Pathophysiological Response to CnTX Voltage-Gated Channel Modulation

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Voltage-gated ion channels are plasma membrane proteins that generate electrical signals following a change in the membrane voltage.

conotoxins | voltage-gated ion currents | sodium

1. Introduction

Marine organisms produce a great variety of toxins. Among them, venomous mollusks cone snails have provided so far more than 6000 different toxins that have been isolated and characterized by more than 100 different species. Cone snails have been the object of huge interest and study since ancient times due to their shell features but mostly for their poison-associated toxins. The main physiological roles of these toxins are for self-defense from predators but also to predate themselves and to compete with other marine species, also thanks to a poisonous sting that may be fatal for humans. The venomous properties of the toxins have been revealed to exert pharmacological bioactivities, especially on a vast variety of pain-associated neurological disorders. Hence, the toxin's level of poisonousness has been used as an efficient and beneficial pharmacological and therapeutic tool, making conids good candidates for new drug design and development [1][2][3]. Conus-derived toxins, known as conotoxins (CnTX), are venomous small peptides consisting of 5–50 amino acid residues with multiple disulfide bonds among cysteines. An old classification divided CnTX into cysteine-rich and cysteine-poor groups. At present, instead, three CnTX groups are classified based on the cysteine framework gene superfamily and the pharmacological family.

Currently, pharmacological family classification is related to the receptor target and the type of CnTX-target interaction. CnTX included in the same superfamily share a similar signal peptide sequence, which undergoes structural and functional differentiation when they become encoded mature peptides [4][5]. Nonetheless, over 10,000 CnTX sequences have been disclosed and published; however, 3D structural and functional information is still lacking and their pharmacological characterizations have not been elucidated. CnTX includes several different pharmacological families that selectively target specific voltage-gated ion channels, G protein-coupled receptors, enzymes, and transporters [6][7][8]. Currently, CnTXs are under evaluation by neuroscientists and drug developers for their peculiar selectivity to mammalian and human targets and, in particular, for their ability to inhibit voltage-gated ion channels. As an example, the μ-CnTX family exerts its activity by combining multiple peptides action known as "cabal", which is aimed to ensure the most effective bioactivity through ion channels modulation. Due to their ability to interact with human ion channels, these toxins are considered "specialists in neuropharmacology"

and given their therapeutic potential, some of them have been involved in human clinical treatments [9][10][11]. Based on their specific selectivity, CnTX represent basic tools to elucidate ion channel function and their involvement in biological mechanisms and processes. In this research, the researchers focus on the main CnTX pharmacological properties and their modulation of voltage-gated sodium (Na_V), calcium (Ca_V), and potassium (K_V) channels acting through different and, sometimes, opposite physiological and pathological mechanisms (**Figure 1**).

