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Drugs from the Sea: Conopeptides as Potential Therapeutics

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Abstract: Marine cone snails from the genus *Conus* are estimated to consist of up to 700 species. These predatory molluscs have devised an efficient venom apparatus that allows them to successfully capture polychaete worms, other molluscs or in some cases fish as their primary food sources. The toxic venom used by the cone shells contains up to 50 different peptides that selectively inhibit the function of ion channels involved in the transmission of nerve signals in animals. Each of the 700 *Conus* species contains a unique set of peptides in their venom. Across the genus *Conus*, the conotoxins represent an extensive array of ion channel blockers each showing a high degree of selectivity for particular types of channels. We have undertaken a study of the conotoxins from Australian species of *Conus* that have the capacity to inhibit specifically the nicotinic acetylcholine receptors in higher animals. These conotoxins have been identified by mass spectroscopy and their peptide sequences in some cases deduced by the application of modern molecular biology to the RNA extracted from venom ducts. The molecular biological approach has proven more powerful than earlier protein/peptide based technique tor the detection of novel conotoxins [1,2]. Novel conotoxins detected in this way have been further screened for their abilities to modify the responses of tissues to pain stimuli as a first step in describing their potential as lead compounds for novel drugs.

This review describes the progress made by several research groups to characterise the properties of conopeptides and to use them as drug leads for the development of novel therapeutics for the treatment of a range of neurological conditions.

I. KINGDOM OF THE SEA: A RICH SOURCE OF NOVEL DRUG LEADS

It has been estimated that over half the drugs currently used for the treatment of cancer are either natural products or drugs derived from natural products. The large diversity of exotic plants in rain forests has provided sources for some of these clinically active compounds [3,4], but many have come from the sea [5] and from marine invertebrates such as sponges, tunicates bryozoans and mollusks [6].

While most of the natural products mined from marine invertebrates are suspected to be secondary metabolites of microbial or algal origin [7], a large and largely untapped source of marine natural products with exquisite selectivity for specific ion channels and receptors has been revealed as endogenous components of the venom of carnivorous marine snails of genus *Conus* [8-11]. These endogenous peptide toxins are yielding new drug leads for the development of pharmaceuticals with applications for the treatment of human neurological disorders including epilepsy, Parkinson`s, Alzheimer`s and chronic neuropathic pain syndromes.

When investigating marine creatures and organisms as possible sources for future drugs, one limitation often encountered in the field is that only a small number of a given species can be found. This severely limits the amount of compound that can be isolated. Some marine sponges have been successfully cultured (e.g. for the production of the anticancer agent Peloruside A [12]), but mariculture is not yet an option for *Conus* due to the complex variety of plankton required at critical stages throughout its development from the fertilized egg to the planktonic veliger to adult stages. Such attempts as there are have only been successful with *Conus pennaceus* and *Conus textile* which hatch live as mature veligers [13, 14]. It is often desirable to produce the molecule synthetically and for small organic molecules, much use has been made recently of the combinatorial chemistry approach combined with molecular modelling [15]. An alternative approach being taken by many laboratories looking for new drugs from the sea is to first isolate the gene that codes for the product of interest and express it in a bacterial culture system and then to deduce the peptide product of the gene and produce it synthetically by solid phase peptide synthesis [1].

II. *CONUS* – A RICH SOURCE OF PHARMACOLOGICALLY ACTIVE PEPTIDES

The genus *Conus* (suborder: Toxoglossa) comprises over 500 (estimated closer to 700) different species. These predatory marine snails (cone shells) inject a potent cocktail of peptide toxins into their prey to immobilise them. They hunt mainly at night and being crepuscular are most active in the period around sunset and sunrise. Some 20 or so *Conus* species hunt fish (piscivorous), a larger number (approx. 80 species) hunt molluscs (molluscivorous) and an

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Table 1. Sites and Modes of Action of Some Conopeptides

