Review Article

Conotoxins: Modulators of Ion Channel and their Applications

R. Samundeswari*, T. Nirmala, G. Mahalakshmi, D. Sivassoupramanien, J. Gopi Sudheer Kumar, S. Kavimani

Department of Pharmacology, Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry-605 006, Tamil Nadu, India.

ABSTRACT

Conotoxin is a group of neurotoxic peptides isolated from the venom of marine snail. It works in a concerted manner to shut down the prey's nervous system. They are potent neurotoxins that target ion channels & receptors, and inhibit pain pathways by blocking calcium and sodium channels. It is classified into α, δ, γ, δ, κ, ω, ρ, χ, σ, μ, conopressin, conantokin, contulakin. Each conotoxin has its own action for example: sodium channel is inhibited by μ-conotoxin where as activated by δ-conotoxins. They are used as tools for research including determining how specific receptors and channels work. More number of potential pharmaceuticals has been derived from conotoxins. ρ-Conopeptide TIA, isolated from the venom of *conus tulipa* is the only known conopeptide with activity at adrenergic receptors selectively inhibits human and hamster α_{1B} receptor in an allosteric manner. Ziconotide is a synthetic version of ω – conotoxin MVIIA, found in the venom of fish hunting marine snail, *conus magus.* Ziconotide is approved by regulatory bodies worldwide as a therapeutic approach for the symptomatic management of severe chronic pain and it is non-addictive and does not appear to induce the devolpment of tolearance. There are an estimated 50,000 to 100,000 conotoxins and approximately 0.1% has been characterized pharmacologically.

Keywords: Activators, blockers, conotoxins, marine snail, non-competitive, reversible, sodium channel, ziconotide

Received 04 Oct 2014 Received in revised form 16 Oct 2014 Accepted 18 Oct 2014

***Address for correspondence:**
R. Samundeswari,
Department of Pharmacology, Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry-605 006, Tamil Nadu, India.
E-mail: sam22.pharma@gmail.com

INTRODUCTION

Conotoxin is a group of neurotoxic pepti des isolated from the venom of the mari ne consnail,genus Conus. Conotoxins, w hich are peptides consisting of 10 to 30 amino acid residues,typically have one o r more disulfide bonds. All cone snails have a venom apparatus and the toxins found in their venom glands have led the field in characterizing peptide toxins from marine snails when venom is injected into a prey, the conotoxin work in a concerted manner to shut down the prey's nervous system. Conotoxin are potent neurotoxins that target ion channels and receptors [1]. The complement of peptide found in any one conus venom is different from that found in the venom of another conus specimen [2]. Conotoxins act at many of the ion channels and a smaller number of receptors found in pain pathways of particular interest are conotoxins that inhibit pain pathways by blocking calcium and sodium channels, the nicotinic acetylcholine receptor, the norepinephrine transporter, the NMDA receptor and the neurotensin receptor [3]. In this review, an attempt is made to explore the conotoxins of cone snail with their detailed mechanism of action and their therapeutic potentials.

Calcium channel inhibitors (ω – Conotoxin)

Ca^{2+} influx into nerve terminals through voltage sensitive calcium channels (VSCCs) is the trigger that initiates neurotransmitter release. These channels have been classified into six

groups according to their electrophysiological and pharmacological properties, termed L-, N-, P-, Q-, T-, and R-types [4]. 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 neurons.

Table 1: Types of Conotoxin

CLASS	ACTION
α conotoxin	Competitively block muscle vertebrate neuronal nicotinic Ach receptor
γ conotoxin	Activate pacemaker cationic channels
δ conotoxin	Activate predominantly sodium channels
κ conotoxin	Block potassium channels
μ conotoxin	Block vertebrate muscle/nerve sodium channel
σ conotoxin	Inhibit $5HT_3$ channel
ω conotoxin	Block N-type or P/Q type vertebrate calcium channels
Conopressin	Vasopressin agonist
Conantokin	Inhibit vertebrate NMDA glutamate channels
Contulakin	Bind to neurotensin receptor
χ conopeptide	Act as reversible noncompetitive inhibitor of the neuronal noradrenaline transporter
Rho conotoxin	Acts as reversible noncompetitive inhibitor of α_1 adrenergic receptor
Ikot-Ikot conopeptide	Enhancement of AMPA receptors

In addition, multiple splice variants of calcium channels exist in central and peripheral tissues [5]. 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 [6, 7].

Since N-type (Cav2.2) VSCCs play a role in the ascending pain pathways 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 [8]. Extensive structure-activity relationship studies have allowed the development of a pharmacophore model for ω-conotoxins [9] 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 106-fold selective for N-type over P/Q-type VSCCs [10]. 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. Subnanomolar bolus intrathecal doses of ω - MVIIA or ω -CVID produce analgesia for up to 24 h in inflammatory [5] and neuropathic [11] 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, while ω -CVID (AM336, AMRAD) is entering Phase II clinical trials for the treatment of chronic pain.

