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Acid-Sensing Ion Channels (ASICs) as Pharmacological Targets for Neurodegenerative Diseases

Zhi-Gang Xiong[1], **Giuseppe Pignataro**[2], **Minghua Li**[1], **Su-youne Chang**[1], and **Roger P. Simon**[1]

1*Robert S. Dow Neurobiology Laboratories, Legacy Research, Portland, OR 97232, USA*

2*Department of Pharmacology, School of Medicine, University of Naples Federico II, Via Sergio Pansini 5, 80131 Naples, Italy*

Abstract

A significant drop of tissue pH or acidosis is a common feature of acute neurological conditions such as ischemic stroke, brain trauma, and epileptic seizures. Acid-sensing ion channels, or ASICs, are proton-gated cation channels widely expressed in peripheral sensory neurons and in the neurons of the central nervous system. Recent studies have demonstrated that activation of these channels by protons plays an important role in a variety of physiological and pathological processes such as nociception, mechanosensation, synaptic plasticity, and acidosis-mediated neuronal injury. This review provides an overview of the recent advance in electrophysiological, pharmacological characterization of ASICs, and their role in neurological diseases. Therapeutic potential of current available ASIC inhibitors is discussed.

Introduction

A stable pH is critical for normal cellular function [1]. In physiological conditions, extracellular pH (pH_o) and intracellular pH (pH_i) are maintained at ~7.3 and ~7.0 through various H^+ transporting mechanisms [1]. In pathological conditions such as tissue inflammation, ischemic stroke, neurotrauma, and epileptic seizure, accumulation of lactic acid due to enhanced anaerobic glucose metabolism and the release of H^+ from ATP hydrolysis result in marked reduction of tissue pH, a condition termed acidosis. During severe ischemia, for example, brain pH can drop to as low as 6.0 [2].

Changes in pH_o have profound influence on the physiology of neurons [3], and in pathological conditions affect the outcome of neuronal injury [4]. Mild acidosis, for example, has been reported to reduce excitatory injury of neurons [5] likely due to proton inhibition of NMDA channels. Severe acidosis, on the other hand, induces neuronal injury [4,6]. For decades, the entity or receptor that detects pH_o changes surrounding neurons and its signal transduction pathway remained elusive. The recent finding that a fall of pH_o activates a distinct class of cation channels, the acid-sensing ion channels (ASICs), in peripheral sensory neurons and in the neurons of the central nervous system, dramatically changed the view of acid signaling and offered new pharmacological targets for neurological diseases [7-12].

Correspondence Author: Xiong, Zhi-Gang (zxiong@Downeurobiology.org).

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Molecular organization of ASICs

Since the first subunit was cloned 10 years ago [13], six ASIC subunit proteins, encoded by four genes, have been identified: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4. All ASICs belong to the degenerin/epithelial Na^+ channel (DEG/ENaC) superfamily, which are Na^+-selective cation channels sensitive to amiloride [13,14]. Though not exclusively, ASICs are highly expressed in peripheral sensory neurons and in the neurons of the central nervous system. In the peripheral sensory system, ASICs are enriched in dorsal root ganglion and trigeminal ganglion, whereas in the brain, high level of ASICs are expressed in cerebral cortex, cerebellum, hippocampus, amygdala, and olfactory bulb [13,15,16].

Based on the biochemical analysis of ENaC [13,17] and the glycosylation studies of ASIC2a subunits [18], the proposed membrane topology of each ASIC subunit consists of two transmembrane domains (TM I and TM II), linked by a large extracellular cystein-rich loop, and intracellular N and C termini. Functional ASICs are believed to be tetrameric assemblies of homomeric or heteromeric subunits [19] (Figure 1). However, based on the stoichiometric studies of ENaC, the possibility that ASICs are assembled with four to nine subunits cannot be excluded [20,21].

