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Chapter 8

Targeting Acid-Sensing Ion Channels by Peptide Toxins

Chengchong Li, Yuhua Wang and Xiang-Ping Chu

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Abstract

Acid-sensing ion channels (ASICs) are proton-gated ion channels that are highly expressed in the nervous system and play important roles in physiological and pathological conditions. They are also expressed in non-neuronal tissues with different functions. The ASICs rapidly respond to a reduction in extracellular pH with an inward current that is quickly inactivated despite the continuous presence of protons. Recently, protons have been identified as neurotransmitters in the brain. Until now, six different isoforms (ASIC1a, 1b, 2a, 2b, 3 and 4) in rodents have been discovered and they can be assembled into homotrimers or heterotrimers to form an ion channel. Peptide toxins targeting ASICs have been found from the venoms of spider Psalmotoxin-1 (PcTx1), sea anemones (APETx2 and PhcrTx1) and snakes (MitTx and mambalgins). They reveal different pharmacological properties and are selective blockers of ASICs, except for MitTx, which is a potent activator of ASICs. In this mini review, the structure, pharmacology and effects of peptide toxins on ASICs will be introduced and their therapeutic potentials for neurological and psychological diseases will be discussed.

Keywords: acid-sensing ion channels, peptide neurotoxins, pain, stroke, depression, neuron

1. Introduction

With great interests in venom toxins, scientists are extremely involved and enthusiastic about this area of research, as applications of these venoms for drugs could bring about a greater understanding of human diseases, potentially changing and advancing human healthcare [61, 65]. Venoms of species like spiders, sea anemones and snakes have been found to target ion channels with highly therapeutic potentials as drug candidates [17, 38]. To explore structure-function, gating mechanisms and tissue localization of many ion channels, animal venom toxins were important pharmacological tools in the ion channel field [28]. Certain peptides even lead to clinical development and venom-based drugs, such as ziconotide, which is an inhibitor of neuronal

voltage-gated calcium channels isolated from *Conus magus*, designed for patients with intractable pain who fail to respond to other drugs [57, 66].

Recently, protons have been identified as neurotransmitters in the brain [26]. One of the candidate targets for proton sensing is called "acid-sensing ion channels" (ASICs). Three decades ago, the proton-activated inward currents were discovered and recorded in neurons isolated from rat spinal ganglia and from the ganglion of trigeminal nerve by the pioneer Krishtal and Pidoplichko [48, 49]. Twenty years ago, Waldmann et al. first cloned the ASICs [80]. ASICs are widely expressed in the nervous system with high density [1, 62, 80]. Molecular cloning of ASICs has identified four genes (*ACCN1–4*) encoding at least six ASIC subunits in rodents (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4) [35, 84]. Structurally, each ASIC subunit consists of 500–560 amino acids with a simple topology: two transmembrane domains, large extracellular loop (370 amino acids) and short intracellular N- and C-terminals (35–90 amino acids). The structure of ASICs is different from traditional ligand-gated G-protein couple receptor, which has seven transmembrane domains. ASICs can form functional ion channels structurally appearing as trimeric complexes of these subunits [44], which form both homomeric and heteromeric channels with different electrophysiological and pharmacological properties [3, 11, 37, 45, 51, 67, 71]. Among all the ASIC subunits, the ASIC2b and ASIC4 subunits do not form functional homomeric proton-gated ion channels by themselves, but they can associate with other ASIC subunits to reveal new pharmacological properties on the heteromeric channels [21, 51, 67].

ASICs are mainly expressed in the central and peripheral nervous systems, chiefly found in neurons [80, 82]. In central nervous system, ASICs contributed to several physiological and pathological conditions, such as learning and memory, fear conditioning, pain, chemoreception, ischemia, seizures, drug addiction and neuroinflammation, where extracellular acidification occurs [5, 9, 12, 82–84]. More importantly, ASICs are involved in synaptic physiology and are neurotransmitter receptors critical for amygdala-dependent learning and memory [26]. In peripheral sensory neurons such as dorsal root ganglia (DRG), ASIC1, 2 and 3 are found. During pathological condition such as inflammation, tumors or wounds, peripheral tissue acidosis associated with pain occurs. ASICs are of particular interest because they are profoundly sensitive to moderate acidifications [18]. They are more sensitive than transient receptor potential vanilloid 1 (TRPV1), another ion channel activated by protons, capsaicin and heat in nociceptive neurons. ASICs can produce sustained depolarizing currents upon prolonged tissue acidification compatible with the detection of non-adapting pain [18]. ASIC currents and/or transcripts have also been found in glia, smooth muscle cells, lung epithelial cells, immune cells, urothelial cells, adipose cells, joint cells and osteoclasts, indicating that ASICs likely play a role in non-neuronal cells as well [18, 32, 35, 50, 59, 70, 86]. The review regarding the effects of peptide toxins on ASICs has also been discussed by previous publications [4, 5, 9, 10, 12, 17].

