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Review Article

Modulation of acid-sensing ion channels: molecular mechanisms and therapeutic potential

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Abstract: Increases in extracellular proton concentrations, which takes place in physiological conditions such as synaptic signaling and pathological conditions such as tissue inflammation, ischemic stroke, traumatic brain injury, and epileptic seizure, activates a unique family of membrane ion channels; the acid-sensing ion channels (ASICs). All ASICs belong to amiloride-sensitive degenerin/epithelial Na^+ channel superfamily. Four genes encoded at seven subunits have been identified. ASICs are expressed primarily in neurons and have been shown to play critical roles in synaptic plasticity, learning/memory, fear conditioning, sensory transduction, pain perception, ischemic brain injury, seizure, and other neurological as well as psychological disorders. Although protons are the primary activator for ASICs, the properties and/or level of expression of these channels are modulated dramatically by neuropeptides, di- and polyvalent cations, inflammatory mediators, associated proteins, and protein phosphorylations, etc. Modulation of ASICs can result in profound changes in the activities and functions of these channels in both physiological and pathological processes. In this article, we provide an up to date review on the modulations of ASICs by exogenous agents and endogenous signaling molecules. A better understanding of how ASICs can be modulated should help define new strategies to counteract the deleterious effects of dysregulated ASIC activity.

Keywords: Acid-sensing ion channel, acidosis, neuron, modulation

1. Introduction

Neuronal function is highly sensitive to variations in proton concentrations. Similar to non-neuronal cells, neurons maintain differences in extracellular vs. intracellular pH levels through various H^+ transporting mechanisms such as Na^+/H^+ and Cl^-/HCO_3^- systems (for review see [1]). In normal brain tissues, for example, extracellular pH (pH_o) is maintained at ~7.3 while intracellular pH (pH_i) is at ~7.0 [1-3]. A marked reduction of tissue pH, a condition termed acidosis, accompanies a variety of neuropathological conditions including tissue inflammation, ischemic stroke, traumatic brain injury, and epileptic seizure [2,4-11].

Changes in proton concentrations affect a variety of membrane receptors and ion channels. For example, the activity of a majority of voltage-gated and ligand-gated ion channels is inhibited by reduced pH_o, and potentiated by increased pH_o, from a baseline of 7.3 - 7.4. For example, N-methyl-D-aspartate (NMDA) receptor-gated cation channels are strongly inhibited by decreases in pH_o [12,13]. Similarly, glycine receptor-gated chloride channels are inhibited by acidic pH [14]. In addition to its modulating effect on other channels, recent studies have demonstrated that lowering pH_o can, by itself, activate a distinct family of ligand-gated cation channels, the acid-sensing ion channels (ASICs), in both peripheral sensory neurons and neurons of the central nervous system (CNS) [15-26]. This finding has shed light on acidosis-associated changes of neuronal activity and function and provided new targets for therapeutic inventions [27-33]. Although protons are the most widely studied agonist for the activation of ASICs, a recent study has identified a non-proton activator for ASIC3 [34]. Moreover, a variety of extracellular and intracellular signaling molecules can modulate the activities of ASICs (**Figure 1**). The present review will focus

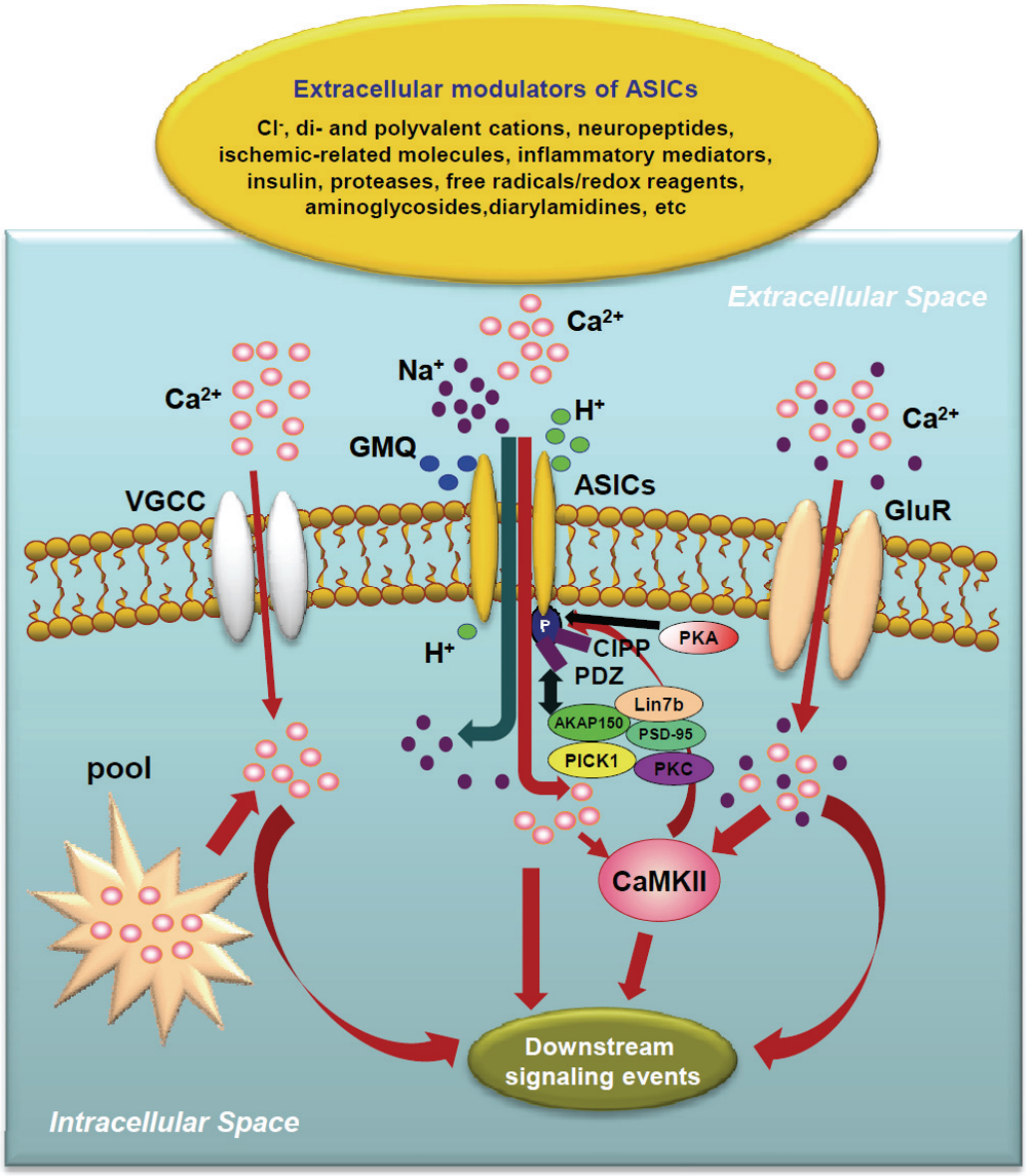

Figure 1. Modulation of ASICs by extracellular and intracellular signaling molecules. ASICs are activated by protons and non-proton molecules such as GMQ (for ASIC3 only). Activation of ASIC1a, voltage-gated calcium channels (VGCCs), glutamate receptors (GluRs) and the releases of intracellular Ca^{2+} pools increases intracellular $[Ca^{2+}]_i$. The resulting increase in $[Ca^{2+}]_i$ triggers downstream signaling events, including CaMKII activation. ASIC activity is also regulated by interactions with CaMKII, PKA, PKC, AKAP150, calcineurin, PICK1 and possibly other PDZ-domain proteins such as CIPP.

on recent advances in our understanding of the modulations of ASICs.

