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Ligand and ASIC Receptor Interactions in a Rat Ischemic Stroke Model

Elena N. Nesmeyanova [a], Galina A. Sroslova [a], Margarita V. Postnova [a], Nikita V. Panin [a], Yuliya A. Zimina [a]

[a] Volgograd State University, Russian Federation

Abstract

One of the current issues of medicine and pharmacology is investigating the factors that induce cerebral ischemia. In the last few years, there has been a growing interest in the role of ion homeostasis in this disease. However, most previous studies have mainly focused on individual ion channel genes that are responsible for cerebral ischemia development, especially, under adverse conditions. Very little is known about how many ion channel genes are involved in the disease development or its prevention, or whether the changes in different ischemia brain conditions may differ. The most important of these factors is extracellular acidosis. ASIC receptors (voltage-independent proton-gated cation channels) are able to detect physiological changes associated with acid concentration increase. A number of authors consider ASICs to be the most promising target to prevent neuronal injury after ischemic stroke. ASIC activation has an impact on pain sensation, ischemic neuronal injury, neuronal degeneration and mechanosensation that allows ASICs to become potential therapeutic targets for manipulating pain sensations and neurological disorders. In addition to screening for new ASIC1a inhibitors, known ASIC inhibitors can undergo structural modification in order to develop new strong and highly specific inhibitors. Inhibitors of small molecules targeting ASICs may serve as promising therapeutic agents for stroke treatment as well as for psychological adjustment. The paper describes mechanisms of ASIC receptors' activation, localization, and role in physiological processes. A model of ischemic stroke in rats has also been observed.

Keywords: Acid-Sensing Ion Channels (ASIC), acidosis, ischemic stroke, ion channels, Ca2+-Permeable Channels.

1. Introduction

For proper functioning, every system of a living organism requires the extracellular environment to be stable, which implies maintenance of an optimal acid-base balance. A process leading to increased acidity in the extracellular environment, termed acidosis, directly affects the nervous system, causing various pathological processes. A decrease in extracellular pH has an impact on cerebral ischemia progression, enhancing it (Chu et al., 2014; Sherwood et al., 2011).

2. Results and discussion

Ischemic stroke is caused by a blockage which interrupts blood flow to the brain (Xiong, 2004). Ischemic stroke contributes to most traumatic brain injuries and remains the common cause of long-term disability and death. Today, there are two ways to treat this disease: liquefaction of blood clots with tissue plasminogen activator (tPA) and endovascular surgery that can be hampered by a number of factors (Kotoda et al., 2017). Recently, new promising candidates for stroke treatment have been discovered. Acid-sensing ion channels (ASICs) are able to predict stroke. However, local decreases in brain pH can cause difficulties that lead to Ca^{2+} excessive overload (Xiong, 2004).

Acid-Sensing Ion Channels are voltage-insensitive cation channels activated by protons and widely spread throughout the central and peripheral nervous systems (1, 9, Kotoda et al., 2017). ASICs are members of the Degenerin/Epithelial Sodium Channel superfamily (DEG/ENaC/EDA(ASIC)) (Chu et al., 2014; Hernandez-Encarnacion et al., 2017; Kotoda et al., 2017). Members of this family share the same topology (Figure 1B) consisting of two hydrophobic transmembrane domains, a large cysteine-rich extracellular loop and short intracellular N- and C-termini (Hernandez-Encarnacion et al., 2017). ASICs are activated by extracellular pH drop. These ASIC channel subunits are located within the cell bodies and sensory nerve terminals which is important for the processes of nociception and mechanosensing. In central neurons, ASICs are located within the cell bodies, dendrites, and dendritic spines, which is important for synaptic plasticity (Chu et al., 2014).

In mammals, there are four ASIC genes encoding six different subunits: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4 (Chu et al., 2014; Xiong, 2004; Kotoda et al., 2017).

Each ASIC subunit has a different sensitivity to pH levels that allows these receptors to detect a wide range of physiological pH values. ASIC1 and ASIC3 are sensitive to mild extracellular acidosis whereas ASIC2a is activated in the conditions of lower pH. Each subunit is remarkable for different properties of its kinetics and ionic selectivity (Hernandez-Encarnacion et al., 2017).