Super Family	Class Conotoxins	ID	S-S bonds	Target	Binding Site *subunit interface*	Type (C/NC)	*Conus* species (type)	Reference
A	**α-**	GI	2	Ligand-gated nicotinic acetylcholine receptor (nAChR), M	α/δ or α/γ*	C	*C. geographus, p*	[8,33,34]
		MI	2		α/δ or α/γ*	C	*C. magus, p*	[32]
		SI	2		α/δ and α/γ**	C	*C. striatus, p*	
		EI	2		α/γ	C	*C. ermineus, p*	
		ImI	2	nAChR, N	α/α [α7/α7]	CBg, VI	*C. imperialis, v*	[32]
		ImII	2		α/α [α7/α7]	NCBg,	*C. imperialis, v*	[35]
		MII	2		α/β [α3β2]	VI	*C.magus, p*	[32]
		EpI	2		α/β [α7 & α3β4]	C	*C. episcopatus, m*	[36]
		AuIB	2		α/β [α3β4]	C	*C. aulicus, m*	[32]
		GIC	2		α/β [α3β2]	C	*C. geographus, p*	[37]
		GID	2		α/β [α7, α3β2, α4β2]	C	*C. geographus, p*	[38]
		Vc1.1	2		α/β	C	C. victoriae, m	[1]
		PIA	2		α/β [α6-containing]		C. purpurascens, m	[118]
A	**αA-**	EIVA/B	3	nAChR, M	α/δ and α/γ**	NC	*C. ermineus, p*	[39]
		PIVA	3				*C. purpurascens, p*	[40]
M	**ψ-**	PIIIE	3	nAChR, M	3rd uncharged loop binds NC to nAChR	NC	*C. purpurascens, p*	[41-43]
		PIIIF	3					
O	**κ-**	PVIIA	3	Voltage-gated K^+ ion channel	Internal pore. Shaker-type K^+ channels in Oocytes not $Kv_{1.1}$ or $Kv_{1.4}$	R	*C. purpurascens, p*	[58-60]
A	**κA-**	SVIA	3	K^+ ion channel	Internal pore		*C. striatus, p*	[58]
A	**κM-**	RIIIK	3	K^+ ion channel	Internal pore. Shaker-type K^+ channels in Oocytes		*C. radiatus, p*	[61]
	κ-?	ViTx	4	K^+ ion channel	Vertebrate K^+ channels $Kv_{1.1}$ and $Kv_{1.3}$ but not $Kv_{1.2}$		*C. virgo, v*	[62]
M	**μ -**	PIIIA	3	Voltage-gated Na^+ ion channel	Internal pore, TTX-sensitive.		*C. purpurascens, p*	[50]
		SmIIIA		(inhibitory action)	TTX-resistant		*C. stercusmuscarum, p*	[51]
O	**δ-**	TxVIA	3	Voltage-gated Na^+ ion channel	Internal pore. Distinct hydrophobic patch. Phe9, Ile12 bind to site 6 and inhibit inactivation.		*C. textile, m*	[52-54]
		GmVIA		(excitotoxic action)			*C. gloriamaris, m*	[55]
		NgVIA						
		PVIA			Bind to a site different to site 6		*C. nigropunctatus,p*	[56]
							C. purpurascens,p	[57]
O	**μO-**	MrVIB	3	Voltage-gated Na^+ ion channel	Internal pore		*C. marmoreus, m*	[50]

(Table 1). contd.....

Super Family	Class Conotoxins	ID	S-S bonds	Target	Binding Site *subunit interface*	Type (C/NC)	*Conus* species (type)	Reference
O	**ω-**	GVIA	3	Voltage-gated Ca^{++} ion channel (N-type)	External pore. Lys2, Tyr13 binds receptor	NR, C	*C. geographus, p*	[44,49]
		MVIIA				R, NC	*C. magus, p*	[46]
		CVID		N-subtype Ca(v) 2.2 specific		R, NC	*C. catus, p*	[25,47]
		CNVII				R, NC	*C. consors, p*	[48]
		MVIIC		P/Q-type specific		R, NC	*C. magus, p*	[77]
	γ	PnVIIA	3	Voltage-gated cation-unspecific channels	"Pacemaker channels", slow and unspecific. PnVIIA is homologous to TxVIIA.		*C. pennaceus, m*	[65]
		TxVIIA					*C. textile, m*	[66]
A	**ρ-**	TIA	2	α1-adrenergic receptor		R, NC	*C. tulipa, p*	[26]
T	**τ-**	(ε)TxIX (tx5a)	2	Voltage-sensitive Calcium channels, (presynaptic)	5 PTMs in 11-17 amino acids.	NC	*C. textile, m*	[63,64]
A	**χ-**	MrIA/B m10a	2	Norepinephrine transporter	NE transporter – No effect on DA or 5HT. Antinociceptive.	R, NC	*C. marmoreus, m*	[26,72,116] [119]
P		gm9a	3	Both are spasmodic when injected into mice	Both are 27 residue peptides with 2 Gla residues	C	*C. gloriamaris, m*	[73]
		tx9a	3			C	*C. textile, m*	[74]
S	**σ-**	GVIIIA	5	Ligand-gated 5-HT receptor	Selective competitive antagonist of the serotonin-gated 5-HT3 receptor. Brominated Trp residue.	C	*C. geographus, p*	[70]
	Contulakins	Cont-G	0	Neurotensin receptor	Cont-G, is a glycosylated 15 amino acid peptide	C	*C. geographus, p*	[71]
	Conantokins	Con-G	0	NMDA ligand-gated receptor	NR2A/NR2B subtypes. Gla3 & Gla4 in Con-G/T bind divalent cation and stabilize α-helix fold.		*C. geographus, p*	[67]
		Con-T	0				*C. tulipa, p*	[68]
		Con-R	1				*C. radiatus, p*	[69]
	Contryphans	Vn	1	not known	Elicits 'stiff tail'syndrome in mice and body tremour and mucous secretion in fish		*C. ventricosus, v*	[11,75]
RFamide	**Conorfa-mides**	SrI	0	not known	When injected ICV, into mice older than 16 days, SrI elicits a hyperactive behaviour.		*C. spurious, v*	[76]