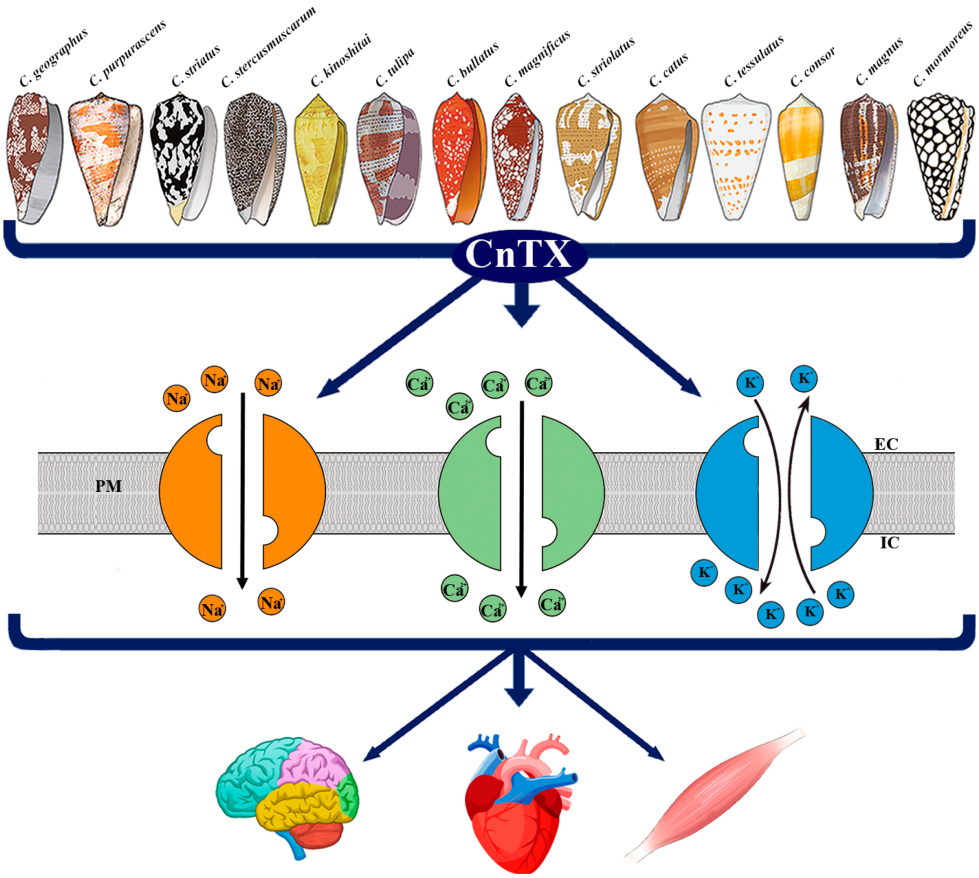

Figure 1. Representative image of CnTX bioactivity via voltage-gated ion channel modulation. Conus derived toxins (CnTX) target numerous and different Na_V and/or Ca_V and/or K_V channel subtypes generating ion current fluxes through neurons of central and peripheral nervous systems as well as in heart and skeletal muscle cells. IC = intracellular compartment; EC = extracellular compartment; PM = plasma membrane.

2. Pathophysiological Response to CnTX Voltage-Gated Channel Modulation

2.1. Na_V Channels

Na_V channels are voltage-gated ion channels responsible for the generation of the rapid RP depolarization known as action potential that, in excitable cells, propagates electrical signals in muscles and nerves in either the central or peripheral nervous system [12]. Since Na_V channels underlie neurotransmission, contraction, excitation coupling, and associated physiological functions [12], mutations and defects in their functional activity are associated with several neurological disturbances and channelopathies [13]. From a structural point of view, Na_V channels are

heteromeric complexes with a pore-forming α subunit of about 260 KDa linked to one or two β subunits of different molecular weights. The α subunit contains the binding site for several neurotoxins and drugs that target the channel and significantly change its activity. The β subunits are involved in different signaling roles in physiological processes such as cell adhesion, gene regulation, and brain development and in the kinetic regulation of channel opening. Both α and β subunits contain the receptors for toxins targeting the channel. Currently, characterized according to the α-pore-forming subunit sequences, nine isoforms of the Na_V channels α subunits (1.1–1.9) have been identified. Specifically, isoforms 1.1, 1.2, 1.3, and 1.6 are predominantly expressed in the central nervous system, whereas isoforms 1.7, 1.8, and 1.9 are mainly expressed in the peripheral nervous system. In addition, skeletal and heart muscles contain 1.4 and the 1.5 isoforms, respectively [14][15][16][17].

Toxins and venom compounds targeting Na_V channels are of particular importance due to their pivotal role played in the neuromuscular system. Together with their pathophysiological action, CnTX have also provided basic information on the molecular structure, function, and subtype-selectivity of Na_V channels [18] (**Table 1**).

Table 1. CnTX subfamilies targeting voltage-gated sodium (Na_V) channel subtypes, functional impact, and pathophysiological activity.

