#### ASSOCIATE EDITOR: ANNETTE C. DOLPHIN

## Conus Venom Peptide Pharmacology

Richard J. Lewis, Sébastien Dutertre, Irina Vetter, and MacDonald J. Christie

Institute for Molecular Bioscience, the University of Queensland in St. Lucia, Brisbane, Queensland, Australia (R.J.L., S.D., I.V.); and Discipline of Pharmacology, the University of Sydney, Sydney, New South Wales,

Australia (M.J.C.)

	Abstract	. 260
I.	Introduction	. 260
II.	ω-Conotoxin inhibitors of voltage-gated calcium channels · · · · · · · · · · · · · · · · · · ·	. 263
	A. Subtype selectivity	. 264
	B. Structure-activity relationships	. 266
	C. Calcium channel inhibition by conotoxins in pain management	. 267
II.	Conotoxins interacting with voltage-gated sodium channels	. 269
	A. μ-Conotoxin inhibitors of voltage-gated sodium channels	. 271
	B. μO-Conotoxin inhibitors of voltage-gated sodium channels	. 272
	C. Other conotoxin inhibitors of voltage-gated sodium channels	. 273
	D. δ-Conotoxin activators of voltage-gated sodium channels	
	E. ι-Conotoxin activators of voltage-gated sodium channels	. 273
	F. Sodium channel inhibition in pain management	. 274
III.	κ-Conotoxins interacting with potassium channels	
	A. κA-Conotoxins	
	B. κO-Conotoxins	
	C. kM-Conotoxins	
	D. κJ-Conotoxins.	
	E. κI(2)-Conotoxins.	
	F. Contryphan-Vn	
	G. Conkunitzin-S1	
IV	Conotoxins interacting with ligand-gated ion channels	
_ , ,	A. Conotoxin inhibitors of nicotinic acetylcholine receptors	
	B. $\sigma$ -Conotoxin inhibitors of serotonin 5-hT $_3$ receptors	
	C. Ikot-Ikot conopeptide enhancers of $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	00
	receptors	283
	D. Conantokin inhibitors of N-methyl-D-aspartate receptors	
	E. Ligand-gated ion channels inhibitors in pain management	
V	Conotoxins Interacting with G protein-coupled receptors and neurotransmitter transporters	
٧.	A. $\rho$ -Conopeptide inhibitors of $\alpha$ 1-adrenoceptors	
	B. $\chi$ -Conopeptide inhibitors of the noradrenaline transporter	
	C. Conopeptides modulating vasopressin/oxytocin receptors	
	D. Neurotensin receptor agonists	
	D. Neurotensin receptor agomsts	. 403

Address correspondence to: Richard J. Lewis, Institute for Molecular Bioscience, the University of Queensland, Q4072, Australia. E-mail: r.lewis@imb.uq.edu.au

This work was supported by a National Health and Medical Research Council of Australia (NHMRC) Research Fellowship (to R.J.L., M.J.C.); an NHMRC Trainee Fellowship (to I.V.), a University of Queensland Postdoctoral Fellowship (to S.D.), and an NHMRC Program Grant (to R.J.L., M.J.C.).

This article is available online at http://pharmrev.aspetjournals.org. http://dx.doi.org/10.1124/pr.111.005322.



	1 ,
2	play libraries (Pande et al., 2010), and
2	(Lewis and Garcia, 2003). Among thes
	peptides provide arguably the largest s
	diversity, being driven by evolutionary
	proved prey capture and/or defense. T
工	arisen multiple times in nature in anin
	sea anemones, jellyfish, centipedes,
	ons, cone snails, cephalopods, echin
	lizards, fish, platypus and arguably
	quitoes, kissing bugs, leeches, ticks, a
	(Fry et al., 2009). Ensuring their succe
_	(11) ct al., 2000). Elisaring men succe

<sup>1</sup>Abbreviations: 5-HT<sub>3</sub>, 5-hydroxytryptamine<sub>3</sub>; ACh, acetylcholine; AChBP, acetylcholine binding protein; Am2766, CKQAG-ESCDIFSQNCCVGTCAFICIE-NH<sub>2</sub>; AMPA, α-amino-3-hydroxy-5methyl-4-isoxazolepropionic acid; AR, adrenergic receptor; Ca, calcium channel; CNS, central nervous system; Con-G, conantokin-G; DRG, dorsal root ganglion; GPCR, G-protein-coupled receptor; nAChR, nicotinic acetylcholine receptor; NET, norepinephrine transporter; NMDA, N-methyl-D-aspartate; NTSR, neurotensin receptor; SNX-482, GVDKAGCRYMFGGCSVNDDCCPRLGCHSLF-SYCAWDLTFSD-OH; TTX, tetrodotoxin.

VI. Conclusions and outlook..... 

Abstract—Conopeptides are a diverse group of recently evolved venom peptides used for prey capture and/or defense. Each species of cone snails produces in excess of 1000 conopeptides, with those pharmacologically characterized ( $\sim\!0.1\%$ ) targeting a diverse range of membrane proteins typically with high potency and specificity. The majority of conopeptides inhibit voltage- or ligand-gated ion channels, providing valuable research tools for the dissection of the role played by specific ion channels in excitable cells. It is noteworthy that many of these targets are found to be expressed in pain pathways, with several conopeptides having entered the clinic as potential treatments for pain [e.g., pyroglutamate1-MrIA (Xen2174)] and one now marketed for intrathecal treatment of severe pain [ziconotide (Prialt)]. This review discusses the diversity, pharmacology, structure-activity relationships,

and therapeutic potential of cone snail venom peptide families acting at voltage-gated ion channels ( $\omega$ -,  $\mu$ -,  $\mu$ O-,  $\delta$ -,  $\iota$ -, and  $\kappa$ -conotoxins), ligand-gated ion channels ( $\alpha$ -conotoxins,  $\sigma$ -conotoxin, ikot-ikot, and conantokins), G-protein-coupled receptors (p-conopeptides, conopressins, and contulakins), and neurotransmitter transporters ( $\chi$ -conopeptides), with expanded discussion on the clinical potential of sodium and calcium channel inhibitors and  $\alpha$ -conotoxins. Expanding the discovery of new bioactives using proteomic/transcriptomic approaches combined with high-throughput platforms and better defining conopeptide structure-activity relationships using relevant membrane protein crystal structures are expected to grow the already significant impact conopeptides have had as both research probes and leads to new therapies.

#### I. Introduction

The interrogation of chemical diversity with therapeutically relevant screens remains the dominant discovery engine for the pharmaceutical industry. Although most success with this approach has been achieved by exploring natural product extracts composed mostly of smaller organic molecules, there is a growing realization that peptides are an underused source of leads for new therapeutics. This realization is now gaining momentum following the disappointments of combinatorial chemistry, increasing failure rates of small molecules in clinical development, and the limitations of small-molecule—based approaches for many druggable targets. Sources of peptides include plant cyclotides (Henriques and Craik, 2010), phage disd venom peptides se sources, venom source of chemical pressure for imhis diversity has nals as diverse as spiders, scorpioderms, snakes, even fleas, mosand vampire bats essful implementation, venom peptides have coevolved with sophisticated and specialized envenomation machinery comprising fangs, barbs, modified teeth, harpoons, nematocysts, spines, or sprays. Not surprisingly, given that most venoms are delivered intravenously, there are multiple examples of convergent evolution of a relatively small number of structural scaffolds that possess the requisite stability to remain intact, both within the venom and in plasma after envenomation. These "privileged" structural frameworks have provided the scaffolds for an explosion in sequence diversity that explains much of the chemical diversity found in present-day venoms.

