Acid-sensing ion channels under hypoxia

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Keywords: acid-sensing ion channel, hypoxia, pain, calcium ion, proinflammatory factor, tumour, cell injury

Abbreviations: ASIC, acid-sensing ion channel; NSAID, non-steroidal anti-inflammatory drugs; PcTx1, Psalmotoxin 1; APETx2, Anthopleura elegantissima toxin 2; DC, dendritic cell; OC, osteoclast; OB, osteoblast; CNS, central nervous system; PNS, peripheral nervous system; DRG, dorsal root ganglion; CA, carbonic anhydrase; HIF-1, hypoxia-induced factor-1; CO₂, carbon dioxide; AA, arachidonic acid; NO, nitric oxide; IL-1, interleukin-1; PLA₂, phospholipases A₂; GBM, glioblastoma; RGC, retinal ganglion cell; AKAP150, a kinase-anchoring protein 150; NHERF-1, Na⁺/H⁺ exchanger regulatory factor-1; CIPP, channel-interacting PDZ domain protein

Hypoxia represents the lack of oxygen below the basic level, and the range of known channels related to hypoxia is continually increasing. Since abnormal hypoxia initiates pathological processes in numerous diseases via, to a great degree, producing acidic microenvironment, the significance of these channels in this environment has, until now, remained completely unknown. However, recent discovery of acid-sensing ion channels (ASICs) has enhanced our understanding of the hypoxic channelome. They belong to the degenerin/epithelial Na⁺ channel family and function once extracellular pH decreases to a certain level. So does the ratiocination emerge that ASICs participate in many hypoxia-induced pathological processes, including pain, apoptosis, malignancy, which all appear to involve them. Since evidence suggests that activity of ASICs is altered under pathological hypoxia, future studies are needed to deeply explore the relationship between ASICs and hypoxia, which may provide a progressive understanding of hypoxic effects in cancer, arthritis, intervertebral disc degeneration, ischemic brain injury and so on.

Introduction

Oxygen homeostasis is a vital principle for understanding metazoan physiology and pathology, while metazoan life relies on the utilization of O₂ for basic metabolic processes. The physiological O₂ concentration to which cells in our body are exposed varies from ~1% to ~21%. Researchers usually maintain cultured cells in 20% O₂ and make this concentration as "normoxia" in spite of the fact that lots of cells in our body are exposed to much lower O₂ levels. Hypoxia is defined as a reduction in the O₂ level available to our body. So, it is a relative term. Hypoxia includes two basic phases: one is "acute phase," in which transient responses are mediated via modification of existing proteins, and another one is chronic phase, in which durable changes are mediated by the changes in gene transcription and protein translation. What’s more, hypoxia can also be systemic.

Local or systematic inflammation is characterized by alteration in the supply and demand of metabolites that results in the lack of O₂, and we call it inflammation-associated hypoxia.3,4 Researches of ambient hypoxia have offered strong evidence that hypoxia itself is able to induce a upregulation in many inflammatory factors. In a word, hypoxia represents a distinctive feature of inflammation, in which cancers are included. Direct evidence of the significance of hypoxia in cancers has been shown most convincingly by the work of Peter Vaupel and colleagues, whose results indicated that hypoxia in tumors was associated with increased malignancy in patients suffering from squamous tumors of the head and neck or breast cancers. Besides cancers, some ischemia-related diseases including cerebral infarction are also greatly characterized by hypoxia, in which cell apoptosis may lead to an unfavorable prognosis.

In the hypoxia-related regulation mechanism, ion channels play a great part, which was gradually accepted after the report in 1988 demonstrated that rabbit carotid body glomus cells could express the O₂-sensitive potassium channel. Since that precursory description of an O₂-related ion channel, more and more work has been produced demonstrating that numerous ion channels spread across the different ion channel families accept the regulation from O₂ and exhibit changes in activity with hypoxia and shifts in channel quantity with prolonged hypoxic challenge.9

Increase in glycolysis and lactate production under hypoxic conditions result in acidosis, which is the most important characteristics of hypoxia.10 When there is a group of ion channels that could be greatly affected by pH, we will conclude the following: these channels will enlarge the channel spectrum related to hypoxia and show us a strategy of clinical treatment for diseases greatly characterized by hypoxia.