2. Molecular profiles of ASICs

ASICs are voltage-independent, amiloride-sensitive, cation-selective, channels which belong to the degenerin/epithelial Na^+ channel (DEG/ENaC) superfamily [35]. To date, seven subunits of ASICs (1a, 1b1, 1b2, 2a, 2b, 3, and 4) encoded by four genes have been identified [15,36]. High-level expression of 1a, 2a, 2b, and 4 has been demonstrated in CNS neurons, while all others ASICs, except ASIC4, are ex-

pressed in peripheral sensory neurons. ASIC genes are also expressed in non-neuronal tissue such as vascular smooth muscle cells [37] and bone [38].

Among all ASICs, the homomeric ASIC1a channel is of particular interest, because of its permeability to Ca^{2+}, whereas other homomeric or heteromeric ASICs are largely impermeable to Ca^{2+} [16,30,33,39]. Each ASIC subunit consists of two transmembrane domains (TM1 and TM2) and a large, cysteine rich extracellular loop, with the pre-TM2 region essential for ion permeability and the gating of these channels [16,36,40]. The crystal structure of chicken ASIC1a channels has revealed a trimeric assembly [41].

The main functions of ASICs in peripheral sensory neurons include nociception [42-48] and mechanosensation [49-51]. ASIC1a and ASIC3, for example, contribute to pain sensation. It has also been suggested that activation of ASICs is involved in taste transduction [52-54]. In CNS, ASIC1a is involved in synaptic plasticity, learning/memory, fear conditioning [19,55], and retinal physiology [56] (also see [57]). ASIC2a is required for the maintenance of retinal integrity [58], baroreceptor sensitivity [59], and survival of neurons following global ischemia [60]. It has also been demonstrated that activation or sensitization of Ca^{2+}-permeable ASIC1a channels is involved in acidosis-mediated neuronal injury [30,61-64]. Furthermore, ASICs, like ENaC, are implicated in the pathology of tumor cells such as malignant glioma [65,66].

The exact mechanisms by which ASICs provide these functions remain unclear. Under experimental conditions (e.g. in patch-clamp recordings), ASICs are activated only by rapid pH drops and the currents of most ASIC subtypes desensitize rapidly in the continuous presence of acidic pH. The questions of whether tissue pH is subject to quick fluctuations of a magnitude sufficient to activate ASICs, and whether the effects of ASIC activation could be long-lasting in vivo, would be crucial in determining the functional significance of these channels. One explanation for this latter concept, of how a transient opening of ASICs can generate a long-lasting effect, is the capacity of endogenous signaling molecules and biochemical changes associated with various pathological conditions to modulate the properties of ASICs. For example, the expression and/or activities of ASICs are dramatically enhanced after peripheral inflammation or global ischemia [61,67,68], exposure to psychological stimulants [69,70], or ischemia related signaling molecules (e.g. arachidonic acid and lactate) [71,72]. In addition, the desensitization of ASICs can be dramatically reduced by FMRFamide and related mammalian peptides (e.g. NPFF and NPSF) [73,74], dynorphin opioid peptides [75], and other endogenous signaling molecules (e.g. spermine) [76]. A detailed understanding of how ASICs are regulated by endogenous modulators is critical to our comprehension of the precise role of these channels in various physiological and pathological conditions.

3. Pharmacology of ASICs

3.1. Nonselective ASIC inhibitors

Amiloride, a previously widely used K^+-sparing diuretic agent, is a nonselective blocker of ENaC. As the member of ENaC superfamily [36], all ASICs (excluding the sustained component of ASIC3 current) are inhibited by amiloride. In general, micromolar concentrations of amiloride inhibit ASIC currents in a concentration-dependent manner [16,30]. Amiloride has also been shown to decrease ASIC-mediated increases in intracellular Ca^{2+}, and attenuate acid-induced membrane depolarization [30,33,47,77]. When combined with other pharmacological or molecular biological approaches, amiloride has been used as a pharmacological tool to identify the involvement of ASICs in physiological and pathological processes. For ASIC1a, ASIC1b, and ASIC2a subunits, the IC_{50} for amiloride inhibition is ~20 μM, whereas slightly higher concentrations of amiloride (IC_{50} of ~60 μM) are needed for the inhibition of transient ASIC3 currents [78-81]. Interestingly, the sustained component of the ASIC3 currents is completely resistant to amiloride blockade. Although the exact site(s) involved in amiloride's effect on individual ASICs remain to be determined, the pre-M2 region is known to be involved in amiloride blockade of epithelial Na^+ channels and ASICs. Mutation of Gly-430 on ASIC2a subunit, for example, dramatically alter the amiloride sensitivity [81]. Amiloride has been shown to have therapeutic effects against acid-induced pain in human [44], and protect CNS neurons against acidosis-induced neuronal damage both *in vitro* and *in vivo* [30].

A-317567, a small molecule ASIC blocker unrelated to amiloride, has recently been described

[82]. A-317567 concentration-dependently inhibits ASIC1a-like, ASIC2a-like, and ASIC3-like currents in rat dorsal root ganglion (DRG) neurons. The IC_{50} values for blocking ASIC1a-like, ASIC2a-like, and ASIC3-like currents are 2.0, 29.1, and 9.5 μM, respectively. Unlike amiloride, A-317567 blocks both the fast and sustained phases of the ASIC3-like current with equal potency. In both *in vitro* studies and *in vivo* pain models, A-317567 appears to be more potent than amiloride [82].

3.2. Subunit-specific inhibitors of ASICs

Two peptides, one derived from the venom of spiders and the other from sea anemones, have been characterized as subunit-selective ASIC inhibitors. These inhibitors are important tools for exploring the functional roles of distinct ASIC subunits in native neurons *in vitro* and *in vivo*.

Psalmotoxin 1 (PcTx1), isolated from the venom of South American tarantulas (*Psalmopoeus cambridgei*), is a specific inhibitor for ASIC1a channels [83]. It contains 40 amino acids cross-linked by three disulfide bridges. PcTx1 potently inhibits the homomeric ASIC1a current with an IC_{50} of 0.9 nM, without affecting other configurations of ASICs. Thus PcTx1 has been used to determine the presence, and function, of homomeric ASIC1a in native neurons [30,56,83,84]. PcTx1 inhibits ASIC1a channels by increasing their apparent affinity for H^+ [85], and the interaction between PcTx1 and ASIC1a depends on the state of the channel [86]. It binds tightly to the channel in open and desensitized states, thus promoting channel inactivation. The binding site for PcTx1, recently analyzed using radio-labeled tools, involves cysteine-rich domains I and II (CRDI and CRDII) of the extracellular loop [87]. Although the post-transmembrane I (M1) and pre-transmembrane II (M2) domains are not directly involved in the binding, they are crucial to the ability of PcTx1 to inhibit channel. The linker domain between CRDI and CRDII also appears to be important by contributing to the correct spatial positioning to form the PcTx1 binding site [87]. In addition to ASIC1a, PcTx1 also interacts with the ASIC1b subunit, a splice variant of ASIC1a. However, it enhances rather than inhibits the activity of ASIC1b. PcTx1 exerts its potentiation of ASIC1b at much higher concentration (>10 nM) than the concentration that inhibits ASIC1a. It binds to the ASIC1b in open state, promoting channel opening [86].