Among the venomous species, cone snails within the family Conidae are unique for their ability to use a diverse array of small disulfide-bridged peptides (conopeptides or conotoxins) for prey capture (Fig. 1). Conopeptides have evolved across all phylogenetic clades and feeding strategies of cone snails from at least 16 genetically distinct superfamilies, many of which are subject to extensive posttranslational modifications (Table 1). This highly evolved hunting strategy, developed in parallel with the deployment of a hollow, barbed harpoon, allows normally herbivorous molluscs to prey on animals as diverse as worms (vermivorous species), fish (piscivorous species), and other molluscs (molluscivorous species). Successful implementation of this strategy has allowed this single genus of widely distributed marine molluscs to evolve into more than 500 Conus species. Cone snails hunt prey mostly at night and once prey is located by smell they skillfully inject small quantities of venom (up to  $\sim 50 \mu l$ ) to paralyze prey within seconds using a rich cocktail of conopeptides that are cleaved from propertides by specialized venom endoproteases (Milne et al., 2003). Their small molecular size (typ-



Downloaded from pharmrev.aspetjournals.org at Thammasart University on December 3, 2012

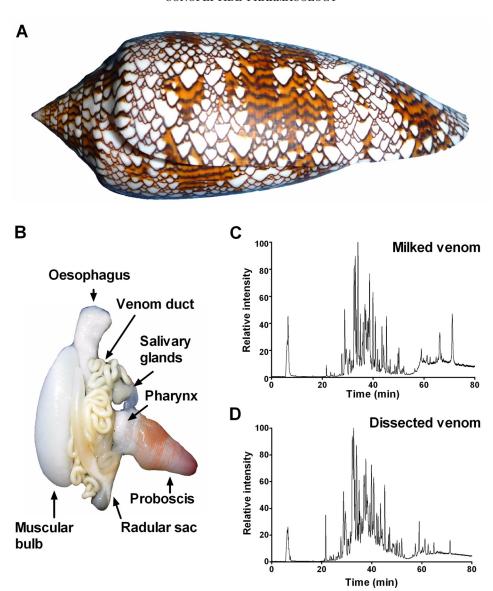


FIG. 1. The cone snail, venom apparatus, and venoms. A, shell of *C. textile*, a typical tented molluscivorous cone snail. B, dissected venom apparatus from *C. textile*, showing the long and convoluted venom duct, radular sac, proboscis, and salivary glands. C, high-performance liquid chromatography/mass spectrometry separation of venom peptides from the milked venom of *C. textile*. D, matching liquid chromatography/mass spectrometry of the venom duct from the same *C. textile*.

ically <5 kDa), relative ease of synthesis, structural stability and target specificity make them ideal pharmacological probes (Adams et al., 1999). This broadly evolved bioactivity provides a unique source of new research tools and potential therapeutic agents, with  $\omega$ -MVIIA [ziconotide (Prialt; Azur Pharma Inc., Philadelphia, PA)] already approved for clinical use in the treatment of severe pain (Miljanich, 2001).

Somewhat surprisingly, many of these classes of conotoxins act on pain targets, allowing the specific dissection of key ion channels and receptors underlying pain and providing new ligands with potential as pain therapeutics (Fig. 2). Each species of cone snail produces in excess of 1000 conopeptides, with an estimated 5% overlap in conopeptides found between species (Davis et al., 2009) (see Fig. 1). At the moment,

only ~0.1% of conopeptides have been characterized pharmacologically, yet many have already been identified with clinical potential, as highlighted in Table 2. The use of advanced transcriptomic/proteomic approaches combined with high throughput and multiplexed high content screens is expected to accelerate ligand and target discovery. One caveat is that many conotoxins will preferentially target prey species over related mammalian targets and are likely to be missed in screens relevant to human diseases. This review expands and updates the last comprehensive review of Conus spp. venoms in 2004 (Terlau and Olivera, 2004). Here we discuss the diversity, pharmacology, structure-activity relationships, and therapeutic potential of cone snail venom peptide families acting at voltage-gated ion channels ( $\omega$ -,  $\mu$ -,  $\mu$ O-,  $\delta$ -,  $\iota$ - and

PHARMACOLOGICAL REVIEWS

TABLE 1 Superfamilies of conopeptides, occurrence in different Conus spp. clades and prey targets, and post-translational modifications (PTMs) Data from Conoserver (http://www.conoserver.org).

Superfamily	Cysteine Framework	Family	Species	Clades	Diet	PTMs
A	I, II, IV, XIV	α, κ, ρ	43	E, I, II, III, V, VI, VII, X, XII, XIV, XV, XVII	V, M, P	O, *, γ, G, Z, Ys
D	XX	α	6	XII	V	Ο, γ
I1	VI/VII, XI	ι	11	E, I, V, X, XIV, XVII	V, M, P	O, BrW, *, D, γ
I2	XI, XIÍ	К	17	I, V, VI, X, XII, XIII, XVII	V, M, P	$0, *, \gamma$
I3	VI/VII, XI		3	XIV	V	
J	XIV	К	2	IX	V	*
$_{ m L}$	XIV	α	2	E	V, P	
M	III, IV, VI/VII, IX, XVI	$\alpha$ , $\iota$ , $\kappa$ , $\mu$	28	E, I, II, III, IV, V, VI, VII, X, XII, XIV, XVI, XVI	V, M, P	O, *, D
01	VI/VII, XII	$\delta,~\gamma,~\kappa,~\mu O,~\omega$	53	E, I, II, III, V, VI, VII, IX, X, XI, XII, XI	V, M, P	O, BrW, *, $\gamma$
O2	VI/VII, XV	γ	15	I, V, VI, VII, X, XII, XIII, XIV, XVI	V, M, P	O, BrW, *, γ
$O_3$	VI/VII	•	6	V, VII, XIV, XVI	V, M, P	, , , ,
P	IX		6	V, X, XVII	V, M	*, y
S	VIII	$\alpha$ , $\sigma$	6	I, II, V, XIV	V, M, P	O, BrW, *, γ
T	I, V, X, XVI	$\chi$ , $\varepsilon$ , $\mu$	24	É, IÍ, IÍI, V, VI, VII, X, XII, XIII, XIV, XVI, XVII	V, M, P	O, BrW, *, γ, G, Z
V	XV		2	XIII	V	*
Ý	XVII		$\bar{1}$	XIV	V	0

E, early clade; P, fish; M, molluscs; V, worms; \*, C-terminal amidation; O, hydroproline; BrW, bromotryptophane; D, D-amino acid;  $\gamma$ ,  $\gamma$ -carboxyglutamate;  $\gamma$ -Val,  $\gamma$ -hydroxyvaline; G, glycosylation; Z, pyroglutamate; Ys, sulfotyrosine.

 $\kappa$ -conotoxins), ligand-gated ion channels ( $\alpha$ -conotoxins,  $\sigma$ -conotoxin, ikot ikot, and conantokins), GPCRs<sup>1</sup> ( $\rho$ -conopeptides, conopressins and contulakins), and neu-

rotransmitter transporters ( $\chi$ -conopeptides), and we discuss the potential of sodium and calcium channel inhibitors and  $\alpha$ -conotoxins to modulate pain pathways.

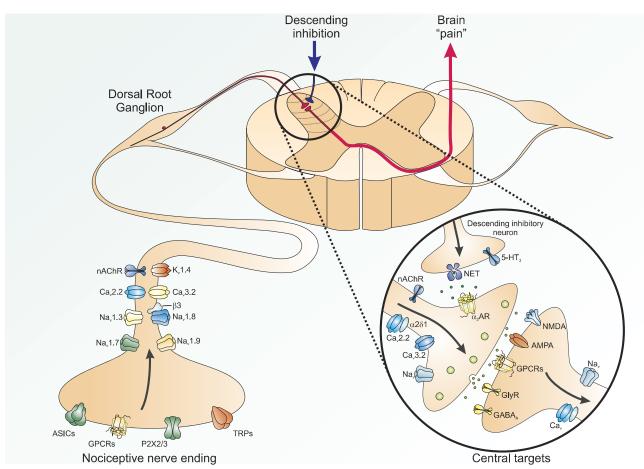


Fig. 2. Peripheral and spinal pain pathways amenable to conopeptide modulation. A number of selected targets on peripheral nociceptive terminals and afferent neurons as well as descending facilitatory and inhibitory pathways originating in the medulla are shown. Therapeutic targets with known conotoxin modulators are highlighted in blue, and those targeted by peptides derived from other species are highlighted in green. Targets with therapeutic potential but no known specific peptide modulators are shown in yellow, and targets that are modulated by venom peptides but in an undesirable manner (e.g., inhibition of  $K_v$ 1.4 by  $\kappa$ -conotoxins and activation of TRPV1 by  $\pi \kappa$ -theraphotoxin-Pc1a) are highlighted in orange. ASIC, acid-sensing ion channel; P2X2/3, purinoceptors 2X2 and 2X3; TRP, transient receptor potential channel;  $\alpha 2\delta 1$ , auxiliary  $Ca_v$  subunit; GlyR, glycine receptor.