Sodium channel inhibitors. (μ - conotoxin)

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 [12]. These include the neuronal TTX-S type I/Nav1.1, type

II/Nav1.2, type III/ Nav1.3, PN1/Nav1.7 and PN4/Nav1.6, and the skeletal muscle TTX-S m1/Nav1.4. The TTX-R sodium channels include the cardiac H1/Nav1.5 which is partially TTX-resistant, and the neuronal TTX-R SNS/PN3/Nav1.8 and NaN/PN5/Nav1.9. A number of these VSSC subtypes are implicated in clinical states such as pain 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. While sodium channel activators are typically toxic (e.g., ciguatoxins), subtype selective inhibitors may have considerable therapeutic potential. Despite this therapeutic potential, little progress has been made towards 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 [13] appear to hold most potential to be subtype selective inhibitors of VSSCs. In contrast, the intramembrane local anesthetic site where many classes of small molecules act is conserved across the different VSSCs and could be more problematic for conferring subtype discrimination. However, state- and frequency-dependent block producing functional selectivity has allowed the therapeutic use of less selective compounds in the treatment of epilepsy, neuropathic pain, and arrhythmias.

Sodium channel activators (δ-conotoxins)

The δ-conotoxins inhibit fast Na_v inactivation and shift the voltage-dependence of activation to more negative potentials, resulting in excitatory activity, prolonged action potentials and persistent neuronal firing [14]. While little is known about the subtype selectivity of δ-conotoxins, and hence their therapeutic potential, subtype-selective Na_v activators might find applications in a range of conditions including arrythmias and certain types of epilepsy [15].

Antagonists of nicotinic acetylcholine receptors (α- conotoxins)

The nicotinic acetylcholine receptors (nAChRs) are a family of pentameric ligand gated cation channels that play a key signalling role at synapses and neuromuscular junctions. The α-conotoxins are a large and growing class of small peptides from cone snails that competitively inhibit specific subtypes of the nAChR [16]. Muscle selective α conotoxins may represent an alternative to the use of small molecule curaremimetic muscle relaxants, which are used during surgery but have slower than ideal recovery periods. Interestingly, the novel α conotoxin, Vc1.1, has been identified as having potential analgesic properties following peripheral administration to rats [17]. This result contrasts the analgesic effects usually attributed to agonists of the nAChR acting centrally and appears to be mediated by either via the $GABA_b$ receptor or the α9α10 receptor [18-20]. Unfortunately, Metabolic has discontinued clinical trials of Vc1.1 due to an anticipated lack of efficacy in humans (affinity for the human form of the receptor is reportedly much lower than for the rat form) Considerable efforts have gone towards improving the understanding of the structural requirements for subtype-selective inhibition of nAChR by α-conotoxins, as well as better synthesis approaches which could improve the biophysical properties of these peptides [21-23]. Such optimized subtype-selective peptides could, in addition to pain, also find applications in smoking cessation and treating diseases such as schizophrenia and Parkinson's disease.

NMDA receptor antagonists (conantokins)

Conantokins are specific inhibitors of the N-methyl-D-aspartate (NMDA) receptor. They are helical peptides that competitively inhibit glutamate activation, especially at NR2B receptors [24]. Analogues of conantokin-G discriminate among different NMDA receptor subtypes in human brain [25]. 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 [26] 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.

Norepinephrine transporter inhibitors (χ- conopeptides)

χ-conopeptides first isolated from *Conus marmoreus* are highly specific, noncompetitive inhibitors of norepinephrine uptake through the NET that produce potent analgesia in rats [27]. A synthetic variant of the χ-conotoxins (Xen2174) is currently being developed as a novel analgesic [28]. Xen2174 is currently being evaluated intrathecally in a Phase I/IIa safety trial in cancer patients suffering otherwise unmanageable pain. Initial results are promising both in terms of safety and efficacy [29]. Interestingly, the binding site for χ-conopeptides on the NET partially overlaps the tricyclic anti-depressant binding site but not the NE binding site, providing clues to the development of noncompetitive small molecule inhibitors [30].

Neurotensin receptor agonists (contulakins)

Cone snails produce a glycosylated neurotensin analogue named contulakin-G [31] that is a potent analgesic in a wide range of animal models of pain [32]. Interestingly, contulakin-G is 100-fold less potent than neurotensin for NTR1, but 100-fold more potent as an analgesic, suggesting an additional mechanism(s) of action besides activation of NTR1. Based on its potency and wide therapeutic window, contulakin-G (CGX-1160) is in early stage clinical development for the treatment of pain.

5-HT_3 receptor antagonists (σ-conotoxins)

5-HT_3 receptors are widely expressed throughout the central and peripheral nervous systems, as well as in non-neuronal tissues, in particular the gut, where they regulate gastrointestinal motility and emesis [33]. The σ-conotoxin GVIIIA, a 41 aminoacid peptide, is the only conopeptide inhibitor of this non-selective cation channel [34]. GVIIIA, which competitively inhibits 5-HT_3, contains 10 cysteines (5 disulfide bonds), an amidated C-terminus and a posttranslationally modified 6-bromoptryptophan. Due to its size and disulfide bond complexity, little is known about the structure-activity and pharmacological properties of this class of conopeptides. While the therapeutic potential of the σ-conotoxins has not been explored, GVIIIA and analogues might find similar applications to small molecule 5-HT_3 antagonists in the treatment of nausea and vomiting, as well as irritable bowel syndrome.