Note on nomenclature: Peptides of the same superfamily share a highly conserved signal sequence in their precursors, and the mature peptides of each family have characteristic arrangements of cysteine residues. In some superfamilies, peptides are found with more than one disulfide framework, but all have the same highly conserved signal sequence. Take for example, chi-MrIA and mr10a which have the same mature peptide sequence. Analysis of a cDNA clone of mr10a indicated that it is a member of the T-superfamily but unlike the other T-superfamily conotoxins it has a divergent arrangement of Cys residues (1-4;2-3) rather than (1-3;2-4), defining a distinct branch of the T-superfamily [119]. Members of one superfamily have a disulfide framework that is distinct from those of other superfamilies [32]. For discussion, see [10, 32, 120].

* In mammals (BC(3)H1 cell receptors) and in the fish *T. mamorata*, MI and GI specifically target the α/δ binding site whereas in the fish *Torpedo californica* these toxins bind preferentially to the α/γ site. Using site-directed mutagenesis of mouse skeletal muscle nAChRs, Sine et al [33] identified 3 amino acids that differ between δ and γ subunits and that are involved in the binding of α-conotoxins.

** SI and both αA-EIVA and αA-EIVB block nAChRs of mammalian skeletal muscle by binding similarly to α/δ and α/γ [78, 79].

Abbreviations : nAChR, nicotinic acetylcholine receptor; M, muscle-type; N, neuronal type; C, competitive ; NC, non-competitive ; R, reversible; NR, irreversible; CBg, competes with α-bungarotoxin; NCBg, does not compete with α-bungarotoxin; VI, voltage-independent ; *p*, piscivorous; *m*, molluscivorous; *v*, vermivorous

even larger group (> 400) hunt marine worms (vermivorous). The piscivorous species of *Conus* are dangerous to humans and there have been over 30 recorded deaths due to envenomation, principally by *Conus geographus*. The first record of a fatal envenomation by a cone snail was reported by Rumphius in 1705 on the island of Banda, in the Molucca islands, on Ambon in the Indonesian archipelago and attributed to *Conus* textile [16].

Immobilisation of prey results from the singular and combined actions of up to 50 conopeptides present in the venom, each one targeting a specific ion channel or receptor (Table **1**). Where the receptor targeted is involved in a vital body function, such as muscle contraction, the result can be fatal. This is the mechanism by which *Conus geographus* brings about death to its victims. One of the components of its venom, an alpha-conotoxin (α-conotoxin GI), is a muscle-type nicotinic acetylcholine receptor antagonist that blocks post-synaptic nicotinic receptors such as those on the diaphragm, and brings about death by asphyxiation. There is no antidote and the only effective treatment is to support

breathing by artificial respiration until the toxins have been metabolised. A more recent report in *The Lancet* [17] tells of a diver admitted to the hospital in Port Moresby, New Guinea, and placed in an iron lung and who survived to tell the tale. He had been carrying a *Conus geographus* in a mesh collecting bag and was envenomated through his wet suit. Not so fortunate was a young man in his 20s who was holidaying on Hayman Island in the Whitsundays, Queensland, and as reported by his mother [18] who was with him at the time, the initial symptoms were of dyplopia, with the victim lapsing into a coma within 20 min. Death by asphyxiation came some 5 hours later as a result of failure of respiration. Examination of the internal organs at autopsy revealed no abnormalities and only a small puncture mark was visible on the hand. The symptoms were much like those of curare poisoning indicating that the conotoxin responsible for bringing about death was a nicotinic acetylcholine receptor (nAChR) antagonist of the kind that targets nAChRs at the neuromuscular junction. A number of such reports record that the victim died a "painless death".