ASIC1a, ASIC2a and ASIC2b channels are commonly located in both the peripheral and central nervous system, whereas ASIC3 and ASIC1b are expressed typically only in the peripheral (Xiong, 2004). ASICs are also known to be located in some nerve cells such as astrocytes, vascular smooth muscle cells and glioma cells (Hernandez-Encarnacion et al., 2017). In rats, ASIC3 is specifically concentrated in the sensory neurons. ASIC3 is expressed in sympathetic cardiac afferents innervating the heart, where they may serve as mediators of cardiac pain. As for ASIC2a, its expression in sensory neurons is lower compared to other ASICs. ASIC2a has also been found in the taste buds of the circumvallate papillae, where the receptor may have a function in sour taste perception. ASIC2b has been found only in rats (Wang, 2013).

A recently resolved crystal structure from chicken (Gallus galllus) has demonstrated that ASIC subunits assemble as trimers to form functional channels. They can consist of different subunits, termed heteromeric, or they can consist of identical subunits, termed homomeric (Xiong, 2004; Chu et al., 2014). The low pH crystal structure of the chicken ASIC1 resembles a bowl-shaped homotrimer with each subunit consisting of short amino and carboxyl termini, two transmembrane helices, and extracellular regions with multiple domains that are enriched with acid residues (Xiong, 2004).

The location of each ASIC1 subunit may be compared to a forearm and a clenched hand. The extracellular domain consists of a palm (transmembrane domains 1 and 2), a β-ball, a knuckle, a finger, and a thumb. Within the extracellular domain, there is an acidic pocket which is charged highly negatively and is formed by the residues of the palm, the β-ball, the finger, and the thumb. The structure of the transmembrane domain may be compared to an hourglass, with each of the transmembrane domains (TM) defined by two long α-helices. Both TM1 and TM2 consist of three subunits that are related by the three-fold axis of crystallographic symmetry. Most of the TM1 helices' contact is located within the phospholipid bilayer, whereas the TM2 helices line the presumed ion channel pore (Xiong, 2004; Leng et al., 2014).

ASICs have previously been reported to play a critical role in such physiological processes as nociception, mechanosensation, behavior, fear, and synaptic plasticity. Also, they have a critical role in pathological conditions, such as ischemic stroke, spasm, multiple sclerosis, and tumor cell migration (Xiong, 2004).

ASICs display a transient proton-activated peak current that lasts from hundreds of milliseconds to seconds, followed by channel desensitization, despite the presence of a solution with low extracellular pH level. The acid-base balance needed for half maximal activation of ASIC currents depends on channel type (Hernandez-Encarnacion et al., 2017).

ASIC1a homomers commonly expressed in the nervous system have been demonstrated to respond to low pH levels, mediating rapid and transient internal currents with a pH threshold of ~ 7.0 (Xiong, 2004). Decrease in pH level during ischemic stroke is caused by hypoxia, which intensifies anaerobic glycolysis and in this way contributes to a buildup of lactic acid and the development of acidosis (Hernandez-Encarnacion et al., 2017; Xiong, 2004). In the conditions of hyperglycemia, pH value may drop to 6.0, whereas it reaches 6.5 at normal blood sugar levels. Such

deviations in pH are sufficient to activate homomeric ASIC1a as well as other brain channels containing ASIC1a. Some functional ASIC1a changes occur during acidosis and oxygen-glucose deprivation, including an increase in current amplitude and a decrease in ASIC desensitization (Xiong, 2004; Chu et al., 2014). These effects potentiate toxic Ca^{2+} loading and may contribute to chronic activation during conditions of brain ischemia (Hernandez-Encarnacion et al., 2017). Moreover, concurrent activation of Ca^{2+}, calmodulin kinase II and N-methyl-D-aspartate (NMDA) contributes to ASIC1a mediated neuronal death. Excessive concentration of intracellular Ca^{2+} may activate a number of enzymes such as phospholipase A2 (PLA2) and nNOS, resulting in excessive generation of reactive oxygen and nitrogen species followed by damage (Xiong, 2004). It proves that acidosis can damage neurons whether or not voltage-gated Ca2+ channels and glutamate receptors are activated. Acid-induced neuronal damage may be mitigated by inhibition of ASICs and a decrease in extracellular Ca^{2+} concentration. It has led us to conclude that ASIC channels may serve as new targets for stroke therapy (Kotoda et al., 2017). Today, stroke research may be focused on approaches to limit of ASIC activity and expression.

