Acid-Sensing Ion Channels: Structure and Function

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In the last two years, increasing evidence has implicated the ASIC channels in an astounding range of physiological functions including pain and taste transduction, learning and memory. In the present review we will summarize some of the recent findings and discuss the potential of the ASIC channels as a target for drug development.

Acid-sensing ion channels (ASICs) are Na+ channels activated by external protons. The channels were cloned based on their similarity to a larger channel family known as epithelial/degenerin Na+ channels (ENaCs). The ASICs rapidly respond to a reduction in extracellular pH with an inward cation current that is quickly inactivated despite the continuous presence of protons in the medium.

Structural aspects and channel properties

The ASIC channels constitute a subfamily within the larger ENaC superfamily. Despite the fact that the ASICs share only about 20-25% identity with the ENaC channels they display all the characteristics of the superfamily, which are, two transmembrane spanning domains, a large extracellular domain and short intracellular N and C termini. The functional channel of the superfamily is composed of 4 subunits. In the case of the ASICs, the channel can be either a homo or heterotetramer that will result in different functional properties. Another shared characteristic of ASICs and ENaCs is their sensitivity to low concentrations of amiloride, a widely used diuretic.1,5

To date six proteins of the ASIC family have been identified that arise from 4 genes (see table): ASIC1a and ASIC1b are splice forms of the ASIC1 gene; ASIC2a and ASIC2b arise from the ASIC2 gene; ASIC3 and ASIC4. The ASIC4 protein does not appear to function as a proton-gated channel and therefore a modulatory role for this subunit has been proposed.13 Similarly, the ASIC2b protein is inactive when expressed alone, but it modifies the properties of ASIC2a and ASIC3 when they are coexpressed.17

When analyzed in heterologous systems the ASICs show different pH sensitivities and inactivation kinetics. The pH of half maximal activation (pH½) is in the range of 5.9-6.5 for ASICs 1a, 1b and 3. ASIC2 is less sensitive with a pH½ of about 4.4.2,13 Following activation the channels are quickly inactivated with kinetics that vary between the different subunits. For example, ASIC1 and ASIC3, desensitize more rapidly than ASIC2. Interestingly, in some conditions (primarily heteromers and homomers containing ASIC3), inactivation of the current is incomplete leaving a residual current that persists during prolonged acidification.2,3,15

ASIC channel function can be modulated by both intra and extracellular factors. For instance the invertebrate neuropeptide FMRFamide (Phe-Met-Arg-Phe amide) and its mammalian counterpart FF (Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe amide) have been shown to modulate ASIC currents by slowing or preventing the channel inactivation following its pH-dependent activation. It has been speculated that additional FMRFamide-like peptides that have yet to be discovered, might modulate ASICs currents in different physiological and pathological conditions.16 Similarly, lactic acid, a compound produced by anaerobic metabolism, enhances ASIC sensitivity to pH by lowering the extracellular concentration of divalent ions.17 Indeed, it was demonstrated that decreasing extracellular Ca++ could open the ASIC3 channel directly. In fact, it was shown that ASIC channels are activated by ion exchange and not by a conformational change, making them the fastest activating ion channels.18

ASIC function is also modulated by intracellular factors. ASIC1 is a direct target of PKA, while ASIC1 and 2 are modulated by PKC through the PDZ domain-containing protein PICK1.19,20 ASIC3, on the other hand, interacts with CIPP, another PDZ domain-containing protein. These interactions appear mainly to regulate surface expression of the channels although they could serve as scaffold proteins that connect ASIC channels to intracellular signal transduction mechanisms.21

The pharmacology of the ASIC channels is rather limited. The channels can be blocked non-specifically by amiloride as previously mentioned. ASIC1a has been shown to be specifically blocked by a tarantula Psalmotoxin22 while several nonsteroid anti-inflammatory drugs (NSAIDs) can directly block different ASIC channels.23

Functional roles

Due to their functional characteristics and specific localization the ASIC channels have been implicated in numerous physiological (and pathological) functions.

In the CNS, mouse knockouts of ASIC1 have implicated this channel in learning and memory.4,5 In the knockout mice, long-term potentiation (LTP) impairment was identified in hippocampal neurons. Impaired LTP has been linked to defects in learning and memory, among others, through the activity of NMDA receptors. It was shown that lack of ASIC currents in hippocampal neurons decreased the activity of NMDA receptors. The exact mechanism by which ASIC currents potentiate NMDA receptors and thus LTP, remains to be addressed. Interestingly, although ASIC1 can form heteromers with ASIC2a (that is also present in the CNS) the latter was unable to form functional channels on its own in the ASIC1 knockouts.