Species	CnTX Subfamilies	Channel Subunit Targeted	Functional Impact	Pathophysiological Activity	References
C. geographus	μ-GIIIA	$Na_V1.4$	block skeletal muscle channels	paralysis	[19]
C. geographus	μ-GIIIB μ-GIIIC	$Na_V1.1$ $Na_V1.2$ $Na_V1.4$ $Na_V1.6$	discriminate between muscle and neuronal channels	-	[20]
C. bullatus *C. catus* *C. consor* *C. magnus* *C. purpurascens* *C. stercusmuscarum* *C. striatus* *C.tulipa*	μ-CnIIIA μ-CnIIIB μ-CnIIIC μ-CIIIA, μ-MIIIA	Na_V1	block channel conductance	paralysis (CIIIA)	[21] [22]
C. purpurascens	μ-PIIIA	$Na_V1.2$ $Na_V1.4$ $Na_V1.7$	inhibit channel modulation	-	[23]
C. stercusmuscarum	μ-SmIIIA		irreversible block of NaV currents	nociceptive role	[24][25]
C. striatus	μ-SIIIA	$Na_V1.2$	block of neuronal	analgesic activity	[26]

Species	CnTX Subfamilies	Channel Subunit Targeted	Functional Impact	Pathophysiological Activity	References
			NaV current		
C. tulipa *C. kinoshitai* *C. striatus*	µ-TIIIA, µ-KIIIA, µ-KIIIB, µ-SIIIB	$Na_V1.1$ $Na_V1.2$ $Na_V1.3$ $Na_V1.4$ $Na_V1.6$	affinity Na_V channels	analgesic activity	[27][28][29]
C. marmoreus	µO-MrVIA, µO MrVIB, µO MfVIA	$Na_V1.8$	inhibit channel activity	analgesic activity	[30]
C. radiatus	í-RXIA [32][33]	$Na_V1.6$	shift channel activation	-	[31]

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(µ- and µO-CnTX) or stimulators (δ- and ι′-CnTX) of Na_V activity. The channel inhibition is underlined by different mechanisms. For example, µ-CnTX blocks ion conductance by binding to the channel's external vestibule, whereas µO-CnTXs are gating modulators that bind the external side of the pore, causing channel closure [34][35]. Similarly, δ- and ι′-CnTX activate channels by two different mechanisms that prolong the channel's opening and shift voltage activation to more hyperpolarized potentials, respectively [36]. µ-CnTX are small peptides composed of 16–22 amino acids and three disulfide bonds that, by inhibiting the α-subunit of Na_V channels, affect neuromuscular transmission causing paralytic to analgesic effects in mammals [37][38]. Currently, among the 12 CnTX known, µGIIIA was the first one to be isolated from *Conus geographus*; by binding the $Na_V1.4$ sub-type channel pore, it exerts the inhibition of rat skeletal muscle channels [19]. From the same venom, the two isoforms µ-GIIIB and µ-GIIIC, differing for only four residues, exhibited a different affinity for the muscle subtype Na_V channels 1.4 and the neuronal $Na_V1.1$, $Na_V1.2$, and $Na_V1.6$ channel subtypes. These µCnTX can discriminate between muscle and neuronal Na_V channels, becoming candidates for therapeutic plans on neurological disorders. Therefore, a wide range of studies was aimed to identify whether interactions between several µ-CnTX and Na_V channel subtypes may act as selective inhibitors of Na_V channels neuronal subtypes, with possible clinical impact on neurological processes [20]. In this light, several µ-CnTXs with affinities for neuronal Na_V channel subtypes were isolated from *Conus bullatus*, *catus*, *consor*, *kinoshitai*, *magnus*, *purpurascens*, *stercusmuscarum*, *striatus*, *striolatus*, and *tulipa*. Among these, µ-CnIIIA, µ-CnIIIB, µ-CnIIIC, µ-CIIIA, and µ-MIIIA can block the conductance of Na_V1 subtypes in amphibian neurons. Some of them, by inhibiting olfactory and sciatic nerve action potentials, modulate pain signals and exert analgesic activity [21]; however, only CIIIA may cause paralysis [22]. µ-PIIIA is a peculiar versatile µ-CnTX that can differentiate Na_V channel subtype isoforms by inhibiting $Na_V1.2$, $Na_V1.4$, and $Na_V1.7$ channel subtypes from rat brain, skeletal muscle, and peripheral nerves, respectively. Furthermore, recent studies revealed that µ-PIIIA is a strong inhibitor of Na_V channels involved in muscle contraction and, specifically, of Na_V neuronal subtypes in both the central and peripheral nervous systems [23][39]. Isolated from the venom of *Conus stercusmuscarum*, µ-SmIIIA, due to its strong affinity for neuronal Na_V channel subtypes, it can irreversibly block Na_V currents in different neurons of either amphibians or rats, demonstrating a nociceptive role [24][25].

