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Cnidarian peptide neurotoxins: a new source of various ion channel modulators or blockers against central nervous systems disease

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Cnidaria provide the largest source of bioactive peptides for new drug development. The venoms contain enzymes, potent pore-forming toxins and neurotoxins. The neurotoxins can immobilize predators rapidly when discharged via modifying sodium-channel-gating or blocking the potassium channel during the repolarization stage. Most cnidarian neurotoxins remain conserved under the strong influence of negative selection. Neuroactive peptides targeting the central nervous system through affinity with ion channels could provide insight leading to drug treatment of neurological diseases, which arise from ion channel dysfunctions. Although marine resources offer thousands of possible peptides, only one peptide derived from Cnidaria: ShK-186, also named dalazatide, has reached the pharmaceutical market. This review focuses on neuroprotective agents derived from cnidarian neurotoxic peptides.

Introduction

Venoms are complex cocktails involving a range of molecules, such as large proteins, small peptides, polyamines and salts, that disrupt the physiology of prey animals upon injection [1]. Venom peptides and proteins have been honed by millions of years of evolutionary pressure to bind their receptors with high affinity, and in many cases exceptional selectivity [2]. Most of the current venom-peptide-derived drugs were studied in several venomous lineages that are relatively younger in evolutionary origin, such as snake venoms, which evolved to target either the neuromuscular system or cardiovascular system [3,4]. By striking contrast, most peptides in cone-snail and arachnid venoms target the nervous system and aim to induce rapid immobilization of prey. Proteomic and transcriptomic analyses have identified that spider and cone-snail venoms comprise >1000 distinct peptides [5,6], whereas scorpion venoms often contain as many as several hundred components [7,8]. Only one extant venom-derived drug: Prialt®, has a nervous system target, whereas many of the venom-derived compounds are currently under development to target enzymes, ion channels, receptors or transporters in the central or peripheral nervous system (e.g., ACV-1, CGX-1007, CGX-1160, CNSB004, RPI-78M, RPI-MN, ShK-186, THA 901 and Xen2174) [9–16].

In the phylum Cnidaria (sea anemones, corals, jellyfish and hydra) all members are venomous [17]. It is typified by the unique venom delivery characteristic known as nematocyst, employed to inject a cocktail of toxins into animals for predation and defense [17–19]. Nematocysts are mostly located on tentacles, which also exist on the surface of the bell, oral arms and the stomach in certain species of jellyfish [20]. The morphological properties of nematocysts are abundant and correlate to body size, specific ecosystems, prey size and selectivity in prey capture [21,22]. The capsule venom is located on the inner surface of the inverted tubule which everts outside during discharging then exposes and injects into the prey [23]. The mechanism of nematocyst discharge in response to external stimuli is generally accepted that the high osmotic pressure exceeds a critical threshold at the end of capsule morphogenesis then triggers the venom discharge [24]. Cnidarian peptide toxins could be classified as enzymes like phospholipase A2 and metalloproteases, pore-forming toxins, including actinoporins and perforin, and neurotoxins including NaTxs, KTxs,

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Kunitz peptides, SCRiPs, acid-sensing ion channel (ASIC) inhibitors and transient receptor potential cation channel subfamily V member 1 (TRPV1) inhibitors [25]. As such, owing to the increasing scientific interest in the discovery and characterization of novel toxins or toxin families, several recent studies were carried out, using genomic, transcriptomic or proteomic approaches, on the cnidarian venom from hydra [26], sea anemone [27,28], box jellyfish [29–32], cannonball jellyfish [33,34], ghost jellyfish [35], sea nettle [36], stony coral [27,28] and zoanthid coral [37].