APETx2, a 42-amino-acid peptide toxin isolated from sea anemones (*Anthopleura elegantissima*), is a selective inhibitor for ASIC3 and ASIC3 containing channels [88]. Similar to PcTx1, APETx2 is cross-linked by three disulfide bonds. It belongs to the disulfide-rich all-beta structural family of peptide toxins commonly seen in animal venoms [89]. APETx2 inhibits transient ASIC3 currents with an IC_{50} of 63 nM, without affecting sustained ASIC3 currents. The affinity of this ASIC3 inhibitor, however, is decreased when ASIC3 is associated with other ASIC subunits. For example, the IC_{50} for ASIC3/ASIC2b is 117 nM, while the IC_{50} for ASIC3/ASIC1a is 2 μM. APETx2 directly inhibits the ASIC3 channel by acting at its external side [88].

4. Modulators of ASICs

Although protons were the only known activator for ASICs when they were discovered, the existence of other ASIC agonist(s) has recently been reported. Yu et al., demonstrated that 2-guanidine-4-methylquinazoline (GMQ), a small molecule containing a guanidinium group and a heterocyclic ring, causes persistent activation of ASIC3 channels at normal pH [34]. They also demonstrated that residues around E423 and E79 of the extracellular "palm" domain of the ASIC3 channels are crucial for the activation by GMQ. Consistent with ASIC3 activation, GMQ activates sensory neurons and induces pain-related behaviors in an ASIC3-dependent manner. Similarly, ASIC1a channels appear to be gated by ammonium independent of changes in proton concentration [90]. Pidoplichko and Dani reported that, in midbrain dopamine neurons and in HEK 293 cells endogenously expressing ASIC1 subunits, ASIC currents are activated by NH_4Cl at millimolar concentrations. Thus, ligands, other than protons, may activate ASICs under physiological and/or pathological conditions through nonproton ligand sensors, resulting in channel activation independent of marked acidosis.

The sensitivity of ASICs to pH_o, and their activation/inactivation kinetics, vary according to the ASIC subtypes and subunit composition of the channel complex, with pH_{50}'s ranging from 4.0 to 6.5, and activation thresholds for most ASICs (excluding ASIC2a) close to pH7.0 [16,36]. However, various factors, discussed below, can modulate ASICs and dramatically change the properties and dynamics of these channels

(**Figure 1**). These include Cl^-, di- and polyvalent cations [91-97], free radicals and redox reagents [98,99], neuropeptides [73,100,101], proteases and ischemia-related signaling molecules [71,72,102,103], intracellular accessory proteins [104-107], and protein kinases [61,84,108,109].

4.1. Extracellular Modulators

4.1.1 Cl^-

Recent crystallization of chicken ASIC1a resulted in the identification of three potential binding sites for Cl^- ions in the extracellular domain [41]. These sites are coordinated by two nearby residues (Arg-310 and Glu-314) on an α-helix of one subunit, and a third residue (Lys-212) from an adjacent subunit, all three of which are conserved among H^+-gated ASIC isoforms. The functional significance of Cl^- binding to ASIC channels, however, was not clear. Recently, Kusama and coworkers investigated the effect of Cl^- substitution on heterologously expressed ASIC1a currents and native ASIC currents in hippocampal neurons [110]. They showed that, replacement of the extracellular Cl^- ions with impermeable and inert anion methanesulfonate ($MeSO_3^-$) accelerates the rapid desensitization of the ASIC1a current but attenuates tachyphylaxis, a phenomenon of slow and gradual decrease of the current amplitude. Replacement of extracellular Cl^- ions with other anions, including Br^-, I^-, and thiocyanate, also alters the kinetics of ASIC1a desensitization and tachyphylaxis. Mutations affecting the amino acid residues that form the Cl^--binding site in ASIC1a abolish the modulatory effects associated with substituting other anions for Cl^-. The results of anion substitution on native ASIC channels in hippocampal neurons mirror those in heterologously expressed ASIC1a, and alter acid-induced neuronal death. Anion modulation of ASICs provides new insight into channel gating, and may prove important in pathological conditions associated with changes in pH and Cl^-.

4.1.2 Di- and polyvalent cations

Ca^{2+}

Divalent cations such as Ca^{2+} are important modulators of various voltage-gated and ligand-gated ion channels including ASICs. The effect of changing extracellular Ca^{2+} on ASICs depends on whether Ca^{2+} is co-applied with acidic solution or pre-applied prior to channel activation. Co-application of Ca^{2+} with acidic solution reduces ASIC currents [16,47,93,96,111]. Similarly, pretreatment followed by continuous presence of the extracellular Ca^{2+} inhibits the ASIC currents [112,113]. However, pretreatment with Ca^{2+}, prior to ASIC activation in the absence of Ca^{2+}, enhances ASIC responses [94,113]. These experiments must be performed and interpreted with caution, since a drop in Ca^{2+} itself may activate separate non-selective cation currents (i.e. non ASIC) in neurons and other cells [114,115]. Investigation into the mechanisms underlying Ca^{2+} modulation of ASICs has led to the finding that Ca^{2+} decreases the affinity of ASICs (e.g. ASIC3) for H^+ [94,96]. It has been proposed that, at a pH of 7.4, ASIC3 channels are closed because of the Ca^{2+} blockade. As the pH_0 is decreased, binding of H^+ to the channel displaces Ca^{2+} from its binding site, leading to opening of the channel [96]. For ASIC1a channels, different models have been proposed [97,111]. Paukert and colleagues showed that two negatively charged residues near the entrance of the channel pore, E425 and D432, are crucial for Ca^{2+} blockade of the ASIC1a channel [111]. They proposed that more than one Ca^{2+} binding site, one that mediates blocking and one mediates modulation, exist on the channel. Based on the data from the single-channel recordings in heterologous expression systems, Zhang et al. suggest that Ca^{2+} modulates the ASIC1 channel in an allosteric manner [97].

Zn^{2+}

A number of studies have demonstrated that extracellular Zn^{2+}, an endogenous trace element released during neuronal activity [116,117], bi-directionally modulates ASIC activities. Chu et al. showed that, at nanomolar concentrations, Zn^{2+} dose-dependently inhibits ASIC currents in cultured mouse cortical neurons [92]. In Chinese hamster ovary (CHO) cells expressing various combinations of ASIC subunits, Zn^{2+} inhibits the currents mediated by homomeric ASIC1a and heteromeric ASIC1a/ASIC2a channels, without affecting currents mediated by homomeric ASIC1b, ASIC2a, or ASIC3 channels. In addition to a reduction in the current amplitude, Zn^{2+} reduces the affinity of ASIC1a for protons. Mutation of lysine-133 in the extracellular domain of the ASIC1a subunit abolishes the high-affinity Zn^{2+} inhibition [92].

At high micromolar concentrations (>100 μM), however, Zn^{2+} also binds to low-affinity site(s) on the ASIC2a subunit, resulting in increased affinity for proton binding and a potentiation of the activity of ASIC2a containing channels [91]. In addition to increasing the current amplitude, higher concentrations of Zn^{2+} also slow down the inactivation kinetics of the ASIC currents in hippocampal neurons [93]. Similar to the effect on the ASIC current, micromolar concentrations of Zn^{2+} facilitate acid-induced membrane depolarization in cultured [91] and acutely dissociated hippocampal neurons [93]. Thus, in regions of the brain that contain large amount of Zn^{2+} (e.g. the hippocampus) where homomeric ASIC1a and heteromeric ASIC1a/ASIC2a channels are the most common configurations of ASICs [16,19,26,84], ASICs might be a physiological target of Zn^{2+}. Site-directed mutagenesis studies demonstrated that two histidine residues, His-162 and His-339 in the extracellular loop of the ASIC2a subunit, are involved in low affinity Zn^{2+} potentiation of the ASIC2a containing channels [91].