2.2. Ca_V Channels

Ca^{2+} is the signaling ion for which its elevation from the resting state is involved in many physiologic processes in most cell types. Ca^{2+} homeostasis is regulated by an intricate connection of intracellular organelles, binding molecules, and transmembrane channels and transporters. Rapid Ca^{2+} entry into the neurons gives rise to action potential propagation along cells, activating a cascade of processes such as enzyme activation and gene regulation. Ca_V channels are at the origin of depolarization evoked by Ca^{2+} entry into excitable cells of either brain or muscle tissues. This, in turn, is responsible for most physiological functions as Ca^{2+} dependent muscle contraction, neurotransmitters release, gene transcription, and others. Dysfunctions in these processes, therefore, may alter neurotransmission and gene transcription generating neuropathic pain and related disease states [40][41]. Ca^{2+} channels are made by 4–5 different subunits, among which the α1 includes the voltage sensor, related apparatus, and the conduction pore [42]. According to the voltage changes and depolarization amplitude needed for their activation, Ca_V channels are organized into two categories: high- and low-voltage activated Ca^{2+} channels. The most known members of Ca_V channels are (i) the high voltage-activated L-type characterized by slow voltage-dependent inactivation involved in cell excitability, contraction, gene expression regulation, and oocyte maturation; and (ii) the P/Q-, N-, and R-types that are more prominently active in fast neuronal signal transmissions [43][44]. T-types are the low voltage-activated channels present in either neurons or smooth and cardiac muscular tissues. N-type $Ca_V2.1$ and $Ca_V2.2$, in particular, play important roles in the transmission of pain signals to the central nervous system. About ten different genes encode for different types of Ca_V subunits that are grouped into three major classes (Ca_V1, Ca_V2, and Ca_V3). Specifically, the Ca_V1 family encodes four different types of L-type channels, Ca_V2 family for 2.1, 2.2, and 2.3 corresponding to P/Q type, N-type, and R-type channels, respectively, whereas the Ca_V3 family includes three different types of T-type Ca_V channels [45]. Following channel gating, once Ca^{2+} ions are released into the cytosol, they behave as second messengers binding a large number of proteins and, in turn, influence multiple cell functions and complete several physiological processes [46]. Although most of the studies have focused on the pivotal involvement of $Ca_V1.2$ and $Ca_V2.2$ isoforms in the modulation of pain states, some hints also revealed the involvement of T-type $Ca_V3.1$–$Ca_V3.3$ with a special focus on the $Ca_V3.2$ knockdown effect in mechanical, thermal, and chemical pain diseases [47] (**Table 2**).

Table 2. CnTX subfamilies targeting voltage-gated calcium (Ca_V) channel subtypes, functional impact, and pathophysiological activity.

Species	CnTX Subfamilies	Channel Subunit Targeted	Functional Impact	Pathophysiological Activity	References
C. pennaceus	ω-PnVIA ω-PVIB	HVA Ca_V	selectively but reversibly block HVA currents	-	[48]
C. textile	ω-TxVII	Ca_V	block Ca_V currents	-	[49]
C. geographus	ω-GVIA	Ca_V	irreversibly block Ca_V	-	[50]