According to the classification proposed in the World Register of Marine Species (WoRMS), class Anthozoa currently includes ten orders and 6989 valid species, occupying about two-thirds of all known cnidarian species. Besides, this class comprises 99% of new marine nature products recorded from Cnidaria [38]. Within Anthozoa, the orders Alcyonacea and Gorgonacea are the ones that contributed with the highest number of promising bioactive marine compounds [39,40]. These compounds include diterpenes, sesquiterpenes, furanoditerpenes, terpenoids, capnellenes and steroids with HIV-inhibitory [41], cytotoxic [42], anti-inflammatory [43], anticancer [44] and antimicrobial activity [45]. The diversity of cnidarian venom components ranges from small molecules (e.g., purines, biogenic amines) to peptides or proteins that evolved over the course of hundreds of millions of years [46]. Interestingly, some toxin types identified previously in other venomous animals comprise similar cnidarian venom arsenals. The most striking example is Kunitz peptides, which are expressed in sea anemones, cone snails, insects, scorpions, spiders, reptiles, ticks and vampire bats [2,47]. A novel Kunitz neurotoxin from zoanthid coral *Palythoa caribaeorum* has also been proposed that could have potential for the development of neuroprotective agents through K_V1.3 blockade [48]. However, to date, the evolution, diversification and peptide toxin composition of ancient venom systems, like those of cnidarians, remain understudied. Another example of convergent expression of toxins is afforded by the peptide blockade with potassium channel K_V1, like BgK, ShK and HsTx1, which evolved convergently in sea anemones and scorpions – species that even use the same residues for blocking the channel pores [49–51]. The neurotoxins from Cnidaria could disrupt ion conductance through modification or blockage of the voltage-gated sodium (Na_V) and potassium (K_V) ion channels. There are 110 peptide or protein toxins isolated from 45 sea anemone species that were manually curated from the Tox-Prot database that could act on ion channels. Three toxin families act on ion channels including sea anemone sodium channel inhibitory toxin family (52 proteins), sea anemone type 3 (BDS) potassium channel toxin family (32 proteins) and sea anemone type 2 potassium channel toxin subfamily (26 proteins) are most common [52]. Briefly, sodium channel toxins (NaTxs) are some of the best characterized cnidarian toxins and are classified into three types based on the cysteine arrangement, amino acid composition and immunological cross reactivity [53–55]. Another type of neurotoxin is potassium ion channel toxin (KTx), which has been classified into five types based on physiological homologs and biochemical characteristics [56–59]. Recently, a novel peptide neurotoxin that consisted of two cysteine residues forming one disulfide bond decreased sodium (Na_V1.7) and potassium (K_V1.4) currents and was isolated from *Palythoa caribaeorum* [60,61]. Using next-generation sequencing, some novel peptide neurotoxins were identified from zoanthid coral transcriptome in our previous studies [37,62].

Neurotoxic peptides have attracted growing interest in drug development for neurological disorder treatment owing to their high affinity to receptors, ionic channels and transporters in the CNS [63]. They can present with either analgesic, anxiolytic, antiepileptic or neuroprotective effects, acting as inhibitors or stimulants in specific structures, such as ion channels, neurotransmitter receptors and transporters [64–66]. In this review, peptide neurotoxins, particularly those acting on the nervous system through their impact on ion channels, are discussed. Also, we provide insight into the characterization of neuroactive peptide sequences in Cnidaria having potential neurotoxic activity, which will serve to inform the development of drugs for the investigation and prospective treatment of neurodegenerative diseases arising from ion-channel dysfunctions.

The role of cnidarian venom in drug discovery

Cnidarian stings can cause minor local irritation, and even systemic reactions including excruciating pain and life-threatening cardiovascular collapse [35,67], owing to their interaction with ion channels in the CNS and transmitter release at the neuromuscular junction [17,53,67,68]. Cnidarian venom can affect ion conductance of the plasma membrane of target cells by altering the activity of resident ion channels or forming new ion permeation pathways following direct insertion of pore-forming proteins in the lipid bilayer [67,69]. These properties mean that cnidarian peptide toxins could contribute to the development of compounds for the treatment of pain and neurodegenerative diseases [70,71]. Ion channels are attractive targets for human disease treatment [72]. The venoms of Cnidaria could act with high potency and selectivity at K_V, Ca_V and Na_V ion channels, taking advantage of the neuronal effects of channel modulation [73]. Importantly, it is believed that venom-derived peptides will make the transition to drugs targeting voltage-gated potassium channels in future [74].

