Peptide Modulation of Acid-Sensing Ion Channels

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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 pain-related mechanisms in the periphery. 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.

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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 should be absent from lower organisms that also express such fundamental behavioral phenotypes. The identification of primitive ASICs has perhaps been hampered by our poor understanding of the 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.

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Peptide Modulation of Acid-Sensing Ion Channels
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Acid-sensing ion channels (ASICs) are homo- or heterotrimeric proton-gated cation channels which are involved in numerous biological processes, including initiation of pain and neuronal death associated with ischemic stroke. A number of neuropeptides and peptide toxins have been shown capable of modulating ASIC function and activity. Thus, these peptides have great potential as therapeutics for the treatment of these disorders, as well as molecular tools for the understanding and control of ASIC function. Despite the recent advances in structure determination of ASIC-peptide complexes, the precise molecular mechanisms underlying peptide modulation of ASICs remain elusive, which hampers the development of new and improved ASIC-modulating compounds. In this study, we aimed to decipher the molecular basis of peptide modulation of ASICs by employing a combination of molecular biology, electrophysiology, and synthetic peptide chemistry. Synthetic peptides and peptide analogs were produced by solid-phase peptide synthesis and their effects on ASICs were measured by two-electrode voltage clamp electrophysiology. In addition, a series of ASIC concatemers were constructed to allow for the introduction of mutation(s) into one or more specific ASIC subunits. By evaluating the effects of modified ASIC-modulating peptides on both wild-type, mutated and concatenated ASICs, we have gained detailed insights into the molecular requirements for peptide modulation of ASIC function. Information gained from this study will allow the rational design of novel peptides with improved potency and selectivity in the future.

Posters: Ion Channel Regulatory Mechanisms I

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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 mutant hERG 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.

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Regulation of hERG C-terminal Isoform Expression by *KCNH2* Intronic Elements
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The *KCNH2* gene encodes the hERG potassium channel that conducts the rapidly activating delayed rectifier current in the heart. Two C-terminal hERG isoforms are expressed in the heart. Splicing of *KCNH2* intron 9 leads to the formation of a full-length, functional hERGa isoform and polyadenylation of intron 9 results in the production of a non-functional, C-terminally truncated hERGa-USO isoform. In the heart, only one-third of *KCNH2* pre-mRNA is processed to hERGa due to inefficient intron 9 splicing. A unique feature of *KCNH2* intron 9 is the presence of a 25 nt adenosine stretch immediately downstream of the weak, noncanonical, polyadenylation site, AGTAAA. Interestingly, non-human primate species contain the AGTAAA poly(A) site, but differ in the length of the downstream poly(A) stretch. It is not known how the length of the adenosine stretch effects the alternative processing of *KCNH2* and the generation of C-terminal hERG isoforms. To test the effect of adenosine stretch length on alternative processing, the *KCNH2* intron 9 poly(A) stretch was changed from 25 nt to 13 nt and 33 nt, corresponding to the sequences of the marmoset and baboon. In the presence of a 13 nt adenosine stretch, intron 9 splicing outcompeted polyadenylation and resulted in two-thirds of *KCNH2* pre-mRNA being processed to hERGa. Introducing the 33 nt adenosine stretch into intron 9 also resulted in increased hERGa expression and concomitantly decreased expression of the hERGa-USO isoform. Whole-cell patch clamp analyses showed increased hERG current in HEK-293 cells transfected with full-length *KCNH2* gene constructs containing 13 nt and 33 nt adenosine stretches. Although the expression of hERGa-USO has not been previously