 $\begin{tabular}{ll} TABLE 2 \\ Clinical potential and representative sequences of major conotoxin classes defined by pharmacology \\ Cysteines involved in disulfide bonds (underlined) connect in discrete overlapping patterns depending on sequence (see Tables 4, 5, 7–9, and 11). \\ \end{tabular}$ 

Class	Mode of Action	Name	Sequence	Clinical Potential
ω	Ca <sub>v</sub> 2.2 inhibitor	MVIIA	CKGKGAKCSRLMYDCCTGSCRSGKC*	Pain (intrathecal; phase IV)
μ	Na <sub>v</sub> inhibitor	SIIIA	ZNCCNGGCSSKWCRDHARCC*	Pain (intravenous)
μO	Na <sub>v</sub> 1.8 inhibitor	MrVIB	ACSKKWEYCIVPIIGFIYCCPGLICGPFVCV	Pain (intrathecal/intravenous)
δ	Na, enhancer	EVIA	DDCIKOYGFCSLPILKNGLCCSGACVGVCADL*	?
К	K, inhibitor	PVIIA	CRIONOKCFOHLDDCCSRKCNNRFNKCV	Cardiac reperfusion
χ	NET inhibitor	Xen2174	ZGVCCGYKLCHOC	Pain (intrathecal; phase II)
α	nAChR inhibitor	Vc1.1	GCCSDPRCNYDHPEIC*	Pain (intravenous) <sup>a</sup>
$\sigma$	5HT <sub>3</sub> receptor	GVIIIA	GCTRTCGGOKCTGTCTCTNSSKCGCRYNVHPSGBGCGCACS*	?
ρ	$\alpha_1$ -Adrenoceptor inhibitor	TIA	FNWRCCLIPACRRNHKKFC*	Cardiovascular/BPH
Conantokin	NMDA-R antagonist	Con-G	GEYYLQYNQYLIRYKSN	Pain/epilepsy (intrathecal) <sup>a</sup>
Conopressin	Vasopressin-R agonist	Cono-G	CFIRNCPKG*	Cardiovascular/mood
Contulakin	Neurotensin-R agonist	Cont-G	ZSEEGGSNAtkkpyIlL	Pain $(intrathecal)^a$

<sup>\*,</sup> C-terminal amidation.

## II. $\omega$ -Conotoxin Inhibitors of Voltage-Gated Calcium Channels

The  $Ca_v$  channels are assemblies of the various pore-forming  $\alpha$ -subunits associated with single transmembrane  $\alpha 2\delta$ -subunits and intracellular  $\beta$ -subunits (Catterall et al., 2005b, 2007) (Table 3). Calcium channel ( $Ca_v$ ) inhibitors are used as antihypertensive, antiarrhythmic, and anti-convulsant agents and in chronic pain management, and other potential uses are in development (Triggle, 2007). The therapeutic potential of  $\omega$ -conotoxins is based on their

ability to selectively inhibit mammalian  $Ca_v$  isoforms (Table 4) expressed in ascending pain pathways (especially  $Ca_v2.2$ , which underlies the N-type current present in sensory neurons) and to produce antinociception in animal models of pain. Our understanding of the pharmacology of  $\omega$ -conotoxins has arisen mostly from studies on GVIA, MVIIA, CVID, and MVIIC. Although these peptides are considered to function as simple pore blockers, their pharmacology is reminiscent of  $\mu$ O-conotoxins, because coexpression of the pore-forming  $\alpha$  subunit with auxiliary  $\beta$  or

TABLE 3
Ca., distribution, toxin inhibitors and potential effects of inhibition

$\mathrm{Ca_v}$ Isoform	Distribution	Selective Toxin Inhibitors	Effects/Side Effects of Inhibition	References
	Skeletal muscle (1.1), (T-tubules), Cardiac muscle (1.2–1.3), smooth muscle (1.2), CNS (1.1– 1.3), DRG (1.2–1.4), retina (1.4) lymphocytes (1.2–1.4), GIT (1.2), cochlea (1.3).	None known (dihydropyridines)	Widespread effects on cardiovascular system, 1.2 implicated in neuropathic pain in spinal cord.	Kim et al., 2001; Catterall et al., 2005b, 2007; Fossat et al., 2010
Ca <sub>v</sub> 2.1 (P/Q type) (CACNA1A) splice variants	Brain, spinal cord, sympathetic neurons, DRG, endocrine cells, contribution to presynaptic neurotransmitter release at CNS, PNS, and neuromuscular junction.	ω-Conotoxins MVIIC ~ MVIID > CVIB > CVIC ≫ MVIIA > GVIA ≫ CVID Spider toxins: ω- agatoxin IVA	Mutations in several neurological disorders, blockers produce partial inhibition of synaptic transmission. Peripheral side effects expected from autonomic and neuromuscular block.	Bourinet et al., 1999; Nudler et al., 2003; Catterall et al., 2005b, 2007 (For selectivity see Table 4.)
$\begin{array}{c} {\rm Ca_v 2.2~(CACNA1} B) \\ {\rm splice~variants} \end{array}$	Brain, spinal cord, sympathetic neurons, DRG, contribution to presynaptic neurotransmitter release in CNS and PNS.	$\omega$ -Conotoxins CVIE = CVID = GVIA $\gg$ CVIA $\sim$ MVIIA $>$ CVIB $\sim$ CVIC	Blockers produce inhibition of synaptic transmission throughout the nervous system.	Catterall et al., 2005b, 2007 (For selectivity see Table 4.)
${ m Ca_v}2.3~({ m CACNA1}E)$ splice variants	Brain, spinal cord, sympathetic neurons, DRG, minor role in presynaptic neurotransmitter release in CNS and PNS, synaptic plasticity.	No conotoxins Spider: SNX-482	Blockers modulate synaptic plasticity at some brain synapses.	Murakami et al., 2004; Catterall et al., 2005b, 2007; Matthews et al., 2007 (For selectivity see Table 4.)
${ m Ca_v}3.1~({ m CACNA1}G)$	Brain neurons localized to soma and dendrites. High expression in cerebellum and thalamus. Modulates action potential firing. Ovary, placenta, heart.	No conotoxins (pimozide, mibefradil, TTA-P2)	Small-molecule modulators may be useful for some CNS neurological disorders.	Catterall et al., 2005b, 2007; Yaksh, 2006; Triggle, 2007; Zamponi et al., 2009
${\rm Ca_v} 3.2 \; ({\rm CACNA1} H)$	CNS, DRG neurons: localized to soma and dendrites. Modulates action potential firing. Also heart, liver, kidney, lung, skeletal muscle, pancreas.	No conotoxins Scorpion: kurtoxin (pimozide, mibefradil, Z123212, <sup>a</sup> TTA-P2)	Pain modulation. Relaxation of coronary arteries, potential side effects from actions in other tissues.	Catterall et al., 2005b, 2007; Yaksh, 2006; Triggle, 2007; Zamponi et al., 2009; Choe et al., 2011
${ m Ca_v} 3.3~({ m CACNA1}I)$	CNS neurons: localized to soma and dendrites. Modulates action potential firing.	No conotoxins (pimozide, TTA-P2, mibefradil very weak)	Presumably many side effects.	Catterall et al., 2005b, 2007; Yaksh, 2006; Triggle, 2007; Zamponi et al., 2009

<sup>&</sup>lt;sup>a</sup> Clinical development suspended.

TABLE 4  $\omega$ -Conotoxins targeting  $Ca_v$  calcium channels (loop formula C6C5-9CC2-4C3-6C)