Species	CnTX Subfamilies	Channel Subunit Targeted	Functional Impact	Pathophysiological Activity	References
			channels		
C. magnus	ω-MVIIA ω-MVIIC	$Ca_V2.2$ P/Q-type $Ca_V2.1$ and $Ca_V2.2$	inhibits channel activity blocks channel activity	analgesic on chronic pain neuroprotective effect	[51][52]
C. moncuri	ω-MoVIA ω-MoVIB	$Ca_V2.2$	channel affinity	-	[53]
C. striatus	ω-SVIA ω-SVIB ω-SO-3	$Ca_V2.2$ $Ca_V2.1$ and $Ca_V2.2$ N-type $Ca_V2.2$	targeting binding affinity inhibition	paralytic effect lethal injection attenuates acute and chronic pain	[54][55]
C. catus	ω-CVIE ω-CVIF ω-CVID	Ca_V N-type $Ca_V2.2$	affinity antagonist activity	inhibition of nociceptive pain; reducing allodynic behaviour alleviates chronic neuropathic pain reduce allodynic behaviour	[56][57][58]
C. fulmen	ω-FVIA	N-type $Ca_V2.2$	inhibition	reduces nociceptive behaviour, neuropathic pain, mechanical and thermal allodynia	[59]
C. textile	ω-CNVIIA	N-type $Ca_V2.2$	inhibition	blocks neuromuscular junction, paralysis, death	[60]
C. pergrandis	α-PeIA	GABAB receptors coupled to N-type Ca_V	blocking activity	analgesic activity	[61]
C. victoriae *C. regius*	α-Vc1.1 α-RgIA α-AuIB α-MI[V]	GABAB receptors coupled to N-type $Ca_V2.2$.	inhibition	analgesic activity on sciatic nerve ligation injury; allodynia relieves	[62][63][64][65]

uld exhibit and their possible similarities disclosed. The in vitro studies of toxin administration and, in particular, their effects on neuropathic disturbs have been progressively evidenced, and the clinical applications for relieving different pathologies were established. The most known CnTX able to modulate Ca_V channels, by occluding the channel pore and, thus, preventing Ca^{2+} entry, is the ω-CnTX family. Typically, ω-CnTX are peptides that are composed of 24–30 amino acids and belong to the superfamily of disulfide-rich conopeptides [66][67]. In the ω-CnTX family, numerous peptides have been isolated from different conid venoms [68]. Depending on the molecular structure, ω-CnTXs that target neuronal N-type Ca_V channels have been identified as potential drugs for chronic pain treatments. The antagonism with the N-type Ca_V also suggested a ω-CnTX-neuroprotective effect through a size reduction in cerebral infarction and the delayed inhibition of neuronal cell death in the hippocampal CA1 area [69].

PnVIA and PnVIB from *Conus pennaceus* were among the first ω-CnTx identified to be able to discriminate subtypes of high voltage-activated (HVA) Ca^{2+} currents in molluscan neurons. In the snail *Lymnaea stagnalis*, they selectively but reversibly blocked transient HVA currents in caudodorsal cells with negligible effects on L-type currents. Although no clear effects on neurological dysfunctions were reported, they were considered useful selective drugs for relevant Ca_V channel subtypes [48].