Tissue distribution and electrophysiological properties of ASICs

Tissue distribution of individual ASICs has been studied using in situ hybridization, immunohistochemistry, and electrophysiology, whereas the properties of individual ASICs have been analyzed largely in herterologous expression systems and by gene knockout approaches. ASIC1a is widely expressed in the neurons of peripheral sensory and the central nervous system [13,16,22]. Homomeric ASIC1a channels respond to low pH_o by mediating a fast and transient inward current with a threshold pH of ~7.0, and the pH for half maximal activation ($pH_{0.5}$) at ~6.2 [13]. In one study, $pH_{0.5}$ of 6.8 has been demonstrated [23]. In addition to conducting Na^+ ions, homomeric ASIC1a channels are permeable to Ca^{2+} ions [10,12,13]. ASIC1b (ASIC1β) is a splice variant of ASIC1a with restricted expression in sensory neurons [24]. Homomeric ASIC1b channels respond to pH_o drop with a similar transient current and a $pH_{0.5}$ of ~5.9 [24,25]. Unlike ASIC1a, homomeric ASIC1b channels do not show Ca^{2+} permeability. ASIC2a is expressed broadly in peripheral sensory and CNS neurons. Homomeric ASIC2a channels have a low sensitivity to protons with a $pH_{0.5}$ of ~4.4 [26,27]. ASIC2b is a splice variant of ASIC2a. Though widely expressed in peripheral sensory and central neurons, ASIC2b subunits do not form functional homomeric channels. However, they may associate with other subunits to form heteromeric ASICs with distinct properties [26]. ASIC3 is predominantly expressed in dorsal root ganglia [28]. Homomeric ASIC3 channels respond to pH_o drops by a biphasic response with a fast desensitizing current followed by a sustained component [28,29]. These channels have a high sensitivity to protons and a $pH_{0.5}$ of 6.7 has been reported [29]. ASIC4 subunits show high level of expression in pituitary gland. Similar to ASIC2b, they do not seem to form functional homomeric channels [30,31].

Although the exact subunit combination and stoichiometry of ASICs in native neurons remain to be determined, the relative contributions by ASIC1a, ASIC2a, or ASIC3 subunit to acid-evoked currents in peripheral sensory and CNS neurons have been examined [10,22,23,32]. In medium-sized DRG neurons, for example, acid-activated currents match those recorded from heterologous cells expressing a mix of ASIC1, ASIC2, and ASIC3 subunits [23]. Deletion of any one subunit did not abolish acid-activated currents, but altered currents in a manner consistent with heteromultimerization of the two remaining subunits, indicating that combinations of two or more ASIC subunits co-assemble as heteromultimeric channels in mouse DRG neurons [23]. In cortical and hippocampal neurons, however, knockout of ASIC1 gene alone almost completely eliminated the acid-activated current [10,22,32], suggesting that

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ASIC1a is a predominant functional ASIC subunit in CNS neurons. Further studies suggest that the acid-activated currents in CNS neurons are largely mediated by a combination of ASIC1a homomeric channels and ASIC1a/ASIC2a heteromeric channels [32,33]. ASIC1a is key in establishing the current amplitude. ASIC2a, on the other hand, has little effect on the amplitude but influences desensitization, recovery from desensitization, and pH sensitivity of the channels [32].

Activation of ASICs induces membrane depolarization and increased intracellular Ca^{2+} in neurons

Since all ASICs are Na^+-selective channels which have a reversal potential near Na^+ equilibrium potential (~ +60 mV), activations of ASICs at normal resting potentials produce exclusively inward currents which result in membrane depolarization and the excitation of neurons [33,34]. For homomeric ASIC1a channels, acid activation also induces Ca^{2+} entry directly through these channels [10,12,13]. In addition, the ASIC-mediated membrane depolarization may facilitate the activation of voltage-gated Ca^{2+} channels and NMDA receptor-gated channels [22], further promoting neuronal excitation and $[Ca^{2+}]_i$ accumulation.