~ .			Sequence Disul	fide Bonded	l ( 1–4/2–	5/3-6)		
Species	Name	Diet	1 2	34	5	6	$Ca_v$ Selectivity	References
C. consors	CnVIIA	Р	<b>C</b> KGKGAO <b>C</b> TRLMYI	)СС-Н	GSCSSS	KGR <b>C</b> *	2.2 > 2.1	Favreau et al., 200
C. catus	CVIA	P	CKSTGASCRRTSYI			_	2.2 > 12.	Lewis et al., 2000
c. carao	CVIB	P	CKGKGASCRKTMYI				$2.2 \sim 12.$ $2.2 \sim 2.1 > 2.3$	Lewis et al., 2000
	CVIC	P	CKGKGQSCSKLMYI				$2.1 \sim 2.2$	Lewis et al., 2000
	CVID	P	CKSKGAKCSKLMYI				2.1   2.2   2.2   2.1	Lewis et al., 2000 Lewis et al., 2000
	CVIE-2**	P				_	2.2 > 2.1 $2.2 > 2.1 > 1.2 \sim 1.3 \sim 2.3$	Berecki et al., 2000
	CVIE-2 CVIF	P	CKGKGASCRRTSYI				$2.2 > 2.1 > 1.2 \sim 1.3 \sim 2.3$ $2.2 > 2.1 > 1.2 \sim 1.3 \sim 2.3$	
0.61			CKGKGASCRRTSYI					Berecki et al., 2010
C. fulmen	FVIA	P	CKGTGKSCSRIAYI				2.2 > 2.1 > 3.2	Lee et al., 2010
C. geographus	GVIA	Р	<b>c</b> <u>k</u> sogss <b>c</b> sots <u>v</u> i	NCC	<u>R</u> S <b>C</b> NO <u>Y</u>	T <u>K</u> R <b>C</b> Y*	2.2 > 2.1	Olivera et al., 1984 Sato et al., 1993 Kim et al., 1994 Kim et al., 1995
	GVIIA	P	<b>C</b> KSOGTO <b>C</b> SRGMRI	)CC	TS <b>C</b> LLY	SNK <b>C</b> RRY	$\rm Mouse \sim fish > frog$	Olivera et al., 1985 Abe and Saisu, 1987
	GVIIB	P	<b>C</b> KSOGTO <b>C</b> SRGMRI	)CC	TS <b>C</b> LSY	SNK <b>C</b> RRY	$\rm Mouse \sim fish > frog$	Olivera et al., 1985 Abe and Saisu, 1987
C. magus	MVIIA	Р	<b>c</b> kgkgak <b>c</b> s <u>rl</u> m <u>y</u> i	) <b>CC</b> -T	GS <b>C</b> RS-	−GK <b>C</b> *	2.2 > 2.1	Olivera et al., 1987 Kim et al., 1995; Nielsen et al., 1999a
	MVIIB	P	CKGKGASCHRTSYI	DCC-T	GSCN	RGK <b>C</b>	N.D.	Olivera et al., 1987
	MVIIC	P	<b>C</b> KGKGAP <b>C</b> R <u>K</u> TMYI	D <b>CC</b> -S	GS <b>C</b> -GR	RGK <b>C</b> *	2.1 > 2.2	Hillyard et al., 1992; Nielsen et al., 1999a
	MVIID	Р	<b>C</b> QGRGAS <b>C</b> R <u>K</u> TMYI	N <b>CC</b> -S	GS <b>C</b> N	RGR <b>C</b> *	$2.1\gg 2.2$	Haack et al., 1993; Monje et al., 1993; Gandía et al., 1997
C. pennaceus	PnVIA	$\mathbf{M}$	GCLEVDYFCGIPFAI	NNGL <b>CC</b> -S	GN <b>C</b> VFV	<b>C</b> TPO	Molluscan N-type	Kits et al., 1996
	PnVIB	$\mathbf{M}$	DDDCEPPGNFCGMIKIO				Molluscan N-type	Kits et al., 1996
C. pulicarius	PuIA	V	-RD <b>C</b> RPVGQY <b>C</b> GIPYER				N.D.	Zhao and Huang, 2000
	PuIIA	V	TCNTPTQYCTLHRH	<b>cc</b> -s	LҮ <b>С</b> НКТ	THACA	N.D.	Zhao and Huang, 2000
C. radiatus	RVIA	P	<b>C</b> KPOGSO <b>C</b> RVSSYI				2.2	Abbott and Litzinger, 1994
C. striatus	SO3	Р	<b>C</b> KAAGKP <b>C</b> SRIAYI	N <b>CC</b> -T	GS <b>C</b> RS-	-GK <b>C</b> *	$\mathrm{Na_v} \sim \mathrm{K_v} \sim 2.2 > 2.1 > 2.3$	Lu et al., 1999; Li et al., 2003b; We et al., 2005
	SO4	P	ATD <b>C</b> IEAGNY <b>C</b> GPTVM				N.D.	Lu et al., 1999
	SO5	P	STS <b>C</b> MEAGSY <b>C</b> GSTTR				N.D.	Lu et al., 1999
	SVIA	P	CRSSGSOCGVTSI-	<b>-CC</b> -G	R- <b>C</b> -Y-	RGKCT*	2.2 > 2.1	Ramilo et al., 1992
	SVIB	P	<b>C</b> KLKGQS <b>C</b> RKTSYI	D <b>CC</b> -S	GS <b>C</b> GRS	−GK <b>C</b> *	2.1 > 2.2	Ramilo et al., 1992 Woppmann et al 1994; Nadasdi et al., 1995
C. tulipa	TVIA	P	<b>c</b> lsogss <b>c</b> sotsyn	4 <b>cc</b>	RS <b>C</b> NOY	SRKC	2.2 > 2.1	Chung et al., 1995 Wang et al., 199
C. textile	TxO1	$\mathbf{M}$	CLDAGEVCDIFFP	г <b>сс</b>	GYCILL	FCA	N.D.	Lu et al., 1999
-	TxO2	M	CYDSGTSCNTGNQ-				N.D.	Lu et al., 1999
	TxO3	M	CYDGGTSCDSGIQ-				N.D.	Lu et al., 1999
	TxO4	M	-YDCEPPGNFCGMIKIO				N.D.	Lu et al., 1999
	TxO5	M	CVPYEGPCNWLTQ				N.D. N.D.	Lu et al., 1999
	TxO6	M	-NYCQEKWDYCPVPFLO				N.D. Molluscan L-type	Lu et al., 1999
	TxVII	M	<b>C</b> KQADEP <b>C</b> DVFSLI	JCC-T	GT <b>C</b> TG≬	CIMW	мониясан 11-гуре	Fainzilber et al., 1996

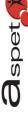
P, fish; M, molluscs; V, worms; \*, C-terminal amidation; N.D., not determined.

 $\alpha2\delta$  subunits can significantly alter their channel affinity (Lewis et al., 2000; Mould et al., 2004; Motin et al., 2007). Such differences may have particular relevance in disease states such as pain, where  $\alpha2\delta$  subunits, particularly  $\alpha2\delta1$ , are up-regulated (see section II.C).  $\omega$ -Conotoxins remain the only class of conotoxins with FDA approval to date, the registration of MVIIA (ziconotide) for the treatment of intractable pain having been approved in 2004. Unfortu-

nately, dose-limiting neurological side effects have limited the clinical applications for  $\omega$ -conotoxins.

### A. Subtype Selectivity

 $\omega$ -Conotoxins with defined activity at mammalian  $\mathrm{Ca_v}$  isoforms have so far been isolated only from piscivorous cone snails, where they are likely to have evolved as part of the motor cabal (Olivera et al., 1985). Based on the behav-



Downloaded from pharmrev.aspetjournals.org at Thammasart University on December 3, 2012

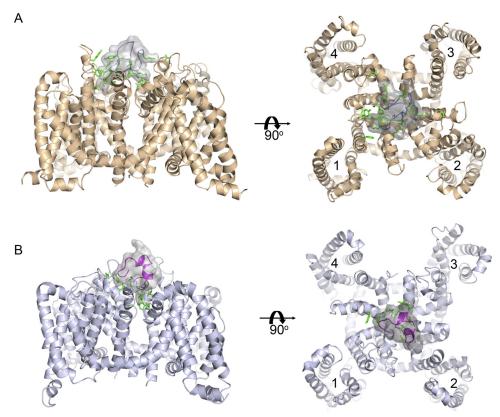


Fig. 3. Docking of conotoxins to homology models of sodium and calcium channels. A,  $\omega$ -MVIIA (dark gray) docked to a homology model of Ca<sub>v</sub>2.2 (light brown) built from a bacterial sodium channel crystal structure (Payandeh et al., 2011). B,  $\mu$ -TIIIA (magenta) docked to a homology model of Na<sub>v</sub>1.4 (light blue) built from the same bacterial sodium channel (Payandeh et al., 2011). Identified likely interacting residues are shown in sticks (green), and the order of subunits is labeled numerically. The highly flexible, unstructured loops (approximately  $50 \sim 100$  amino acids long) between transmembrane helices 5 and P1 in subunits 1 and 3 of both Ca<sub>v</sub>2.2 and Na<sub>v</sub>1.4 were removed for clarity. The docking simulation outcomes reveal large contact areas and extensive ionic interactions networks between conotoxins and all four subunits of voltage-gated ion channels that probably explain their high binding affinities.

ioral phenotype elicited after intracerebral injection in mice they were termed "shaker" peptides. It is now appreciated that  $\omega$ -conotoxins represent some of the most selective known inhibitors for the neuronal Ca<sub>v</sub> isoforms 2.2 and 2.1. Specifically,  $\omega$ -conotoxins CVID, CVIE, CVIF, GVIA, and MVIIA are particularly selective for Ca<sub>v</sub>2.2, whereas MVIIC and MVIID show a preference for Ca<sub>v</sub>2.1 underlying P/Q-type calcium currents (Monje et al., 1993; Woppmann et al., 1994; Nadasdi et al., 1995; Nielsen et al., 1996; Flinn et al., 1999; Lewis et al., 2000; Berecki et al., 2010). The binding site of  $\omega$ -conotoxins to the Ca<sub>v</sub> subtypes has been mapped primarily to the external vestibule of the channel in the domain III S5-S6 region (Ellinor et al., 1994), consistent with preliminary results obtained from docking of ω-MVIIA to a homology model of Ca<sub>v</sub>2.2 (Fig. 3A) built from the recently reported crystal structure of a bacterial sodium channel (Payandeh et al., 2011). Residues immediately outside this region seem to also contribute to Ca, 2.2 inhibition by GVIA, most notably Gly1326, which contributes to the reversibility of  $\omega$ -conotoxin block (Feng et al., 2001). Intracellular domains (Kaneko et al., 2002; McDonough et al., 2002) are also reported to modulate binding kinetics and ω-conotoxin affinity for Ca<sub>v</sub>2.2 and, in addition, the presence of subunits seems to not only modify Ca<sub>v</sub> gating characteristics and

kinetics but also the interaction of  $\omega$ -conotoxins with the channel.

