Chapter 13

# Investigation of the Structure and Function of Acid-Sensing Ion Channels

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**Abstract**

Acid-sensing ion channels (ASICs) are members of the epithelial sodium channel/degenerin (ENaC/DEG) superfamily and are encoded by five distinct genes, giving rise to seven different subunits. These subunits predominantly assemble into trimeric ion channels that, upon activation by extracellular protons, generate a transient inward current, thereby enhancing cellular excitability. These ion channels, which are activated particularly by extracellular acidification (pH decrease), regulate intracellular ion balance and electrical activity. ASICs exhibit a broad range of tissue distributions and display diverse biophysical characteristics. Moreover, their capacity to form both homomeric and heteromeric trimers adds further complexity to their functional and pharmacological properties. Certain modulators have been identified that lower the proton concentration required for ASIC activation, thereby sensitizing these channels. The roles of ASICs in neurological diseases, pain mechanisms and psychiatric disorders are attracting increasing interest. Substantial evidence from transgenic mouse models and pharmacological investigations indicates that ASICs represent a promising target for therapeutic intervention in various pathological conditions. Further investigation of the molecular mechanisms of ASICs may enable the development of new therapeutic strategies in disease models. This review aims to summarize the current understanding of ASIC function, explore their physiological and pathological roles, discuss mechanisms of modulation, and identify critical gaps in knowledge that warrant further investigation.

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## INTRODUCTION

### Acid-Sensitive Ion Channels

Acid-sensitive ion channels were first discovered in rat sensory neurons in 1980 by Krishtal and Pidoplichko using the voltage clamp technique (Krishtal & Pidoplichko, 1980). Krishtal and Pidoplichko argued that these acid-evoked currents were mediated by a previously unidentified ion channel and thought that they might be acid-evoked Ca2+ channels. Lazdunski and colleagues later showed that these channels were sensitive to amiloride and permeable to Na+ ions, and described acid-sensitive ion channels for the first time (Waldmann, Champigny, Bassilana, Heurteaux, & Lazdunski, 1997). In 2007, the crystal structure of ASIC1a in chicken was described, and they showed that ASICs consist of trimers, i.e., three different subunits (Jasti, Furukawa, Gonzales, & Gouaux, 2007). Acid-sensitive ion channels, a subgroup of the voltage-insensitive proton-gated degenerin/epithelial sodium channel (DEG/ENaC) superfamily, were shown to be widespread in both the central and peripheral nervous systems (Grunder & Chen, 2010; Waldmann & Lazdunski, 1998). In subsequent studies, the basic properties of these channels were determined. These channels generally open with decreasing pH (Krishtal & Pidoplichko, 1980), are permeable to Na+ allow (Krishtal & Pidoplichko, 1981a), and are blocked by amiloride (Krishtal & Pidoplichko, 1981b). In normal cells, extracellular pH is between 7.3-7.4 (Calorini, Peppicelli, & Bianchini, 2012). ASICs are rapidly activated and desensitized when extracellular pH falls below the normal physiological value (approximately pH=7.4) ) (Hesselager, Timmermann, & Ahring, 2004). ASIC subunits have different pH sensitivities, activation kinetics and desensitization rates (Korkushko & Kryshtal, 1984). ASIC members are named as "X-NaC". The abbreviation "X" means that it is related to a basic property of the proteins (DEG, degenerin protein), while the abbreviation "C" means 'channel'. For example; epithelial Na+ channel (ENaC), FMRF amide (Phe-Met-Arg-Phe-NH2) gated Na+ channel (FaNaC), Drosophila gonad Na+ channel (dGNaC), human intestinal Na+ channel (hINaC), and brain, liver, and intestine Na+ channel (BLINaC) (Grunder & Chen, 2010). At least 6 ASIC subunits encoded by 4 different genes have been identified in rodents. There are 6 known ASIC subgroups: ASIC1a (Waldmann, Champigny, et al., 1997), ASIC1b (Chen, England, Akopian, & Wood, 1998), ASIC2a (Price, Snyder, & Welsh, 1996), ASIC2b (Lingueglia et al., 1997), ASIC3 (Waldmann, Bassilana, et al., 1997) and ASIC4 (Akopian, Chen, Ding, Cesare, & Wood, 2000). ASIC subtypes can exist as homomers or heteromers. However, ASIC2b and ASIC4 subtypes are not functional as homomers; they function by forming heteromers with other ASIC subtypes.

All ASIC subunits consist of two hydrophobic transmembrane domains (TM1 and TM2), a large extracellular loop and short intracellular N- and C-terminal regions. The typical structure of ASICs and their localization in the cell membrane are shown in Figure 1 (Osmakov, Andreev, & Kozlov, 2014).