The Ca^{2+}-permeability of ASICs in CNS neurons have been characterized using fluorescent Ca^{2+} imaging and ion-substitution protocols [10,12]. In mouse cortical and hippocampal neurons, activation of ASICs by decreasing pH_o induces increases of $[Ca^{2+}]_i$. This acid-induced increase of $[Ca^{2+}]_i$ could be recorded in the presence of a cocktail blocking other voltage-gated and ligand-gated Ca^{2+} channels [10], indicating Ca^{2+} entry directly through ASICs. The acid-induced increase of $[Ca^{2+}]_i$ is eliminated by specific and non-specific ASIC1a blockade, or by ASIC1 gene knockout [10,12]. Consistent with the finding of fluorescent imaging, acid-activated inward current is activated when extracellular solution contains Ca^{2+} as the only conducting cation [10]. Thus, homomeric ASIC1a channels constitute an additional and important Ca^{2+} entry pathway for neurons.

ASIC1a activation in acidosis-mediated and ischemic neuronal injury

During ischemia, increased anaerobic glycolysis due to reduced oxygen supply leads to lactic acid accumulation [2]. Accumulation of lactic acid, alone with increased H^+ release from ATP hydrolysis, causes a decrease in brain pH, or acidosis. During brain ischemia, pH_o falls to 6.5 or lower [2,35].

Acidosis has long been recognized to play an important role in ischemic brain injury [36,37]. However, the cellular and molecular mechanism remained unclear. The widespread expression of ASIC1a in the brain, its activation by pH drop to the level commonly seen in ischemic brain, and its demonstrated permeability to Ca^{2+} strongly suggested that activation of ASIC1a might be involved in the pathology of brain injury. Indeed, a series of recent studies have clearly demonstrated a role for ASIC1a activation in acidosis-mediated and ischemic brain injury [10,12,38,39]. In cultured mouse cortical neurons, activation of ASICs by brief acid incubation induces glutamate receptor-independent Ca^{2+}-dependent neuronal injury that is inhibited by specific and non-specific ASIC1a blockade, and by ASIC1 gene knockout [10]. Reducing $[Ca^{2+}]_o$, which lowers the driving force for Ca^{2+} entry through ASICs, also decreases the acid-induced neuronal injury. Intracerebroventricular injection of an ASIC1a blocker in rodents reduces the infarct volume induced by transient or permanent focal ischemia by up to 60% [10,39]. Similarly, ASIC1 gene knockout produces significant neuroprotection in vivo [10]. The protection by ASIC1a blockade has an time window of efficacy of up to 5 hours, and the protection persists for at least 7 days [39]. Attenuating brain acidosis by intracerebroventricular administration of $NaHCO_3$ is also protective, further suggesting that acidosis is the effector of injury.

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Since activation of NMDA receptors and subsequent Ca^{2+} toxicity has been known to play an important role in ischemic brain injury, the outcome of co-application of both blockers has also been investigated. Compared to ASIC1a or NMDA blockade alone, co-application of NMDA and ASIC blockade produces additional neuroprotection, and the presence of ASIC1a blockade prolongs the time window of effectiveness of NMDA blockade [39]. Therefore, Ca^{2+}-permeable ASIC1a represents a novel pharmacological target for ischemic brain injury.

ASIC activation and epileptic seizure activity

A significant drop of brain pH during intense neuronal excitation or seizure activity [55-58] suggests that ASIC activation might play a role in the generation/maintenance of epileptic seizures. In a cell culture model of epilepsy, brief withdrawal of the NMDA antagonist kynurenic acid induces a dramatic increase in the firing of action potentials, in addition to a sustained membrane depolarization [1]. ASIC blockade by amiloride and the selective ASIC1a blocker PcTX1 significantly inhibits the increase of neuronal firing and the sustained membrane depolarization (SY Chang et al, abstract in *Soc Neurosci Abstr* 2007, 257.5). In hippocampal slices, high frequency electrical stimulation or removal of extracellular Mg^{2+} triggers spontaneous seizure-like bursting. Bath perfusion of amiloride and PcTX1 decrease the amplitude and the frequency of these seizure-like bursting activities. Slices prepared from the brains of ASIC1a knockout mice demonstrate reduced sensitivity to low $[Mg^{2+}]_o$-induced or stimulation-evoked seizure activities. In an *in vivo* model of epilepsy, intra-amygdala injection of kainic acid induces sustained polyspike activity on EEG followed by dramatic injury of CA3 neurons. In this model, intracerebroventricular injection of PcTX1 reduces both the electrographic seizure activity and the CA3 neuronal injury (SY Chang et al, abstract in *Soc Neurosci Abstr* 2007, 257.5). Together, these data suggest that activation of ASICs, particularly the ASIC1a channels, is involved in the generation or maintenance of seizure activity and resultant seizure-mediated neuronal injury.