Peptide toxins (molecular weight <10 kDa) contained in venoms are of great interest as a source of drug leads because of their high potency and selectivity [75]. Most venom peptides have cysteine-rich architectures that lead to extreme stability in the gastrointestinal tract for sufficient residence and potent resistance to proteases [76]. Most of the known cnidarian peptide toxins belong to class Anthozoa. To date, ~200 proteinaceous (proteins and peptides) toxins have been identified among sea anemones [59,77]. In general, peptide toxins from sea anemones can be classified into three major groups: phospholipase A2, cytolysins and neurotoxins [78]. The neurotoxins include cysteine-rich peptides distributed across eight unique structural scaffolds, mainly voltage-gated sodium-channel toxins and voltage-gated potassium toxins, as mentioned above.

One of the most famous peptide toxins from phylum Cnidaria is *Stichodactyla* (ShK) toxin, a potassium-channel inhibitor, which was first discovered in the sea anemone *Stichodactyla helianthus* [51,79]. ShK toxin can induce antiobesity activity and reduce insulin resistance through blockade of K_V1.3 [80,81]. Blockers of K_V1.3 channels in lymphocytes preferentially inhibit the activation of these cells and therefore show considerable potential as therapeutics for autoimmune diseases, such as multiple sclerosis,

type 1 diabetes mellitus and rheumatoid arthritis [82]. Thus, most studies on the development of ShK toxin analogs as therapeutic agents focus on targeting K_V1.3 ion channels in autoimmune processes and diseases [82]. There are several ShK derivatives that have been developed to improve their selectivity to K_V1.3. Briefly, ShK-Dap22 could possess a highly selective and potent T lymphocyte channel-blocking effect, when the Lys22 of ShK was replaced by diaminopropionic acid [83]. ShK-170, also named ShK-L5, containing an N-terminal phosphotyrosine extension, is a potent and selective blocker. Notably, it can prevent neurotoxicity by radiation-activated microglia [84]. Peng *et al.* proved that ShK-170 suppressed radiation-induced production of the proinflammatory factors interleukin (IL)-6, cyclooxygenase (COX)-2 and tumor necrosis factor (TNF)-α by microglia. Moreover, ShK-170 inhibited neurotoxicity mediated by radiation-activated microglia and promoted neurogenesis by increasing the proliferation of neural progenitor cells [84]. However, it showed minor pH-related hydrolysis and oxidation byproducts that were exacerbated by increasing temperatures [14]. Dalazatide, previously referred to as ShK-186, is currently in Phase Ib clinical trials for the treatment of autoimmune diseases such as psoriasis [85]. The Phase Ib clinical trial proved dalazatide treatment was well tolerated without serious adverse events; and is the first K_V1.3 inhibitor to be tested in an autoimmune disease clinical trial [86]. However, there are new peptides identified in anemone transcriptomes that contain a ShKT domain and are structural homologs of ShK and BgK but not active against K_V1 channels. Recently, a peptide AsK132958 from *Anemonia sulcate*, with an ShKT cysteine framework and similar disulfide connectivities and structural scaffold to ShK, showed no activity against any of the K_V channels tested [87].