ASICs have been reported to play an important role in ischemic stroke models (Sherwood et al., 2011; Wang, 2013). In a focal ischemia model, the pharmacological inhibition of the ASIC1a activity or disruption of the ASIC1a gene protects the brain from ischemic injury (Leng, Xiong, 2013; Chassagnon, 2017; Leng et al., 2014; Wu et al., 2013; Simard et al., 2007). It has been demonstrated that agents potentiating ASIC1a activity such as spermine, dynorphin and nitric oxide exacerbate acidosis-mediated neuronal injury and brain ischemia. It has also been shown that increasing surface expression of ASIC1a (e.g., by means of inhibition of ASIC1a internalization) exacerbates acidosis-induced neuronal damage (Leng et al., 2014).

In a mouse model of focal ischemia, the activation of either ASICs or glutamate receptors both contributed to brain injury (Leng et al., 2014). A recent study in a rat cardiac model of global ischemia has shown that amiloride medication inhibiting ASICs and blocking NMDA receptor is able to reduce neurodegeneration (Simard et al., 2007). It might be evidence that, in some brain ischemia models, ASICs have a more important role than NMDA receptors in the mediation of neuronal damage.

The common response of ASIC1a receptors is characterized by a transient inward current. In electrophysiological recordings in vitro, the ASIC1a current is usually desensitized within several seconds. Moreover, pre-exposure of ASIC1a with a small decrease of the pH level, which is not sufficient for channel activation, inhibits the channel activation upon followed large pH decreases (Xiong, 2004). These results raise an important question as to what a role the ASIC1a channel activation plays in ischemic brain injury. According to one explanation, endogenous modulators exist and are able to prevent the ASIC desensitization and/or potentiate ASIC responses (Li et al., 2016). At that point, a number of recently-identified endogenous molecules are able to markedly modulate the ASIC properties.

Lactate has the ability to enhance, but not to activate ASIC channels. In addition, it can chelate divalent cations (Zn^{2+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Ba^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, and Gd^{3+}), modulating ion channels (Hernandez-Encarnacion et al., 2017; Li et al., 2016; Xiong, 2004). Most of the divalent cations listed inhibit ASICs, but only Zn^{2+} has a complex modulatory function on the channels containing ASIC1a and ASIC2a (Xiong, 2004). At physiological concentrations, Zn^{2+} enhances acid activation of homomeric or heteromeric ASIC2a channels, raising the activation dependence on pH up to higher values (Hernandez-Encarnacion et al., 2017).

Arachidonic acid is known to enhance the ASIC activity. Addition of 5 or 10 μM of the acid has led to a significant potentiation of the ASIC current in Purkinje cells from the rat cerebellum. Moreover, arachidonic acid has enhanced or induced a sustained ASIC current. A possible explanation for this phenomenon may be that arachidonic acid potentiates ASICs by stretching the cell membrane upon insertion (Li et al., 2016; Xiong, 2004).

Spermine is known to inhibit esensitization in open-state ASIC1a. However, it accelerates ASIC1a recovery after desensitization in response to repeated acid stimulation (Hernandez-Encarnacion et al., 2017; Li et al., 2016). In addition, ASIC1a activity can be modulated by serine proteases. Protease exposure increases the activity of ASIC1a. Moreover, protease treatment has been proven to accelerate ASIC1a recovery from desensitization (Kellenberger et al., 2015). Calmodulin II (CaMKII), nitric oxide (NO) and dynorphins also play an important role in ischemic injury and ASIC modulation (Hernandez-Encarnacion et al., 2017; Xiong, 2004). ASIC1a and

ASIC2 overlap each other in brain expression (Kotoda et al., 2017). Aside from homomeric ASIC1a, heteromeric ASIC2b/1a is also permeable to Ca^{2+} ions and contributes to neuronal injury induced by acidosis. Homomeric ASIC2b cannot form functional channels, whereas heteromeric ASIC2b/1a is known to produce proton-gated currents (Xiong, 2004).

