Conotoxins as Selective Inhibitors of Neuronal Ion Channels, Receptors and Transporters

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Cone snails have evolved a vast array of peptide toxins, known as conotoxins, for prey capture and defence. These peptides are directed against a wide variety of pharmacological targets, making them an invaluable source of ligands for studying the properties of these targets in normal and diseased states. A number of these peptides have shown efficacy *in vivo*, with several having undergone pre-clinical or clinical development for the treatment of pain.

Introduction to venom peptides

Venom peptides are usually found in animal venoms associated with specialised envenomation apparatus. This allows their delivery into the soft tissue of animals via subcutaneous, intramuscular or intravenous routes. Most venoms comprise a highly complex mixture of peptides often with diverse and selective pharmacologies. Despite this diversity, venom peptides appear to have evolved from a relatively small number of structural frameworks that are well suited to addressing the critical issues of potency and stability (1). It is this evolved biodiversity that makes venom peptides a unique source of leads and structural templates from which new therapeutic agents may be developed. In this review, the small and highly structured peptides found in the venom of marine cone snails (**Table 1**) called conotoxins or conopeptides are discussed in relation to their pharmacology and therapeutic potential.

Pharmacology of cone snail venom peptides

Venom peptides target a wide variety of membrane-bound protein channels and receptors, many of which contribute to disease pathology. The targets and therapeutic potential of a selection of cone snail venom peptides are described below.