The high-affinity block of Ca, 2.2 by CVID was found to be reversible in heterologous systems expressing only the pore-forming  $\alpha$ -subunit, but irreversible in DRG neurons, an effect that is mimicked in expression systems where the  $\alpha$ -subunit is coexpressed with auxiliary  $\beta$ 3 and  $\alpha$ 2 $\delta$ 1 subunits (Lewis et al., 2000; Mould et al., 2004; Motin et al., 2007). Likewise, inhibition of Ca<sub>v</sub>2.2 by CVIE and CVIF was found to be virtually irreversible in the presence of  $\beta$ 3 subunits but reversible in systems coexpressing the  $\beta 2$ subunit (Berecki et al., 2010). Intriguingly, these effects parallel modification of channel characteristics by auxiliary subunits, suggesting that altered block characteristics of  $\omega$ -conotoxins in the presence of auxiliary subunits reflect altered channel kinetics. In addition to altered binding kinetics, many ω-conotoxins also have lower affinity for Ca<sub>v</sub>2.2 in the presence of auxiliary subunits, especially  $Ca_{\nu}\alpha 2\delta 1$  (Lewis et al., 2000; Berecki et al., 2010). However, the precise mechanisms contributing to these intriguing characteristics of  $\omega$ -conotoxins, as well as the effect of these pharmacological differences on the therapeutic potential of these peptides, remain to be elucidated.

Although the vast majority of  $\omega$ -conotoxins isolated to date are selective, to varying degrees, for mammalian

Ca<sub>y</sub>2.1 and Ca<sub>y</sub>2.2, only TxVII, isolated from the molluscivorous Conus textile, blocks molluscan dihydropyridinesensitive channels, which are homologous to mammalian L-type voltage-gated calcium channels (Fainzilber et al., 1996). However, this  $\omega$ -conotoxin seems to display phylic selectivity, as TxVII was found to be inactive at rat L-type channels expressed in PC12 cells (Fainzilber et al., 1996; Sasaki et al., 1999). Likewise, PnVIA and PnVIB were isolated from the molluscivorous Conus pennaceus, but in contrast to TxVII, they inhibit the molluscan dihydropyridine-insensitive high-voltage activated Ca<sup>2+</sup> current (Kits et al., 1996). Not surprisingly, the sequences of these related peptides are distinct from conotoxins from piscivorous cone snails targeting mammalian channels. Indeed, molluscan channels are usually insensitive to GVIA, suggesting distinct phylum-selective pharmacologies resulting from these divergent sequences. Accordingly, ω-conotoxins from molluscivorous cone snails seem to represent a distinct subclass of  $\omega$ -conotoxins with specificity for molluscan calcium channels.

Other structurally diverse  $\omega$ -conotoxins include PuIA and PuIIA from the worm-hunting *Conus pulicarius*, as well as several peptides identified from the molluscivorous *C. textile* and piscivorous *Conus striatus*. The precise pharmacological target and subtype selectivity of these conotoxins remains to be determined; however, their homology to the functionally promiscuous SO3, which also inhibits sodium and potassium channels in hippocampal neurons (Li et al., 2003b; Wen et al., 2005), suggests that their pharmacological classification as  $\omega$ -conotoxins may be premature.

#### B. Structure-Activity Relationships

The pharmacological activity of  $\omega$ -conotoxins has been attributed primarily to Tyr13 and Lys2, both of which are highly conserved in  $\omega$ -conotoxins derived from piscivorous cone snails (Table 4) and are proposed to form a two-point pharmacophore (Sato et al., 1993; Kim et al., 1994; Lew et al., 1997; Flinn et al., 1999) (Fig. 4). The hydroxyl group of Tyr13 in particular seems critical for high-affinity binding of  $\omega$ -conotoxins to Ca<sub>v</sub>2.2, although the aromatic moiety also seems to contribute to binding (Kim et al., 1994; Flinn et al., 1999). The positive charge of the second most important residue, Lys2, which is conserved in all ω-conotoxins except MVIID and TVIA, also contributes significantly to activity (Sato et al., 1993; Lew et al., 1997; Flinn et al., 1999). Detailed structure-activity relationship studies in GVIA showed that the precise location of this positive charge is less important, both lengthening and shortening of the side chain being tolerated. However, bulkier substitutions in this position greatly reduced activity (Flinn et al., 1999).

In addition, several other residues in loop 2 and 4 also contribute to varying degrees to  $\omega$ -conotoxin affinity and selectivity (Gandía et al., 1997; Lew et al., 1997; Nielsen et al., 1999a). Loop 4 residues with a significant effect on  $\omega$ -conotoxin affinity include Arg21 in MVIIA and Arg17,

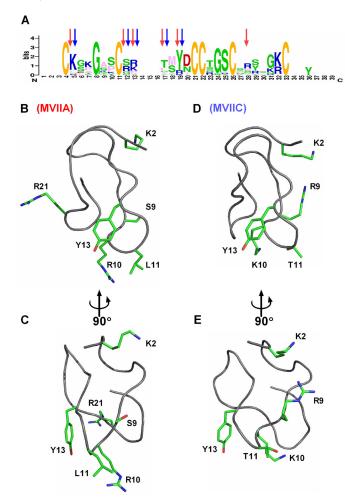


Fig. 4. Structure activity relationship of  $\omega$ -conotoxins. A, consensus sequence for  $\omega$ -conotoxins. B and C, structure of  $\omega$ -MVIIA showing important residues for interaction with the N-type calcium channel. D and E, structure of  $\omega$ -MVIIC showing important residues for interaction with the P-type calcium channel.

Tyr22, and Lys24 in GVIA, whereas the residue at position 10 in loop 2 seems to play a key role in determining subtype selectivity (Nielsen et al., 1999a). Biotinylation of Lys10 in the P/Q-selective MVIID significantly decreased binding affinity (Haack et al., 1993), and the importance of this residue for Ca<sub>v</sub> subtype selectivity was also confirmed by several independent studies (Nadasdi et al., 1995; Nielsen et al., 1996). The residue at position 10 is consistently a lysine in  $\omega$ -conotoxins with high affinity for Ca<sub>2</sub>2.1, but often an arginine or hydroxyproline in Ca<sub>2</sub>2.2selective peptides (Nadasdi et al., 1995; Nielsen et al., 1996). However, it remains to be determined if the subtype selectivity of Ca, 2.1-selective MVIIC and MVIID are significantly altered by a K10R swap. It is noteworthy that an arginine in position 10 was reported to reduce recovery from Ca<sub>v</sub>2.2 block for both MVIIA and CVID, suggesting that the effect of this residue on subtype selectivity could be due to altered recovery characteristics (Mould et al., 2004).

Although the pharmacophore of  $\omega$ -conotoxins responsible for affinity and subtype selectivity at  $Ca_v$ s is becoming



Aspet

increasingly clear, residues responsible for any splice variant selectivity of  $\omega$ -conotoxins have not been determined to date. Nonetheless, our understanding of these structure-activity relationships are being applied to the design of small molecule, orally active  $\omega$ -conotoxin peptidomimetics with the hope of engineering novel analgesic molecules based on the unique pharmacological properties of  $\omega$ -conotoxins (Schroeder et al., 2004; Andersson et al., 2009).