2. Targeting ASICs by peptide toxins

2.1. Psalmotoxin-1 (PcTx1)

Among all the peptide toxins, PcTx1 is the first peptide discovered for the ASICs. PcTx1 was identified from venom of the South American tarantula *Psalmopoeus cambridgei* [30, 31]. It is a

potent and selective inhibitor for both homomeric ASIC1a and heteromeric ASIC1a/2b channels [31, 67]. Structurally, this toxin has 40 amino acids crosslinked by three disulfide bridges [31]. Pharmacologically, the IC_{50} of PcTx1 is 0.9 nM for homomeric ASIC1a channels [30, 31] and 2.6 nM for heteromeric ASIC1a/2b channels [67] in *Xenopus oocytes* expressed homomeric ASIC1a or heteromeric ASIC1a/2b channels. In our previous studies, PcTx1 at a concentration of 10 nM significantly inhibits ASIC currents in majority of cultured striatal and cortical neurons, respectively [45, 89]. At concentrations that effectively inhibit the homomeric ASIC1a current, it has no effect on the currents mediated by other configurations of ASICs such as heteromeric ASIC1a/2a channels [31] or known voltage-gated Na^+, K^+, Ca^{2+} channels as well as several ligand-gated ion channels [89]. Unlike amiloride, which is a blocker of epithelial sodium channel and directly blocks the ASICs, PcTx1 acts as a gating modifier [9, 35]. PcTx1 shifts the channel from its resting state toward the inactivated state by increasing its apparent affinity for protons [5, 17].

Purified PcTx1 or venom toxin was the first peptide used to explore the function of ASICs in neurological, psychological and other diseases [5]. Our previous studies have shown that PcTx1 reveals neuroprotective effects on mouse cultured cortical neurons subjected to extracellular acidosis as well as oxygen and glucose deprivation [88, 89]. In a rodent experimental stroke model (middle cerebral artery occlusion), central injection of venom toxin or PcTx1 significantly reduces the infarct volume by 60% and the protection by PcTx1 treatment lasts 1 week [60, 89]. Consistent with our findings, similar effect by application of PcTx1 was also found in a model of traumatic spinal cord injury in rats [39]. Venom toxin also shows certain protection in a mouse model of multiple sclerosis associated with axonal degeneration [33] as well as in the mouse MPTP model of Parkinson's disease [2]. Moreover, PcTx1 decreases the acidosis-mediated cell death in cultured retinal ganglion cells [74]. Collectively, all the results support that PcTx1 might be a potential therapeutic agent for neurological disease [9, 12, 81, 87, 88].

ASIC1a is highly expressed in the amygdala, a brain region critical for fear, arousal and emotions [82, 84, 85]. Central injection of venom toxin reduces mouse innate fearing [14, 16], mouse depression-related behavior [15] and stress-induced elevation in core body temperature of mice [29]. The mechanisms of fear reduction, antidepressant and anxiolytic effects by PcTx1 are likely mediated by inhibition of ASIC1a-containing channels in the amygdala.

PcTx1 has also been used to study pain modulation in rodents [54]. Treatment by PcTx1 was shown to induce a potent analgesic effect in acute pain, inflammatory and neuropathic pain models in mice [54].

ASICs are involved in the central chemoreception [40, 71, 72]. Central injection of PcTx1 in the lateral hypothalamus (LH), nucleus of the solitary tract (NTS) and rostral ventrolateral medulla (RVLM) inhibits the acid-induced stimulating effect on respiration [40, 71, 72]. Thus, ASICs in the LH, NTS and RVLM contribute to central regulation of respiration.

ASICs are also expressed in non-neuronal tissue, including but not limited to smooth muscle cells (VSMC) from arteries, where they might play a role in mechanotransduction of the myogenic response and VSMC migration [25]. ASIC currents recorded in acutely dissociated mice cerebral artery smooth muscle cells are potentiated by PcTx1 in majority of the cells [13]. PcTx1 also reduces store-operated calcium entry in VSMCs in rat pulmonary arteries. By using PcTx1, ASIC1a-containing channels are involved in the vascular mechanotransduction.

PcTx1 itself cannot cross the blood-brain barrier. Therefore, the critical importance is how to deliver the PcTx1 to its correlated damaged specific brain region and to search a small molecule with similar effect as PcTx1 [9].

