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Review

Cnidarian Toxins Acting on Voltage-Gated Ion Channels

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Abstract: Voltage-gated ion channels generate electrical activity in excitable cells. As such, they are essential components of neuromuscular and neuronal systems, and are targeted by toxins from a wide variety of phyla, including the cnidarians. Here, we review cnidarian toxins known to target voltage-gated ion channels, the specific channel types targeted, and, where known, the sites of action of cnidarian toxins on different channels.

Keywords: Cnidaria; ion channels; toxin; sodium channel; potassium channel.

Abbreviations: K_V channel, voltage-gated potassium channel; Na_V , voltage-gated sodium channel; Ca_V , voltage-gated calcium channel; ApA, Anthopleurin A; ApB, Anthopleurin B; ATX II, *Anemone sulcata* toxin II; Bg II, *Bunodosoma granulifera* toxin II; Sh I, peptide neurotoxin I from *Stichodactyla helianthus*; RP II, polypeptide toxin II from *Radianthus paumotensis*; RP III, polypeptide toxin III from *Radianthus paumotensis*; RTX I, neurotoxin I from *Radianthus macrodactylus*; PaTX, toxin from *Paracicyonis actinostoloides*; Er I, peptide toxin I from *Entacmaea ramsayi*; Da I, peptide toxin I from *Dofleinia armata*; ATX III, *Anemone sulcata* toxin III; ShK, potassium channel toxin from *Stichodactyla helianthus*; BgK, potassium channel toxin from *Bunodosoma granulifera*; AsKS, kalciceptine from *Anemonia sulcata*; HmK, potassium channel toxin from *Heteractis magnifica*; AeK, potassium channel toxin from *Actinia equina*; AsKC 1-3, kalcicludines 1-3 from *Anemonia sulcata*; BDS-I, BDS-II, blood depressing toxins I and II

from *Anemonia sulcata*; APETx1, potassium channel toxin 1 from *Anthopleura elegantissima*; SNTX, sea nettle toxin; E, glutamic acid; D, aspartic acid; K, lysine; R, arginine; Y, tyrosine; HERG, human *ether-a-go-go*-related gene.

Introduction

Voltage-gated ion channels underlie electrical excitability in cells, and they also play important roles in non-excitabile cells [1]. Voltage-gated channels open in response to changes in membrane potential, allowing ions to flow down the electrochemical gradient across the cell membrane. They are thus gated (by voltage), and they form an ion-selective pore. Voltage-gated channels represent a protein superfamily that comprises more than 140 members, and is part of the even larger ion channel superfamily [1,2].

The main, pore-forming protein of voltage-gated ion channels is the α (or α_1) subunit. It consists of four homologous domains (I-IV), each containing 6 predicted transmembrane regions (S1-S6), with a loop between S5 and S6 that reenters the membrane. These four domains surround a central pore, with this S5-S6 loop thought to serve as the ion selectivity filter of the pore. Voltage-gated potassium (K_v) channels consist of single domains that form a tetramer of α subunits in the membrane [3]. In contrast, voltage-gated sodium (Na_v) and calcium (Ca_v) α (or α_1) subunits contain all four homologous domains in a monomeric structure [1,4,5].

The simplest ion channels consist of a tetrameric structure forming a central pore and selectivity filter [1,2,6]. These channels contain equivalents of the S5-S6 transmembrane regions along with the S5-S6 loop. The voltage-gated channel family is thought to have arisen through addition of an N-terminal voltage-gating domain (S1-S4) to this basic structure, with the four-domain Na_v and Ca_v α subunits arising presumably *via* two rounds of gene duplication [1,7,8]. Incorporation of regulatory domains at the intracellular, C-terminus of the basic channel structure yields sites for gating and modulation of voltage-gated and voltage-gated-like channels by intracellular factors.