In contrast to ASIC2a containing channels, high concentrations of zinc inhibits ASIC3 channels [118,119]. In CHO cells expressing ASIC3 channels, ASIC3 currents are inhibited by pretreatment with zinc in a concentration-dependent manner with an IC_{50} of 61 μM. Zinc inhibits ASIC3 in a pH- and Ca^{2+}-independent manner and the inhibition of ASIC3 currents is dependent upon the interaction of zinc with binding sites in the extracellular domain(s) of ASIC3. Thus, at physiological concentrations, zinc is an important negative regulator of ASIC3 channels [119]. Like ASIC3, ASIC1b is inhibited by zinc with an IC_{50} of 26 μM. Cysteine 149 in the extracellular finger domain of the ASIC1b subunit is involved in this inhibition [120].

Mg^{2+}

The effect of Mg^{2+} on ASICs is comparable to that of Ca^{2+}. In a heterologous expression system, sequential application of Mg^{2+} and pH drop produces concentration-dependent and voltage-independent potentiation of ASIC1 [94]. For ASIC3, Mg^{2+} concentration-dependently reduces the channel activity when co-applied with acidic solutions, comparable to the effect of Ca^{2+} [96].

Cu^{2+}

Cu^{2+} is the third most abundant trace element in human body. A recent study has shown that this divalent cation has a modulatory effect on ASICs in cultured hypothalamic, hippocampal and cortical neurons [121]. It concentration-dependently reduces the amplitude of ASIC currents, but slows down the current desensitization. Consistent with the inhibition of ASIC current, micromolar concentrations of Cu^{2+} attenuate acid-induced membrane depolarization. Thus Cu^{2+} may represent another endogenous modulator of ASIC channels in the CNS that could negatively modulate increased neuronal excitability caused by ASIC activation.

Pb^{2+}

Pb^{2+} is a toxic metal ion that has well documented detrimental effects on the CNS. A variety of membrane receptors and ion channels, including ASICs, have been proposed to mediate the toxicity of Pb^{2+}. In acutely dissociated CNS neurons, Pb^{2+} inhibits ASIC currents in a concentration-dependent manner [113]. Pb^{2+} also decreases ASIC-mediated increases in intracellular Ca^{2+}, and attenuates acid-induced membrane depolarization. In CHO cells transfected with various ASIC subunits, currents mediated by ASIC1a, ASIC1b, and ASIC3 subunits were inhibited by Pb^{2+}. Since ASIC activation contributes to normal synaptic function, Pb^{2+} induced neurotoxicity may be partially explained by its inhibition of ASIC function.

Ni^{2+} and Cd^{2+}

Ni^{2+} and Cd^{2+}, like Pb^{2+}, are heavy metal ions that affect various membrane ion channels and receptors, and are neurotoxic. Both Ni^{2+} and Cd^{2+} reversibly inhibit the ASIC activity, in a concentration-dependent manner [122]. Ni^{2+} and Cd^{2+} also decrease the pH-sensitivity of ASICs. In CHO cells transfected with multiple ASIC subunits, 1 mM Ni^{2+} selectively inhibits homomeric ASIC1a and heteromeric ASIC1a/ASIC2a channels, whereas Cd^{2+} inhibits homomeric ASIC2a, ASIC3, and heteromeric ASIC1a/ASIC2a, ASIC1a/ASIC3, and ASIC2a/ASIC3 channels. Both the fast and the sustained phases of the ASIC3 current are sensitive to Cd^{2+} inhibition [122].

Gd^{3+}

Gd^{3+}, a trivalent metal ion, has been reported to inhibit both the fast phase and the sustained component of the currents mediated by homo-

meric ASIC3 and heteromeric ASIC2a/ASIC3 channels [95]. Since Gd^{3+} has previously been used to block stretch activated responses in neurons [123], the inhibition of ASIC3 and ASIC2a/ASIC3 currents by Gd^{3+} might suggest an involvement of ASIC3 containing channels in the transduction of mechanosensory stimuli [95].

Spermine

Spermine is a polyvalent cation whose extracellular concentration fluctuates significantly within the nervous system. Studies by Babini and colleagues showed that spermine potentiates the activities of ASIC1a and ASIC1b channels through a shift in the steady-state inactivation toward more acidic pH and stabilization of the resting state [94]. Consistent with the potentiation of ASIC1a by spemine, Duan et al., recently showed that extracellular spermine exacerbated ischemic neuronal injury through sensitization of ASIC1a channels to extracellular acidosis [76]. Pharmacological blockade of ASIC1a or deletion of the ASIC1 gene greatly reduced the enhancing effect of spermine on ischemic neuronal damage, both in cultures of dissociated neurons and in a mouse model of focal ischemia. Spermine also reduced desensitization of ASIC1a in the open state and accelerating recovery from desensitization in response to repeated acid stimulation. Functionally, enhanced channel activity was accompanied by increased acid-induced neuronal depolarization and cytoplasmic Ca^{2+} overload, which may partially explain the exacerbation of neuronal damage caused by spermine. Thus, extracellular spermine contributes to ischemic neuronal injury, at least in part, by enhancing ASIC1a activity [76].

In summary, endogenous and exogenous di- and polyvalent cations represent the largest categories of ASIC modulators. Understanding the mechanisms underlying the modulation of ASICs by these cations may provide valuable insights that can facilitate the design of novel therapeutic agents for protecting neurons against acidotoxic damage.

4.1.3 Redox reagents and free radicals

Redox status is known to affect the function of various voltage-gated and ligand-gated ion channels [124-128]. This oxidation/reduction-dependent modulation of ion channel function is important because redox status can change dramatically in physiological/pathological conditions [129-131]. Recent studies demonstrated that ASIC currents in both central and peripheral neurons are potentiated by reducing agents, and inhibited by oxidizing agents [98,99,132]. Consistent with their effects on ASIC currents, reducing agents increased, while oxidizing agents decreased, acid-induced membrane depolarization and intracellular Ca^{2+} accumulation [99]. In CHO cells expressing various ASICs, redox modulation was found to be ASIC1a selective [99]. Site-directed mutagenesis studies showed that cysteine 61 and lysine 133, located in the extracellular domain of the ASIC1a subunit, are involved in the modulation of ASICs by oxidizing and reducing agents, respectively. These data suggest that the redox status of ASIC1a subunits is important in determining the overall physiological function and pathological role of ASICs within the CNS. Given that excessive activation of ASIC1a is associated with acidosis-induced neuronal injury [30,33,61], targeting redox modulating sites of ASIC1a subunits is a tempting strategy for developing novel therapeutic agents.

The mechanism underlying the modulation of ASIC activity by redox reagents is not fully understood. Recently, Zha et al demonstrated that ASIC1a forms inter-subunit disulfide bonds and that H_2O_2 increases this link between subunits [133]. They found that Cys-495 near the C terminus of ASIC1a is particularly important for inter-subunit disulfide bond formation. Inter-subunit disulfide bond formation reduced the proportion of ASIC1a located on the cell surface, contributing to H_2O_2-induced decreases in H^+-gated currents. Thus, H_2O_2 and likely other oxidants, decrease ASIC activity by increasing formation of disulfide bonds between intracellular ASIC1a subunits, which in turn suppress their expression on the cell surface.