2.3. K_V Channels

K_V channels are plasma membrane proteins allowing the selective outside/inside flux of K^+ ions in response to the membrane depolarization. K_V current activity plays a crucial role in many biological processes and functions such as RP and cell volume regulation, propagation of action potential in nerves, cardiac and skeletal muscles, cell proliferation, differentiation, and apoptosis [70]. Furthermore, K_V s are essential in the regulation of Ca^{2+} six transmembrane helices (S1–S6) embedded in the lipid bilayer, forming the voltage sensor domain (S1 to S4) and the pore-forming domain (S5, S6). The voltage sensor domain induces a channel conformational change by sensing RP changes, whereas the interaction with the pore generates K^+ ion current fluxes [71]. K_V channels include 12 different channel families among which a pivotal role is played by the K_V1 family, which contains up to eight isoforms ($K_V1.1$–$K_V1.8$). Among them, $K_V1.3$ was first detected in T-cells and, hence, is considered as a possible target for treating autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and psoriasis. Subsequently, $K_V1.3$ has been proved to be widely distributed in organs and tissues and mainly expressed in both nervous and immune systems participating in several signaling pathways of either normal and/or cancer cells. In particular, $K_V1.3$ channel expressions and/or alterations are involved in numerous pathophysiological processes, such as insulin and apoptosis sensitivity, neoplastic malignancy, inflammatory diseases, cognitive alterations, and anxiety [72]. More recently, it has been shown that the inhibitors of the $K_V1.3$ channel reduce neuroinflammation in rodents together with Alzheimer's and Parkinson's disease and trauma derived brain injury likely by enabling microglia to resist depolarization stimuli [73]. Being involved in overmentioned pathologies, the $K_V1.3$ channel and its blockers have been considered as safe pharmacological tools for chronic inflammatory disease therapies such as type II diabetes mellitus, obesity, and cancer [74]. Among the CnTX studied so far, a few can modulate K_V channels (**Table 3**).

Table 3. CnTX subfamilies targeting voltage-gated potassium (K_V) channel subtypes, functional impact, and pathophysiological activity.

Species	CnTX Subfamilies	Channel Subunit Targeted	Functional Impact	Pathophysiological Activity	References
C. striatus	kA-SIVA	K_V	block	spastic paralytic symptoms	[75]
C. purpurascens	K-PVIIA	$K_V1.3$	inhibition	therapeutics for multiple sclerosis, rheumatoid arthritis, diabetes, and dermatitis	[76][77]

Species	CnTX Subfamilies	Channel Subunit Targeted	Functional Impact	Pathophysiological Activity	References
C. radiatus	kM-RIIIK K-CnTX RIIIJ	Human $K_V1.2$ $K_V1.2$–$K_V1.5$	block target	cardio-protective action no activity	[78][79]
C. striatus	K-Conk-S1; K-Conk-S2	$K_V1.7$	target	therapeutics for diabetes	[80]
C. capitaneus *C. miles* *C. vexillum* *C. striatus* *C.imperialis*	I-superfamily conus peptides	$K_V1.1$ $K_V1.3$	block	-	[81][82]
C. virgo	ViTx	$K_V1.1$ $K_V1.3$	inhibition	-	[83]
C. purpurescens	CGX-1051	K_V	inhibition	cardioprotective	[84]

rified and
ar spastic paralytic symptoms in fish and repetitive action potential oscillations in amphibian nerve-muscle tissues in response to exposure and injection [75]. The latter κ-CnTX PVIIA, belonging to a different family, was first described to bind and block K^+ channels [76]. This peptide possesses a disulfide bridge pattern similar to those of ω- and δ-CnTX [85].

In addition, $K_V1.3$ blockers can ameliorate several harmful diseases, such as rheumatoid arthritis, diabetes, and dermatitis in animal models with a safety profile in rodents and primates [77]. Accurate molecular simulation techniques aimed to disclose the interaction between PVIIA and shaker K_V channels demonstrated the existence of two clusters of amino acids that are critical for the binding between the toxin and the ion channel. The consistency of this binding model and the experimental data indicate that the interaction between PVIIA-K_V and shaker K_V channels may be useful for the development of new therapeutic agents [86].

kM-CnTX RIIIK is a 24 amino acid peptide isolated from *Conus radiatus* venom identified as the first CnTX able to block human $K_V1.2$ channels. Although structurally similar to μ-CnTX GIIIA, RIIIK inhibits shaker K_V expressed in Xenopus oocytes, whereas it showed no affinity with the mammalian $K_V1.1$, $K_V1.3$, and $K_V1.4$ subtypes [78]. When administered before reperfusion, RIIIK significantly reduced the in vivo infarct size in rat hearts demonstrating a potential cardioprotective action. On the contrary, another K-CnTX RIIIJ from the same conid venom did not exert any clear cardio-protective effects when targeting $K_V1.2$–$K_V1.5$. However, both isoforms were suggested to provide new hints for understanding the biological mechanism of cardioprotection [79].

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