AMPA receptor enhancers (ikot-ikot conopeptides)

AMPA receptors are non-NMDA ionotropic glutamate receptors that mediate fast synaptic transmission in the central nervous system (CNS) and may be involved in several neurological conditions, including epilepsy, pain, depression and schizophrenia [35]. They consist of 4 subunits which are arranged as a dimer of dimers to form a functional channel, and desensitize rapidly through a process involving conformation rearrangement of the glutamate binding subunits [36]. A novel polypeptide named con-ikot- ikot from *Conus striatus* inhibits AMPA desensitization and greatly enhances current magnitudes. Con-ikot-ikot is a large, 86 amino acid polypeptide with 13 cysteines, corresponding to a molecular weight of 9432 Da. The functional form of con-iko-ikot assembles as a dimer of dimers and potentially locks the receptor in the active configuration through simultaneous association with each of

the AMPA receptor subunits [37]. While the therapeutic potential of con-ikot-ikot has not been explored, enhanced AMPA receptor activation or decreased desensitization has been proposed as a therapeutic strategy for the treatment of cognitive dysfunction [38].

α_1-adrenoceptor antagonists (ρ - conopeptides)

The actions of norepinephrine (noradrenaline) on blood pressure, myocardial and smooth muscle contractility and CNS function are mediated by several classes of adrenoceptors, which are broadly categorized as α_1- α_2- and β-adrenoceptors based on anatomical localization and functional properties [39]. Each of these groups of adrenoceptors is further subclassified, based on pharmacological and molecular characteristics (α_{1A}, α_{1B} and α_{1D}; α_{2A} , α_{2B} and α_{2C}; β_1, β_2 and β_3) [40,41]. The small ρ-conopeptide TIA, isolated from the venom of *Conus tulipa* [27], is the only known conopeptide with activity at adrenergic receptors. This nineteen amino acid (2 disulfide bonds) peptide selectively inhibits human and hamster α_{1B} receptors in an allosteric (insurmountable) manner [42]. TIA is also one of the most subtype-selective α_{1B} adrenergic antagonists known, with approximately 10-fold selective for human α_{1B} over α_{1A} and α_{1D} [43]. The residues responsible for the high affinity interaction of TIA with the 1B receptor include N2, W3, R4, L7 and I8 [42]. Interestingly, the I8A analogue did not only display reduced affinity for the 1B receptor, but also functioned as a competitive rather than an allosteric antagonist [43]. The F18A (and F18N) analogues of TIA had increased selectivity for α_{1B} over other receptor isoforms, making these peptides useful probes for distinguishing the role of particular α1-adrenoceptor subtypes in native tissues. The therapeutic potential of L-conopeptides remains to be established. α_{1B}-adrenoceptors mediate contractions in a number of tissues, including the human prostate, but may be less important for maintenance of basal blood pressure [44], suggesting ρ-TIA may be useful for the treatment of benign prostatic hypertrophy, with minimal adverse effects on orthostatic hypotension or reflex tachycardia. In addition, overexpression of the α_{1B} adrenoreceptor was associated with apoptotic neurodegeneration [45], suggesting that subtype selective antagonists may find applications in neurological disease.

Vasopressin and oxytocin agonists and antagonists (conopressins)

The oxytocin and vasopressin receptors belong to the GPCR family (A class of 7-transmembrane receptors) that is activated by oxytocin and vasopressin respectively. The oxytocin receptor is highly expressed on the myoepithelium of the mammary gland as well as uterine myometrium and endometrium. Accordingly, activation of the oxytocin receptor stimulates the contraction of uterine and mammary myocytes during parturition and lactation, respectively [46]. In addition, oxytocin receptors are also expressed in the central nervous system, where they contribute to complex social behaviours including maternal care, social attachement and stress related behaviour [47]. In contrast, at least three different isoforms of vasopressin receptors have been described: V1 (or V1a), V2 and V3 (or V1b) [48]. V1, which is coupled to activation of phospholipase C, is involved in blood pressure regulation and vasoconstriction, while the adenylate cyclase coupled V2 regulates water homeostasis and produces antidiuresis. V3, which also couples to activation of phospholipase C, is responsible for corticotrophin release from the pituitary gland. Accordingly, these receptors are promising therapeutic targets in a range of diseases. However, the high sequence homology of oxytocin and vasopressin, in conjunction with conservation of the extracellular binding domains of the oxytocin and vasopressin receptors, results in poor selectivity of these

peptides at their respective receptors [49,50]. Functional specificity is thus not conferred through pharmacological selectivity, but rather through complex physiological processes involving spatial and temporally restricted receptor expression, controlled peptide release and rapid breakdown [51]. Conopressins were isolated from the venom of fish hunting cone snails [52] and likely represent endogenous invertebrate homologues due to the high sequence similarity of vasopressin to conopressin-G and conopressin-T. However, based on the discovery of divergent conopressins from other cone snail species, continued evolution as a venom peptide appears likely [53-55]. Interestingly, conopressin-T was a V1 antagonist and a partial agonist at OTR [54], with a single substitution to V9 replicating this functional switch in vasopressin and oxytocin [54]. This single change missed during SAR analysis of the oldest class of bioactive peptide, highlights the potential of these conopeptides as templates for subtype-selective modulators of oxytocin and vasopressin receptors. Given that intranasal delivery of oxytocin and vasopressin produces therapeutic systemic effects, subtype specific peptidic conopressin analogues could be particularly promising therapeutic leads.