An Overview of the Acid-Sensing Ion Channels

Acid-sensitive ion currents were first reported in sensory neurons, and it has been known that the acid-sensitive ion channels
ASICs, to a great degree, were responsible for this current. ASICs, which are sensitive to Amiloride and voltage-independent, belong to degenerin/epithelial Na+ channel family. In addition to Na+, ASICs are also permeable to K+, Li+, Ca2+ and H+. Six subunits have been identified: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4 (Table 1), forming homomeric or heteromeric channel complexes. They share a similar structure with approximately 500 amino acids in sequences arranged into two transmembrane domains, a large extracellular loop (350 amino acids) and short intracellular N- and C-termini. Neither ASIC2b nor ASIC4 can form functional homomeric channel, but ASIC2b has been shown to alter the properties of other functional ASICs when co-expressed.

Pharmacologically, Amiloride may be the most common non-specific inhibitor for ASICs, also including non-steroidal anti-inflammatory drugs (NSAIDs), A-317567 and so on. There are also some toxin peptides that have more specific inhibiting effect on ASICs: Mambalgins and Psalmotoxin 1 (PcTx1) for ASIC1a and Antholeura elegansima toxin 2 (APETx2) for ASIC3. A recent report indicated another toxin peptide from the Texas coral snake, called MitTx, which is highly selective for the ASIC1 subtype at neutral pH and massively potentiates proton-evoked activation of ASIC2a channels under more acidic conditions (pH < 6.5).

Significance of this channel family depends crucially on its distribution range and expression pattern in our bodies. ASICs were first discovered and mainly distributed in nervous system. They are widely expressed in neurons of the central and peripheral nervous system. Furthermore, it has been found that ASIC1, 2 and 3 are expressed in isolated mouse dendritic cells (DCs), T cells and B cells at mRNA and protein levels. Isolated human monocytes also express ASIC1, 2 and 3. Being especially interesting in bone, ASIC2 and ASIC3 are most abundant; while in chondrocytes, it is ASIC1. Recently, relative researches indicate the existence of ASIC3 and ASIC 2b both in annulus fibrous and nucleus pulposus cells of intervertebral disc. For intracellular aspect, the stable distribution pattern reminds us that there may be a transporting regulatory system, and recently, a series of regulatory factors have been identified. It has been reported that by regulating the amount of the channel on the plasma membrane, PSD95 decreases the maximal ASIC3 peak current while lin7b, Na+/H+ exchanger regulatory factor-1 (NHERF-1) and Channel-interacting PDZ domain protein (CIPP) increase it, both in a PDZ-dependent way. As for ASIC1 and ASIC2, Chai and his colleagues indicated that A kinase-anchoring protein (AKAP) 150 increases amplitude of acid-evoked currents of ASIC1 and ASIC2.

ASICs under Hypoxia

More and more evidences recently remind us of the clinical significance of ASICs, especially when talking about hypoxia. This review will focus on recent researches that characterize the shifts in expression, activity and function of ASICs under hypoxia. A wide range of “bridges” between ASICs and hypoxia have now been identified, and we are also now in a position to hypothesize about the roles played by ASICs in hypoxia-related diseases including cancers, arthritis and cerebral infarction.

Hypoxia Upregulates the Expression of ASICs

The work from Jernigan showed that exposure to chronic hypoxia resulted in a significant increase in pulmonary arterial ASIC1 protein. This may be a direct hint for us to make the hypothesis that the hypoxia is a positive but indirect regulator for ASICs.

Regional hypoxia is a key environmental stressor in inflammatory diseases, and it induces a condition characterized by immune cell invasion and increased expression of inflammatory substances, including arachidonic acid (AA), nitric oxide (NO) and interleukin-1 (IL-1), which, as reported recently, could act on ASICs.

