



## Indirect Determinants of Ion Selectivity in Acid-Sensing Ion Channels and Epithelial Sodium Channels

Sheikh, Zeshan P.; Lynagh, Timothy; Kristensen, Anders S.; Pless, Stephan A.

DOI:

[10.1016/j.bpj.2018.11.626](https://doi.org/10.1016/j.bpj.2018.11.626)

Publication date:

2019

Document version

Publisher's PDF, also known as Version of record

Document license:

[CC BY](#)

Citation for published version (APA):

Sheikh, Z. P., Lynagh, T., Kristensen, A. S., & Pless, S. A. (2019). *Indirect Determinants of Ion Selectivity in Acid-Sensing Ion Channels and Epithelial Sodium Channels*. 110A-110A.

<https://doi.org/10.1016/j.bpj.2018.11.626>

molecular basis of these disorders. In the CNS, activation of microglial P2X7 receptors (P2X7Rs) triggers neuroinflammation, which is intricately linked to some mental disorders. P2X7Rs belong to the family of adenosine triphosphate (ATP)-gated ion channels, which are trimeric receptor assemblies. Interestingly, the P2X7R displays an unusually high number of single nucleotide polymorphisms (SNPs) across the population, resulting in numerous SNP-containing receptor variants, some of which are linked to diseases, such as mental disorders. Notably, the functional and pharmacological consequences of P2X7R SNPs remain unexplored. To gain a comprehensive understanding of the function of pathophysiologically relevant P2X7R SNP variants, we study halotypes of the human P2X7R found in patients suffering from mental disorders. The functional and pharmacological characterization of these halotypes is performed both in human microglia and HEK cells, using patch-clamp and high-throughput fluorescence assays. Additionally, both wild type P2X7Rs and SNP variants have been suggested to play diverse and sometimes contradictory roles in different cell types. We hypothesize that this differential role is caused by cell-specific protein-protein interactions (PPIs) between the P2X7R N- and/or C-terminal domains and intracellular proteins, which we aim to identify and characterize using mass spectrometry. Together, this starts to disentangle the molecular basis of disease-linked microglial P2X7R variants, leading to a better understanding of mental disorders.

#### 548-Pos

##### Stomatin Dependent Regulation of the Acid Sensing Ion Channels

Robert C. Klipp, John Bankston.

Physiology and Biophysics, University of Colorado Anschutz Medical Campus, Aurora, CO, USA.

In the peripheral and central nervous systems, Acid Sensing Ion Channels (ASICs) are the primary mediators of enhanced neuronal activity due to extracellular acidification. Despite ASICs importance in regulating pathophysiological conditions of tissue acidosis, many of the molecular mechanisms underlying ASIC function, such as its incorporation into higher order ion channel complexes, remain poorly elucidated. Stomatin, a member of the SPFH family of integral membrane proteins has previously been shown to complex with ASIC isoforms 1, 2 and 3, while having differential regulation of all 3. Stomatin shows a near complete inhibition of ASIC3 function when recombinantly expressed in mammalian cells. However, in DRG sensory neurons, where Stomatin and ASIC3 are known to interact, ASIC3 activates to moderate acidifications. Here, we present our preliminary findings investigating several aspects of the molecular mechanisms underlying Stomatin's regulation of ASIC3. First, we seek to localize both regulatory and non-regulatory Stomatin binding sites, creating a systematic series of truncated, chimeric, and point mutant channels. Mutant channels are recombinantly expressed into mammalian cells and patch clamp electrophysiology is utilized to examine channel function in the presence and absence of Stomatin. We use FRET to pair mutant channel function with Stomatin binding using the respective fluorescently labelled constructs. Finally, we investigate the role that domains on Stomatin play in the dynamic regulation of ASIC3.

#### 549-Pos

##### Indirect Determinants of Ion Selectivity in Acid-Sensing Ion Channels and Epithelial Sodium Channels

Zeshan P. Sheikh<sup>1</sup>, Timothy Lynagh<sup>2</sup>, Anders S. Kristensen<sup>1</sup>, Stephan A. Pless<sup>1</sup>.

<sup>1</sup>Drug Design and Pharmacology, Univ Copenhagen, Copenhagen, Denmark,

<sup>2</sup>Univ Copenhagen, Copenhagen, Denmark.