Pharmacological characterization of ASICs

Amiloride

Amiloride, a diuretic agent known to block Na^+/H^+, Na^+/Ca^{2+} exchangers and ENaC [40,41], is a non-specific blocker for ASICs. It reversibly inhibits the ASIC currents with an IC_{50} of 10 - 50 μM [10,13,24,28,42]. Similar to its effect on the ASIC currents, amiloride inhibits acid-induced increase of $[Ca^{2+}]_i$ and membrane depolarization [10,12,43,44]. The sustained component of the ASIC3 current, on the other hand, is much less or completely insensitive to amiloride [28,45]. A recent study has shown that in cardiac sensory neurons, the small sustained ASIC3-like current activated at pH 7.0 is in fact increased by amiloride [46].

Based on the studies of ENaC [47], it is believed that amiloride inhibits the ASIC current by a direct blockade of the channel, and that the pre-TM II region of the channel is critical for its effect. Mutation of Gly-430 in this region, for example, dramatically increases the sensitivity of ASIC2a current to amiloride [48].

Therapeutic potential—In peripheral sensory system, amiloride has been shown to suppress acid-induced pain [49-52], whereas in CNS neurons, it reduces acid-mediated and ischemic neuronal injury [10,12]. However, due to its nonspecificity for various ion channels and ion exchange systems, it has low potential to be used as a future analgesic or neuroprotective agent in human subjects. In a lower or similar concentration range to that inhibits ASICs, amiloride also blocks other ion channels (e.g. ENaC, T-type Ca^{2+} channels) and ion exchange systems (Na^+/H^+, Na^+/Ca^{2+} exchanger). Recent studies have suggested that the normal activity of Na^+/Ca^{2+} exchanger, for example, is critical for maintaining cellular Ca^{2+} homeostasis and the survival of neurons against delayed calcium deregulation and injury

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caused by glutamate receptor activation [53]. Conversely, inhibition of Na^+/Ca^{2+} exchanger by amiloride is expected to compromise normal neuronal Ca^{2+} handling, which may transform the Ca^{2+} transient elicited by non-toxic glutamate concentrations into a lethal Ca^{2+} overload. The inhibition of Na^+/Ca^{2+} exchanger by amiloride may partially explain its reduced neuroprotective efficacy *in vivo* compared with the ASIC1a-specific blocker PcTX1 [10]. It may also explain the finding that prolonged incubation with amiloride (e.g. 5h) itself induces injury of cultured mouse cortical neurons [10]. Although amiloride itself is unlikely to be used as a neuroprotective agent for neurological conditions, one direction for future drug development could be to chemically modify the structure of amiloride (Figure 2) to achieve a molecule that specifically blocks ASICs, or at least has more selectivity for these channels.

A-317567

A-317567, a small molecule unrelated to amiloride (Figure 2), is a new non-selective ASIC blocker [52]. It inhibits the ASIC1a-like, ASIC2a-like, and ASIC3-like currents in rat DRG neurons with IC_{50} of 2 - 30 μM. Unlike amiloride, A-317567 blocks both the fast and the sustained components of the ASIC3-like currents. In a rat thermal hyperalgesia model, A-317567 is fully efficacious at a dose 10-fold lower than amiloride. It is also effective in a skin incision model of post-operative pain. A-317567 does not show diuresis or natriuresis activity [52], suggesting that it is more specific for ASICs than amiloride.