Any particular ion channel family (e.g., Na_v channels) typically comprises a variety of different subtypes with particular physiological, pharmacological, and structural characteristics. For example, there are ten members of the mammalian Ca_v channel gene family, each of which has distinct roles in cellular signaling. The potential for expression of α_1 splicing variants adds to this diversity, as does modulation of channel function by auxiliary subunits and second messenger pathways [9-14].

Voltage-gated channels are critical to normal neuromuscular transmission. Disruption of their normal function can lead to rapid paralysis. As such, they represent excellent targets for toxins from a variety of organisms including snakes [15], arthropods [16], molluscs [17,18,19], and, the subject of this review, cnidarians [20]. These toxins can serve as remarkably powerful molecular probes of channel structure-function relationships, as well as models for studying molecular evolution of toxins and targets in predators and prey [21]. Some ion channel toxins may also prove to have therapeutic value, as is the case for toxins from cone snails that target a particular Ca^{2+} channel subtype and are currently being investigated as candidate drugs for their antinociceptive and other potential beneficial effects [18,19,22].

Cnidarian Ion Channel Toxins

The cnidarians are the earliest extant organisms with a neuromuscular system. They are simple carnivores that are radially symmetrical, with a mouth surrounded by tentacles. These tentacles contain high concentrations of stinging cells called nematocysts (cnidocysts), which are specialized epidermal cells that produce, store, and inject toxins used for protection and predation. Venoms contain a bouquet of substances, including peptides, proteins, phospholipids, phospholipases, glycoproteins, sterols, bioactive amines, and carbohydrates [23]. Thus, the cnidarians represent a rich source of venoms and toxins. The phylum comprises four extant classes: the hydrozoans; the cubozoids, or box jellies; the scyphozoans, or true jellyfish; and the anthozoa, the sea anemones and corals.

Sea anemone toxins dominate in terms of number identified and they are also the best characterized in terms of mechanism of action [20]. To date, many cnidarian toxins that act on Na_v and various K_v channels have been described in detail; toxins from cnidarians that clearly act on Ca_v channels have thus far not been characterized.

Na_v Channel Toxins

Some of the most thoroughly studied cnidarian toxins are polypeptide toxins from sea anemones that target Na_v channels [20,24,25,26]. These toxins are polypeptides of ~5 kDa, and more than 50 different sea anemone Na_v channel toxins have been isolated or cloned from a large variety of species. Particular toxins, including ATX II from *Anemonia sulcata* [27], and Anthopleurin A (ApA) [28] and Anthopleurin B (ApB) [29] from *Anthopleura xanthogrammica*, have proven especially useful as probes of Na_v channel structure and function. Furthermore, recent studies have provided evidence for *in vivo* epileptogenic effects of toxins (e.g., cangitoxin from *Bunodosoma cangicum*) [30].

Norton [25] described the classification of anemone Na_v channel toxins into different types based on their amino acid sequences (Fig. 1). Type 1 and Type 2 toxins are polypeptides of 46-49 amino acids; Type 3 peptides, from the genus *Entacmaea* (family Actiniidae), are shorter, containing 27-32 amino acids. Calitoxins I and II from *Calliactis parasitica* [31,32] are long peptides that do not fit clearly into Types 1 or 2, and may constitute a fourth class. Members of the Type 1 and Type 2 classes show extensive sequence similarity within each class (≥60%), but sequence similarity between members of the two classes is significantly lower (~30%), and there is no immunological cross-reactivity between the two groups. Members of both classes contain six conserved cysteine residues that are cross-linked by three disulfide bridges, and both have basic C-terminal sequences, though more markedly for Type 2 toxins (Fig. 1). The Type 1 and 2 toxins are largely distributed according to taxonomy. Toxins from the Actiniidae family are Type 1, while both types of toxins can be found in the family Stichodactylidae [20,25]. A single species can produce toxins from both classes, as is seen with Gigantoxin II and Gigantoxin III from *Stichodactyla gigantea* [33].