It is reported that hypoxia would raise the amount of AA via upregulating the production of phospholipases A2 (PLA2). It is reported that an increasing amount of AA is liberated from neoplasms cells and from cultures of rat hippocampal slices upon ischemic injury. AA acts as a structural lipid substance combined with phospholipid in blood, liver, muscle and other organs, and it is known to positively regulate ASIC currents.

Table 1. Basic characteristics of ASICs in physiological condition

| Subunits | Distribution | Subcellular location tendency | Activated pH/pH0.5 | Reference |
|----------|--------------|-----------------------------|-------------------|----------|
| ASIC1a   | CNS, PNS, DC, OC, OB | Cytoplasma (peri-nuclear, ER) | 6.7/6.2 | 21       |
| ASIC1b   | PNS, DC, OC, OB | Cytoplasma | 6.6/5.9 | 22 and 23 |
| ASIC2a   | CNS, PNS, DC, OC, OB | Plasma membrane | 6.0/4.4 | 24 and 25 |
| ASIC2b   | CNS, PNS, DC, OC, OB, NPC | Plasma membrane | No activity | 26       |
| ASIC3    | DRG, DC, OC, OB, NPC | Cytoplasma (Mitochondria) | 7.0/6.7 | 27       |
| ASIC4    | PG            | No research                | No activity       | 114      |

The distribution of these subunits indicates their vital significance in nervous system. The recent researches also underline the existence and significant roles of ASICs in immune-related cells such as DC, OC. Different affinity to H+ may explain the various functions of the 6 subunits.
Protons Produced under Hypoxia Directly Activate ASICs, which is also Responsible for the Acidosis-Induced Pain

Glycolysis is an adaptive pattern for cells under hypoxia, and accumulation of lactate during glycolysis leads to the extracellular acidosis. With it, carbonic anhydrase (CA) will overexpress in response to hypoxia-induced factor-1 (HIF-1). CA9 is a number of CA, being able to remit the intracellular acidosis via catalyzing the hydration of extracellular carbon dioxide (CO₂) to generate HCO₃⁻ and H⁺. The control of pH has been postulated to be the transportation of HCO₃⁻ into intracellular compartment through anion exchanger, which diminishes intracellular acidosis while the proton is pumped out into the extracellular compartment. The accumulation of extracellular H⁺ activates the ASICs, which then display relevant effects. In a word, hypoxia leads to accumulation of H⁺, directly activating the ASICs (Fig. 1).

More than one third of the people in the world experience persistent or recurrent pain. Pain may be one of the most common and feared complications in patients with any diseases, which would cause discomfort, depression and anxiety. As a well-known inducement of pain, acidosis caused by hypoxia deservedly attracts much attention when talking about ASIC-related clinical manifestation. ASICs have been proposed to be a sensor for extracellular acidification occurring in pathological conditions such as inflammation, ischemia, hematomas, fractures and lesions as well as in postoperative states, and the activation of ASICs could fire the pain-sensation transduction. Just like the report from Benson, which implies a critical role for ASIC in sensation associated with myocardial ischemia. Accordingly, researchers have found that there is an upregulation in mRNA expression of ASIC1a, ASIC1b and ASIC3 in dorsal root ganglia (DRG) following complete Freund adjuvant-induced inflammation adjacent to bone. This may be a mechanism for the existence of severe bone pain induced by peripheral inflammation.

Among ASICs, ASIC3 is most important in pain sensation because of its highest affinity to H⁺, which makes the body quickly react to harmful stimuli. Supportably, both APETx2 and the knockdown of ASIC3 with a specific siRNA could have potent analgesic effects against primary inflammation-induced hyperalgesia in rat.
Bivalent Cations Inhibit ASICs and Hypoxia may Remove it via Chelation

Cations with two positive charges—particularly calcium ions (Ca\(^{2+}\))—are crucial in this regulation of ASICs.\(^{31}\) As lactate is a strong chelator for bivalent cations and bivalent cations, such as Ca\(^{2+}\) or Zn\(^{2+}\), affect the ASICs greatly, it deserves a deeper investigation.