In comparison, mouse knockouts of ASIC2 and ASIC3 revealed less straightforward defects, probably due to compensation with other ASIC channels. Nevertheless, ASIC2 and ASIC3 knockouts showed defects in light touch perception and mechanosensation.5,24

Recently, a heteromeric ASIC2a/ASIC2b channel has been implicated in sour taste sensing which is essentially a “proton taste”. Activation of the receptor by acid leads to depolarization of taste cells and transmitter release onto gustatory afferent neurons. It is important to note that only the heteromeric ASIC channel, and not the homomer, exhibited the required characteristics in terms of pH sensitivity and amiloride insensitivity.7

Interestingly, the involvement of ASIC channels in tumor development has been recently reported. Constitutive large amiloride-sensitive Na+ currents have been detected in aggressive brain tumors while in normal and low-grade astrocytic tumors there is none. It was shown that normal
Compelling evidence links ASIC3 function to pain perception. Mouse models of ASIC3 knockouts showed that ASIC3 was necessary for pain sensation, primarily high intensity pain produced by heat and acid.25, 26 Other studies have shown a strong correlation between ASIC3 function and cardiac pain. During a heart attack the heart receives insufficient blood supply, causing release of several compounds such as lactic acid that reduce the pericardial pH to as low as 6.7. Given the fact that lactic acid can modulate tissue acidosis has been directly linked to the feeling of pain in inflammation. Recently it has been shown that proinflammatory mediators such as serotonin, IL-1, bradykinin and especially NGF, can enhance ASIC3 expression at the transcriptional level which is reflected by an increase in ASIC current in sensory neurons.25, 26

As mentioned above, the ASIC subunits can assemble to form either homo or heterotetramers. The heteromers have distinct activation and desensitization characteristics, pharmacological properties, and different abilities to interact with intra and extracellular factors. This characteristic makes the ASIC channels an even more appealing drug target, since it may allow exquisite selectivity. For instance, a heteromer of ASIC3 and ASIC1b may be involved in inflammatory pain transmission, but not in other sensory functions, therefore a drug targeted to this particular channel configuration may alleviate pain sensation caused by inflammation, but not affect other functions mediated by ASICs.

The fact that the ASIC channels are involved in diverse physiological and pathological conditions makes them an attractive target for compounds seeking to modulate pain, learning and memory.

### Characteristics of ASIC Channels

<table>
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<tr>
<th>Protein name</th>
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<tr>
<td></td>
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<td>MDEG2, BNaC1p</td>
<td>CNS, SN (++), taste buds</td>
<td>Sour taste sensing</td>
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<tr>
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Abbreviations: CNS - central nervous system, SN - sensory neurons.

### References:

Related Products

**Anti-ASIC1**

(Amiloride-sensitive brain Na+ channel, BnaC2, ACCN2)

**Product #: ASC-014**

Host: Rabbit.

Epitope: Peptide CQKEAKRSSADKGVALSLDD corresponding to residues 469-488 of rat ASIC1.

Putative epitope location: Intracellular, C-terminus.

Homology with other species:
- Human-identical, mouse – 19/20 residues identical.

Reactivity Confirmed: Rat.

Applications:
- Western Blotting:

  1. Anti-ASIC1 antibody (ASC-014) (1:200).
  2. Anti-ASIC1 antibody, preincubated with the control peptide antigen.

**Anti-ASIC2a**

(Acid Sensitive Ion Channel 2, BNaC1, Brain Na+ Channel 1, BNC1, MDEG1, Amiloride-Sensitive Neuronal Cation Channel 1, Accn1)

**Product #: ASC-012**

Host: Rabbit.

Epitope: Peptide DLKESPSEGS LQPSS IQC, corresponding to residues 2-18 of human ASIC2a.

Putative epitope location: Intracellular, N-terminus.

Homology with other species:
- Rat, mouse - identical.

Specificity: The antibody is specific for ASIC2a and does not recognize ASIC2b.

Reactivity Confirmed: Rat.

Applications:
- Western Blotting:

  1. Anti-ASIC2a antibody (ASC-012) (1:200).
  2. Anti-ASIC2a antibody, preincubated with the control peptide antigen.

**Anti-ENaCγ**

(Epithelial Na+ channel γ subunit, Amiloride-sensitive Na+ channel γ subunit, SCNN1G)

**Product #: ASC-011**

Host: Rabbit.

Epitope: Peptide (C)YGVKESRKRREAGS, corresponding to amino acid residues 129-142 of rat ENaCγ.

Putative epitope location: Extracellular.

Homology with other species:
- Human-13/17 residues identical.

Reactivity Confirmed: Rat.

Applications:
- Western Blotting:

  2. Anti-ENaCγ antibody, preincubated with the control peptide antigen.