Figure 1. Alignments of representative sea anemone Na_v channel toxins.**A. Type 1.**

		*		20		*		40																																												
ApA	:	G	V	S	C	L	C	D	S	D	G	P	S	V	R	G	N	T	L	S	G	T	L	W	L	Y	P	S	G	C	P	S	G	W	H	N	C	K	A	H	G	P	T	I	G	W	C	C	K	Q	:	49
ApB	:	G	V	P	C	L	C	D	S	D	G	P	R	P	R	G	N	T	L	S	G	I	L	W	F	Y	P	S	G	C	P	S	G	W	H	N	C	K	A	H	G	P	N	I	G	W	C	C	K	K	:	49
ATX II	:	G	V	P	C	L	C	D	S	D	G	P	S	V	R	G	N	T	L	S	G	I	I	W	L	-	-	A	G	C	P	S	G	W	H	N	C	K	K	H	G	P	T	I	G	W	C	C	K	Q	:	47
Bg II	:	G	A	S	C	R	C	D	S	D	G	P	T	S	R	G	N	T	L	T	G	T	L	W	L	I	-	G	R	C	P	S	G	W	H	N	C	R	G	S	G	P	F	I	G	Y	C	C	K	Q	:	48
Cangitoxin	:	G	V	A	C	R	C	D	S	D	G	P	T	V	R	G	N	S	L	S	G	T	L	W	L	T	-	G	G	C	P	S	G	W	H	N	C	R	G	S	G	P	F	I	G	Y	C	C	K	K	:	48

B. Type 2.

		*		20		*		40																																											
Sh I	:	A	A	C	K	D	D	E	G	P	D	I	R	T	A	P	L	T	G	T	V	D	L	G	S	C	N	A	G	W	E	K	C	A	S	Y	Y	T	I	I	A	D	C	C	R	K	K	K	:	48	
Rp II	:	A	S	C	K	C	D	D	D	G	P	D	V	R	S	A	T	F	T	G	T	V	D	F	W	N	C	N	E	G	W	E	K	C	T	A	V	Y	T	P	V	A	S	C	C	R	K	K	K	:	48
Rp III	:	G	N	C	K	C	D	D	E	G	P	N	V	R	T	A	P	L	T	G	Y	V	D	L	G	Y	C	N	E	G	W	E	K	C	A	S	Y	Y	S	P	I	A	E	C	C	R	K	K	K	:	48
RTX I	:	A	S	C	K	C	D	D	D	G	P	D	V	R	S	A	T	F	T	G	T	V	D	F	A	Y	C	N	A	G	W	E	K	C	L	A	V	Y	T	P	V	A	S	C	C	R	K	K	K	:	48

C. Type 3.

		*		20		*																																
PaTX	:	A	G	G	K	S	T	C	C	P	C	A	M	C	K	Y	T	A	G	C	P	W	G	Q	C	A	H	H	C	G	C	S	-	-	-	-	:	31
Er I	:	A	G	G	K	S	T	C	C	P	C	A	M	C	K	Y	T	A	G	C	P	W	G	Q	C	A	H	H	C	G	C	S	E	-	-	-	:	32
Da I	:	-	G	G	K	A	T	C	C	P	C	F	M	C	S	Y	T	A	G	C	P	W	G	Q	C	A	H	H	C	G	C	D	-	-	-	-	:	30
ATX III	:	-	-	-	R	-	S	C	C	P	C	-	-	-	Y	W	G	G	C	P	W	G	Q	N	C	Y	P	E	G	C	S	G	P	K	V	:	27	

Identical residues are shaded black. Na_v toxins shown are: ApA and ApB from *Anthopleura xanthogrammica* [28,29]; ATX II from *Anemonia sulcata* [27]; Bg II from *Bunodosoma granulifera* [51]; Cangitoxin from *Bunodosoma cangicum* [30]; Sh I from *Stichodactyla helianthus* [52]; Rp II and Rp III from *Radianthus paumotensis* [53,54]; RTX I from *Radianthus macrodactylus* [55]; PaTX from *Parascyonis actinostoloides* [56]; Er I from *Entacmaea ramsayi* [33]; Da I from *Dofleinia armata* [33]; and ATX III from *Anemonia sulcata* [57].