2.2. APETx2

The peptide toxin APETx2 was isolated from sea anemones (*Anthopleura elegantissima*) and is a selective inhibitor for ASIC3 and ASIC3-containing channels [22]. Structurally, APETx2 contains 42 amino acids crosslinked by three disulfide bonds, a compact disulfide-bonded core with a four-stranded beta-sheet. APETx2 possesses the disulfide-rich all-beta structural family of peptide toxins usually seen in animal venoms. Pharmacologically, APETx2 inhibits both homomeric ASIC3 channels and heteromeric ASIC3-containing channels in heterologous expression systems as well as primary cultures of sensory neurons in rodents. It inhibits the transient component of ASIC3 currents with an IC_{50} of 63 nM, without affecting sustained component of ASIC3 currents [22]. However, the affinity of this particular ASIC3 inhibitor is reduced when ASIC3 is associated with other ASIC subunits [22]. For instance, the IC_{50} for ASIC3/ASIC2b is about 117 nM, whereas the IC_{50} for ASIC3/ASIC1a is around 2 μM [22]. By acting at this external side, APETx2 directly inhibits the ASIC3 channel, and it does not modify the channel unitary conductance [5].

ASIC3 and ASIC3-containing channels are widely expressed in peripheral sensory neurons and play a critical role in pain modulation [8]. During chronic inflammation, the expression level of ASIC3 was upregulated in rat sensory neurons [52, 53, 77], which might be critical for the sensitization of cutaneous nociceptors during inflammation. Consistent with these findings, a reduction in pH in the skin of human volunteers was involved in non-adapting pain [73], and this cutaneous acid-induced pain is largely mediated by ASIC channels, because it is inhibited by amiloride [46, 56, 76]. Additionally, the non-amiloride ASIC blocker, A-317567 exhibits distinct in vitro and in vivo activities over amiloride [27]. Furthermore, by using APETx2, ASIC3 was identified as a sensor of cutaneous acidic pain and postoperative pain and as an integrator of molecular signals released during inflammation in rat, where it is involved in primary thermal hyperalgesia [18–20]. In correlation with this result, local peripheral application of APETx2 was found to attenuate mechanical hypersensitivity in a cutaneous inflammatory pain rat model [47].

ASIC3 is mainly expressed in small muscle afferents in rat [19, 58] and in more than 30% of sensory neurons innervating the knee joint in mouse [42]. The expression level of ASIC3 in sensory neurons is enhanced in models of muscle inflammation [79] and acute arthritis [42] in mice. The application of APETx2, in comparison with ASIC3 knockout and knockdown mice, revealed a critical role for ASIC3 in the generation of secondary mechanical hyperalgesia associated with central sensitization achieved in a mouse model of non-inflammatory muscular pain triggered by repeated acid injections into the muscle [63, 68] and in a mouse model of joint inflammation [41]. Consistent with these findings, peripheral application of APETx2 was also found to decrease mechanical hypersensitivity in a non-inflammatory muscular pain in rat [47]. Furthermore, ASIC3 is also involved in the development of primary cutaneous mechanical hyperalgesia induced by muscle inflammation [69, 78]. In a rat model

of osteoarthritis, continuous intra-articular injections of APETx2 reduced pain-related behavior and secondary mechanical hyperalgesia [43]. An increase in ASIC3 expression was also seen in afferent sensory neurons of the knee joint [43].

APETx2 significantly reduces the exercise pressor reflex mediated by contracting skeletal muscle in rodents [36, 55, 75]. This is supported by the expression of ASIC3 in muscle metaboreceptors [58]. By using ASIC3 knockout mice, researchers have found minor changes in normal cutaneous mechanical sensitivity [8, 63], whereas other studies did not reveal a significant contribution to mechanosensory function [24]. By using selective inhibitor of ASIC3, ASIC3 has been shown to be a neuronal sensor for the skin vasodilation response to direct pressure in both humans and rodents and for skin protection against pressure ulcers in mice [34]. Thus, APETx2 reduces local vascular tone control through blockade of ASIC3 or ASIC3-containing channels.