Phylogeny and evolution of cnidarian peptide neurotoxins

Neurotoxins play an extremely important part in the venoms of cnidarians, to rapidly immobilize prey animals by disrupting ion conductance through the modification or blocking of ion channels [17,46]. Neurotoxins in Cnidaria have been characterized at the protein level only within Anthozoan, and most of them were characterized from sea anemones [88]. In sea anemone, the sodium-channel toxins are better defined within the cnidarian toxin groups [52]. It was found that the cysteine arrangement for the 3D structure in type III potassium-channel toxins share a strong resemblance to that in sodium-channel toxins [89]. However, evidence shows that sodium-channel toxin and type III potassium-channel toxin are specific to their respective ion-channel targets. Many type III potassium-channel toxins appeared to act on various ion channels [90–92]. For example, the peptide BDS-I could modify K_V3-family subunits and Na_V1.7 [92,93]. In addition, a neurotoxin, AdE-1, which was identified in *Aiptasia diaphana*, could be active on K_V and Na_V channels [91]. Phylogenetic tree reconstruction for neurotoxin genes in sea anemone concluded that a similar cysteine framework between sodium-channel toxins and type III potassium-channel toxins resulted from their shared ancestry, which was clarified as the first example of neofunctionalization once the gene duplication event was initiated [46]. Collectively, molecular evolutionary assessments of cnidarian toxin families indicated most cnidarian neurotoxins evolve under strong influence of negative selection whereas only type III potassium-channel toxins seem to evolve rapidly under positive Darwinian selection [46]. Cnidarian neurotoxins can affect ion channels (Fig. 1) including the voltage-gated sodium (Na_V) channel, voltage-gated potassium (K_V) channel, sodium-selective ASIC channels, human ether-á-go-go-related gene (hERG) voltage-gated potassium channels and TRPV1. The various cnidarian neurotoxic peptides are summarized in Table 1.