Anemone Na_v channel toxins were initially characterized as cardiac stimulants [34] and neurotoxins [35], with their predominant effect based on relative affinities for cardiac or neuronal Na_v isoforms. They were subsequently shown to interact with neurotoxin receptor site 3 of Na_v channels, with their action being the delay of channel inactivation [36-40]. The open state of the channel is thus prolonged during depolarization. Neurotoxin receptor site 3 is one of at least six neurotoxin receptor sites on Na_v channels. It is also a receptor for α-scorpion toxins, which do not share sequence homology with the anemone toxins. Receptor site 3 is a complex receptor site that includes the extracellular loop between transmembrane segments S3 and S4 in domain IV of the channel [41]. The toxins bind to this site *via* electrostatic interactions, predominantly with the negatively charged E1613 residue in rat brain Na_v channels [42] and D1612 in cardiac Na_v channels [43]. Contributions from other, nearby residues [44] and unidentified contacts in the IS5-S6 and IVS5-S6 loops [45,46] are also

likely involved in interaction with these toxins. Interestingly, unlike toxins that interact with receptor site 4, ApB and other site 3 toxins do not bind phospholipids [47].

Work on the ApB toxin has defined a region critical for interaction with Na_v channels. ApB, like other Type 1 toxins, has a conformation that is primarily a β -structure, with a four-stranded antiparallel β sheet linked by β -turns and loops with no α -helix [48]. A disordered region corresponding to residues 8-17 of ApB and found in all active sea anemone toxins is referred to as the Arg-14 loop [49]. This region appears to be critical for interaction with receptor site 3 of the Na_v channel. Furthermore, the relative non-selectivity of ApB for cardiac and neuronal channels *versus* the relative selectivity of ApA for cardiac channels is associated with two cationic residues (R12, K49) found in ApB but not in ApA [50].

K_v Channel Toxins

There have been at least 11 K_v channel toxins that have been identified in various sea anemones to date. These peptide toxins fall into three classes based on structural and functional differences (Table 1, Fig. 2).

Table 1. K_v channel toxins.

Toxin	Species	Type	Reference
ShK	<i>Stichodactyla helianthus</i>	1	[67]
AsKS (kaliseptine)	<i>Anemonia sulcata</i>	1	[64]
BgK	<i>Bunodosoma granulifera</i>	1	[68,69]
HmK	<i>Heteractis magnifica</i>	1	[70]
AeK	<i>Actinia equine</i>	1	[71]
AsKC 1-3 (kalcicludines 1-3)	<i>Anemonia sulcata</i>	2	[64]
BDS-I, BDS-II	<i>Anemonia sulcata</i>	3	[65]
APETx1	<i>Anthopleura elegantissima</i>	3	[66]

Type 1 toxins potently block K_v1 (*Shaker*) channels. They contain 35-37 amino acids that form two nearly perpendicular stretches of helices and include three disulfide bridges [58, 59]. A conserved dyad of lysine (K22 in ShK) and tyrosine (Y23 in ShK) appear to be essential residues required for interaction with rat brain K_v channels [60]. A similar result is found for the BgK toxin [56, 58, 59]. Recently, use of ShK and analogs to selectively block K_v1.3, a lymphocyte K_v channel that is important for the activation of terminally differentiated effector memory (TEM) T cells, is being investigated as a candidate treatment for multiple sclerosis and other autoimmune diseases [63].

The Type 2 K_v channel toxins, represented by the AsKCs, are made up of 58 or 59 amino acid residues, and also block K_v1 channels, though with less potency than Type 1 toxins [64]. They have sequence homology to dendrotoxins, K_v channel toxins from snake venom; they also show homology to Kunitz-type protease inhibitors, and exhibit protease inhibitory activity.