MEDICAL APPLICATION

Conotoxins are employed in both basic science investigations and for therapeutic explorations. Conotoxins are used as tools of research including determining how specific receptors and ion channels work. Conotoxins have potential roles in the direct treatment of disease. The ω-conotoxins are used in neuroscience research to study calcium channel subtypes [80]. A variety of conotoxins are used to understand specific sodium channels. A number of potential pharmaceuticals are being derived from conotoxin. κ-conotoxin PVIIA may have cardio protective effects [62]. Analogues of α-CTx MII, a centrally acting nicotinic blocker derived from *Conus geographus*, may have a role in treating Parkinson's disease. Other centrally acting α-conotoxins could be useful in treating Alzheimer's disease, nicotine addiction, and in pain management [57]. Prospective therapeutic or research uses of conantokins include pain, epilepsy, stroke, and Parkinson's disease. The ψ- family inhibits norepinephrine transport and thus is potential treatments for attention-deficit/hyperactivity disorder or depression. Conotoxins that block Nav1.6 and Nav1.2 channels may mitigate the inflammatory process in multiple sclerosis. Other conotoxins may have applications for studying schizophrenia [81]. There are an estimated 50,000 to 100,000 conotoxins and approximately 0.1% have been characterized pharmacologically [62].

Ziconotide

It is also known as SNX-111, is a novel non-opioid analgesic drug. It is a synthetic version of ω-conotoxin MVIIA (ω-MVIIA), which is a peptide that is found in the venom of the fish-eating marine snail, *Conus magus*. Ziconotide was recently approved by regulatory bodies worldwide as a therapeutic approach for the symptomatic management of severe chronic pain, particularly in patients who are refractory to treatment with morphine and for whom intrathecal therapy is a viable option. The "Ziconotide Intrathecal Infusion" product is marketed by Elan Pharmaceuticals as Prialt®.It's mechanism of action involves potent and selective block of presynaptic neuronal N-type calcium channels in the spinal cord. In fact, it is the only selective N-type channel blocker that is currently approved for clinical use. Evidence suggests that ziconotide delivers its antinociceptive efficacy by reducing the release of pronociceptive neurotransmitters in the dorsal horn of the spinal cord, thereby inhibiting pain signal transmission. Importantly, ziconotide is non-addictive and does not appear to induce the development of tolerance [82].

Table 2: Source and Site of Action of Therapeutic Conopeptides

CONOPEPTIDE	CONUS SPECIES	TARGET	REFERENCE
α - conotoxin VC 1.1	*Conus victoriae*	Competitive blocker of selected neuronal type nicotinic Ach receptor	18
α - conotoxin Vc1.1	*Conus victoriae*	Inhibit N- type calcium channel	56
α - conotoxin GI α - conotoxin GIA α - Conotoxin GII	*Conus geographus*	Nicotinic Ach receptor	57
α - conotoxin CTXIMI	*Conus imperidis*	Nicotininc Ach receptor	58
α - conotoxin PIVA	*Conus purpurascens*	Nicotinic Ach receptor	59
α - conotoxin RgIA	*Conus regius*	Inhibit α9α10 nAch receptor	60
δ - conotoxin PVIA	*Conus purpurascens*	Delays sodium channel inactivation	61
δ - conotoxin SVIE	*Conus striatus*	Slow the inactivation kinetics of VGSCs	61
δ - conotoxin TXVIAI	*Conus textile*	Inhibits Na current inactivation	62
δ - conotoxin Ng VIA	*Conus nigropunctatus*	Inhibits Na current inactivation	63
κ - conotoxin PVIIA	*Conus purpurascens*	K channel inhibitor	18
μ - conotoxin PIIA	*Conus purpurascens*	Blocks sodium channel	59
μo - conotoxin Mr VIA	*Conus marmoreus*	Blocker of sodium channel	64
ω - conotoxin MVIIA	*Conus magus*	Block N- type ca^{2+} channel specific sub type	65
ω - conotoxin SVIB	*Conus striatus*	P/Q-type	66
ω - conotoxin MVIIC	*Conus magus*	P/Q/N-type	67
ω - conotoxin CVIA	*Conus catus*	N-type	7
ω - conotoxin CVIB	*Conus catus*	N/P/Q-type	7
ω - conotoxin CVIC	*Conus catus*	N/P/Q-type	7
ω - conotoxin CVID	*Conus catus*	Block N-type calcium channel	7
ω - conotoxin TVIA	*Conus tulipa*	N-type	68
ω - conotoxin TxVII	*Conus textile*	L-type	69,70
ω - conotoxin SVIA	*Conus striatus*	Block calcium channel subtypes	66
ω - conotoxin GVIA	*Conus geographus*	N-type	71
χ conopeptide	*Conus marmoreus*	Acts as reversible noncompetitive inhibitor of neuronal NA transporter	27
Contulakin-G	*Conus geographus*	Binds to neurotensin receptor	26
Conantokin-G	*Conus geographus*	Selective inhibitor of the NMDA receptor(NR2B)	26
Conantokin-T	*Cous tulipa*	Selective inhibitor of the NMDA receptor(NR2A)	26
Conopressin-S	*Conus striatus*	Vasopressin antagonist	72
Conopressin-G	*Conus geographus*	Vasopressin antagonist	52
Conopressin-T	*Conus tulipa*	Vasopressin antagonist, partial agonist at oxytocin receptor	73
Conotoxin Pn IVB	*Conus pennaceus*	Blocker of sodium channel	74
Ψ conotoxin PIIIF	*Conus purpurascens*	Antagonist of nicotinic Ach receptor	75
Ψ conotoxin PIIE	*Conus purpurascens*	Inhibitor of nicotinic Ach receptor	76
Conotoxin So3	*Conus striatus*	N- type	77
Rho conotoxin TIA	*Conus tulipa*	Inhibitor of α1 adrenergic receptor	27
μ-conotoxin (geographutoxin I& II)	*Conus geographus*	Binds to sodium channel	78,79