As for ASIC1a, it has been proposed that Ca\(^{2+}\) inhibits ASIC1a activation and protons activate ASICs by facilitating Ca\(^{2+}\) unbinding,\(^{7,77}\) the same as ASIC3.\(^{66}\) On another surprising aspect, some researches find that Ca\(^{2+}\) lightly enhanced proton sensitivity of ASIC2a.\(^{38}\) Structurally, it has been determined that there is an area of extracellular domain, which has been found to be amino acids 197–233 in ASIC1a, is required for Ca\(^{2+}\)-dependent regulation of ASICs activation.\(^{79}\)

Zn\(^{2+}\) is the second most prevalent trace element in human body,\(^{79}\) which typically binds to proteins and functions as a signaling molecule that regulates a variety of neuronal and non-neuronal functions.\(^{80}\) What’s more, Zn\(^{2+}\) regulates the activity of voltage- and ligand-gated ion channels, including ASICs.\(^{16,81,82}\) Zn\(^{2+}\) potentiates the acid activation of homomeric and heteromeric ASIC2a-containing channels but not of homomeric ASIC1a and ASIC3.\(^{83}\) In contrast, few reports have shown that Zn\(^{2+}\) inhibits ASIC1a and ASIC1a/2a channels with high-affinity.\(^{34}\) Homomeric ASIC3 channels are sensitive to inhibition by bath application of Zn\(^{2+}\) at physiologically relevant concentrations.\(^{16}\) In addition, Zn\(^{2+}\) and Ca\(^{2+}\) bind to distinct sites on ASICs, which means that Zn\(^{2+}\) inhibition is independent of Ca\(^{2+}\).\(^{16}\)

Lactate acid is produced by anaerobic hypoxic metabolism when cell gets insufficient oxygen. This and the fact that normally lactate acid would decrease the amount of bivalent cations, including Ca\(^{2+}\) and Zn\(^{2+}\), via chelation suggest that lactic acid intervenes the Ca\(^{2+}\)/Zn\(^{2+}\)-related regulation of ASICs.\(^{85}\) It is shown that lactate acid could dramatically increase activity of an acid-sensing ion channel. Just as follows: a change in extracellular pH from 7.4 to 7.0 evoked a large, Amiloride-sensitive Na\(^{+}\) current mediated by ASIC3, and when 15 mM lactate acid was present, decreasing extracellular pH to 7.0 depolarized the neurons about 70% more than pH 7.0 alone, often triggering or increasing action potential activity.\(^{86}\)

ASICs, Hypoxia and Relative Clinical Diseases

Cancer. Cancer is the malignant disease greatly characterized by its hypoxic microenvironment in virtue of the aberrant blood vessels and the poor blood flow. Although hypoxia is toxic to both cancer cells and normal cells, cancer cells undergo genetic and adaptive changes that allow them to survive and even proliferate in a hypoxic condition.\(^{37}\) As we said before, is there a upregulation of ASICs mediated by hypoxia in cancer cells? Accordingly, researchers had identified that a novel Amiloride-sensitive inward Na\(^{+}\) current appears to be constitutively activated in malignant gliomas but neither in low grade tumors nor normal astrocytes.\(^{38}\)

Thus, it is reasonable to hypothesize that ion-transporting systems specifically expressed by glioma cells are intimately related to and indeed may define the unique growing and migratory ability of these cells. Some reports indicated that high-grade glioma cells functionally express ASIC current because of the lack of ASIC2 in the plasma membrane.\(^{89}\) In another word, surface expression of ASIC2 inhibits the Amiloride-sensitive current to decrease the migratory ability of the glioma cells.\(^{69,90}\) Supportively, it is reported that knockdown of ASIC1 would inhibit GBM whole cell current and cell migration.\(^{32}\) But we, for the moment, cannot identify the accurate role played by hypoxia in the upregulation of ASICs in cancer cells.