These observations lead several research groups to search for analgesic components in the venom. The first such principle was found in the fish hunting cone *Conus magus* and proved to be an N-type calcium channel blocker [19,20]. This conopeptide (ω-conotoxin MVIIA) has been developed as an analgesic for the treatment of neuropathic pain [21] and is currently completing a repeat of Stage III clinical trials for the treatment of cancer pain [122]. Ziconotide has also been tested for neuroprotection [22, 23] where its efficacy has been tested against ischaemic stroke and in coronary bypass surgery. However these human neuroprotection trials have been disappointing and have been abandoned [24]. More recently, other conopeptides with analgesic activity have been identified in the venom of the fish-eating cone, *Conus catus* [25], and the molluscivorous cones, *Conus marmoreus* [26] and *Conus victoriae* [1]. It would appear that the genus *Conus* has adopted the general strategy of including an analgesic component among its more lethal components of its venom in order to pacify its victim as part of the immobilisation strategy.

The finding that these marine invertebrate neurotoxins and analgesic peptides from *Conus* are effective in humans has opened a Pandora's box of potential drugs from the sea for commercial development as clinical pharmaceuticals. In the short review that follows the different molecular targets of these conopeptides are identified together with their potential clinical applications. This review focuses on publications since 1999. A number of reviews detailing earlier studies on conotoxins and their clinical applications [6, 9, 27-30] should also be consulted.

III. ION-CHANNEL SELECTIVITY OF CONOTOXINS WITH THERAPEUTIC POTENTIAL

The conotoxin family of peptides (for review see [8]) selectively targets receptors and ion-channels in the nervous system [31, 32, 121] (Table **1**).

The conotoxins α- [1,8,32-38], αA- [39,40] and ψ- [41-43] target nicotinic acetylcholine ligand gated ion channel receptors (nAChRs), the ω-conotoxins target voltage-gated ion channels such as the N-type voltage gated calcium channels (VGCCs) [25,44-49] while other classes of conotoxins (μ- [51], μO- [50] and δ- [52-65]) target voltage-gated sodium channels, κ- [58-60], κA- [58], κM- [61] and κViTx- [62] potassium channels, τ- [63, 64] pre-synaptic voltage-sensitive calcium channels, and γ- [65, 66] unspecific voltage-gated "pacemaker channels". Other conopeptides (Table **1**) target (NMDA type) glutamate ligand-gated ion channels [67-69], σ- [70] ligand-gated 5HT receptor, contulakins [63, 71] the neurotensin receptor, χ- [26, 72] the noradrenaline transporter and ρ- [26] the alpha-1 adrenoreceptor. Still other conopeptides which interact with cryptic or unidentified receptors include two P-class conotoxins (gm9a- [73] and tx9a- [74]), contryphans Vn [11, 75] and the conorformide SrI [76].

The selectivity of ω-conotoxin MVIIA (SNX-111, Ziconotide, Prialt) for pre-junctional N-type voltage-gated calcium channels has been developed clinically as a treatment for chronic neuropathic pain in patients for whom opiates are no longer effective (for reviews see [9, 28, 29, 80, 81]). However, although the ω-conotoxins have demonstrated efficacy, they have a low therapeutic index and in human trials of Ziconotide, unwanted side effects have been reported [82, 83] resulting in the FDA requesting Elan to repeat stage III clinical trials for cancer pain [121]. Another N-type calcium channel blocker, conotoxin GVIA (SNX-124), has been studied extensively in animals [49, 84] and has been studied as a potential analgesic and neuroprotective agent [85]. Smith and coworkers [47], reported a differential effect of morphine tolerance on the antinociceptive effects of intrathecally administered Ziconotide and ω-conotoxin CVID (AM336) suggesting that they interact with different subtypes of the N-type calcium channel. Adams and coworkers [25] recently reported that AM336 appears to target an N-type (Ca(v)2.2) calcium channel variant.