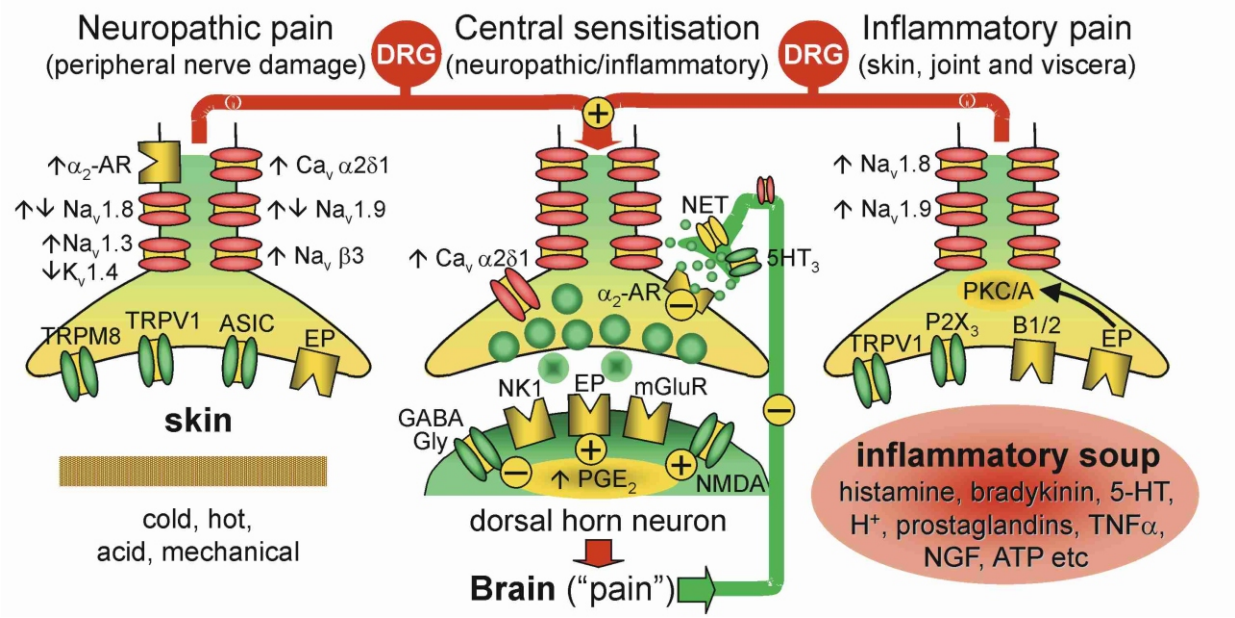

Fig. 1. Pain pathways: highly tuned complex processing networks of excitatory and inhibitory neurones.
Illustrated are a number of selected membrane protein targets that are known to be expressed by dorsal root ganglion (DRG) nerves that respond to painful stimuli [e.g. $Na_V1.8$ sodium channel, vanilloid receptor 1 (TRPV1), a related temperature-sensitive channel TRPM8, acid-sensing ASIC channels, prostaglandin E_2 receptor (EP), and α_2-adrenergic receptors], presumed pain responsive dorsal horn neurones [e.g. γ-aminobutyric acid (GABAG), glycine, NK1 neurokinin, EP, metabolic glutamate (mGluR), and *N*-methyl-D-aspartate (NMDA) receptors], and nerves involved in detecting inflammation [e.g. TRPV1, an ATP-gated channel ($P2X_3$), protein kinases A and C (PKA/C), bradykinins B1 and B2, and EP] and descending facilitatory and inhibitory modulatory pathways originating in the medulla. Many of these targets are up- or down-regulated in different pain states, and thus could be modulated by cone snail venom peptides to produce analgesia. Examples include the ω-conotoxins (N-type calcium channel inhibitors) which inhibit ascending pain pathways, and the χ–conopeptides (noradrenaline transporter inhibitors) that enhance descending inhibitory pathways. These classes of conotoxins are currently in development as novel analgaesic for chronic pain. Both need to be given intrathecally to be effective.

Table 1. Sequence and pharmacological diversity among different classes of conopeptides.

Class	Name[a]	Sequence[b]	Disulfide connectivity[c]	Target of class
ρ	TIA	FNWR**C**CLIPA**C**RRNHKKF**C***	A–C, B–D	α_1-adrenoceptor inhibition
χ	MrIA	NGV**CC**GYKL**C**HO**C**	A–D, B–C	noradrenaline transport inhibitor (nc)
α[d]	GI	E**CC**NPA**C**GRHYS**C***	A–C, B–D	α/δ nicotinic AchR inhibitor
α[d]	PnIB	G**CC**SLPP**C**ALSNPDY**C***	A–C, B–D	α7 nicotinic AchR inhibitor
αA[d]	PIVA	G**CC**GSYONAA**C**HO**C**S**C**KDROSY**C**GQ*	A–E, B–C, D–F	α/δ and α/γ nicotinic AchR inhibitor
μ	PIIIA	RL**CC**GFOKS**C**RSRQ**C**KOHR**CC***	A–D, B–E, C–F	plug TTX-sensitive VSSC
ω	MVIIA	**C**KGKGAK**C**SRLMYD**CC**TGS**C**RSGK**C***	A–D, B–E, C–F	N-type calcium channel inhibitor
κ	PVIIA	**C**RIONQK**C**FQHLDD**CC**SRK**C**NNRFNK**C**V	A–D, B–E, C–F	plug *Shaker* potassium channel
δ	PVIA	EA**C**YAOGTF**C**GIKOGL**CC**SEF**C**LPGV**C**FG*	unknown	delay inactivation of VSSC
γ	PnVIIA	D**C**TSWFGR**C**TVNSγ**CC**SNS**C**DQTY**C**γLYAFOS	unknown	activate pacemaker cation channel
σ	GVIIIA	G**C**TRT**C**GGOK**C**TGT**C**T**C**TNSSK**C**G**C**RYNVHP SGBG**C**G**C**A**C**S*	unknown	5-HT_3 channel inhibitor
Conopressin	Conopressin-S	**C**FIRN**C**PRG*	A–B	vasopressin receptor agonist
Conantokin	Conantokin-G	GEγγLQγNQγLIRγKSN		inhibit spermine activation of NMDA-glutamate receptors