Type 3 K_v channel toxins include BDS-I and BDS-II from *A. sulcata* [65], and APETx1 from *Anthopleura elegantissima* [66]. BDS-I and II are selective blockers of K_v3 (*Shaw*) channels, predominantly the fast-inactivating K_v3.4 channel. APETx1 is highly similar (54%) to the BDS toxins.

However, unlike the BDS toxins, it selectively inhibits *ether-a-go-go*-related gene (ERG) K_V channels such as the human ERG (HERG; K_V11.1) channel, an essential component of cardiac cells that controls the duration of the plateau phase of the action potential. APETx1 acts by modifying the voltage-dependence of HERG gating [66].

Figure 2. Alignments of sea anemone K_V channel toxins.

A. Type 1.

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                *           20           *
ShK      : RSCIDTIPKSRCTAFQ----CKHSMKYRLSFCRKTCGTC : 35
BgK      : -VCRDWFKETACRHA KSLGNCRTSQKYRAN-CAKTCELC : 37
AsKS     : -ACKDNFAAATCKHVKENKNCG-SQKYATN-CAKTCGKC : 36
AeK      : -GCKDNFSANTCKHVKANNNCG-SQKYATN-CAKTCGKC : 36
HmK      : RTCKDLIPVSECTDIR----CRTSMKYRLNLCRKTCGSC : 35
  
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B. Type 2.

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                *           20           *           40           *
AsKC 1   : INKDCLLPMDVGRCRASHPRYYNSSSKRCEKFIYGGCRGNANNFHTLEECEKVCGVR- : 58
AsKC 2   : INKDCLLPMDVGRCRARHPRYYYNSSSRRCEKFIYGGCRGNANNFITKKECEKVCGVR- : 58
AsKC 3   : INGDCLEPKVVGRCRARFPRYYNLSRRCEKFIYGGCGGNANNFHTLEECEKVCGVRS : 59
  
```

C. Type 3.

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                *           20           *           40
BDS-I    : AAPCFCSGKPGRGDLWILRGTCPGGYGYTSNCKYKWPNICCYPH- : 43
BDS-II   : AAPCFCPGKPDRGDLWILRGTCPGGYGYTSNCKYKWPNICCYPH- : 43
APETx1   : GTTCYC-GKT-IGIYWFGTKTCP SNRGYTGSCGYFLGICCPVD : 42
  
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Identical residues are shaded black. K_V toxins shown are: ShK from *Stichodactyla helianthus* [67]; BgK from *Bunodosoma granulifera* [69]; AsKS (kaliseptine), AsKC (kaliclodines 1-3), BDS-I, and BDS-II from *Anemonia sulcata* [64, 65]; AeK from *Actinia equina* [71]; HmK from *Heteractis magnifica* [70]; APETx1 from *Anthopleura elegantissima* [66].

Concluding remarks

Although sea anemone toxins have provided a wealth of pharmacological probes, toxins from only a relatively few cnidarian species have thus far been characterized. Novel venoms from other anemone species as well as other cnidarians have the potential to provide even more powerful tools, as well as possible pharmaceutical applications. For example, Ca_V channels are critical components of the neuromuscular junction and of neurosecretory activity, and are targeted by toxins from a wide variety of organisms. Interestingly, however, purified cnidarian toxins with clear selectivity for Ca_V channels have not yet been described. A potential candidate Ca_V toxin may be the Bainh toxin isolated from *Bunodosoma granulifera* [72]. This toxin increases the force of contraction in mammalian ventricular

muscle preparations, an effect that appears to be mediated by a voltage-independent, toxin-induced increase in L-type Ca_v currents.

Several non-anemone venoms produce perturbations in ion transport across excitable membranes [73], and potential Ca_v toxins may be present in the crude venoms of the hydrocoral *Millepora complanata* [74] and the sea nettle *Chrysaora quinquecirrha* [75]. SNTX, a toxin also from *C. quinquecirrha*, creates large cation-selective channels that open and close spontaneously and show voltage-dependence of open probability [76]. It is thus likely that as more venoms from different cnidarian species are examined in greater detail, a wider diversity of ion channel toxins will emerge.

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