Among the different ASIC channels, desensitization may vary. E.g., ASIC1 and ASIC3 desensitize faster than ASIC2. Under certain conditions, inactivation of the current carried by some ASIC heteromers is incomplete. It leaves a sustained residual inward current following the fast transient current. This current might be found under the conditions of prolonged acidification in cells that express ASIC3 homomultimers or the heteromers ASIC2a/ASIC2b, ASIC2a/ASIC3, and ASIC2b/ASIC3. Interestingly, the sustained currents in ASIC-expressing cells have different biophysical and pharmacological features from the transient currents. The reasons for these functional differences have not been satisfactorily explained. Transient and sustained currents might involve different types of ASIC channels. It is also possible that sustained currents represent leakage currents due to ASIC overexpression (Hernandez-Encarnacion et al., 2017).

Kinetics of the desensitization changes when ASIC1a subunits are combined with other ASIC subunits, e.g., ASIC2b. When ASIC1a/2b heteromers are combined, the dependence of steady-state desensitization on pH level drops to lower pH values (7.28 for ASIC1a/2b and 7.18 for ASIC1a homomers). The drop in desensitization pH leads to a decrease of ASIC activity after slight decreases in baseline pH. This shift may be neuroprotective in nerve cells or in regions that express a higher amount of ASIC1a/2b heteromers (Kotoda et al., 2017). This has led us to conclude that like homomeric ASIC1a channels, ASIC2b/1a heteromeric channels might contribute to neuronal injury mediated by acidosis. It is also important to note that activation of ASIC2b/1a might result in intercellular Ca^{2+} overload and cell death, which was thought to be mediated only by homomeric ASIC1a (Li et al., 2016; Kotoda et al., 2017). Recently, human ASIC1a channels have been found to have two desensitization states: short- and long-lasting. One of desensitization forms is prevented by high frequency stimulation that somewhat models usage dependent stimulation. Desensitization properties are also determined by the duration of stimulation. However, long term repetitive stimulation results in gradual irreversible loss of channel activity (Kotoda et al., 2017). Having only eight residues distinct from chicken ASIC1a, human ASIC1a has a number of desensitization properties found in chicken, mouse and rat ASIC1a. Surprisingly, the replacement of transmembrane domain 1 (TM1) of hASIC1a has demonstrated the largest effect on the current desensitization compared to individual amino acid mutations.

Homomeric and heteromeric ASICs can be modified by serine proteases such as trypsin, chymotrypsin, and proteinase K (Kellenberger et al., 2015). In addition, endogenous agents can also limit ASIC desensitization. Neuropeptides have recently been found to enhance ASIC1a activity preventing sustained desensitization (Li et al., 2016; Kotoda et al., 2017). Dynorphins are among the basic neuropeptides and they are widely expressed throughout the central nervous system, including areas with high concentration of ASIC1a that are located in stroke-injured regions. Focal accumulation of dynorphin in these regions is suggested to enhance neuronal injury due to increased ASIC activation and subsequent accumulation of Ca^{2+} ions (Kotoda et al., 2017).

ASIC inhibition is often caused by neuroprotective medications. Amiloride is known to be an important pharmacological tool for all known members of the ASIC ion channel family (Kotoda et al., 2017), as it has been previously reported to be able to inhibit ASICs and reduce neurodegeneration (Simard et al., 2007). Amiloride can inhibit all combinations of ASIC subunits (with the exception of the sustained part of ASIC3 current). Intracerebroventricular amiloride injection in mice before transient middle cerebral artery occlusion (MCAO) has been demonstrated to decrease ischemic injury that might be caused by the wide spread of ASIC inhibition (Kotoda et al., 2017). Another research has tested amiloride in a model of a transient ischemic attack caused by middle cerebral artery occlusion (MCAO). A decrease in infarct volume has been observed after intracerebroventricular injection of ASIC1a, amiloride, and PcTx1 blockers (Hernandez-Encarnacion et al., 2017).

3. Conclusion

In summary, ASICs play an important role in cerebral ischemia. ASIC members might become promising pharmaceutical agents for ischemia therapy due to their relatively high

permeability to the blood-brain barrier, the ability to be activated by ligands, and the processes such as mechanotransduction, ion selectivity, or blocking by pharmacological ligands.

ASIC1a can be considered an effective neuroprotective therapeutic target for cerebral ischemia and neuronal degeneration.

4. Conflict of interest

No risk of a conflict of interest.

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