Amino acid sequences of representative conopeptides from each class are shown.
[a]Conopeptides were isolated from fish hunters *C. geographus* (G) *C. magus* (M), *C. purpurascens* (P), *C. striatus* (S) or *C. tulipa* (T), or mollusc hunters *C. marmoreus* (Mr) or *C. pennaceus* (Pn).
[b]B, 6-bromotryptophan; O, *trans*-4-hydroxyproline; γ, γ-carboxyglutamic acid; *, amidated C-terminus.
[c]Cysteines involved in disulfide bonds (shown in bold) are labelled along the sequence, sequentially A through F.
[d]Three representative α-conotoxins highlight sequence differences that contribute to AChR subtype selectivity, while ρ-and χ-conotoxins have similar cysteine patterns (CC…..C…..C).
Abbreviations: AchR, acetylcholine receptor; 5-HT, 5-hydroxytryptamine (serotonin); nc, non-competitive; NMDA, *N*-methyl-D-aspartate; VSSC, voltage-sensitive sodium

Calcium channel inhibitors. It has long been established that Ca^{2+} influx into nerve terminals through voltage-sensitive calcium channels (VSCCs) is the trigger that initiates neurotransmitter release. In recent years, much has been learned about the nature of VSCCs. These channels have been classified into six groups , termed L-, N-, P-, Q-, T, and R-types, according to their electrophysiological and pharmacological properties (2). Studies investigating the role of VSCCs in neurotransmitter release have suggested that the release of a particular neurotransmitter is coupled to the activity of different calcium channel subtypes in different neurones. In addition, multiple splice variants of calcium channels exist in central and peripheral tissues [8]. Given this diversity, considerable opportunity exists to develop selective inhibitors of VSCCs.

ω-Conotoxins are unique tools with which to identify and determine the physiological role of different neuronal VSCCs (3,4). Since N-type ($Ca_V2.2$) VSCCs play a role in the ascending pain pathways (see **Fig. 1**), and are upregulated in the spinal cord in chronic pain states, it is not surprising that ω-conotoxins specific for N-type VSCCs are potent analgesics (5). Extensive structure-activity relationship studies have allowed the development of a pharmacophore model for ω-conotoxins (6) that may allow the rational development of specific N-type VSCC inhibitors. Recently, ω-CVID was found to inhibit an otherwise resistant VSCC found in parasympathetic nerve terminals despite being ~ 10^6-fold selective for N-type over P/Q-type VSCCs (7). The implications of inhibiting this R-type calcium channel for pain conditions are unclear, but these neurons arise from cell bodies in the spinal cord that could play a role in spinal signal processing. Sub-nanomolar bolus intrathecal doses of ω-MVIIA or ω-CVID produce analgesia for up to 24 hours in inflammatory (8) and neuropathic (9) pain models, with ω-CVID displaying a wider therapeutic index than ω-MVIIA. ω-MVIIA (SNX111, Ziconotide or Prialt, Elan) is in late Phase III clinical trials, while ω-CVID (AM336, AMRAD) is entering Phase II clinical trials for the treatment of chronic pain.

Sodium channel toxins. Like the structurally related VSCCs, voltage-sensitive sodium channels (VSSCs) play a key role in the nervous system. Based on their susceptibility to block by tetrodotoxin (TTX), which acts at site 1 in the P-loop region of the α-subunit, VSSCs can be divided into TTX-sensitive (TTX-S) and TTX-resistant (TTX-R) classes. Members of both classes share considerable sequence homology and are closely related structurally (10). These include the neuronal TTX-S type I/$Na_V1.1$, type II/$Na_V1.2$, type III/$Na_V1.3$, PN1/$Na_V1.7$ and PN4/$Na_V1.6$, and the skeletal muscle TTX-S μ1/$Na_V1.4$. The TTX-R sodium channels include the cardiac H1/$Na_V1.5$ which is partially TTX-resistant, and the neuronal TTX-R SNS/PN3/$Na_V1.8$ and NaN/PN5/$Na_V1.9$. A number of these VSSC subtypes are implicated in clinical states such as pain (see **Fig. 1**), stroke and epilepsy. Given their critical role in the central

and peripheral nervous system, it is not surprising that a number of marine venoms from sea anemone, coral and cone snails have evolved to target these channels.