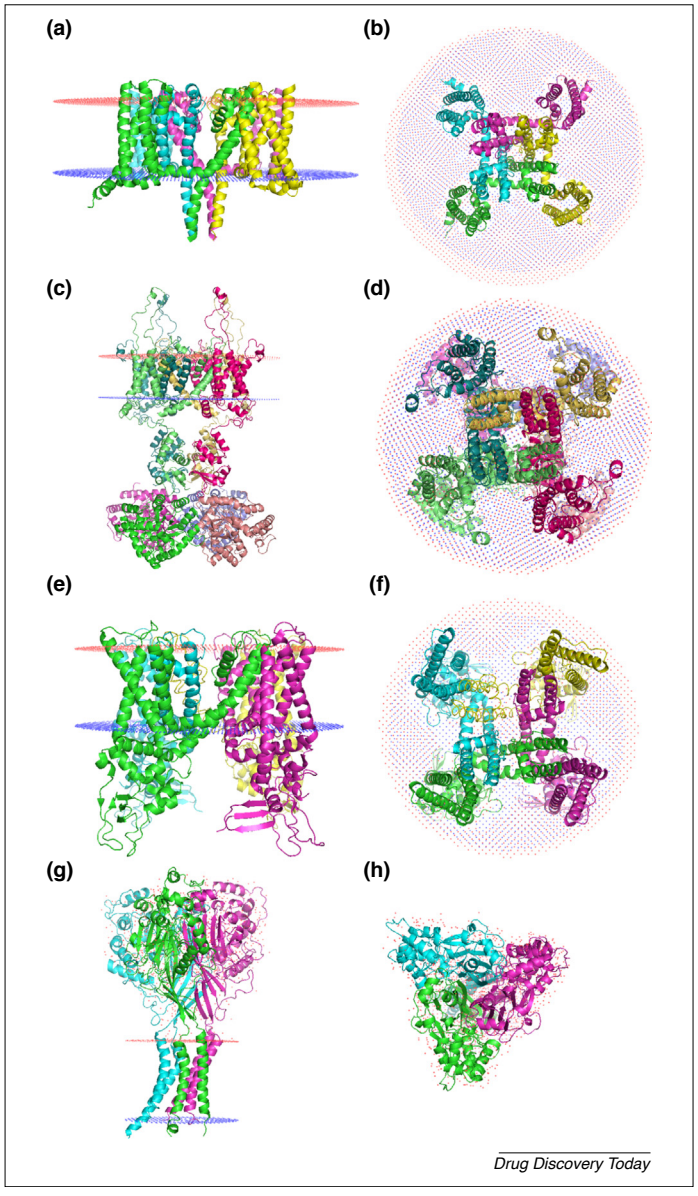

FIGURE 1

Ion channels affected by cnidarian neurotoxic peptides. The structures were obtained from the OPM database. The dashed line highlighted in red indicates the extracellular side of the eukaryotic plasma membrane and the blue is the cytoplasmic side. All structure visualization was achieved using VMD program v1.9.2. Na_V channel (PDB ID: 5EK0) viewed from the transmembrane side **(a)** and extracellular side **(b)**. K_V channel (PDB ID: 3LUT) viewed from the transmembrane side **(c)** and the extracellular side **(d)**. TRPV1 channel (PDB ID: 5IRX) viewed from the transmembrane side **(e)** and the extracellular side **(f)**. ASIC channel (PDB ID: 2QTS) viewed from the transmembrane side **(g)** and the extracellular side **(h)**.

TABLE 1

Summary of cnidarian neurotoxic peptides

Toxin type	Toxin	Source of toxin	Activity	Number of residues	Molecular targets	Potential application	Refs
Sodium channel inhibitor							
NaTx (type I, II)	ATX I, Anthopleurin-A (AP-A), Anthopleurin-B (AP-B)	*Anemonia, Anthopleura*	Neurotoxic, cardiotoxic, insecticide	46–49	Na_V1	Neuroprotection, antiepileptic seizures	[156,157]
NaTx (type III)	ATX III	*Anemonia*		27–32	Na_V1		[158]
NaTx (type IV)	Calitoxins I, calitoxins II	*Calliactis*		46	Na_V1		[159]
novel NaTx	A novel neurotoxin	*Palythoa*	Neurotoxic	32	$Na_V1.7$		[160,161]
Potassium channel inhibitor							
KTxs (type I)	BgK	*Bunodosoma*	Neurotoxic, hypotensive, cardiotoxic, analgesic, antimicrobial, immunosuppressive	37	$K_V1.1$, $K_V1.2$, $K_V1.3$, $K_V1.6$, IKCa1	No description	[162,163]
	ShK	*Stichodactyla*		35	$K_V1.3$, $K_V3.2$, IKCa1	Antiobesity, multiple sclerosis treatment	[164–168]
	HmK	*Heteractis*		36	$K_V1.1$, $K_V1.2$, $K_V1.3$	No description	[169]
	AsKS (Kaliseptine)	*Anemonia*		36	$K_V1.2$	No description	[170]
KTxs (type III)	BDS-I	*Anemonia*		43	$K_V3.1$, $K_V3.2$, $K_V3.4$, $Na_V1.7$	Anti-pain	[171–173]
	BDS-II	*Anemonia*		43	$K_V3.1$, $K_V3.2$, $K_V3.4$, $Na_V1.7$	Anti-pain	[171–173]
	APETx1	*Anthopleura*		42	$K_V11.1$, $K_V11.3$, $Na_V1.2$, $Na_V1.3$, $Na_V1.4$, $Na_V1.5$, $Na_V1.6$, $Na_V1.8$	Antiepileptic seizures, anti-pain	[174–177]
Kunitz peptides (type II KTxs)	PcKuz3	*Palythoa*	Paralytic, neurotoxic, serine protease inhibitor	52	$K_V1.1$, $K_V1.2$	Neuroprotection	[178]
	AsKC1 (kalicludine 1)	*Anemonia*		58	$K_V1.2$	No description	[170]
	AsKC2 (kalicludine 2)	*Anemonia*		58	$K_V1.2$	No description	
	AsKC3 (kalicludine 3)	*Anemonia*		59	$K_V1.2$	No description	
Acid-sensing ion-channel inhibitor							
Acid-sensing ion-channel inhibitor	APETx 2	*Anthopleura*	Analgesic	42	ASIC3, $K_V3.4$, $K_V11.1$	Anti-pain	[174,179–183]
TRPV1 inhibitor							
TRPV1 inhibitors	APHC1	*Heteractis*	Analgesic, serine protease inhibitor	51	TRPV1	Anti-pain, anti-epilepsy, neuroprotection	[184–188]
	APHC2	*Heteractis*		56	TRPV1		
	APHC3	*Heteractis*		56	TRPV1		
	HCRG21	*Heteractis*		56	TRPV1	Anti-pain	