A number of natural and engineered changes to conotoxin primary sequence influence conotoxin function. Apart from the disulfide bonding regions which are highly conserved, the amino acids comprising the loops between the cysteine bridges are hypervariable. Even small changes in amino acid composition of the conotoxins can bring about large changes in their specificity and/or efficacy at their receptors as evidenced by early studies on the interactions of alpha-conotoxin MI and GI with the muscle-type nicotinic acetylcholine receptor [33] and more recently by the following three examples : 1) The hydrophobic amino acid leucine present at position 10 in alpha-conotoxin PnIB confers selectivity for the neuronal nicotinic response compared to PnIA which has an alanine [86-88]. 2) Alpha-conotoxin ImI and ImII are closely related, having 9 out of 12 amino acids identical. Both ImI and ImII functionally inhibit heterologously expressed rat alpha7 nAChRs with similar IC_{50} values. Furthermore, the biological activities of intracranially applied ImI and ImII are similar over the same dose range and are consistent with alpha7 nAChR inhibition. However, unlike ImI, ImII does not block the binding of alpha-bungarotoxin to alpha7 nAChRs [34, 92]. It is known that the binding site for ImI overlaps that of alpha-bungarotoxin and that yet there is no competition with ImII and no overlap between functional binding sites of ImI

and ImII in homomeric alpha7 receptors expressed in oocytes. While this is somewhat unexpected and puzzling, it indicates that ImII may be useful as a probe for a novel antagonist binding site, or microsite, on the alpha7 nAChR [34]; 3) High affinity inhibition of the CVID-sensitive calcium channel was enhanced when position 10 of the ω-conotoxin was occupied by the smaller residue Lysine, as found in CVID instead of an Arginine as found in MVIIA [88].

Changes in the disulfide connectivity have profound effects on biological activity of the conotoxins. For example, the three disulphide isomers of conotoxin GI differ over 50 fold in activity on the classical rat neuromuscular junction preparation, the rat phrenic nerve – hemidiaphragm, the native 3:5 loop isomer being 40 times more active than the other two structural isomers [89]. In a recent study, Dutton *et al.* [90] showed that a synthetic ribbon disulfide isomer of alpha conotoxin AuIB, while being less well defined structurally, exhibited 10-times greater potency than the native peptide. This is the first demonstration that a non-native disulfide bond isomer of a conotoxin exhibits greater biological activity than the native isomer.

In addition several amino acids in native conopeptides are found to be post-translationally modified [11]. This is particularly so for the contryphans and contulakins [11, 71]. The types of posttranslational modifications that have been found include, C-terminus amidation, proline hydroxylation, tryptophan bromination and isomerization, tyrosine sulphation, O-glycosylation and glutamic acid γ-hydroxylation [11, 36]. Gamma-glutamyl carboxylation which contributes directly to the stability of the α-helix in conantokins is an extracellular mechanism of post-translational modification that antedates the divergence of molluscs, arthropods and chordates [91].

IV. THERAPEUTIC TARGETS FOR *CONUS* PEPTIDES

Neuropathic Pain and Cancer Pain

Chronic pain, resulting from cancer, AIDS, arthritis or injury, afflicts some 60% of people in the developed world at some time in their life. It is a major health problem. Chronic neuropathic pain is predominantly treated with morphine. However, morphine causes dependence and at the higher doses is required to maintain an appropriate level of analgesia; it produces undesirable side effects such as constipation, respiratory depression and dysphoria. Many physicians are loath to prescribe morphine, the "gold-standard" of opioid drugs, for chronic neuropathic pain. Opioids benefit only some people with neuropathic pain, hence the need for a complement to or substitute for morphine that would be free of these unwanted side-effects and be free of tolerance and dependence. The initial observation that a young man envenomated by *Conus geographus* died a "painless death" [18] stimulated a search some half a century later by several groups of researchers for the active analgesic component present in the deadly venom.

Wang and colleagues [93] found that intrathecal administration of ω-conotoxin MVIIA (Ziconotide) and morphine produce apparent synergistic analgesic effects in rats. Ziconotide did not prevent morphine tolerance to analgesia. They concluded that: 1) acute intrathecal administration of Ziconotide and morphine produced additive or synergistic analgesic effects; 2) chronic intrathecal morphine infusion resulted in tolerance to analgesia but did not produce cross-tolerance to Ziconotide; 3) chronic intrathecal Ziconotide administration produced neither tolerance nor cross-tolerance to morphine analgesia; and 4) intrathecal Ziconotide did not prevent or reverse morphine tolerance. Although there have been some problems experienced in clinical trials in achieving stable dose levels and some patients have had adverse reactions to long-term Ziconotide treatment, this N-type calcium channel antagonist has provided welcome relief in the short term to many patients with neuropathic pain for whom morphine was no longer an option.