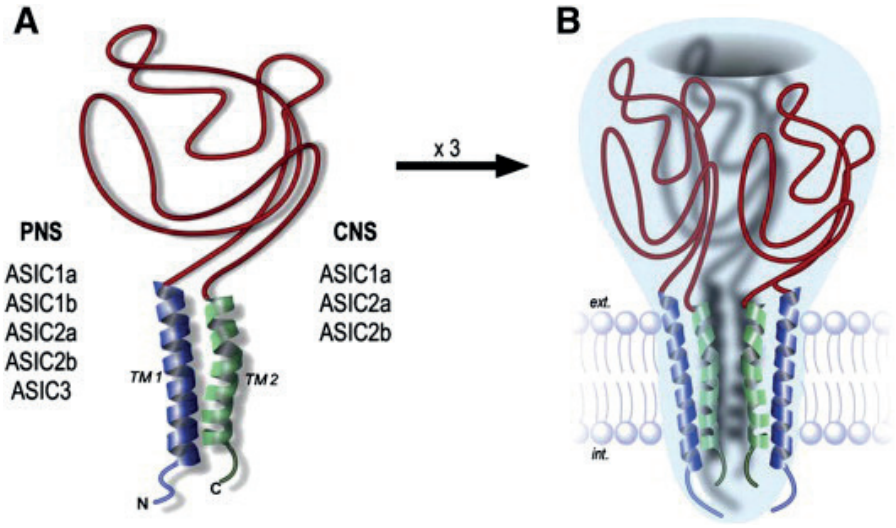


***Figure 1. A. Schematic representation of ASIC subunits B. Trimeric structure of the ASIC channel (Osmakov, Andreev, & Kozlov, 2014).***

ASICs have been shown to be present in most regions of the mammalian brain and in all sensory ganglia. However, it has been reported that ASIC channels are found in neurons but not in glial cells. Studies have shown that ASIC1a, ASIC2a, and ASIC2b genes are more expressed in the central nervous system, while ASIC1b and ASIC3 genes are more expressed in the peripheral nervous system (Chu et al., 2011). ASIC subtypes exhibit different electrophysiological and pharmacological properties (Hesselager, Timmermann, & Ahring, 2004). While all ASICs only conduct Na+ ions, ASIC1a conducts calcium ions along with sodium (Waldmann, Champigny, Bassilana, Heurteaux, & Lazdunski, 1997). Zinc (Zn2+) enhances homomeric and heteromeric ASIC2a currents, while attenuating other ASIC currents (110). Psalmotoxin1 is a specific ASIC1a inhibitor (Baron et al., 2001). Lead (Pb2+) inhibits ASIC1a currents but not other ASICs (Wang at al., 2006). Salicylic acid blocks only the ASIC3 subunit (Voileyet al., 2001). The kinetic properties of the ion currents generated by ASIC subunits are different from each other (Figure 2) (Osmakov, Andreev, & Kozlov, 2014).

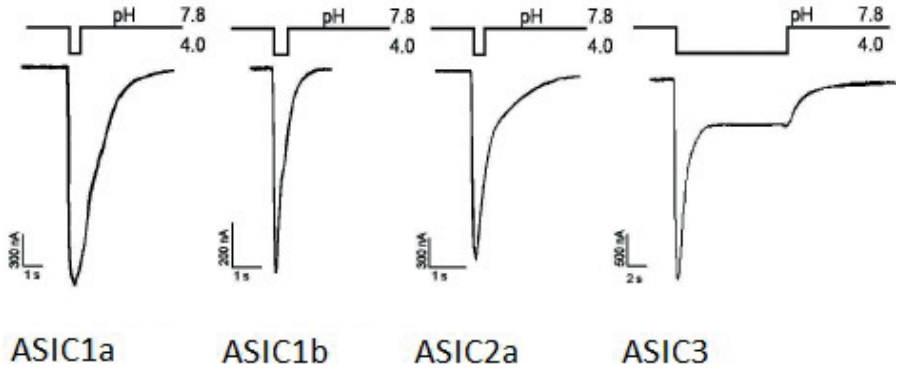


***Figure 2. Currents generated by ASIC subunits expressed in frog (Xenopus laevis) oocyte cells (Osmakov, Andreev, & Kozlov, 2014).***

Studies have shown that acid-sensitive ion channels play a role in many physiological events such as nociception (Chen et al., 2002), touch (Price et al., 2001), taste and smell transmission (Lin et al., 2002), long-term potentiation (LTP), synaptic transmission, memory/learning (Wemmie et al., 2002), sensory transmission and retinal integrity (Ettaiche et al., 2004) and in many pathological events such as pain (Duan et al., 2007), ischemia-related brain damage (Xiong et al., 2004), stroke and epilepsy (Biagini et al., 2001). In the auditory system, the presence of ASICs has been shown in hair cells in the cochlea (Ugawa et al. 2006), spiral ganglion neurons (Peng et al., 2004), vestibular organs (Mercado et al., 2006) and inferior colliculus neurons (Zhang et al., 2008). It has been determined that the ASIC2a subunit is related to noise sensitivity in mice (Peng et al., 2004), and that the absence of ASIC2 does not impair hearing (Roza et al., 2004). In a study conducted on mice that did not express the ASIC3 gene, hearing loss was found in mice lacking the ASIC3 subunit (h,ldebrand et al., 2004).