CONCLUSION

Conotoxin plays an important role in medical field, particularly in pain management. They block pain neural pathways by blocking the voltage gated calcium channel (N- type). The values of conopeptides are increasingly apparently, with both naturally occurring as well as in synthetic form. Some of these new drug candidates have entered clinical trials, several of them targeted to the treatment of intractable neuropathic pain. To develop this potential, future work needs to incorporate novel approaches to take better advantages to the unique pharmacological properties of conotoxins.

REFERENCES

1. Terlau H, and Olivera B.M. Conus venoms: A rich source of ion channel-targeted peptides. Physiol. Rev. 2004;84:41–68.
2. Romeo C, Di Francesco L, Oliverio M, Palazzo P, Raybaudi Massilia G, Ascenzi P et al . Conus ventricosus venom peptides profiling by HPLC-MS: A new insight in the intraspecific variation. J Sep Sci . 2008;31:488–498.
3. Richard lewis. Conotoxin venom peptide therapeutics in Pharmaceutical Bio-technology, Carrors A Cruzman and Giora Z Feuerstein, Editor 2009, Lands bioscience and springer science.
4. Randall A. D. The molecular basis of voltage-gated Ca2+ channel diversity: time for T? J. Memb. Biol. 1998;161:207–213.
5. Smith M, Cabot P. J, Ross F. B, Robertson A. D, and Lewis R. J. The novel N-type calcium channel blocker, AM336, produces potent dose-dependent anti-nociception after intrathecal dosing in rats and inhibits substance P release in rat spinal cord slices. Pain. 2002;96:119–127.
6. Olivera B. M, McIntosh J. M, Cruz L. J, Luque F. A, Gray W. R. Purification and sequence of a presynaptic peptide toxin from Conus geographus venom. Biochemistry. 1984;23:5087–5090.
7. Lewis R. J, Nielsen K.J, Craik D. J, Loughnan M. L, Adams D. A,Sharpe I. A, et al. Novel ω-conotoxins from Conus catus discriminate among neuronal calcium channel subtypes. J. Biol. Chem. 2000;275:35335–35344.
8. Malmberg A. B, Yaksh T. L. Effect of continuous intrathecal infusion of ω-conopeptides, N-type calcium-channel blockers, on behaviour and antinociception in the formalin and hot- plate tests in rats. Pain .1995;60:83–90.
9. Nielsen K, Schroeder T, and Lewis R. J. Structure-activity relationships of ω-conotoxins. J. Mol. Recognition. 2000;13:55–70.
10. Adams D. J, Smith A. B, Schroeder C. I, Yasuda T, Lewis R. J. ω-Conotoxin CVID inhibits a pharmacologically distinct voltage-sensitive calcium channel associated with transmitter release from preganglionic nerve terminals. J. Biol. Chem. 2003; 278:4057–4062.
11. Scott D. A, Wright C. E, and Angus J. A. Actions of intrathecal ω-conotoxins CVID, GVIA, MVIIA, and morphine in acute and neuropathic pain in the rat. Eur J Pharmacol. 2002;451:279–286.
12. Goldin A.L, Barchi R.L, Caldwell J.H, Hofmann F, Howe J.R, Hunter J.C, Kallen R.G,et al. Nomenclature of voltage-gated sodium channels. Neuron. 2000;28: 365-368.
13. Shon K. J, Olivera B.M, Watkins M, Jacobsen R. B, Gray W. R, Floresca C.Z ,Cruz L.J , et al. m-Conotoxin PIIIA, a new peptide for discriminating among tetrodotoxin-sensitive Na channel subtypes. J Neuroscience. 1998;18: 4473–4481.
14. Leipold, E.; Hansel, A.; Olivera, B.M.; Terlau, H.; Heinemann, S.H. Molecular interaction of δ-conotoxins with voltage-gated sodium channels. FEBS Letter. 2005;579:3881-3884.
15. Wingerd J.S, Vetter I, Lewis R.J. Voltage Gated Sodium Channels as Therapeutic Targets., in Therapeutic Targets: Modulation, Inhibition, and Activation, E. Luis M. Botana and Mabel Loza, Editor. 2011, John Wiley & Sons: New Jersey.
16. Nicke A, Wonnacott S, Lewis R.J. α-Conotoxins as tools for the elucidation of structure and function of neuronal nicotinic acetylcholine receptor subtypes. Eur J Biochem. 2004;271: 2305-2319.
17. Sandall D.W, Satkunanathan N, Keays D.A, Polidano M.A, Liping X, Pham V, Down J.G, Khalil Z et al. A novel α-conotoxin identified by gene sequencing is active in suppressing the vascular response to selective stimulation of sensory nerves in vivo. Biochemistry. 2003;42:6904-6911.
18. Lewis R.J, Dutertre S, Vetter I, Christie M.J. Conus venom peptide pharmacology. pharmacol Rev, 2012;64:259-298.
19. Callaghan B, Haythornthwaite A, Berecki G, Clark R.J, Craik D.J, Adams D.J. Analgesic α-conotoxins vc1.1 and Rg1A inhibit N-type calcium channels in rat sensory neurons via GABAb receptor activation. J. neuroscience. 2008;28: 10943-10951.
20. Satkunanathan N, Livett B, Gayler K, Sandall D, Down J, Khalil Z. α-Conotoxin Vc1.1 alleviates neuropathic pain and