Nitric oxide (NO) is a short-lived mediator whose release is strongly enhanced by inflammation. NO regulates the protein function by two main pathways. An indirect mechanism involves the production of cGMP and the activation of protein kinase G. NO can also directly modify the tertiary structure of proteins by *S*-nitrosylating the thiol side-chains of cysteine residues, leading to the formation of disulfide bonds between neighboring cysteine residues [134]. Due to the

large number of cysteine residues on their extracellular loop, ASICs are a potential target by NO. Consistent with this idea, Cadiou et al., found that proton-gated currents of both sensory neurons and ASIC isoforms are potentiated by NO. Intracellular pathways modulated by NO are not involved in the enhancement of acid-gated currents, and the mechanism of the effect is instead via a direct action on the extracellular surface of the ion channel [135].

4.1.4. Neuropeptides

Dynorphin opioid peptides are abundantly expressed in the CNS. Recently, Sherwood and Askwith reported that dynorphin A and big dynorphin potentiate acid-activated currents in cortical neurons and in CHO cells expressing homomeric ASIC1a subunits [75]. The potentiation of the ASIC1a activity was mediated through a limitation of steady-state desensitization of the channel. The potentiation of ASIC1a activity by dynorphin was not mediated by opioid or bradykinin receptor activation but through a direct interaction with ASIC1a. Alteration of steady-state desensitization by dynorphins enhanced ASIC1a-triggered neuronal injury during prolonged acidosis. Thus, ASIC1a is a new non-opioid receptor target for dynorphins, and that dynorphins can enhance ischemic brain injury by preventing steady-state desensitization of ASIC1a channels.

FMRFamide and structurally related peptides are abundant in invertebrate nervous systems where they function as neurotransmitters and neuromodulators. Although FMRFamide itself has not been isolated in mammals, several FMRFamide-related peptides exist in the mammalian nervous system. FMRFamide and related peptides are generally thought to exert their physiological roles through G-protein coupled receptors [136]. However, two ionotropic receptors involved in the function of these peptides have recently been identified. FMRFamide -gated Na^+ channel (FaNaC), which is a neuronal Na^+ channel in invertebrates, is directly activated by micromolar concentrations of FMRFamide and RFamide-related peptides (RFRPs) [136]. In addition, ASICs, which share significant structure and sequence homology with FaNaC in the mammalian nervous system, can be modulated by FMRFamide and RFRPs. FMRFamide, and RFRPs such as neuropeptide FF were incapable of generating any ASIC currents on their own, but significantly potentiated ASIC currents in sensory neurons and in heterologous expression systems [73,100,101]. In addition to their effects on the amplitude of ASIC currents, FMRFamide and RFRPs also reduced the rate of current desensitization [73,100,101]. Studies using knockout mice have suggested that ASIC3 plays a major role in RFamide modulation of proton-gated currents in sensory neurons [100, 137]. In contrast, the capacity of these peptides to modulate ASIC currents appears to be only modestly dependent on the ASIC1a, and essentially independent of ASIC2a [100]. These data are consistent with the effects of FMRFamide and related peptides on homomeric ASIC1a and ASIC3 channels expressed in a heterologous system [73]. Though ASIC2a is not directly modulated by these peptides, the presence of ASIC2a subunits has been shown to enhance the capacity of these peptides to modulate both ASIC1a-containing and ASIC3-containing channels [101,138].

As a further complication, different ASICs show distinct responses to different FMRFamide related peptides. For example, FMRFamide and NPFF have a different effect on the inactivation of ASIC3 currents than neuropeptide SF [139]. Similarly, FRRFamide and FMRFamide have comparable effects on ASIC1a, but ASIC1b currents are inactivated much more rapidly by FRRFamide than by FMRFamide [73]. FMRFamide also drastically slows down the desensitization of ASIC1b/3 heteromers [140]. The potentiation of ASIC activity by endogenous FMRFamide neuropeptides likely contributes to the response of sensory and central neurons to noxious acidosis [73,136]. An extensive review of the modulation of ASIC by FMRFamide and related peptides is presently available [136].

4.1.5. Non-steroid anti-inflammatory drugs and inflammatory mediators

Decreases in tissue pH to levels well below the threshold for ASIC activation is a common feature of inflammation [10,11]. It is therefore expected that ASICs, particularly the ASIC1a and ASIC3 channels which are highly sensitive to moderate pH changes, are activated during tissue inflammation and participate in pain sensation. This notion was supported by the finding that local injection of acid activates nociceptors and produces pain in human subjects [141-144]. Moreover, acid-induced pain could be inhibited by amiloride, a non-selective ASIC

blocker [44,141,144]. Furthermore, chronic hyperalgesia induced by repeated acid injections into muscle is abolished by the loss of ASIC3 [45]. Together, these findings strongly suggest that ASIC activation contributes to the transduction of pain sensation.

Non-steroid anti-inflammatory drugs (NSAIDs) constitute a diverse group of chemicals that are commonly used to treat a variety of inflammatory conditions [27]. Although inhibition of cyclooxygenase enzymes (COXs) and consequent reductions in prostaglandin synthesis constitutes the main mechanism by which most NSAIDs inhibit inflammation, abundant evidence suggests that mechanisms other than COX inhibition contribute to their analgesic effect. For example, COX knockout mice still showed sensitivity to analgesic action of NSAIDs [145]. It is also known that both S- and R-enantimeres of flurbiprofen are effective in pain relief. Unlike the S-form, which inhibits COX and prostaglandin synthesis, the R-form inhibits prostaglandin synthesis with much lower efficiency, but retains its analgesic properties [146]. It has also been shown that diclofenac has an analgesic effect independent of COX inhibition [147], and that NSAIDs can reduce acid induced cutaneous pain in the absence of inflammation [148].

This collective evidence, in conjunction with earlier findings that protons activate depolarizing inward currents in nociceptors [42,43] and the recent discovery of ASICs as proton receptors in sensory neurons, suggests a strong link between NSAIDs and ASICs. Indeed, studies by Voilley et al demonstrated that NSAIDs directly inhibit the activity of ASICs in DRG neurons and in CHO cells at concentrations relevant to their analgesic effects [68]. Ibuprofen and flurbiprofen, for example, inhibit ASIC1a containing channels with an IC_{50} of 350 μM. Aspirin and salicylate inhibit ASIC3 containing channels with an IC_{50} of 260 μM, whereas diclofenac inhibits the same channels with an IC_{50} of 92 μM. In addition to a direct inhibition of the ASIC activity, NSAIDs largely prevent inflammation induced increases of ASIC expression in sensory neurons [68]. Thus, ASICs represent novel targets in the search for new analgesic agents.

Proinflammatory mediators such as nerve growth factor (NGF), TNF, histamine, bradykinin, serotonin, interleukin-1, substance P, and PGE2 are pro-nociceptive agents, as they sensitize nociceptive fibers [149]. Though details regarding the mechanisms underlying this sensitization remain unclear, earlier studies have demonstrated that the excitatory effects of protons on sensory neurons are potentiated by these pro-inflammatory mediators [150,151]. In addition, NGF is required for long-term up-regulation of proton-induced excitation of DRG neurons [152]. These findings suggest that the pro-inflammatory mediators may have an effect on the expression and/or activity of ASICs in nociceptors. Indeed, studies by Mamet and colleagues demonstrated that the majority of pro-inflammatory mediators stimulate the expression, and increase the activity, of ASICs [67]. At concentrations relevant to tissue inflammation, NGF, serotonin, IL-1, and bradykinin induced a dramatic (up to 10 fold) increase in ASIC1a, ASIC1b, ASIC2b, and ASIC3 expression in cultured DRG neurons [67]. Electrophysiological recordings confirmed the increase of ASIC current density in DRG neurons following the treatment with pro-inflammatory mediators [67].