Most cancer patients in the advanced stages experience chronic pain of moderate to severe intensity. This may be due to invasion by the tumour into surrounding tissues or to chemotherapy or radiotherapy. In up to 15% of these, the pain is intractable or refractory to management with oral opioids and non-opioid medication. The situation is further complicated by the fact although opioids relieve the pain, they can cause unwanted side effects such as respiratory depression, constipation, nausea, dysphoria, sedation clouded thinking and fatigue [82]. Management of the pain condition becomes difficult and clinicians (and patients) become reluctant to use opioids because of addiction. Elan Corp. has recently embarked on a repeat of Stage 3 clinical trials with Ziconotide for use in controlling chronic cancer pain. The encouraging results of this clinical trial have recently been published [122].

In addition to the ω-conotoxin Ziconotide, a number of other conotoxins and conopeptides have been found effective as analgesics in animal studies and some of these have progressed to clinical testing. Somewhat surprisingly, these include α-conotoxins [1] and conantokins [71], that target the acetylcholine ligand-gated ion channels and NMDA glutamate channels respectively, as well as conotoxins that target voltage-gated sodium ion channels. The discovery of a new μ-conotoxin, which is a potent inhibitor of tetrodotoxin-resistant sodium channels [51], provides a new and potentially useful tool to investigate the functional roles of TTX-resistant voltage-gated sodium channels, including those found in sensory nerves responsible for conveying nociceptive information associated with pain perception to the brain. Still other conopeptides effective as analgesics target the G-protein-linked α-1 adrenergic receptors [72], a neurotensin-like receptor [71] and the neuronal noradrenaline transporter [72].

In addition to Elan Corp (USA and Ireland) and Cognetix Inc. (Salt Lake City, UT, USA), both US companies, three Australian companies, Xenome Ltd., Amrad Corp. and Metabolic Pharmaceuticals Ltd. are involved in the development of cone shell venom components for the treatment of pain (Table **2**).

Xenome Ltd., based in Brisbane, Australia, announced (6 March 2002) that it had signed an agreement with Ionix Pharmaceuticals Ltd., a European analgesic drug discovery company, to search for selective antagonists of a sodium channel drug target that is expressed in the peripheral

Table 2. Conopeptides Being Developed for the Treatment of Neurological Conditions

Name	Conopeptide	*Conus* Species (*)	Target	Stage	Company **	Comment	Reference
ACV1	α-Conotoxin Vc1.1	*Conus victoriae (m)*	Competitive blocker of selected *neuronal-type* nicotinic ACh receptors	Preclinical	Metabolic Pharmaceuticals Ltd, Melbourne, Vic, Australia	Effective against peripheral neuropathic pain in animal models and accelerates functional recovery of injured neurons	[1, 94]
	rho- Conotoxin TIA	*Conus tulipa (p)*	Reversible noncompetitive inhibitor of _-1 adrenergic receptors	Preclinical	Xenome, Ltd., Brisbane, Qld., Australia	Acts as a reversible noncompetitive inhibitor of alpha-1 adrenergic receptors	[72]
AM336	ω-conotoxin CVID	*Conus catus (p)*	Blocks N-type calcium channel, Ca((v)2.2) calcium channel variant.	Stage II	AMRAD Corp. - under license from Univ. of Queensland	Being developed for neuropathic pain. Reported to have a better therapeutic index than Prialt	[25]
SNX-III, C1002, Ziconotide, Prialt	ω-conotoxin MVIIA	*C. magus (p)*	N-type calcium channels	Stage III	Elan Corporation (Elan Pharmaceuticals), CA, USA	Significant pain relief to patients in clinical trials. Side-effects in some patients. Hence call for repeat of Stage III clinical trials for cancer pain. Also trialed (as C1002) for neuro-protection in ischemic stroke and coronary bypass surgery but results were disappointing and trials have been abandoned.	[21, 110] [82, 83, 111,113] [22-24]
Xen2174	χ-conopeptides (chi-CTX MrIA/B)	*Conus marmoreus (m)*	Acts as reversible noncompetitive inhibitor of the neuronal noradrenaline transporter	Preclinical (Stage I to begin in 2004)	Xenome, Ltd., Brisbane, Qld., Australia	Being developed to "treat certain types of pain, for which there is currently a lack of effective treatment" neuropathic pain.	[72]
CGX-1160	Contulakin-G	*Conus geographus (p)*	Binds to neurotensin receptor	Stage II	Cognetix Inc, Salt Lake City, USA	Short term management of post-operative pain	[71]
CGX-1007	Conantokin-G	*Conus geographus (p)*	Selective inhibitor of the NMDA receptor (NR2B subtype)	Stage II	Cognetix Inc, Salt Lake City, USA	Potent antinociceptive effects in several models of injury-induced pain. Also, control of seizures in intractable epilepsy	[71]
CGX-?	Conantokin-T	*Conus tulipa (p)*	Selective inhibitor of the NMDA receptor (NR2A and NR2B) subtypes	Stage II	Cognetix Inc, Salt Lake City, USA	Potent antinociceptive effects in several models of injury-induced pain	[71]