accelerates functional recovery of injured neurons. Brain Res. 2005;1059:149-158.
21. Luo S, Akondi K.B, Zhangsun D, Wu Y, Zhu X, Hu Y, Christensen S, et al. Atypical α -conotoxin LtIA from Conus litteratus targets a novel microsite of the 32 nicotinic receptor. J Biol Chem.2010; 285:12355-12366.
22. Grishin A.A, Wang C.I, Muttenthaler M, Alewood P.F, Lewis R.J,Adams D.J. Alpha-conotoxin AuIB isomers exhibit distinct inhibitory mechanisms and differential sensitivity to stoichiometry of 34 nicotinic acetylcholine receptors. J Biol Chem.2010;285:22254-22263.
23. Dekan Z, Vetter I, Daly N.L, Craik D.J, Lewis R.J, Alewood P.F.α -Conotoxin ImI incorporating stable cystathionine bridges maintains full potency and identical three-dimensional structure. J Am Chem Soc. 2011;133:15866-15869.
24. Donevan S. D, and McCabe R. T. Conantokin G is an NR2B selective competitive antagonist of N-methyl-D-aspartate receptors. Mol. Pharmacol. 2000;58:614–623.
25. Ragnarsson L, Mortensen M, Dodd P. R, and Lewis R. J. Spermine modulation of the glutamate NMDA receptor is differentially responsive to conantokin in normal and Alzheimer's disease human cerebral cortex. J. Neurochem. 2002;81:765–779.
26. Malmberg A. B, Gilbert H, McCabe R. T, and Basbaum A. I. Powerful antinociceptive effects of the cone snail venom derived subtype-selective NMDA receptor antagonists conantokin G and T. Pain. 2003;101: 109–116.
27. Sharpe I.A, Gehrmann J, Loughnan M.L, Thomas L, Adams D.A, Atkins A, Palant E et al. Two new classes of conopeptides inhibit the α_1-adrenoceptor and noradrenaline transporter. Nat Neurosci. 2001;4: 902-907.
28. Nielsen C.K, Lewis R.J, Alewood D, Drinkwater R, Palant E, Patterson M, Yaksh T.L, et al. Antiallodynic efficacy of the χ-conopeptide, Xen2174, in rats withneuropathic pain. Pain. 2005;118: 112-124.
29. Lewis R.J. Discovery and development of the χ-conopeptide class of analgesic peptides. Toxicon.,2012;59:524-528.
30. Paczkowski F.A, Sharpe I.A, Dutertre S, Lewis R.J. χ-Conotoxin and tricyclic antidepressant interactions at the norepinephrine transporter define a new transporter model. J Biol Chem.2007;282:17837-17844.
31. Craig A.G, Norberg, T, Griffin D, Hoeger C, Akhtar M, Schmidt K, Low W, et al. Contulakin-G, an O-glycosylated invertebrate neurotensin. J Biol Chem. 1992;274:13752-13759.
32. Allen J.W, Hofer K, McCumber D, Wagstaff J.D, Layer R.T, McCabe R.T, Yaksh T.L. An assessment of the antinociceptive efficacy of intrathecal and epidural contulakin-G in rats and dogs. Anesth Analg . 2007;104:1505-1513.
33. Machu T.K. Therapeutics of 5-HT_3 receptor antagonists: current uses and future directions. Pharmacol Ther. 2011;130:338-347.
34. England L.J, Imperial J, Jacobsen R, Craig A.G, Gulyas J, Akhtar M, Rivier J, Julius D, Olivera B.M. Inactivation of a serotonin-gated ion channel by a polypeptide toxin from marine snails. Science. 1998;281:575-578.
35. Mellor I.R. The AMPA receptor as a therapeutic target: current perspectives and emerging possibilities. Future Med Chem. 2010;2:877-891.
36. Armstrong N, Jasti J, Beich-Frandsen M, Gouaux E. Measurement of conformational changes accompanying desensitization in an ionotropic glutamate receptor. Cell. 2006;127:85-97.
37. Walker C.S, Jensen S, Ellison M, Matta J.A, Lee W.Y, Imperial J.S, Duclos N, et al. A novel Conus snail polypeptide causes excitotoxicity by blocking desensitization of AMPA receptors. Curr Biol. 2009;19:900-908.
38. Lynch G. AMPA receptor modulators as cognitive enhancers. Curr Opin Pharmacol. 2004;4: 4-11.
39. Koch W.J, Rockman H.A, Samama P, Hamilton R.A, Bond R.A, Milano C.A, Lefkowitz, R.J. Cardiac function in mice overexpressing the β-adrenergic receptor kinase or a β ARK inhibitor. Science. 1995;268:1350-1353.
40. Bylund D.B, Eikenberg D.C, Hieble J.P, Langer S.Z, Lefkowitz R.J, Minneman K.P, Molinoff P.B, et al. International Union of Pharmacology nomenclature of adrenoceptors. Pharmacol Rev. 1994;46:121-136.
41. Graham R. M, Perez D.M, Hwa J, Piascik M. T. α_1-Adrenergic receptor subtypes. Molecular structure, function, and signaling. Circ Res. 1996;78:737-749.
42. Sharpe I.A, Thomas L, Loughnan M, Motin L, Palant E, Croker D.E, Alewood D, et al. Allosteric α_1-adrenoreceptor antagonism by the the conopeptide ρ-TIA. J Biol Chem.2003;278:34451-34457.
43. Chen Z, Rogge G, Hague C, Alewood D, Colless B, Lewis R.J, Minneman K.P. Subtype-selective noncompetitive or competitive inhibition of human α 1-

