the ibuprofen-induced conformational changes. These observations might help decipher the recently proposed allosteric inhibition mechanism of ibuprofen. The peptide toxin PcTx1 inhibits ASICs by inducing steady-state desensitization in ASIC1a. Here we show that it induces global conformational changes in the extracellular domain that are modulated by the proton concentration in the extracellular solution. Mutations of F350 have previously been shown to abolish PcTx1 inhibition, but our VCF work shows that the PcTx1-induced conformational changes persist despite severely reduced PcTx1 inhibition of the mutant channel. Similar to our observations with ibuprofen, this strongly suggests that PcTx1 still binds to and interacts with ASIC1a, despite the mutation-induced reduction of channel inhibition. Together, these findings provide new insight on inhibitor-induced conformational changes in ASIC1a.

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Evolution of Acid-Sensing Ion Channels
Timothy Lynagh, Janne M. Colding, Stephan A. Pless.
University of Copenhagen, Copenhagen, Denmark.

Each major lineage in the animal kingdom possesses genes from the ENaC/DEG channel family, a broad family of functionally diverse ion channels. A small sub-family is that of the acid-sensing ion channels (ASICs), protonated sodium channels that contribute to the neuronal signals underlying, for example, learning and nociception in mammals. Although ASICs have only been described in vertebrates, it seems peculiar that such a signaling protein should be absent from lower organisms that also express such fundamental behavioral phenotypes. The identification of primitive ASICs has perhaps been hampered by the lack of understanding of molecular mechanism of proton-sensing. Here, we use molecular phylogenetics, conventional mutagenesis, unnatural amino acid incorporation and electrophysiological recordings to explore proton-sensing in a broader sample of ASICs than previously possible, in light of recent genomic data. We find that ASICs are not confined to the vertebrate lineage, and a comparison of this extended ASIC family with other ENaC/DEG channels points towards somewhat surprising determinants of proton-sensing. Using unnatural amino acids, we were able to dissect some of the chemical interactions behind the molecular mechanism of proton-sensing. Finally, we provide an explanation for the loss of proton sensitivity in ASIC4 of the mammalian lineage. Together, this combined evolutionary and biochemical approach throws new light on the mechanism of proton-sensing and the distribution of ASICs within the ENaC/DEG family.