4.1.6. Aminoglycosides

Aminoglycosides (AGs) are a group of antibiotics that have been shown to block Ca^{2+} channels, excitatory amino acid receptors, and transient-receptor-potential V1 channels. Recently, Garza et al demonstrated that aminoglycosides (AGs) (streptomycin, neomycin and gentamicin) have modulating effects on ASIC currents [153]. In rat DRG neurons, streptomycin and neomycin produced a reversible reduction in the amplitude of proton-gated currents in a concentration -dependent manner. In addition, streptomycin and neomycin slowed down the desensitization rates of ASIC currents. In HEK-293 cells endogenously expressing hASIC1a channels, streptomycin produced a significant reduction in the amplitude of the proton-gated current, whereas neomycin and gentamicin had no effect. Reduction of extracellular Ca^{2+} concentrations enhanced the action of streptomycin and neomycin on the desensitization of ASIC currents. These results indicate that ASICs are molecular targets for AGs, which may explain, in part, their effects on excitable cells.

4.1.7. Diarylamidines

Diarylamidines have been widely used for the treatment of protozoan diseases such as try-

panosomiasis and leishmaniasis since 1930s. Recently, Chen and colleges found that four members of the diarylamidines, 4', 6-diamidino-2-phenylindole, diminazene, hydroxystilbamidine and pentamidine strongly inhibit ASIC currents in hippocampal neurons with apparent affinities of 2.8 μM, 0.3 μM, 1.5 μM and 38 μM, respectively. Sub-maximal concentrations of diminazene also accelerate the desensitization of ASIC currents. In CHO cells expressing different ASIC subunits, diminazene blocks ASIC1a, 1b, 2a, and 3 currents with a rank order of potency 1b > 3 > 2a > 1a. This study indicates that diarylamidines constitute a novel class of non-amiloride ASIC blockers and suggests that diarylamidines as small molecules may be developed as therapeutic agents in the treatment of ASIC-involved diseases [154].

4.1.8. Proteases and ischemia-associated molecules

Neurological diseases, such as brain ischemia and neurotrauma, are associated with a variety of biochemical changes in addition to decreases in pH. Alterations in ion homeostasis (Ca^{2+}, K^+ for example), increased protein and lipid metabolism and generation of lipid metabolites (e.g. arachidonic acid), changes in the level/activity of proteases, kinases, and phosphatates, are among the common biochemical changes associated with brain ischemia. Many of these changes have been shown to modulate the activities of various voltage-gated and ligand-gated channels. For example, the activities of NMDA receptor-gated channels are modulated by protease [155], arachidonic acid [156], and protein phosphorylation [157,158]. Similarly, the activities of ASICs are modulated by ischemia-related signaling molecules.

Proteases

Brain injury is accompanied by increased protease activity [159]. Blood-derived proteases such as thrombin, tissue plasminogen activator, and plasmin can gain access to CNS interstitial spaces due to a compromised blood-brain barrier [159,160]. Previous studies have demonstrated that proteases modulate the activities of various ion channels, including ENaC, which belongs to the same family as ASICs [155,161,162]. Similarly, recent studies by Poirot and colleagues demonstrated modulation of the ASIC1a function by serine proteases [102]. The exposure of CHO cells stably expressing ASIC1a channels to trypsin or other serine proteases (e.g. proteinase K and chymotrypsin) shifted the pH dependence of activation and steady-state inactivation of the ASIC1a channels to more acidic pH values. As a consequence, protease exposure leads to a decrease in ASIC1a activity when the current is activated by a pH drop from 7.4. Interestingly, if the channel is activated from a basal pH of 7, protease exposure increases, rather than decreases, the ASIC1a activity. In addition, protease treatment dramatically accelerates the recovery rate of ASIC1a channels from desensitization [102]. The effects of proteases on ASICs appear to involve proteolysis of the channel protein, as the capacity of trypsin to modulate ASIC1a was diminished with soybean trypsin inhibitor or modification of trypsin's catalytic site with TLCK. TLCK is a small reagent that modifies irreversibly a histidine residue in the catalytic site of trypsin. Cleavage of the channel protein was confirmed by Western blot analysis showing reduction of a 64-kDa ASIC protein band to a lower molecular weight band of 49 kDa [102]. Further studies demonstrated that trypsin cleaves ASIC1a subunits at Arg-145 in the N-terminal part of the extracellular loop. The cleavage site is between a highly conserved sequence and a sequence that is critical for ASIC1a inhibition by PcTx1 [103]. Since activation of ASIC1a is involved in acidosis-mediated ischemic brain injury [30,33], modulation of ASIC1a by proteases could be relevant to its pathophysiology role in brain ischemia.

Matriptase is an 80-90-kDa type II transmembrane protease of epithelial cells that belongs to the S1 family of trypsin-like serine proteases. It is crucial for epidermal barrier formation and is involved in hair follicle growth and thymocyte development. In addition, matriptase has been implicated in many epithelial cancers. Due to the expression of ASICs in malignant tumor cells, Clark et al tested whether matriptase can modulate the activity of ASIC1 channels [163]. They showed that Matriptase decreases ASIC1 currents recorded in *Xenopus* oocytes. This effect is mediated by cleavage of ASIC1 by Matriptase. Inactivated matriptase, due to an S805A mutation, does not cleave ASIC1 and has no effect on ASIC1 currents. The effect of matriptase on ASIC1 is specific, as it does not affect ASIC2 currents. Three matriptase recognition sites have been identified in ASIC1 (Arg-145,

Lys-185, and Lys-384); site-directed mutagenesis of these sites prevents cleavage of ASIC1 by matriptase.

Arachidonic acid

Arachidonic acid (AA) is a major metabolite of membrane phospholipids, which is involved in a variety of physiological processes [164,165] and pathophysiology of several neurological disorders [165-167]. During brain ischemia, for example, the rise of $[Ca^{2+}]_i$ leads to the activation of phospholipase A2 which results in increased production of AA [165,166,168]. Earlier studies have shown that AA has effects on a variety of voltage-gated and ligand-gated ion channels [169-175]. For example, it potentiates the opening of NMDA-gated channels [156, 169,175]. Recent studies have shown that AA also enhances ASIC currents in rat cerebellar Purkinje and DRG neurons [72]. The potentiation of the ASIC currents appears to be produced by AA itself and not by its derivatives, since an agent known to block the breakdown of AA did not affect its capacity to potentiate ASIC currents [72]. The molecular mechanism for AA potentiation of ASICs is controversial. One potential explanation, similar to that proposed for NMDA channels, is that insertion of AA into the membrane induces membrane stretch and that the ASICs are stretch-sensitive [156]. This explanation is supported by the finding that perfusion of neurons with hypotonic saline, which causes cell swelling and membrane stretch, mimicked the potentiation of ASIC currents by AA [72]. Studies by Smith et al, however, suggested that AA potentiates ASIC activation by a direct mechanism [176]. They showed that inhibition of AA metabolism had no effect on the potentiation of ASIC1a, and that potentiation of single ASIC2a channels by AA could be observed in cell-free patches.