adrenergic receptors by ρ-TIA. J Biol Chem. 2004;279:35326-35333.
44. Docherty J.R. Subtypes of functional α_1-adrenoceptor. Cell Mol Life Sci. 2010;67: 405-417.
45. Zuscik M.J, Sands S, Ross S.A, Waugh D.J, Gaivin R.J, Morilak, D, Perez D. M. Overexpression of the α_{1B}-adrenergic receptor causes apoptotic neuro-degeneration: multiple system atrophy. Nat Med . 2000;6:1388-1394.
46. Petersson M. Cardiovascular effects of oxytocin. Prog Brain Res. 2002;139:281-288.
47. Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. Physiol Rev. 2001;81:629-683.
48. Birnbaumer M. Vasopressin receptors. Trends Endocrinol Metab. 2000;11:406-410.
49. Chini B, Mouillac B, Balestre M.N, Trumpp-Kallmeyer S, Hoflack J, Hibert M, et al. Two aromatic residues regulate the response of the human oxytocin receptor to the partial agonist arginine vasopressin. FEBS Letter. 1996;397:201-206.
50. Kimura T, Makino Y, Saji F, Takemura M, Inoue T, Kikuchi T, Kubota Y, Azuma C, Nobunaga T, Tokugawa Y et al. Molecular characterization of a cloned human oxytocin receptor. Eur J Endocrinol.1994;131: 385-390.
51. Mitchell B.F, Fang X, Wong S. Oxytocin: A paracrine hormone in the regulation of parturition? Rev Reprod. 1998;3: 113-122.
52. Cruz L.J, de Santos V, Zafaralla G.C, Ramilo C.A, Zeikus R, Gray W.R, Olivera B.M. Invertebrate vasopressin/oxytocin homologs. Characterization of peptides from Conus geographus and Conus straitus venoms. J Biol Chem. 1987;262: 15821-15824.
53. Moller C, Mari F. A vasopressin/oxytocin-related conopeptide with γ-carbo-xyglutamate at position 8. Biochem J.2007;404:413-419.
54. Dutertre S, Croker D, Daly N.L, Andersson A, Muttenthaler M, Lumsden N.G, et al. Conopressin-T from Conus tulipa reveals an antagonist switch in vasopressin-like peptides. J Biol Chem. 2008;283: 7100- 7108.
55. Ueberheide B.M, Fenyo D, Alewood P.F, Chait B.T. Rapid sensitive analysis of cysteines rich peptide venom components. Proc Natl Acad Sci. 2009;106: 6910-6915.
56. Brid C, Alison H, Richard J, David J.C, David J.A et al. Analgesic α-ConotoxinsVc1.1andRg1A Inhibit N-Type Calcium Channels in Rat Sensory Neurons via GABAB Receptor Activation. The Journal of Neuroscience.2008;28: 10943–10951.
57. Gray WR, Luque A, Olivera BM, Barrett J, Cruz LJ. Peptide toxins from conus geographus venom. J. biol chem. 1981; 256: 4734-470.
58. Azam L, Mcintosh JM. Alpha conotoxins as pharmacological probes of nicotinic acetylcholine receptors Acta Pharmacol sin. 2009;30:771-783.
59. Terlau H, Shon KJ, Grilley M, Stocker M, Stuhmer W et al. Strategy for rapid immobilization of prey by a fish hunting marine snail. Nature.1996;381: 148-151.
60. Ellison M, Haberlandt C, Gomez-Casati M.E, Watkins M, Elgoyhen A.B, McIntosh J.M, Olivera B.M . α-RgIA: A novel conotoxin that specifically and potently blocks the $\alpha_9\alpha_{10}$ nAChR. Biochemistry. 2006;45:1511–1517.
61. Peter J. West, Grzegorz Bulaj, and Doju Yoshikami. Effects of δ-Conotoxins PVIA and SVIE on Sodium Channels in the Amphibian Sympathetic Nervous System. J Neurophysiol. 2005;94: 3916–3924.
62. Fainzilber M, Kofman O, Zlotkin E, Gordon D. A New Neurotoxin Receptor Site on Sodium Channels Is Identified by a Conotoxin That Affects Sodium Channel Inactivation in Molluscs and Acts as an Antagonist in Rat Brain. J. Biol. Chem. 1994;269: 2574-2580.
63. Fainzilber M, Lodder J.C, Kits K.S, Kofman O, Vinnitsky I, Van Rietschoten J, Zlotkin E and Gordon D. A new conotoxin affecting sodium current inactivation interacts with the δ – conotoxin receptor site. J. Biol. Chem. 1995;270: 1123-1129.
64. McIntosh J.M, Hasson A, Spira M.E, Gray W.R, Li W, Marsh M, Hillyard D.R et al. A new family of conotoxins that block voltage gated sodium channels. J. Biol. Chem. 1995;270: 16796-16802.
65. Olivera B. M, Cruz L. J, de Santos V, LeCheminant G. W, Griffin D, Zeikus R et al. Neuronal calcium channel antagonists. Discrimination between calcium channel subtypes using ω-conotoxin from Conus magus venom. Biochemistry. 1987;26: 2086-2090.
66. Ramilo C. A, Zafaralla G. C, Nadasdi L, Hammerland L. G, Yoshikami, D, Gray W, K et al. Novel α- and ω-conotoxins from Conus striatus venom. Biochemistry. 1992;31: 9919-9926.
67. Hillyard D. R, Monje V. D, Mintz I. M, Bean B. P, Nadasdi L, Ramachandran J et al. A new Conus peptide ligand for mammalian presynaptic Ca^{2+} channels. Neuron. 1992;9:69-77.
68. Miljanich G. P, Ramachandran J. Antagonists of neuronal calcium channels:

structure, function, and therapeutic implications. Annu. Rev. Pharmacol. Toxicol.1995;35: 707-734.
69. Fainzilber M, Lodder J. C, van der Schors R. C, Li K. W, Yu Z, Burlingame A. L, Geraerts W. P, Kits K. S. A novel hydrophobic ω-conotoxin blocks molluscan dihydropyridine- sensitive calcium channels. Biochemistry. 1996;35: 8748-8752.
70. Sasaki T, Feng Z. P, Scott R, Grigoriev N, Syed N. I, Fainzilber M, Sato K. Synthesis, bioactivity, and cloning of the L-type calcium channel blocker ω-conotoxin TxVII. Biochemistry. 1999;38:12876-12884.
71. Olivera B. M, McIntosh J. M, Cruz L. J, Luque F. A, Gray W. R. Purification and sequence of a presynaptic peptide toxin from Conus geographus venom. Biochemistry. 1984;23: 5087-5090.
72. Lourdes J. Cruzs, Victoria de Santoso,Glenn C. Zafaralla, Cecilia A. Ramiloso et al. Invertebrate vasopressin/oxytocin homologs. Chara-cterization of peptides from conus geographus and conus straitus venoms. The journal of biological chemistry. 1987; 262:15821-15824.
73. Sebastien Dutertre, Daniel Croker, Norelle L. Daly, Asa Andersson, Markus Muttenthaler Natal et al. Conopressin-T from Conus tulipa Reveals an Antagonist Switch in Vasopressin-like Peptides. The journal of biological chemistry. 2008;283:7100–7108.
74. Fainzilber M, Nakamura T, Gaathon A, Lodder J.C, Kits K.S, Burlingame A.L, Zlotkin E. A new cysteine framework in sodium channel blocking conotoxins. Biochemistry. 1995;34: 8649-8656.
75. Ryan M, Van Wagoner, Richard B. Jacobsen, Baldomero M. Olivera, and Chris M. Ireland. Characterization and Three-Dimensional Structure Determination of ψ-Conotoxin PIIIF, a Novel Noncompetitive Antagonist of Nicotinic Acetylcholine Receptors Biochemistry.2003;42:6353-6362.
76. Shon K. J, Grilley M, Jacobsen R. B, Cartier G. E, Hopkins C, Gray W. R, Watkins M et al. A noncompetitive peptide inhibitor of the nicotinic acetylcholine receptors from conus purpurascens. Biochemistry. 1997;36:9581-9587.
77. Wen L, Yang S, Qiao H. F, Liu Z. W, Zhou W. X, Zhang Y. X, Huang P. T. SO-3, a new O-superfamily conopeptide derived from Conus striatus, selectively inhibits N-type calcium currents in cultured hippocampal neurons. Br. J. Pharmacol. 2005;145: 728-739.
78. Cruz L.J, Gray W.R, Olivera B.M, Zeikus R.D, Kerr L, Yoshikami D, Moczydlowski E. Conus geographus toxins that discriminate between neuronal and muscle sodium channels. J. Biol. Chem. 1985;260:9280-9288.
79. Nakamura H, Kobayashi J, Ohizumi Y. & Hirata Y. Isolation and amino acid composition of geographutoxin I and II from the marine snail Conus geographus Linne. Experientia. 1983;39:590-591.
80. Becker S, Teralu H, Toxins from snails, properties, application's, biotechno-logical production. Applied microbiology and biotechnology. 2008;79: 1-9.
81. Essack M, Bajic VB, Archer JA. conotoxins that confer therapeutic possibilities. Marine drugs. 2012;10: 1244-1265.
82. McGivern J.G. Ziconotide: A review of its pharmacology and use in the treatment of pain Neuropsychiatric Disease and Treatment.2007;3:69–85.