Voltage-gated sodium channel toxins

Na_V channel transmembrane complexes comprise a pore-forming α subunit (a single polypeptide chain that folds to form four homologous repeats, named domain I to IV) and an auxiliary β subunit that facilitate membrane localization and modulate channel properties [94]. The first Cnidaria toxin, also the first NaTx, is ATX II, suggesting that the positive inotropic effects of ATX II and increased stimulation frequency could be induced by a similar mechanism in guinea pig papillary muscle [95]. It has been suggested that NaTxs have evolved under the influence of negative selection by computing omega values for each clade independently [46]. This finding suggested that favoring the conservation of structurally important and catalytic residues owing to filtering out mutations leads to loss of stable structure and function, because synthesis and secretion of proteins is an energetically expensive process [46]. Anthozoan NaTxs, and several other groups of toxins from scorpions and spiders, bind to loop S3–S4 in domain IV of Na_V channels, locking the S4 segment in its inward position and thus inhibiting conformational changes for channel fast inactivation, resulting in neurotransmitter release in synapses [53,55]. The NaTxs were classified into four groups: type I NaTx; type II NaTx (consists of 46–49 residues crosslinked by three disulfide bridges, except for AeI from *Actinia equina* that is 54 amino acids long); type III (which contains 27–32 residues and can have three or four disulfide bridges); and type IV (which is represented by calitoxins I and II, which are isolated from *Calliactis parasitica*, and contains 46 amino acids and three disulfide bonds) [58,96]. Furthermore, there are peptides as short as ~30 amino acids in length in the sea anemone that showed similar activity against NaTxs, even though they lack shared motifs [53]. Lastly, a novel neurotoxin forming only one disulfide bond was isolated and characterized and could target Na_V and K_V; it does not resemble any previously reported toxin [61].

Voltage-gated potassium channel toxins

Within sea anemone, KTxs were categorized into five groups based on their sequence similarity and binding affinity toward different K_V channel families [56,58]. The first KTx acts as a $K_V3.4$ blocker: BgK, a peptide purified from *Bunodosoma granulifera* [97]. Briefly, type I peptides have 35–37 amino acid residues and three disulfide bridges, including BgK and ShK. They inhibit the potassium current through K_V1, K_V3 and subfamilies

and intermediate conductance calcium-activated potassium channels [57,79,98,99]; type II peptides were composed of 58 or 59 amino acid residues crosslinking with three disulfide bridges, which were similar to Kunitz-type protease inhibitors. They were remarkable in their dual activity that inhibits trypsin and chymotrypsin proteases to prevent rapid degradation of the venom protease [100–102] by endogenous enzymes of the animals themselves, or of the prey, as well as possessing K_V-blocking activity like snake dendrotoxins [103]; type III peptides consisted of 41 or 42 amino acid residues and three disulfide bonds. They were not active on K_V1 subunits but could block either K_V3-containing ($K_V3.4$) channels belonging to rapidly inactivating K_V channels like BDS-I and BDS-II or ether-à-go-go ($K_V10.1$, $K_V11.1$) channels like APETx1 [104,105]. Notably, type III KTxs evolved from NaTxs under the regime of positive selection [46] but do not result in loss of blocking activity against the sodium ion channel [106]; type IV (SHTX I, SHTX II) were structurally novel peptides from *Stichodactyla haddoni* displaying crab paralysis activity and are crosslinked with two disulfide bridges [57]; type V KTxs were active on *Drosophila* Shaker IR channels, which were identified in *Bunodosoma caissarum* and crosslinked by four disulfide bonds [59].