Lactate

Anaerobic metabolism of glucose during ischemia leads to increased production of lactate. Following ischemia, concentrations of lactate between 12-20 mM have been reported in the extracellular space, dramatically higher than the ~ 1 mM lactate in the normal conditions [177,178]. In sensory neurons that innervate the heart, Immke & McCleskey demonstrated that addition of 15 mM lactate dramatically increased the amplitude the ASIC current activated by a moderate pH drop to ~7.0 [71]. Applications of the same concentration of lactate at pH values that do not activate ASICs (8.0 or 7.4) caused no response. Thus, lactate acts by potentiating but not activating the ASICs. In COS -7 transfected with different subunit of ASICs, both ASIC3 and ASIC1a currents were potentiated by lactate [71]. The effect of lactate does not require second messenger or signaling cascade because the potentiation persists in excised membrane patches. Since lactate has the ability to chelate the divalent cations including Ca^{2+} and Mg^{2+}, and the concentration of extracellular divalent cations, particularly Ca^{2+}, has a modulatory role on various membrane receptors and ion channels [179-181], the authors hypothesized that potentiation of the ASICs may be due to the chelation of divalent cations. Indeed, adjusting the concentrations of Ca^{2+} and Mg^{2+} eliminated the effect of lactate, whereas reducing the divalent concentrations mimicked the effect of lactate [71]. Other monocarboxylic acids which have the divalent cation chelation ability also potentiated the ASIC current. Similar to the cardiac sensory neurons, potentiation of the ASIC current by lactate has been reported in other neurons such as cerebellar Purkinje neurons [72].

4.1.9. Insulin

Insulin is present throughout the brain. Although insulin generally functions in glucose uptake, brain insulin is involved in neuronal growth and maturation and modulation of surface expression of various ion channels and neurotransmitter receptors. Recent studies by Chai et al demonstrated that insulin also regulates membrane trafficking of ASIC1a channels [182]. In CHO cells expressing ASIC1a subunit, serum depletion induces a significant increase in ASIC1a surface expression that results in the potentiation of ASIC1a activity. Among the components of serum, insulin is identified as the key factor that maintains a low level of ASIC1a on the plasma membrane. Similarly, neurons subjected to insulin depletion increase the surface expression of ASIC1a with resultant potentiation of ASIC1a currents. In the normal condition, ASIC1a is predominantly localized to the endoplasmic reticulum. Under conditions of serum or insulin depletion, the intracellular ASIC1a is translocated to the cell surface, increasing the surface expression level [182]. These results reveal an important function of insulin in regu-

lating the trafficking of ASIC1a subunit.

4.1.10. Psychoactive drugs

The striatum is a key structure in reward circuits involved in biological actions of addictive drugs. Recent studies indicated that ASIC1a is the predominant subtype of ASICs in the medium spiny neurons of striatum [183]. Since homomeric ASIC1a channel is involved in synaptic plasticity [19], it is likely that changes in the expression and/or activity of ASIC1a channels may contribute to the synaptic modification during drug addiction. To test this possibility, Zhang et al investigated the possible effect of psychostimulatant administration on basal ASIC gene expression in the striatum and other forebrain regions *in vivo* using cocaine sensitization model [69]. They found that ASIC1, but not ASIC2, is a target of cocaine as its protein expression can be readily regulated by the drug. Chronic cocaine exposure induces a profound increase in ASIC1, but not ASIC2, protein level in the striatum (CPu and NAc), indicating a subunit-dependent modulation by cocaine. In contrast to striatum, ASIC1 and ASIC2 protein levels in other forebrain regions such as median prefrontal cortex (mPFC) and hippocampus remaine relatively stable after chronic cocaine injections, suggesting a region-specific modulation by chronic cocaine. Similar to chronic cocaine exposure, Suman et al., showed that ASIC1 protein levels is increased in the defined surface and intracellular pools in the striatum (both CPu and NAc) in chronic amphetamine treated, but not saline-treated rats [70]. ASIC2 proteins, however, remain stable in the striatum. In mPFC, repeated amphetamine administration has no effect on ASIC1 expression in either surface or the intracellular compartment. However, amphetamine selectively reduces the surface expression of ASIC2 in this region. The mechanism underlying the alternation of ASIC protein expression in response to chronic drug exposure is unclear and requires further investigation.

4.2. INTRACELLULAR MODULATORS

4.2.1. Protein kinases and phosphatases

Besides direct modulations by the extracellular factors discussed above, ASICs can be regulated by a variety of intracellular modulators (**Figure 1**). Baron et al. first showed that protein kinase C (PKC) activator OAG enhances the ASIC2a current in the presence of protein interacting with C kinase 1 (PICK1). The PICK1-dependent and PKC-induced phosphorylation does not change the kinetics, pH dependence, and the unitary conductance of ASIC2a channels but induces an increase of the channel open probability [84]. Deval et al., demonstrated that ASIC3-like currents in cultured DRG neurons are increased by PKC activator phorbol ester PDBu and pain mediators, such as serotonin, which are also known to activate PKC through their binding to G protein-coupled receptors [109]. Interestingly, PKC regulation of ASIC3 activity involves the ASIC2b subunit, since heteromultimeric ASIC3/ASIC2b channels, but not homomeric ASIC3 channels, are potentiated by PKC. The increase of ASIC3/ASIC2b current is accompanied by a shift in H^+ dose-response toward more physiological pH values. The up-regulation by PKC requires the presence of PICK1, a PDZ domain-containing protein, which interacts with C-terminus of ASIC1a, ASIC2a, and ASIC2b, but not ASIC3 [105,106, 109]. Point mutations identified involvement of Thr-40 and Ser-523 on ASIC3 subunit in PICK1-dependent PKC modulation of the ASIC3/ASIC2b channels [109]. Association with PICK1 likely provides a mechanism for selective targeting of PKCα to ASIC3 phosphorylation sites. In this process, ASIC2b subunits appear to play an essential regulatory role as a link between the PICK-1/PKC complex and the ASIC3 subunits [109].

In contrast to other subunits of ASICs, ASIC1a is not phosphorylated by PKC but by cAMP-dependent protein kinase A (PKA). Leonard et al showed that PKA phosphorylates Ser-479 of ASIC1a. Phosphorylation of ASIC1a by PKA reduces PICK1 binding and attenuates the cluster of ASIC1a in hippocampal neurons. These results suggest that the delivery of ASIC1 subunits is regulated by a combination of protein interactions and posttranslational modifications. Regulation of this interaction by phosphorylation may provide a mechanism to control the cellular localization of ASIC1 [108].

Under pathological conditions such as brain ischemia, a novel form of regulation of ASIC1a by calcium/calmodulin-dependent protein kinase II (CaMKII) has been reported [61]. Studies by Gao et al., demonstrated that global brain ischemia in rats results in an increased

phosphorylation of ASIC1a by CaMKII at Ser-478 and Ser-479 of its C-terminal. This phosphorylation sensitizes the channel to low pH, exacerbating cell death by allowing increased calcium conductance [61]. The phosphorylation is a result of activation of NR2B-containing N-methyl-D-aspartate subtype of glutamate receptors (NMDARs) and increase of intracellular Ca^{2+} during ischemia. In addition to serine/threonine phosphorylation, tyrosine kinase may also be involved in the modulation of ASIC expression/function, though not directly [184]. It has been shown that the level of basal expression of ASIC3 depends largely on the constitutive activation of trkA, the NGF-specific receptor, followed by activation of the PLC/PKC pathway [184].