Recently, the identification of ASICs in adenoid cystic carcinomas makes an excellent example we should follow.\(^{90}\) There must be ASICs expression in many types of cancers, which would greatly influence their malignancy. Considering what we talked above, some mediators induced by hypoxia, especially in cancers, must be meaningful in regulating the distribution change of ASICs, and this may provide us a new therapeutic guide line in management of cancers.

ASICs mediate the cell injury under pathological hypoxia. Lactate accumulated under hypoxia strongly acidifies the microenvironment and this local acidosis will activate the homomullimeric ASIC1a channels, which are permeable to Ca\(^{2+}\), leading to the ischemic injuries.\(^{55}\) Excessive Ca\(^{2+}\) in the cell activates a cascade of cytotoxic event leading to activation of enzymes that breaks down proteins, lipids and nucleic acids.

Cerebral infarction. Ischemia and hypoxia are both vital characteristics of cerebral infarction, which may cause a bad prognosis. The hypothesis that ASIC1a contributes to ischemic toxicity was recently tested in cells heterologously expressing ASIC1a and in hippocampal neurons.\(^{34,91,92}\) Neurons lacking ASIC1a and cells treated with Amiloride or PcTxI resisted acidosis-induced injury.\(^{14}\) In normal brain tissue, extracellular pH is maintained at 7.3 while intracellular pH is at 7.0.\(^{93,94}\) In brain ischemia, hypoxia, due to the lack of blood supply, results in increased anaerobic glycolysis, which leads to accumulation of lactate.\(^{96,97}\) and the extracellular pH typically falls to 6.0–6.5, which is able to activate the ASIC1a.\(^{93,98,101}\) Clinically, post-ischemic administration of an ASIC1a blocker may prove to be an effective neuroprotective strategy for stroke patients.\(^{102}\)

Arthritis. In addition to neurons, articular cartilage is also vulnerable under pathological hypoxia.\(^{103}\) For normal condition, chondrocytes in articular cartilage are already submerged in an ischemic environment, leading to its acidosis. When inflammation appears (e.g., arthritis), the physiological hypoxia develops into pathological hypoxia that aggravates the acidosis.\(^{104}\) The same hypoxia upregulates the ASIC1a and leads to the injury of chondrocytes.\(^{105}\) Focus are also concentrated clinically, drugs are developed to target at ASIC1a to protect chondrocytes under inflammation.\(^{106}\)

Retina degeneration. Retinal ischemia is a serious and common clinical problem,\(^{107}\) which will finally cause the degeneration of retina. Transient global retinal ischemia shares many similarities with transient global cerebral ischemia.\(^{108,112}\) Just like the apoptosis of the neurons in cerebral infarction, loss of retinal ganglion cells (RGCs) represents the final common pathway in the etiology of the disease.\(^{113}\) In the present study,
it has been demonstrated that ASIC1a channels in RGCs were essential for ischemia-induced cell death. After hypoxia in cultured RGCs, the expression and function of ASIC1a channels were upregulated, and the α/-T(3) could reduce the RGC death in vitro.107

**Concluding Remarks**

The past 10 y have seen enormous advances in our understanding of ASICs physiology and pathology. Functional, quantitative immunohistochemical and transcriptomic techniques have increased the rate of identification of ASICs in different tissues or organs. Simultaneously, deeper exploration of hypoxia has revealed many “bridges” connecting ASICs and hypoxia. Cells under hypoxia would transform aerobic respiration into glycolysis, leading to the accumulation of lactate in both intracellular and extracellular space and acidification of extracellular environment. When the pH decreases to a certain level, ASICs opened. H+ is basament of the connections, and all of the known crosstalks between these two factors are functioning around it. Therefore, the changes in activation, expression or distribution of ASICs well define the mechanisms of hypoxia-related pathological processes, more exactly. Similarly, that would also make great sense in dealing with hypoxia-related clinical disease, among which the most important is cancer.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
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