Acid-sensing ion-channel inhibitors

ASICs are proton-activated cation channels that are expressed throughout neuronal and non-neuronal mammalian tissues [107]. In a rat model of postoperative pain, peripheral ASICs formed depolarizing channels that could be activated by extracellular protons [108]. There are now ten ASIC modulators that have been described in animal venoms, whereas the modulators from sea anemone preferentially target ASIC3 [109]. APETx2 was a 42-residue peptide isolated from *Anthopleura elegantissima*, and was the first potent and ASIC3-selective inhibitor discovered [110]. The use of APETx2 *in vivo* has been key in establishing the role of ASIC3 as a sensor of acid-induced and postoperative pain, also demonstrating its involvement in inflammatory pathways [108,111]. It has been reported that APETx2 was analgesic in rat models of inflammatory and osteoarthritic pain [112]. Recently, several other peptide toxins targeting ASICs were isolated from different sea anemones. Hcr 1b-1, an analog of APETx2 with 51% identity, was isolated from *Heteractis crispa* [113]. Although it was ~35-fold less potent than APETx2, it shares identity with much of the APETx2 pharmacophore for ASIC3, indicating less of an off-target effect. A recent review summarized that APETx2 could act on hASIC3 with an IC_{50} value of 175 nM and hetero ASIC3/1b with IC_{50} value of 900 nM. Meanwhile, Hcr 1b-1 was more selective although less potent than APETx2 against hASIC3 with an IC_{50} value of 5.5 μM [114]. Ugr 9-1 was isolated from *Urticina grebelnyi*, which completely blocked transient hASIC3 current but was inactive against ASIC1a, ASIC1b and ASIC2a, potentially making it a more selective ASIC3 inhibitor [68]. Another ASIC toxin is PhcrTx1, which was the first ICK peptide isolated from *Phymanthus crucifer*. This inhibits the ASIC currents in rat sensory neurons and produces a moderate but significant K_V current blockade effect, with no activity on Na_V [115]. ASICs are sensors of acid-induced and postoperative pain [108], so Cnidaria venom peptides acting as ASIC inhibitors could be therapeutic lead candidates developed for pain treatment [114].

TRPV1 inhibitors

TRPV1 is a nonselective cationic ion channel primarily expressed in nociceptors, and can be activated by capsaicin, noxious heat, protons, lipid messengers and exogenous ligands [116–118]. TRPV1 is involved in the progress of different pathological conditions, such as diabetic painful neuropathy, peripheral neuropathic pain, cancer pain and epilepsy [119–122]. The toxins APHC1–3, which are derived from the sea anemone *Heteractis crispa*, were the first peptides to inhibit TRPV1 [123]. Functionally, APHC3 was the most potent by inhibiting 71% of 3 μM capsaicin with the lowest IC_{50} value of 18 nM [124]. *In vivo* tests suggest APHC1 and APHC3 could produce a dose-dependent inhibition of thermal nociception in pain models without causing hyperthermia [125]. Interestingly, APHC1 was a more potent TRPV1 inhibitor than APHC3 and it was effective in increasing paw withdrawal latency from a hot plate at doses as low as 0.01 mg/kg, whereas minimum effective dose of APHC3 was 0.05 mg/kg [125]. Introduction of TRPV1 antagonists to the clinic as new analgesics was slow because of the adverse effects like hyperthermia and insensitivity-causing scalding heat [126,127]. Thus, APHC1 provided an interesting insight into the possible effects of partially inhibiting TRPV1 [128].

Relationship between ion channel modulators and neurotoxicity

Ion channels are vital contributors to cellular communication that regulate the ion permeability of the cell membrane and generate electrical signals that disseminate vital information across the human body [129]. The cnidarian neurotoxins prolong the action potential of the excitable and nonexcitable membranes in sensory neurons and cardiac and skeletal muscle cells by modifying the ion channels (e.g., pore-blocking toxins bind to the outer vestibule or ion conduction pore; and gating-modifier toxins alter channel conformation during opening or inactivation) during the repolarization stage [56,130–132]. By contrast, the vast majority of peptides in cone snails and arachnid venoms target the nervous system or cardiovascular system to immobilize the prey rapidly [52,129]. Animal toxins have provided important insights into development of clinical leads as antagonists against voltage-gated ion channels, such as mechanosensitive and chloride ion channels, acetylcholine, NMDA and G-protein-coupled receptors [133–137].