4.2.2. Postsynaptic scaffolding proteins

Similar to most membrane receptors and ion channels, ASICs reside in macromolecular complexes. Several ASIC-interacting proteins have been recently identified which regulate the localization and the function of ASICs. For example, the PDZ domain of PICK1 interacts directly with the C-terminal of ASIC1a, ASIC2a and ASIC2b, but not ASIC3 [84,105,106]. Interaction with PICK1 facilitates phosphorylation of the ASIC2a subunit and the enhancement of the ASIC2a current by PKC [84], whereas the interaction between ASIC1a and PICK1 affects the cellular localization of this subunit. The synaptic localization of PICK1 and its interaction with ASICs raised the question of whether ASICs are also localized at the synapse. Indeed, co-localization of ASIC1a with PICK1 has been demonstrated at the synapses of cultured hippocampal neurons [106]. A recent study by Zha and coworkers, performed in hippocampal slices, further confirmed that ASIC1a subunit localizes at dendritic spines, the postsynaptic site of most excitatory synapses [185]. It was demonstrated that, the expression of ASIC1a affects the density of spines. Suppressing the ASIC1a expression reduces the number of spines, whereas over-expressing ASIC1a increases the number of spines. ASIC1a-dependent elevation of intracellular Ca^{2+} concentration at dendritic spines and an increased activation of Ca^{2+}/calmuduling dependent protein kinase II signaling pathway is likely responsible for the increase of the spine density [185]. Recent studies by the same authors also identified the localization of ASIC2a in dendrites, dendritic spines, and brain synaptosomes. This localization of ASIC2a at dendritic spines largely relies on ASIC2a binding to PSD-95. Through its interaction with PSD-95, ASIC2 increased the localization of ASIC1a, which does not coimmunoprecipitate with PSD-95, in dendritic spines. Thus, it appears that ASIC2a and ASIC1a subunits work in concert to regulate neuronal function [186].

The C-terminus of ASIC3 shares homology with type I PDZ-binding motifs. Channel-interacting PDZ domain protein (CIPP), which contains four PDZ domains, is reported to interact with the ASIC3 C-terminus and increase H^+-gated current [187]. The interaction with CIPP enhances the surface expression of ASIC3, and may bring together ASIC3 and functionally related proteins at the membrane of sensory neurons. Additionally, the PDZ-binding motif at the ASIC3 C-terminus also interacts with four other proteins that contain PDZ domains: PSD-95, Lin-7b, MAGI-1b, and PIST [107]. Like ASIC3, these interacting proteins are expressed in DRG and spinal cord. However, the outcome of interactions varies with different PDZ-containing proteins. For instance, co-expression with PSD-95 reduces the amplitude of ASIC3 currents, whereas expression of Lin-7b increases the current. PSD-95 and Lin-7b alter the current density by decreasing or increasing, respectively, the amount of ASIC3 expressed on the cell surface [107].

A kinase-anchoring protein 150 (AKAP150), the neuron specific rodent ortholog of human AKAP79, is present in the postsynaptic density in association with cAMP-dependent PKA, PKC, and calcineurin. These enzymes are associated with AKAP79/150 in inactive states, and the interaction with the anchoring protein has different consequences for the activity of these enzymes. Using pulldown assays and mass spectrometry, Chai and coworkers have identified AKAP150 and the protein phosphatase calcineurin as binding proteins to ASIC2a [188]. Extended pulldown and co-immunoprecipitation assays showed that these regulatory proteins also interact with ASIC1a. Transfection of rat cortical neurons with constructs encoding green fluorescent protein- or hemagglutinin-tagged channels show expression of ASIC1a and ASIC2a in punctate and clustering patterns in dendrites that co-localized with AKAP150. Inhibition of PKA binding to AKAPs by Ht-31 peptide

reduces ASIC currents in cortical neurons and CHO cells, suggesting a role for AKAP150 in association with PKA in ASIC function. They also demonstrate a regulatory function for calcineurin in ASIC1a and ASIC2a activity. Cyclosporin A, an inhibitor of calcineurin, increases ASIC currents in CHO cells and in cortical neurons, suggesting that activity of ASICs is inhibited by calcineurin-dependent dephosphorylation. These data imply that ASIC down-regulation by calcineurin could play an important role under pathological conditions accompanying intracellular Ca^{2+} overload and tissue acidosis to circumvent harmful activities mediated by these channels.

4.2.3. Intracellular pH

Along with pH_o, changes in pH_i takes place in both physiological and pathological conditions [2,6]. Although pH_i drops during ischemia in general, the degree of pH_i changes is not homogeneous across all brain regions, and an alkalization of pH_i has been demonstrated in cortical penumbra regions following focal ischemia [3]. Changing the level of pH_i has been shown previously to modulate the activities of several ion channels [189-191]. Similarly, changes in pH_i also affect the activities of ASICs. In cultured mouse cortical neurons, Wang et al., demonstrated that the overall activities of ASICs, including channel activation, inactivation, and recovery from desensitization, are tightly regulated by the level of pH_i [192]. They showed that, bath perfusion of quinine, an agent known to cause intracellular alkalization, dramatically increases the amplitude of the ASIC current. In contrast, manipulations that cause intracellular acidification, for example, addition and withdrawal of NH_4Cl [189,193] or perfusion of propionate [194,195], produce an opposite effect on the ASIC current. The effects of intracellular alkalizing/acidifying agents are also mimicked by using intracellular solutions with the pH directly buffered at high/low values. In addition to affecting the current amplitude, increasing pH_i induces a shift in H^+ dose-response curve toward less acidic pH but a shift in the steady-state inactivation curve toward more acidic pH. Furthermore, alkalizing pH_i induces an increase in the recovery rate of ASICs from desensitization. Therefore, the overall effect of decreasing pH_i is an inhibition of ASIC activity, whereas the effect of increasing pH_i is an enhancement of ASIC activity. Consistent with the changes in ASIC current, acid-induced increases of intracellular Ca^{2+} concentration, membrane depolarization, and acidosis-mediated neuronal injury are dramatically affected by the level of pH_i.

Considering the variable, non-uniform changes of pH_o and pH_i in ischemic brain [3], this study suggests that the activities of ASICs in ischemic brain and ASIC-mediated neuronal injury are non-uniform in different brain regions. In regions where the drop of pH_o is accompanied by a normal or alkaline pH_i, the activities of ASICs and ASIC-mediated cell injury would be greater than the regions where both pH_o and pH_i decrease.

5. CONCLUSION

ASICs represent new biological components in peripheral sensory and CNS neurons. Increasing evidence indicates the involvement of these channels in both physiological and pathological processes such as nociception, mechanosensation, taste transduction, synaptic plasticity, learning/memory, and acidosis-mediated neurodegeneration. By combining biochemical, molecular biological, structural, and functional studies, much progress has been made in recent years in the comprehension of the mechanisms by which extracellular and intracellular signaling molecules can affect the ASIC activity [196]. Although in most electrophysiological recordings ASIC responses appear to be transient in nature, various biochemical changes, largely occur in pathological conditions such as brain ischemia, dramatically enhance the amplitude, reduce the desensitization, or increase the recovery of ASICs from desensitization. Delineating the mechanisms underlying the modulation of ASICs by signaling molecules is, with no doubt, critical for understanding the physiological/pathological roles of these novel channels and for establishing future therapeutic interventions.

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