## C. Calcium Channel Inhibition by Conotoxins in Pain Management

There is strong evidence that Ca, 2.2 channels on nociceptive primary afferent nerves are an target for pain management. Some of the most effective nonopioid analgesics for chronic pain management, gabapentin and pregabalin, interact with  $Ca_{\nu}$  channel  $\alpha 2\delta$  subunits, which is thought to disrupt trafficking of Ca<sub>v</sub>2.2 channels (Bauer et al., 2010). Indeed, in the spinal cord, opioids act predominantly on  $Ca_{\nu}2.2$  channels via  $\mu$ -opioid receptors expressed on nociceptive primary afferent nerve terminals to produce presynaptic inhibition and analgesia (Heinke et al., 2011). Many  $\omega$ -conotoxins exhibit high potency and selectivity for different subtypes of voltage-gated calcium channels (Olivera et al., 1994). The development of the N-type (Ca<sub>v</sub>2.2) antagonist, ω-conotoxin MVIIA (SNX-111; ziconotide), as an intrathecal analgesic to manage moderate to severe chronic pain, provided the first validation of the use of ω-conotoxins and established Ca<sub>v</sub>2.2 as new target for pain therapeutics (Lewis and Garcia, 2003; Winquist et al., 2005). Although ziconotide is potent and does not induce addiction or tolerance as opioids do (Malmberg and Yaksh, 1995; Bowersox et al., 1996), its use is limited because of the need for intrathecal administration combined with significant dose-limiting neurological side effects (Penn and Paice, 2000). This side-effect profile likely arises, at least in part, from the spread of peptide from the intrathecal space to higher (supraspinal) brain centers including cerebellum and the dependence of a significant component of synaptic neurotransmission on Ca<sub>v</sub>2.2 at many CNS synapses, including inhibitory spinal synapses (Penn and Paice, 2000; Schmidtko et al., 2010). However, as discussed below, other properties of  $\omega$ -conotoxins could additionally contribute to their side-effect profile and therapeutic index. The distribution of Ca<sub>v</sub> channel types (Catterall et al., 2005b, 2007), potential actions of inhibitors, as well as selective conotoxins and other inhibitors are summarized in Table 3. The role of  $Ca_v 1$  to 3 and  $Ca_v \alpha 2\delta$  in pain pathways and their potential for selective targeting by conotoxins is outlined below.

The  $\mathrm{Ca_v}1$  channel  $\alpha$ -subunits encode L-type channels but evidence for their involvement in pain states is still very limited (Yaksh, 2006). L-type calcium channel subtypes are expressed in sensory neurons and dorsal horn of the spinal cord, where they could potentially modulate the development of neuropathic pain (Kim et al., 2001). Intrathecal L-type channel antagonists have mixed effects in pain models (see Yaksh, 2006), possibly because nonselec-

tive Ca<sub>v</sub>1 inhibitors have little effect on afferent substance P release (Takasusuki and Yaksh, 2011) or the role of Ca<sub>v</sub>1 changes in different models of pain. More recently, antisense knockdown and microRNA regulation of the Ca, 1.2 in spinal cord has been shown to reverse the development of mechanical signs of neuropathic pain, hyperexcitability of deep dorsal horn neurons, phosphorylation of cAMP response element-binding protein, and induction of cyclooxygenase-2 mRNA in the spinal cord (Fossat et al., 2010), consistent with Ca. 1.2 expression predominantly in neuronal somata and dendrites and its role in gene regulation and plasticity (Murakami et al., 2004; Zhang et al., 2006a). These findings suggest that specific peptide inhibitors of Ca<sub>v</sub>1.2 may be useful for management and perhaps reversal of central adaptations responsible for maintenance of neuropathic pain when applied directly to the dorsal spinal

 $Ca_{\nu}2.1$  encodes the  $\alpha$ -subunit of high-voltage activated P/Q-type calcium channels, with alternative splicing generating the P and Q phenotypic variants (Bourinet et al., 1999). Together with Ca, 2.2 and Ca, 2.3, P/Q-type channels contribute to the calcium influx, triggering synaptic neurotransmission at many synapses including nociceptive synapses (Heinke et al., 2004; Rycroft et al., 2007) and thus may be considered as a target for potential pain therapeutics (e.g., Vanegas and Schaible, 2000; Yaksh, 2006). However, P/Q channels are more densely expressed in non-nociceptive than in nociceptive sensory neurons and are the major Ca<sub>v</sub> channels mediating synaptic neurotransmission at neuromuscular synapses (Uchitel et al., 1992; Westenbroek et al., 1998; Nudler et al., 2003). P/Q channel inhibitors would therefore be expected to produce neuromuscular and non-nociceptive sensory side effects after systemic or intrathecal administration. This might suggest a greater role in sensory modalities other than nociception. Indeed, intrathecal administration of the highly selective Ca<sub>v</sub>2.1 inhibitor, agatoxin IVA, in a variety of pain models has produced mixed results (Malmberg and Yaksh, 1994; Vanegas and Schaible, 2000; Yaksh, 2006).

The most promising calcium channel regulating pain pathways is arguably  $Ca_{\nu}2.2$ , which encodes the  $\alpha$ -subunit of high voltage activated N-type calcium channels. Ca, 2.2 inhibitors have been developed as pain therapeutics, because Ca<sub>2</sub>2.2 is the predominant channel supporting nociceptive primary afferent neurotransmission in the dorsal horn of the spinal cord (Fig. 2). Although Ca, 2.2 is sometimes considered the only calcium channel mediating synaptic transmission at nociceptive synapses, other Ca, channels, including P/Q-type, R-type, and to a small extent L-type, contribute ~50% of neurotransmitter release at nociceptive synapses (Heinke et al., 2004; Rycroft et al., 2007). Among a diverse group of  $\omega$ -conotoxins, MVIIA was established to be highly efficacious after intrathecal administration in a range of animal pain models (Malmberg and Yaksh, 1994; Scott et al., 2002; Yaksh, 2006), although severe behavioral side effects of apparent neurological origin were observed within the effective dose-range for pain

relief (Smith et al., 2002). In humans, optimal use of ziconotide (MVIIA) requires slow and careful titration of dose and rate of intrathecal infusion. The adverse effects of ziconotide on the central nervous system include dizziness, nystagmus, confusion, abnormal gait, somnolence, speech difficulties, amblyopia, ataxia, amnesia, and abnormal thought processes (Penn and Paice, 2000; Schmidtko et al., 2010). Small-molecule Ca<sub>v</sub>2.2 inhibitors may suffer from similar side-effect issues in addition to the peripheral side effects observed after intravenous administration of ziconotide. These include orthostatic hypotension, dizziness, sinus bradycardia, rhinitis, and nausea (McGuire et al., 1997). Preclinical testing of other  $\omega$ -conotoxins suggest improvements are possible. For example, CVID (AM336, CNSB004, leconotide) has potency to inhibit Ca., 2.2 channels and relieve neuropathic pain signs in animal models comparable to that of MVIIA but has been reported to exhibit a better (approximately 5-fold) therapeutic index for analgesia versus side effects (Scott et al., 2002; Smith et al., 2002). Some of the potential reasons for improved therapeutic index and potential applications of novel  $\omega$ -conotoxins are explored below. Although CVID has similar potency at Ca, 2.2 as MVIIA,

Although CVID has similar potency at Ca<sub>v</sub>2.2 as MVIIA, it is approximately 100-fold more selective for N-type than P/Q type channels (Lewis et al., 2000). This has been suggested as a reason for its improved side effect profile both in the CNS and periphery (Smith et al., 2002; Kolosov et al., 2010). However, MVIIA is already nearly 10,000-fold selective for N-type versus P/Q-type channels based on radioligand-binding studies using rat brain membrane (Lewis et al., 2000), so this might not be the explanation for better selectivity of CVID versus MVIIA and GVIA. Further systematic investigation of the relationship between N and P/Q selectivity and therapeutic index will be required to determine whether this is important.