Members of the ENaC/DEG superfamily of ion channels are Na<sup>+</sup>-selective ion channels with a common trimeric architecture. Each of the three subunits has two transmembrane helices (M1 and M2), of which M2 lines the

pore. Members of this family include the acid-sensing ion channels (ASICs), formed by identical or homologous subunits that mediate excitatory Na<sup>+</sup> currents in the nervous system (relative Na<sup>+</sup>/K<sup>+</sup> permeability of approx. 10/1). The epithelial sodium channels (ENaCs) are obligate heterotrimers and display a 10-fold higher Na<sup>+</sup> selectivity than ASICs. The most recent findings indicate that this discrepancy might be due to different selectivity filter (SF) locations; while the ENaC SF is likely formed by the conserved G/S-X-S motif in the center of the pore, we have recently shown the mouse ASIC1a SF to be composed of two acidic side chains in the lower part of M2, namely E18' and D21'. In order to elucidate if other parts of the channel contribute to the stark differences in Na<sup>+</sup> selectivity between ASICs and ENaCs, we used conventional and non-canonical amino acid substitutions to probe the contribution of M1 residues to ion selectivity. Our results show that aromatic residues in ASIC M1 are important for ion selectivity. ENaC contains additional aromatic residues in M1. We hypothesize that these aromatics are similarly important for ion selectivity and that pore diameter plays an important role in both channels. Furthermore, the intracellular domains have previously been suggested to contribute to ion selectivity. Using a novel split intein-based approach we fuse partial ASIC1a constructs with recombinant or synthetic peptides corresponding to the N- or C-terminus of the full length protein. This enables us to introduce non-canonical amino acid substitutions, including post-translational modifications, into these less-studied regions of the channel.

#### 550-Pos

##### Molecular Basis for Ion Selectivity in Heteromeric Acid-Sensing Ion Channels

Zeshan P. Sheikh<sup>1</sup>, Timothy Lynagh<sup>2</sup>, Emelie Flood<sup>3</sup>, Celine Boiteux<sup>4</sup>, Toby W. Allen<sup>5</sup>, Stephan A. Pless<sup>1</sup>.

<sup>1</sup>Drug Design and Pharmacology, Univ Copenhagen, Copenhagen, Denmark,

<sup>2</sup>Univ Copenhagen, Copenhagen, Denmark, <sup>3</sup>RMIT Univ, Brunswick,

Australia, <sup>4</sup>Hlth Innovat Res Inst, RMIT Univ, Melbourne, Australia, <sup>5</sup>Sch of Sci, RMIT Univ, Melbourne, Australia.

Acid-sensing ion channels (ASICs) occur throughout the nervous system and open in response to proton binding. Most ASICs are ~10-fold selective for Na<sup>+</sup> over K<sup>+</sup>, and we recently showed that in homomeric ASIC1a channels, preferential Na<sup>+</sup> conduction is primarily controlled by glutamate (E18') and aspartate (D21') side chains at the intracellular end of the pore. Additionally, two leucine residues (L7' and L14') and a constriction termed the GAS belt (G10'-S12') also contribute to ion selectivity. However, it remained unclear if this mechanism extends to other ASIC isoforms, including heteromeric channels. Here, we investigated the molecular determinants of ion selectivity in homomeric ASIC2a and heteromeric ASIC1a/ASIC2a channels, using site-directed mutagenesis, electrophysiology and molecular dynamics simulations. In contrast to ASIC1a, L7'A mutation had no effect on ion selectivity in ASIC2a. L14' and, especially E18' mutations, however, substantially decreased selectivity of ASIC2a, while G10' and S12' mutations rendered channels non-functional, as in ASIC1a. Coexpression of mutant ASIC2a with WT ASIC1a (and vice versa) led to functional heteromeric channels containing 1-2 mutated subunits. In these heteromers, E18' mutations had stronger effects on ion selectivity than any other position tested here. This was consistent with simulations, showing favorable interactions of E18' side chains with Na<sup>+</sup> ions in both homomeric ASIC2a and heteromeric ASIC1a/ASIC2a channels. Furthermore, simulations provided an explanation for the reduced role of L7' in ASIC2a channels, revealing a distinct free energy profile above the central GAS region, due to attractive interactions with carboxylate-containing residues at the upper end of the pore, rendering the L7' site non-rate determining. These results suggest a more pronounced role of the external mouth of the pore and the GAS belt in ASIC2a compared to ASIC1a, but confirm that E18' is crucial to ion selectivity in various ASIC isoforms.