*Prey preference for *Conus* species: *p = piscivorous* (fish-hunting); *m = molluscivorous* (mollusk hunting); *v = vermivorous* (worm-hunting).
Note: This list does not attempt to be comprehensive. For other examples of *Conus* peptides being investigated for therapeutic potential see the excellent reviews by Heading [21,23].
**Websites of commercial developers: Elan Corporation http://www.elan.com ; Cognetix Inc. http://www.cognetix.com; Xenome Pty Ltd: http://www.xenome.com; Amrad Corp. http://www.amrad.com.au; Univ. of Melbourne: Cone Shell and Conotoxin HomePage http://grimwade.biochem.unimelb.edu.au/cone/; Metabolic Pharmaceuticals Ltd http://www.metabolic.com.au

nervous system. Xenome and Ionix will collaborate on the design, synthesis and screening of toxins and derivatives for evaluation as potential inhibitors of proprietary Ionix drug targets for pain and other neurological disorders.

Amrad Corp. in Melbourne, Australia, announced (28 Feb, 2002) that positive findings from its Phase I/II clinical trials with patients with chronic severe pain have led to early completion of the study enabling the Company to advance

AM336 (based on omega-conotoxin CVID from *Conus catus*) to the next stage of development involving additional safety studies and a full Phase II clinical trial.

Xenome have also carried out pre-clinical testing and commenced clinical trials with Xen2174, a χ-conotoxin based on chi-conotoxin MrIA/B from *Conus marmoreus.* This conopeptide targets the neuronal noradrenaline transporter (NET) and in pre-clinical tests in animals was effective in experimental models of human neuropathic and inflammatory pain. Xen2174 is administered intrathecally. No unwanted side effects were seen. NET regulates the biological effects of norepinephrine on the body. Xen2174 binds selectively to NET and by a process of allosteric modulation, abolishes its ability to transport NE from the synapse back into the nerve ending. In episodes of pain, inhibition of NET by Xen2174 elevates the levels of NE leading to the activation of inhibitory pathways thereby preventing pain signals from reaching the brain.

Xenome reported (July 2003) that "The preclinical efficacy and toxicity studies completed to date show that Xen2174 has a high therapeutic index, indicating that there is a broad margin between doses of Xen2174 that result in pain relief and doses at which mild side effects begin to emerge." Xenome has now commenced a series of tests to confirm efficacy, to define the metabolic processing of the molecule and has commenced formal toxicity studies in animals as part of the preclinical testing program and a prelude to human clinical trials scheduled to begin in 2004. In a more recent report issued 15 July 2003, Xenome Ltd. announced that researchers from UCSD had confirmed the therapeutic potential of Xen2174 in an animal model of human neuropathic pain. Neuropathic pain is commonly experienced by patients with nerve damage resulting from shingles, diabetic neuropathy, chronic back pain HIV/AIDS and cancer.