The target residence time of a drug can strongly influence its therapeutic index (Copeland et al., 2006). The rate of reversal of inhibition of Ca<sub>v</sub>2.2 by ω-conotoxins certainly influences the extent to which side effects can be managed clinically (Penn and Paice, 2000). Many ω-conotoxins, including MVIIA and GVIA, seem to bind nearly irreversibly to the N-type channel. However, reversibility can be induced by hyperpolarizations to potentials more negative than the minimum of the steady-state inactivation curve; i.e., ω-conotoxins bind with higher affinity to the inactivated state of the N-channel than the resting state (Stocker et al., 1997). Such voltage-dependent recovery from block varies for different  $\omega$ -conotoxins and is strongly influenced for some by the  $\alpha 2\delta$ - and  $\beta$ -subunits, in particular  $\alpha 2\delta 1$ ,  $\beta 2a$ , and  $\beta 3$ , coexpressed with the  $\alpha$ -subunit (Mould et al., 2004; Berecki et al., 2010). Reversibility of GVIA and MVIIA is only weakly influenced by  $\alpha 2\delta 1$ - and  $\beta$ 2a- or  $\beta$ 3-subunit coexpression, and both appear to have a narrower therapeutic index than for CVID, which is strongly influenced by the presence of these subunits (Mould et al., 2004; Berecki et al., 2010). Two more recently identified  $\omega$ -conotoxins from Conus catus, CVIE and

CVIF, show greater voltage-dependent reversal in the presence of  $\alpha 2\delta 1$ - and  $\beta$ -subunits than CVID (Berecki et al., 2010). The implications of this property for modulation of primary afferent nociceptive neurotransmission will remain uncertain until the therapeutic indices of these novel  $\omega$ -conotoxins are determined. The importance of this property in nerve terminals is also uncertain because Ca, 2.2 channels in some nerve terminals seem to be resistant to voltage-dependent inactivation (Chan et al., 2007). Nonetheless, it is of interest that GVIA and MVIIA apparently irreversibly inhibit primary afferent synaptic neurotransmission, whereas CVID is partly reversible. CVIE and CVIF are fully reversible after removal of the peptides but show similar efficacy to CVID in pain models (Motin et al., 2007; Berecki et al., 2010). If the therapeutic indices for CVIE and CVIF were significantly better than for both CVID and MVIIA, it would suggest that reversibility of block is an important factor in the rapeutic index. A novel ω-conotoxin, FVIA from Conus fulmen, may be useful because it is fully reversible within the normal steady-state inactivation range of Ca<sub>v</sub>2.2 (-80 mV). However, its voltage- and  $\alpha 2\delta - \beta$ -subunit dependence and therapeutic index have not been determined (Lee et al., 2010).

 $Ca_{\nu}\alpha 2\delta$  subunits also strongly influence the potency of some  $\omega$ -conotoxins but not others, independently of  $\beta$ -subunit coexpression (Mould et al., 2004; Motin et al., 2007; Motin and Adams, 2008; Berecki et al., 2010). The potency (with coexpression of  $\beta$ -subunits) of MVIIA, CVID, CVIF, and MVIIA are reduced approximately 100-fold by coexpression of  $\alpha 2\delta 1$ -subunits in oocytes, whereas the potency of CVIE is only reduced approximately 10-fold (Mould et al., 2004; Berecki et al., 2010). This may be important because α2δ1-subunit expression is profoundly upregulated in the central terminals of primary afferent nerves in neuropathic pain (Bauer et al., 2010) so that the potency of some  $\omega$ -conotoxins in pain states might be strongly modulated. Consistent with such an effect, CVID and MVIIA at maximal effect doses are less effective at nociceptive primary afferent synapses in a rat inflammatory pain model (Rycroft et al., 2007). Other conotoxins and neuropathic models remain to be examined at this level.

The highly specific binding of  $\omega$ -conotoxins to Ca<sub>v</sub>2.2 suggests that specific interaction with splice variants may be possible. The Ca<sub>v</sub>2.2 gene contains several alternative splicing sites (Lipscombe et al., 2002). If these are differentially expressed in normal nociceptive nerves or in pain states more selective  $\omega$ -conotoxins may be developed to target them. The best studied e37a and e37b variants underlie expression of two mutually exclusive isoforms of N-type channels. The e37a isoform is enriched in nociceptive sensory neurons, undergoes distinct modulation by both G-protein  $\beta\gamma$ -subunits and tyrosine phosphorylation, and influences N-type calcium current density (Bell et al., 2004; Raingo et al., 2007). The e37a isoform is selectively involved in nociceptive responses in pain models (Altier et al., 2007) and confers novel pharmacological responses to opioids (Andrade et al., 2010). Because the splice site en-



codes a distinct pair of 32 amino acid sequences in the intracellular, C-terminal region of the channel, the prospects for developing selective  $\omega$ -conotoxins for these isoforms may be limited given these peptides bind extracellularly (see Fig. 3A). The function and tissue distribution of other splice variants have been less extensively studied. A long C-terminal splice variant of Ca, 2.2 has been identified in presynaptic membranes (Gardezi et al., 2010) and other variants lacking a synaptic protein interaction site within the intracellular SII-SIII linker domain are presumably not targeted to synaptic nerve terminals (Kaneko et al., 2002). It is noteworthy that the  $\Delta 1$  variant (SII-SIII domain truncation) was less sensitive to inhibition by both MVIIA and GVIA (Kaneko et al., 2002), suggesting that it may be possible to selectively target  $\omega$ -conotoxins to Ca. 2.2 splice variants, even when sequence variations are within intracellular domains.

The efficacy of  $\omega$ -conotoxins in pain management is generally considered to be due exclusively to inhibition of N-type channels at primary afferent synapses in the dorsal horn of the spinal cord. Until recently, the possibility that pain relief could be achieved with systemic administration was not considered because MVIIA produces serious systemic cardiovascular and other side effects mediated by the peripheral nervous system (McGuire et al., 1997). However, in conscious rabbits and isolated, sympathetically innervated tissues, CVID was much less potent than MVIIA at producing sympathetic and cardiovascular effects (Wright et al., 2000), suggesting it may be safer after systemic administration. Indeed, intravenous CVID has recently been shown to produce modest antihyperalgesia in pain models at doses producing no cardiovascular effects (Kolosov et al., 2010) and synergizes strongly with other analgesics, including morphine (Kolosov et al., 2010; Kolosov et al., 2011). The reasons for the greater therapeutic index of systemic CVID are not known. It could be due to greater N- versus P/Q-channel selectivity (but see selectivity section above) or to influences of auxiliary subunits expressed selectively in sympathetic neurons (Kolosov et al., 2010). Using GVIA and CVID to probe N-channels, different contributions of N-channels to vasoconstrictor versus vasodilator neurons have been noted (Jobling et al., 2004; Morris et al., 2004) but sensitivities to conotoxins differently influenced by auxiliary subunit expression, such as CVD, CVIE, and CVIF, have not been examined. Resolution of differential effects in relevant sympathetic nerve terminal preparations may produce a more rational approach to the development of safe, systemically active  $\omega$ -conotoxin pain therapeutics.

In contrast to  $Ca_v2.2$ ,  $Ca_v2.3$  has not been extensively explored in pain models and no highly selective  $\omega$ -conotoxins targeting this channel are known. R-type channels do contribute to nociceptive primary afferent synaptic transmission (Heinke et al., 2004; Rycroft et al., 2007), and the selective  $Ca_v2.3$  inhibitor SNX-482 from the tarantula,  $Hysterocrates\ gigas$ , has been shown to be antinociceptive after intrathecal administration (Murakami et al., 2004)

and to inhibit nociceptive neural responses in the spinal cord dorsal horn in vivo in a neuropathic pain model (Matthews et al., 2007).

Conotoxins acting at other calcium channel types, notably Ca, 3 subtypes, could also be developed as pain therapeutics (Yaksh, 2006; Triggle, 2007; Zamponi et al., 2009).  $Ca_{\nu}3$   $\alpha$ -subunits encode the family of low-voltage-activated calcium channel currents that modulate action potential burst firing in CNS neurons and contribute to Ca<sup>2+</sup>mediated plasticity. Ca. 3.2 is the predominant type expressed in nociceptive and mechanosensitive sensory neurons (see Todorovic and Jevtovic-Todorovic, 2011). In neuropathic pain models, T-type channel current density is up-regulated, and this contributes to sensory neuron hyperexcitability (Jagodic et al., 2007, 2008). T-type channels are also expressed by neurons in the dorsal horn of the spinal cord (see Todorovic and Jevtovic-Todorovic, 2011) and have been shown to be crucial for induction of plasticity at nociceptive primary afferent synapses that could contribute to persistent pain (Ikeda et al., 2003, 2006). Consistent with this role, knockdown of Ca<sub>v</sub>3.2 in sensory neurons reduced signs of thermal and mechanical pain in neuropathic pain models (Bourinet et al., 2005; Messinger et al., 2009). Nonselective, small-molecule T-type channel inhibitors also reduce neuropathic pain signs in animal models, and antisense knockdown experiments in sensory neurons have confirmed that this effect is mediated by Ca, 3.2 (Dogrul et al., 2003; Choi et al., 2007; Choe et al., 2011). Thus, evidence implicating Ca<sub>v</sub>3.2 in sensory neuron hyperexcitability in neuropathic pain suggests that peripherally administered conotoxins selectively targeting Ca<sub>v</sub>3.2, if they can be identified, may be useful therapeutics.