The association between ion channel inhibition and neuroprotective effects

Neurotoxins are biomolecules that exert a lethal effect in the nanogram dose range or lower, by targeting the nervous system with affinity to Na_V or K_V channels [138,139]. Growing evidence suggests that ion channels could be promising targets for neurodegenerative disease therapeutics [140,141]. Na_V channels are thought to have an important role in neurodegeneration and neuroinflammation [142]. In the CNS, factors such as nitric oxide, inflammation-induced ischemia and mitochondria impairment reduce energy production, leading to decreasing Na^+/K^+-ATPase pump activity, membrane depolarization and abnormal persistent accumulation of Na^+ influx. Intracellular Na^+ overload drives the Na^+/Ca^{2+} exchanger to import Ca^{2+} into axons, triggering a pathogenic loop that causes further mitochondrial damage and activation of a harmful cocktail of pro-

teases, lipases and nitric oxide synthase, finally causing neuron injury and neuronal loss [142–145]. Thus, blockade against Na_V channels might ameliorate neurodegenerative disorders through inhibition of Na^+ overload [142].

K_V channels are key regulators of excitability in neurons and nonexcitable tissues such as microglia [146]. Microglia are the key inflammatory cells in Alzheimer's disease (AD), which could be activated by amyloid-β (Aβ) producing reactive oxygen species (ROS) leading to AD pathogenesis [147]. It was demonstrated that microglia priming and ROS production were abolished by 5-iodo-resiniferatoxin and charybdotoxin, which are TRPV1 and $K_V1.3$ inhibitors, respectively [148]. In addition, it was reported that $K_{Ca}3.1$ was upregulated in reactive astrocytes of $Tg^{APP/PS1}$ mice and AD patients compared with wild-type mice and control humans [149]. Moreover, $K_{Ca}3.1$ blockade inhibited astrocyte activation and reduced brain levels of IL-1β, TNF-α, iNOS and COX-2 [149].

ASICs have vital roles in many physiological processes including sodium homeostasis, synaptic plasticity, sensory transduction and neurodegeneration [150]. ASIC1 is the predominant ASIC expressed in the CNS and is believed to contribute to apoptosis under pathological conditions owing to a robust increase of Ca^{2+} influx and acidic environment (pH 6.5) in cerebral tissues [151]. The overexpression of ASIC1a was found in dopaminergic neurons of Parkinson's disease patients and rodent models [152].

Again, ion channels such as Na_V, K_V, and ASIC could be targets of neurodegenerative diseases. There are peptide neurotoxins isolated from terrestrial animals displaying neuroprotective activities. Chassagnon *et al*. reported that Hi1a, a peptide isolated from tarantula *Psalmopoeus cambridgei*, which was homologous to psalmotoxin, an ASIC1a inhibitor, could inhibit ASIC1a activation in a pH-independent and slowly reversible manner, resulting in a marked reduction of infract size as well as amelioration of neurological and motor function in a focal model of ischemic stroke in rat [153]. However, the use of marine natural products to treat neurodegenerative diseases is largely underexploited and only a few studies reported that compounds originated from marine neuronal models [154,155]. As summarized above, Cnidaria neurotoxic peptides could be a rich resource of various ion-channel blockers, providing drug development insights for therapeutics that target ion channels to treat neurodegenerative diseases.

Concluding remarks

Marine bioprospecting holds significant potential for the discovery of novel drugs, nutritional supplements and industrial biotechnology applications. Members of the phylum Cnidaria, including sea anemones, corals, jellyfish and hydra, are venomous Eumetazoan. High-throughput methods combining transcriptomic and proteomic profiling could yield a holistic overview of the complexities of the venom from Cnidaria, revealing many uncharacterized peptides. Many cnidarian toxins remain to be discovered and their potential as novel sources of therapeutic substances should be investigated. Neuroactive peptides targeting the CNS through their affinity with ion channels could provide an insightful perspective to characterize drug leads for the treatment of neurodegenerative diseases or epilepsy, which arise from ion-channel dysfunction.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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