Sodium channel activators are typically toxic (e.g. ciguatoxins). While subtype-selective inhibitors may have considerable therapeutic potential, little progress has been made in the development of peptides that are subtype-selective inhibitors of VSSCs. Given the latent pharmacology revealed by TTX, pore blockers such as the μ-conotoxins (11) appear to the most promising as subtype-selective inhibitors of VSSCs. In contrast, the intra-membrane local anaesthetic site where many classes of small molecules act is conserved across the different VSSCs. This could be more problematic for subtype discrimination. However, state- and frequency-dependent block has allowed the therapeutic use of less selective compounds in the treatment of epilepsy, neuropathic pain, and arrhythmias.

Toxins inhibiting nicotinic acetylcholine receptors. The α-conotoxins are a rapidly growing class of small peptides that competitively inhibit nicotinic acetylcholine receptors (nAChRs). Like the snake α-neurotoxins which have been intensively studied, α-conotoxins bind at the interface between specific subunits, allowing them to discriminate among different nAChR subtypes (12). Muscle-selective α-conotoxins (e.g. GI, see **Table 1**) may represent an alternative to the use of small molecule curare-mimetic muscle relaxants, which are used during surgery but have slower than ideal recovery periods. A novel α-conotoxin, Vc1.1, has been recently identified as having potential analgesic properties (13).

Noradrenaline transporter (NET) inhibitors. The NET plays a key role in reducing levels of neuronally released noradrenaline, and as a consequence influences learning, memory, endocrine and autonomic functions. Drugs that inhibit the NET have antidepressant and/or psychostimulant effects and produce antinociception through the enhancement of descending inhibitory pathways in the spinal cord, and may also be useful in the treatment of cardiovascular disorders and urinary incontinence. χ-Conopeptides are highly specific, non-competitive inhibitors of noradrenaline uptake by human and rat NET (14). The pharmacology of the χ-conopeptides was first identified in rat vas deferens contractility studies, which are sensitive to inhibition by NET. A variant of of χ-MrIA (Xen2174), is currently being developed as a novel analgesic by Xenome Ltd. Interestingly, the binding site for χ-conopeptides on the NET partially overlaps the tricyclic antidepressant binding site (15).

N-*methyl-D-aspartate (NMDA) receptor antagonists.* Conantokins are specific inhibitors of the NMDA receptor. They are helical peptides that competitively inhibit glutamate activation, especially at NR2B receptors (16). Analogues of conantokin-G discriminate among different NMDA receptor subtypes in human brain (17). The anti-epileptic effects of the conantokins have been explored by Cognetix Inc. Reflecting a likely role of NMDA receptors in pain neuroplasticity, Malmberg *et al.* (18) showed that intrathecal conantokin-G or -T also have analgesic activity in pain models of tissue damage (formalin test), nerve injury (partial sciatic nerve ligation) and inflammation (complete Freund's adjuvant) in mice at doses that were ~20-fold lower than those required to impair motor function. Thus, subtype-specific inhibitors of the NMDA receptor also have therapeutic potential in the management of pain.

Neurotensin receptor (NTR) agonists. Cone snails produce a glycosylated neurotensin analogue named contulakin-G (19) that is a potent analgesic in a wide range of animal models of pain (20). Interestingly, contulakin-G is 100-fold less potent that neurotensin for NTR1, but ~100-fold more potent as an analgesic, suggesting an additional mechanism(s) of action. Based on its potency and wide therapeutic window, contulakin-G (CGX-1160) is in early stage clinical development by Cognetix Inc. for the treatment of pain.

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