Acid-sensitive ion channels help in the transduction of stimuli in various physiological and pathophysiological conditions (Askwith et al., 2001). ASIC inhibitors are isolated from animal venoms, which are natural peptide toxins, as non-specific molecules (Diochot et al., 2007). Pepsin toxins, neuropeptides, organic compounds and some di/trivalent cations obtained from some animal venoms are involved in the modulation of ASICs.

## MATERIAL AND METHODS

### Material

Albino BALB/c mice aged 14-17 days were used in patch clamping studies. Cochlear nucleus brain sections were prepared to record patch clamping. Patch clamp recordings were taken using patch clamp micropipettes. These procedures were performed in constantly oxygenated normal CSF fluid.

### Methods

*Patch clamp technique*

The patch clamp technique is a widely used electrophysiological technique to study ion currents of channels in the cell membrane. The patch clamp technique is based on measuring voltage changes in cells via the electrode in the pipette. This technique is important for determining the biophysical, physiological and pharmacological properties of ion channels. When the patch clamp technique was first discovered, it was used to control the voltage of a small piece of cell membrane. Now, it is used for both voltage clamping and current clamping on the membrane with the help of a micropipette.

*Statistical Analysis*

Statistical evaluation in this study; It was done using the Statistical Package for Social Science (SPSS) Version 23.0 (SPSS inc, Chicago, USA) package program. In patch clamping studies, it was examined whether there was a difference between the recordings taken before the application of the acidic solution and the recordings taken during the application of the acidic solution. Student's t test was used to determine the difference during acidic solution. Descriptive statistics for numerical variables were expressed as group mean ± standard error (S.H.). In statistical evaluation, $p<0.05$ value was accepted as significant.

## RESULTS

To investigate acid-induced currents in neurons of the cochlear nucleus, the responses of these neurons to a decrease in pH in the extracellular solution were recorded using the whole-cell patch-clamp technique. In the present experiments, the average resting membrane potential of stellate cells was found to be – 63.7 ± 0.71 mV (n = 42 ). Therefore, recordings were made by keeping the cells at – 62 mV holding potential. The acidic solution stimulated most of the neurons by generating inward currents. The amplitudes of acid-induced currents showed high sensitivity to pH (Figure 3). Acidic solutions with pH ranging from 7.4 to 4 were applied. The

relationship between the peak values of the currents occurring in different acidic solutions and the pH values obtained was shown graphically.

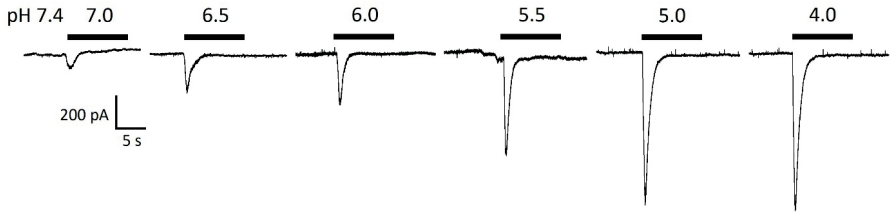


***Figure 3. Typical traces showing the inward currents that are activated by extracellular solutions with different pH.***

## DISCUSSION AND CONCLUSION

Acid-sensitive ion channels (ASICs) are voltage-insensitive sodium channels that are activated by acidification of the extracellular environment. ASICs are widely expressed in the central and peripheral nervous systems. There are reports that ASICs activated by acid exposure play a role in various physiological mechanisms and pathophysiological events. Although local pH decreases in various cellular structures are important enough to activate ASICs, it is thought that pH in tissues is tightly regulated by homeostatic mechanisms (Chesler & Kaila, 1992). For example, synaptic vesicles are generally acidic and have a pH of 5.7 (Yuste et al., 2000). Studies in hippocampal neurons have shown that the extracellular pH in the synaptic cleft temporarily drops below 6 after vesicle release in synapses showing intense synaptic activity (Miesenbock et al., 1998). Again, studies in retinal cone receptors have shown that ASICs can affect synaptic transmission (DeVries SH., 2001). It is thought that many cellular stresses in the cochlea (e.g. ischemia and inflammation) can induce local acidosis. Although the cause is not well understood, it is known that inflammation or ischemia results in sudden hearing loss (Roza et al., 2004). In this context, it is possible that ASICs may underlie some hearing loss caused by non-mechanical causes. It is also thought that ASICs can act as a sensor against harmful stimuli and may have an important role in some pathological cases. It has been shown that intercellular acidosis affects and activates ASICs, and ASICs trigger excessive excitatory activities associated with epileptic seizures and ischemia. Therefore, it is thought that ASICs play a role in the pathogenesis of these diseases (Varming T. 1999).

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**Conflict of Interest**

The authors have disclosed that they have no competing interests.

**Author Contributions**

ZC: Conceptualization, Project administration, Resources, Visualization, Data curation, Formal Analysis, Software, Resources, Writing – original draft, Writing.