Therapeutic potential—Compared with amiloride, A-317567 appears to have better potential to be established as a future analgesic agent. Its inhibition of sustained ASIC3 current suggests that it might be useful in suppressing acidosis-mediated chronic pain. Currently, it is unknown whether A-317567 is also an effective neuroprotective agent. Future study would be helpful to demonstrate whether it is efficient in reducing acidosis-induced neuronal injury in cell culture models and infarct volume in animal models of ischemia. When applied peripherally, A-317567 shows minimal brain penetration in normal conditions [52]. It would be interesting to know whether it can reach the brain in pathological conditions (e.g. ischemia) where blood-brain-barrier may have been compromised. Additional studies may be helpful to elucidate the mechanism underlying A-317567 inhibition of ASIC activity and the binding site (s) where it interacts with the channels. It is also important to know whether A-317567 affects the activities of other ion channels and ion exchanger systems.

PcTX1

Psalmotoxin 1 (PcTX1) is a peptide toxin which specifically inhibits the ASIC1a current [54]. It was isolated from the venom of South American tarantula *Psalmopoeus Cambridge*. The toxin contains 40 amino acids cross-linked by three disulfide bridges (Figure 3). In heterologous expression systems, PcTX1 potently and specifically inhibits the acid-activated current mediated by homomeric ASIC1a subunits in a nanomolar concentration range (IC_{50} < 1 nM), without affecting the currents mediated by other configurations of ASICs [54]. At concentrations that effectively inhibit the ASIC1a current, it has no effect on voltage-gated Na^+, K^+, Ca^{2+} channels, nor an effect on several other ligand-gated ion channels tested [10]. Thus, PcTX1 is so far the best known specific blocker for ASICs and an indispensable pharmacological tool for the studies of ASIC1a-mediated processes [10,54-56].

Unlike amiloride which directly blocks the channel, PcTX1 acts as a gating modifier. It shifts the channel from its resting towards the inactivated state through an increase of its apparent affinity for protons [57]. Interestingly, this PcTX1-induced shift of the pH-dependent inactivation of ASIC1a is Ca^{2+}-dependent, where increasing extracellular Ca^{2+} results in a decrease of the PcTX1 inhibition [57]. This finding implies that, in neurological conditions (e.g. brain ischemia) where a significant drop of extracellular Ca^{2+} concentration occurs [58, 59], the potency for PcTX1 inhibition of the ASIC1a channels would increase.

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The binding site for PcTx1 has recently been analyzed using radiolabelled tools [60]. It binds principally on cysteine-rich domains I and II (CRDI and CRDII) of the extracellular loop. Although the post-transmembrane domain I and pre-transmembrane domain II regions are not involved in the binding, they are crucial for the ability of PcTx1 to inhibit the ASIC1a current [60].

Therapeutic potential—Targeting Ca^{2+} permeable ASIC1a by intracerebroventricular administration of PcTX1 has been demonstrated to be effective in reducing ischemic brain injury in rat and mouse models of ischemia [10,39]. A preliminary study also suggested that blocking these channels is effective in suppressing epileptic seizure activity and seizure-induced neuronal injury (SY Chang et al, abstract in *Soc Neurosci Abstr* 2007, 257.5). In mouse models of focal ischemia, it was demonstrated that blocking ASIC1a by PcTX1 has a neuroprotective time window of 5h [39]. These findings suggest that a specific ASIC1a blocker is useful as a neuroprotective agent for various neurological diseases (Figure 4). However, as discussed below, PcTX1 itself may not be an ideal pharmacological agent for human subject: (1) PcTX1 consists of 40 amino acids linked by three disulfide bonds. Synthesis of this toxin in a large quantity might be a challenge for the pharmaceutical companies. (2) Due to the presence of three disulfide bonds, which are subjected to modification by oxidizing/reducing conditions, the long-term stability of the toxin could be an issue. (3) The large molecule of this toxin (~5 Kd) makes it difficult to across the blood-brain-barrier (BBB) thus preventing its use by conventional routine of administration (e.g. i.v. or i.p.). Indeed, it has not been demonstrated in animal studies that a peripheral administration of this toxin (i.v.) is sufficient to reduce ischemic brain injury [39]. Future efforts may consider making shorter or truncated peptides which can pass BBB, have long-term stability, but still block the ASIC1a channels. Additional efforts should focus on the search for new small molecules that specifically blocks the ASIC1a channels. Since activation of ASIC1a is also involved in the processes of learning, memory and fear [22,56], prolonged blockade of these channels in patients may induce some behavior changes.

