Inhibitor-Induced Conformational Changes in ASIC1A
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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 brain-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 psalmotoxin 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.

Evolution of Acid-Sensing Ion Channels

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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), proton-gated 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 is 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.

Intragenic Rescue of the Function of Long QT Syndrome-Causing hERG Mutant Channels

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The human-ether-a-go-go-related gene (hERG) encodes the pore forming subunit of the rapidly activating delayed rectifier K⁺ current (I_Kr), which is important for the repolarization phase of cardiac action potentials. A reduction in I_Kr due to a loss of hERG function can lead to long QT syndrome (LQTS). There are a variety of loss-of-function hERG mutations known to cause LQTS, the majority of which are thought to be trafficking deficient, including G601S. It has been shown that the plasma membrane (PM) expression of the G601S hERG mutant can be rescued by reduced temperature culture. We have shown that wild-type (WT) hERG channels require extracellular K⁺ for their stability in the PM. They undergo internalization under 0 mM K⁺ culture conditions, which can be prevented by reduced temperature culture. We also identified that the S624T mutation makes hERG insensitive to extracellular K⁺, thereby preventing hERG channel internalization under 0 mM K⁺ culture conditions. We hypothesize that certain hERG mutants, including G601S, may not be able to sense extracellular K⁺, making them unstable in the PM, leading to a loss of PM expression. In this study, we added a secondary mutation, S624T, which does not depend on extracellular K⁺ for its PM expression, to the G601S hERG mutant channel. The addition of S624T suppressed the loss-of-function G601S phenotype, and rescued its current to a level similar to WT channels. The addition of S624T also rescued LQTS-causing hERG mutants T474I and P596R. Our data reveals the most effective intragenic rescue of LQTS-causing hERG mutants to date and lends insight into the mechanisms through which these mutations confer the loss-of-function phenotype.