2.3. Mambalgins

The two peptides of mambalgins (mambalgin-1 and mambalgin-2) were recently found from the venom of the snake *Dendroaspis polylepis polylepis* [23]. Structurally, these two toxins contain 57 amino acids and include eight cysteines linked by four disulfide bridges. Pharmacologically, mambalgins inhibit ASIC-like currents in cultured neurons of hippocampus and spinal cord. Furthermore, mambalgins inhibit homomeric ASIC1a, 1b, heteromeric ASIC1a/2a, 1a/2b and 1a/1b channels with IC_{50} between 50 and 200 nM. Functionally, mambalgins reveal analgesic effects *in vivo* in models of acute and inflammatory pain through either inhibition of ASIC1a and ASIC1a/2a channels in central nervous system or inhibition of ASIC1b channels in peripheral nervous system [5, 23]. Interestingly, the central analgesic effect of mambalgins revealed strong effect similar to morphine but produces less unwanted side effects [4, 23]. Further studies are needed to explore the cellular and molecular mechanisms responsible for such pain pathways, but brain ASICs appear as promising therapeutic targets for novel analgesic drugs [5]. It is also interesting to know whether mambalgins have other effects in brain besides pain modulation [9].

2.4. PhcrTx1

PhcrTx1 represents a newly discovered peptide, which was isolated from the sea anemones *Pseudacris crucifer* [64]. Structurally, it contains 32 amino acid residues. This peptide reveals an inhibitor cystine knot scaffold, which has been found in other venomous organisms, such as spider, scorpions and cone snails. Pharmacologically, PhcrTx1 inhibits peak ASIC currents in DRG neurons of rats with an IC_{50} of 0.1 μM. It does not affect the sustained component of the ASIC current or its desensitization rate. Furthermore, the toxin shows its effect in a closed state of the ASICs rather than an open state. PhcrTx1 also inhibits voltage-gated K^+, but not voltage-gated Na^+, currents in rat DRG neurons with an IC_{50} of 3.4 and 3.5 μM for peak and steady-state component, respectively. However, PhcrTx1 inhibits voltage-gated K^+ currents in DRG neurons, but with significantly lower potency and efficacy than its ability for inhibition on ASIC currents. Thus, PhcrTx1 represents the frontrunner of a novel structural group of sea anemone toxin that acts on both ASICs and Kv channels with high and low potency, respectively [64]. It is interesting to know whether PhcrTx1 plays any functional role in ASICs.

2.5. MitTx

In 2011, MitTx was discovered from the venom of the Texas coral snake *Micrurus tener tener* [6]. Structurally, peptide MixTx contains two subunits (MitTx-α and MitTx-β) with a β-bungarotoxin-like structure. The MitTx-α subunit has a 60 amino-acid Kunitz-type peptide and the MitTx-β subunit consists of a 120 amino-acid phospholipase A2-like protein. They can associate with each other in a 1:1 ratio (Kd: 12 nM), but this interaction is non-covalent, unlike the β-bungarotoxins that are linked by an interchain disulfide bond. Pharmacologically, MitTx, unlike other inhibitory toxins for ASICs, strongly activates several homomeric and heteromeric ASICs [6, 7]. MitTx produces long-lasting profound effects on homomeric rodent ASIC1a and ASIC1b currents (EC_{50}: 9 and 23 nM, respectively) and a much lower effect on ASIC3 current ($EC_{50:}$ 830 nM). During physiological pH condition (e.g. pH 7.4), MitTx reveals subtle effects on ASIC2a current, but potently enhances the ASIC current by shifting its activation curve toward less acidic pH. The effects of MitTx on sensory ganglion neurons from ASIC1a knockout mice were disappeared. Collectively, the data further suggest that effects of MixTx depend on ASIC1a-containing channels [6].

MitTx triggers a strong ASIC current in cultured sensory neurons in wild-type mice; these currents are lost in neurons from ASIC1a-knockout, but not from ASIC3-knockout mice. Consistent with this idea, injection of MitTx in the mice hindpaw displays a strong pain-related behavior (licking response). This effect is reduced in ASIC1a knockout mice but persists in ASIC3 knockout mice, suggesting the contribution of peripheral ASIC1a-containing channels in cutaneous pain [6]. It is needed to explore why MitTx produces lost-lasting effects in physiological concentration of pH on ASICs.

3. Conclusion

PcTx1 was the first peptide toxin found to block homomeric ASIC1a and heteromeric ASIC1a/2b channels. APETx2 was the second ASIC-targeting peptide discovered, and it inhibits ASIC3 channels. MitTx was discovered in 2011 and is a strong activator of ASICs during physiological conditions. Mambalgins have strong inhibition on ASIC1 channels. Another sea anemone peptide PhcrTx1 inhibits ASIC currents in DRG neurons. These peptide toxins have been very important to better understand the structure-function relationships of ASICs and their implication in physiological and pathological processes [5, 17]. ASIC-targeting peptides isolated from animal venoms that selectively block this class of channels are therefore not only instrumental as pharmacological tools to explore their function but also represent molecules of great potential therapeutic value [5]. ASIC channels appear therefore as targets for drug development in a variety of pathophysiological conditions [9].

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