APETx2

APETx2 is a 42 amino-acid peptide toxin isolated from sea anemone Anthopleura elegantissima. It is a potent and selective inhibitor for homomeric ASIC3 and ASIC3 containing channels [61]. It reduces transient peak acid-evoked currents mediated by homomeric ASIC3 channels in heterologous expression systems and in primary cultures of sensory neurons [61]. In contrast to the peak ASIC3 current, the sustained component of the ASIC3 current is insensitive to APETx2. In addition to homomeric ASIC3 channels (IC_{50} = 63 nM for rat and 175 nM for human), APETx2 inhibits heteromeric ASIC3/1a (IC_{50} = 2 μM), ASIC3/1b (IC_{50} = 900 nM), and ASIC3/2b (IC_{50} = 117 nM). Homomeric ASIC1a, ASIC1b, ASIC2a, and heteromeric ASIC3/2a channels, on the other hand, are not sensitive to APETx2. Similar to PcTX1, APETx2 is cross-linked by three disulfide bonds (Figure 3)[61]. However, it does not show any sequence homologies with PcTX1. At present, the mode of action for APETx2 is still unknown.

Therapeutic potential—Since activation of ASIC3 has been implicated in various pain processes [46,51,62,63], APETx2 may be a useful analgesic agent in the treatment or prevention of pain in peripheral sensory system. However, its lack of inhibition of sustained ASIC3 current suggests that it may not be effective in suppressing the chronic pain stimuli. It is also unknown whether it inhibits inflammation-induced increased expression of ASICs. The lack of inhibition of ASIC expression, and likely the COXs activity, would suggest that APETx2 will not be as effective as NSAIDs (see below) in suppressing the pain by tissue inflammation.

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Non-steroid anti-inflammatory drugs

Non-steroid anti-inflammatory drugs (NSAIDs) are the most commonly used anti-inflammatory and analgesic agents. The well accepted mechanism for the effect of NSAIDs is the inhibition of the synthesis of prostaglandins (PGs), a main tissue inflammatory substance. However, exceptions to the correlation of PG synthetase inhibition with anti-inflammatory activity have been noted, suggesting additional mechanism(s) may be involved. A recent study demonstrated that various NSAIDs also inhibit the activity of ASICs at therapeutic doses for analgesic effects [64]. 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 only 92 μM. In addition to a direct inhibition of the ASIC activity, NSAIDs also prevent inflammation induced increase of ASIC expression in sensory neurons [64].

Therapeutic potential

Tissue acidosis is a common feature of many painful states. During tissue inflammation, ischemia, and infection, or in tumors, hematomas, and blisters, pH_o can drop from 7.4 to as low as 5.0 [65,66]. The combined inhibition of NSAIDs on PG synthesis, ASIC currents, and ASIC expression make them ideal for a large spectrum of pain conditions, particularly the pain caused by tissue inflammation. In the acute phase of tissue inflammation, for example, the rapid inhibition of ASIC currents by NSAIDs blocks the activation of pain-sensing neurons by inflammatory acidosis. Later, the NSAIDs suppress the inflammation and pain by their effect on COXs, limiting the production of prostaglandins. In the chronic phase, they may reduce the sensitization to pain by combined inhibition of COXs, ASIC currents, and ASIC expression.

Concluding Remarks

ASICs represent new biological components and therapeutic targets in peripheral sensory and CNS neurons. Increasing evidence supports the involvement of ASIC activation in physiological processes such as synaptic plasticity, and in neurological diseases such as brain ischemia and epileptic seizures. On-going studies are expected to identify the involvement of ASIC activation or changes in ASIC expression in other physiological processes and neurological disorders. Future development of potent and specific blockers for individual ASIC subunits will dramatically advance our understanding of the role of these channels in physiological and pathological processes, and for establishing novel therapeutic strategies for neurological diseases. In addition to channel blockers/inhibitors discussed here, alternative therapeutic strategies may consider targeting endogenous signaling molecules/proteins which closely modulate the activities of ASICs [67], for example, A kinase-anchoring protein 150 [68].