Metabolic Pharmaceuticals Ltd., a peptide pharmaceutical company in Melbourne, Australia, has commenced development of ACV1, a 16 amino acid peptide whose sequence was deduced from an α-conotoxin-like genomic sequence from *Conus victoriae* [1]. This conopeptide inhibits the neuronal-type nicotinic acetylcholine receptor (nAChR) response but has no effect on the nAChR response at the mammalian (or avian) neuromuscular junction. ACV1 has been shown effective in preventing pain in several experimental animal models of human pain syndromes, including post-surgical and neuropathic pain. In addition, it has the unique property that it appears to accelerate the rate of recovery from a nerve injury [1, 94]. In contrast to Ziconotide and AM336, which target the N-type calcium channel, ACV1 targets the neuronal-type nAChR. Studies to date indicate that it is highly selective for the neuronal-type nAChR [1], and in a commercial screening (by Pan Laboratories, Taiwan) ACV1 had no significant interaction with a range of other neurotransmitter receptors and ion channels. ACV1 also has no effect upon systemic blood pressure in the conscious rat [94]. The mechanism by which ACV1 brings about its analgesic effects is thought to be by interaction with neuronal nAChR receptors present on peripheral sensory nerves that convey nociception to the brain. By preventing Na^+ and Ca^{++} ion flux through these ligand-gated ion channels, ACV1 is thought to prevent the activation of cation-activated voltage-gated ion channels. It is known that that mRNA for α3, α4, α5, α7 and β4 nAChR subunits is expressed on rat and chick sensory nerves [95, 96] and that ion channel and receptor expression increases in a neuroma. The particular subunit combination(s) targeted by ACV1 and the receptor(s) responsible for pain relief and for acceleration of functional nerve recovery have not yet been identified. In tests on *Xenopus oocytes* expressing rat α7 subunit nAChRs or rat heterologous α3b4 subunit nAChRs, ACV1 had no effect on the ion current (M. Collins, C. Chu and B.G. Livett, unpublished data). However, it is thought likely that ACV1 may target heterologous α3β4* (e.g. α3α7β4 or α3α5β4) nAChRs since ACV1 inhibits functional nAChR responses in mammalian primary monolayer cell cultures containing these receptor subunit combinations [92, 97-99]. In support of this proposal about α-conotoxin interaction with heterologous nAChRs, a recent paper from McIntosh's group [118] indicates that α-conotoxin PIA from *Conus purpuascens* is selective for α6 subunit-containing nAChRs.

Other Targets

Alzheimer`s

Both conantokins and conotoxins have potential applications in Alzheimer's disease. With regards to the conantokins, it has been proposed that over excitation mediated by specific NMDA receptors might contribute to localised brain damage in Alzheimer's disease. Modified conantokins are useful for identifying the NMDA receptors involved and may have potential as protective agents [117]. With respect to potential applications of conotoxins, it is of interest that glutamatergic axon terminals in human neocortex and in rat striatum possess alpha7* nicotinic heteroreceptors mediating enhancement of glutamate release [100]. Release-enhancing cholinergic autoreceptors in human neocortex are nAChRs with a pharmacological profile compatible with the alpha4beta2 subunit combination. Hence the interest in the potential of subunit receptor specific conotoxins as therapeutics for these conditions has been raised.

Parkinson`s

The recent identification of alpha-5 and alpha-6 subunits contributing to the nAChRs expressed on striatal dopaminergic terminals [101] opens up the possibility of developing selective nAChR ligands active on dopaminergic systems and associated diseases, such as Parkinson's disease. In addition, loss of nicotinic receptors in monkey striatum after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment is due to a decline in alpha-conotoxin MII sites [102].

Epilepsy

Conantokins are NMDA receptor antagonists present in *Conus* venoms and are currently being tested as potential anticonvulsants. Conantokin-R is a highly potent anticonvulsant with a protective index of 17.5 when tested on an audiogenic mouse model of epilepsy. Conantokin-L [103], from *Conus lynceus*, is homologous to Conantokin-R except in for the amino acids at the C-terminus. Of interest it is far less potent as an anticonvulsant suggesting that

sequences at the C-terminus are major determinants of the anticonvulsant potency.

V. CONCLUSIONS

As pointed out by Haefner [6] the fact that the sea should have provided a number of potential drug leads should not come as such a surprise given that the molecular physiology of eukaryotic cells evolved from our early marine ancestors and that it is highly conserved molecular mechanisms that are targeted by many novel drug candidates. The rapid and continuing evolution of *Conus* is reflected in the diversity and specificity of its venom peptides [104-108, 120, 121]. Given the large number of different receptors and ion channels specifically targeted by known conopeptides, one can reasonably expect that orphan receptors will find partners in the large number of conopeptides yet to be characterised. Over 200 patents have been lodged on conopeptides [109]. The potential for drug development is high and the potential benefits great providing that delivery systems are optimised for peptide delivery. In the near future one can expect that pain management by morphine will be supplemented or replaced by co-administration with conotoxins or their derivatives thereby overcoming the dependence and other unwanted side effects of the opioids.

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