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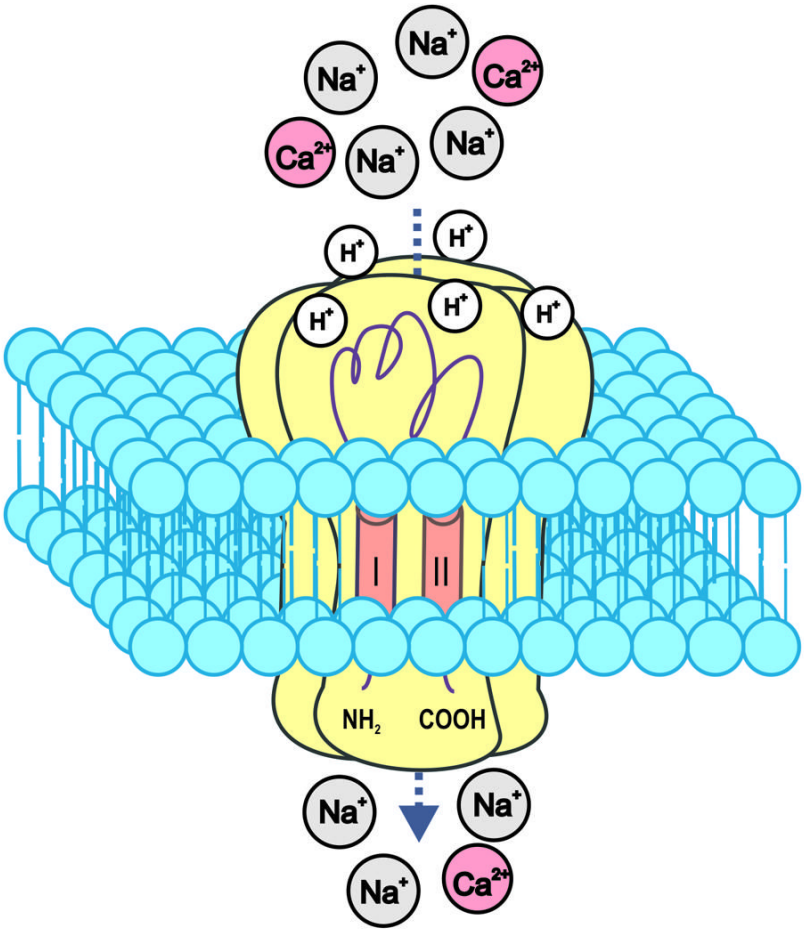

Figure 1.

Proposed tetrameric structure of ASICs. Each channel is assembled by 4 identical or different subunits. Each subunit consists of two transmembrane domain (I & II) linked by large cycteine rich extracellular domain with intracellular N- and C- termini. For homomeric ASIC1a channels, activation of the channels by H^+ binding induces entry of Na^+ and Ca^{2+} ions.

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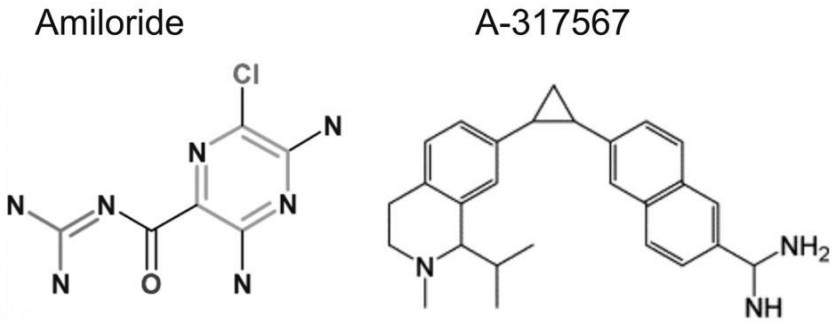

Figure 2.
Chemical structure of amiloride and A-317567.

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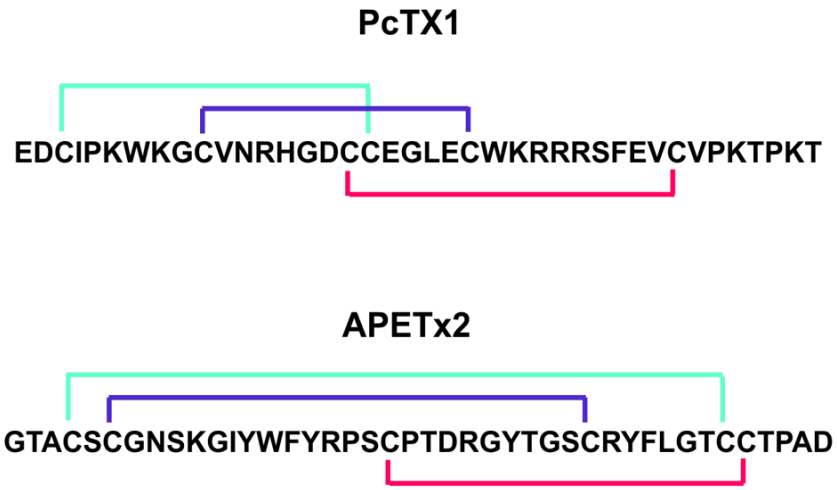

Figure 3.
Amino acid sequence and disulfide linkage for PcTX1 and APETx2.

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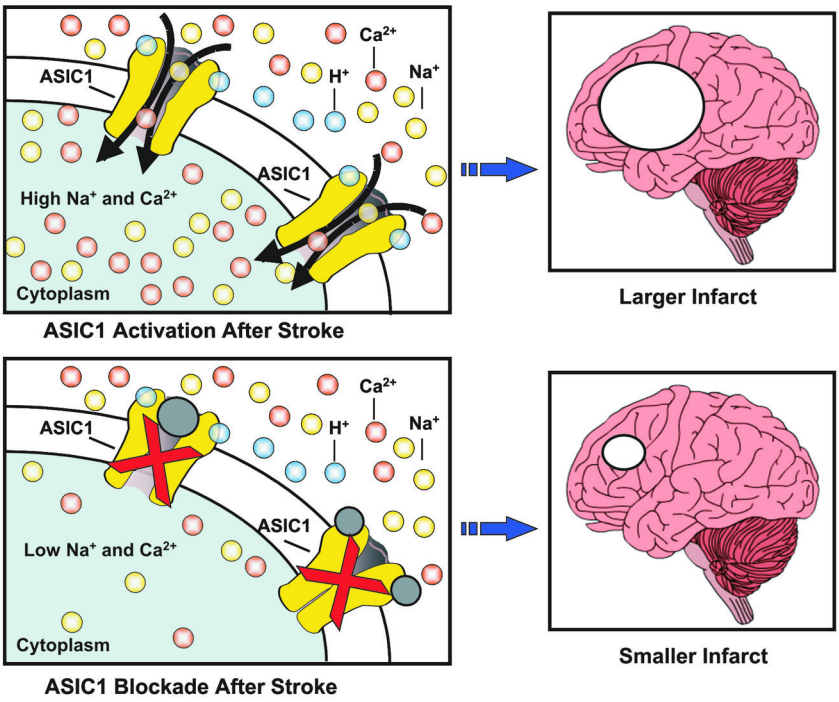

Figure 4.

Simplified diagram demonstrating the role of ASIC1a activation in ischemic neuronal injury and the neuroprotection by ASIC1a blocker/inhibitor. Upper left panel represents neurons in ischemic conditions where the concentration of extracellullar protons is high. Binding of protons opens the channels resulting in large influx of Na^+ and Ca^{2+} ions. Overloading neurons with Ca^{2+} induces neuronal injury and large infarct volume of the brain (upper right panel). Lower left panel represents neurons in ischemic conditions but in the presence of ASIC blocker or inhibitor. Influx of Na^+ and Ca^{2+} ions are reduced due to a direct blockade of the channel (e.g. by amiloride) or an alteration of channel gating (e.g. by PcTX1), resulting in neuroprotection and small infarct volume (lower right panel).