## II. Conotoxins Interacting with Voltage-Gated Sodium Channels

To date, four classes of conotoxins targeting Na, channels ( $\mu$ ,  $\mu$ O,  $\delta$ , and  $\iota$  conotoxins) have been isolated from cone snail venom (Cruz et al., 1985; Fainzilber et al., 1994b; McIntosh et al., 1995; Fiedler et al., 2008). Although these venom peptides exhibit activity at the same pharmacological targets, they are structurally distinct and vary in their mechanism of action, with two families producing inhibition ( $\mu$ - and  $\mu$ O-conotoxins) and two families activation ( $\delta$ - or  $\iota$ -conotoxins) of Na<sub>v</sub> channels (Table 5). The μ-conotoxins elicit Na, inhibition through direct pore block overlapping with tetrodotoxin at site 1, consistent with preliminary docking of A15-TIIIA to a homology model of Na, 1.4 built from the crystal structure of a bacterial sodium channel (Fig. 3B). In contrast, µO-conotoxins seem to interfere with the voltage sensors in domain II of Na, to restrict channel opening, δ-conotoxins cause a delay in Na, inactivation by binding to site 6 resulting in action potential prolongation and persistent neuronal firing, whereas the  $\iota$ -conotoxins enhance channel opening by shifting the voltage dependence of Na, activation to more hyperpolarTABLE 5  $\mu\text{-} \ and \ \mu\text{O-Conotoxins inhibiting Na}_v 1.x \ sodium \ channels \ (\mu\text{-}conotoxin \ loop formula \ CC1-8CC3-4C2-5C)}$  Cysteines are bolded and aligned separately for  $\mu\text{-}$  and  $\mu\text{O-conotoxins}$ , with residues affecting sodium channel affinity underlined.

Species	Name	Diet	Sequence (Disulfide Bonded 1–4/2–5/3–6)	Na <sub>v1</sub> .x Selectivity	Reference
Species	Name	Diet	12 3 4 5 6	Na <sub>v1</sub> .x Selectivity	Keierence
μ-Conotoxins C. bullatus	BuIIIA	P	VTDRCC-KGKRECGRW-CRDHSRC-C*	$2 \sim 4 > 1 \sim 3 > 6 > 5 > 7 \sim 8$	Holford et al., 2009 Wilson et al.,
	BuIIIB	P	VGERCCKNGKRGCGRW-CRDHSRC-C*	$4 > 2 > 3 > 1 > 6 > 5 > 7 \sim 8$	2011a Holford et al., 2009 Wilson et al., 2011a
C. catus	BuIIIC CIIIA	P P	IVDRCCNKGNGKRGCSRW-CRDHSRC-C*GRCCE-GPNGCSSRWCKDHARC-C*	4 > ? Amphibian skeletal/ cardiac, TTX-R	Holford et al., 2009 Zhang et al., 2006b
C. consors	CnIIIA	P	GRCCDVPNACSGRWCRDHAQC-C*	$2 \sim 4 > 6 \sim 5 > 3 > 1 > 7 \sim 8$	Zhang et al., 2006k Wilson et al., 2011a
	CnIIIB	P	ZGCCGEPNLCFTRWCRNNARC-CRQQ	Amphibian TTX-R (< 25%)	Zhang et al., 2006k
C. geographus	GIIIA	P	RDCCTOO <u>K</u> KC <u>K</u> D <u>RQ</u> CK- <u>OQ</u> RC-CA*	$4 > 1 > 6 > 2 > 3 \sim 5 \sim 7 \sim 8$	Cruz et al., 1985; Wilson et al., 2011a
	GIIIB	P	RDCCTOORKCKDRRCK-OMKC-CA*	4 > 2 > 3 > 5 > 7 > 8 > ?	Cruz et al., 1985; Lewis et al., 200
	GIIIC	P	RDCCTOOKKCKDRRCK-OLKC-CA*	4 > 2 > N.D.	Cruz et al., 1985
C. kinoshitai	KIIIA	P	CNCSS <u>KW</u> CRDHSRC-C*	$2 > 4 > 6 > 1 \sim 7 >$ 3 > 5 > 8	Bulaj et al., 2005; Zhang et al., 2007; Wilson et al., 2011a
C. magus	MIIIA	P	ZGCCNVPNGCSGRWCRDHAQC-C*	$4>2>3>6\sim 1> 5>7\sim 8$	Zhang et al., 2006b Wilson et al., 2011a
C. purpurascens	PIIIA	P	-ZRLCCGFOKSC <u>RSR</u> QCK-O <u>H</u> RC-C*	$4 > 6 \sim 1 > 2 > 3 > 5 \sim 7 \sim 8$	Shon et al., 1998a; Wilson et al., 2011a
C. pennaceus	PnIVA	M	CCKYGWTC-LLGC-SPCGC	Molluscan	Fainzilber et al., 1995b
	PnIVB	M	CCKYGWTC-WLGC-SPCGC	Molluscan	Fainzilber et al., 1995b
C. striatus	SIIIA	P	zn <b>cc</b> ngg <b>c</b> ss <u>kw</u> c <u>rdh</u> a <u>r</u> c-c*	$2 > 4 > 6 > 1 \sim 3 > 7 > 5 > 8$	Bulaj et al., 2005; Yao et al., 2008; Leipold et al., 2011; Wilson et al., 2011a
	SIIIB	P	ZNCCNGGCSSKWC <u>KG</u> HARC-C*	4>2>?	Schroeder et al., 2008
C. stercusmuscarum	SmIIIA	P	ZRCCNGRRGCSSRWCRDHSRC-C	$4 > 2 > 1 > 3 > 6 > 5 \sim 7 > 8$	West et al., 2002; Wilson et al.,
C. striolatus	SxIIIA	P	RCCTGKKGSCSGRACKN-LKC-CA*	$\begin{array}{c} 4 > 1 > 6 > 2 > 3 \sim \\ 5 \sim 7 \sim 8 \end{array}$	2011a Walewska et al., 2008; Wilson et
	SxIIIB	P	ZKCCTGKKGSCSGRACKN-LRC-CA*	N.D.	al., 2011a Walewska et al., 2008
C. tulipa	TIIIA	P	-RHG <b>CC</b> KGOKG <b>C</b> SS <u>RE</u> CR-OQHC-C*	$4>2>1>3>6> \ 5\sim 7\sim 8$	Lewis et al., 2007; Wilson et al., 2011a
$\mu$ O-Conotoxins					20114
C. litteratus	LtVIIA	V	GECLGWSNYCTSHSICCSGECILSYCDIW	N.D.	Pi et al., 2007
	LtVd	V	-DCCPAKLLCCNP	TTX-S > TTX-R	Liu et al., 2007a
C. marmoreus	LtVIC MrVIA	$_{ m M}^{ m V}$	WPCKVAGSPCGLVSECCGT-CNVLRNRCV -ACRKKWEYCIVPIIGFIYCCPGLICGPFVCV	$egin{aligned}  ext{N.D.} \ 8 > 7 > 4 > 2 > \end{aligned}$	Wang et al., 2008 Fainzilber et al.,
				TTX-S	1995c; McIntosh et al., 1995; Safo et al., 2000; Daly et al., 2004; Zorr et al., 2006
	MrVIB	M	-ACSKKWEYCIVPILGFVYCCPGLICGPFVCV	$8 > 4 > 2 \sim 3 \sim 5 \sim 7 > 9$	Fainzilber et al., 1995b; Fainzilbe et al., 1995c; McIntosh et al., 1995; Ekberg et al., 2006; Zorn et al., 2006
${\it C. geographus}$	Conotoxin-GS	P	-ACSGRGSRCOOQCCMGLRCGRGNPQKCIGAHγDV	4 > 2 >  N.D.	Yanagawa et al., 1988; Hill et al., 1997

Downloaded from pharmrev.aspetjournals.org at Thammasart University on December 3, 2012

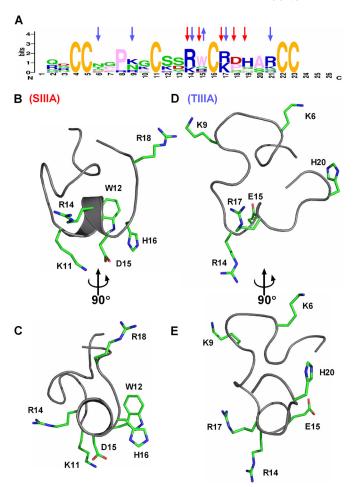


FIG. 5. Structure activity relationship of  $\mu$ -conotoxins. A, consensus sequence for  $\mu$ -conotoxins. B and C, structure of the smaller  $\mu$ -SIIIA showing important residues for interaction with the sodium channel. D and E, structure of larger  $\mu$ -TIIIA showing important residues for interaction with the sodium channel.

ized potentials. It is noteworthy that conotoxins provide some of the most subtype-selective  $Na_v$  modulators, especially for  $Na_v1.4$ ,  $Na_v1.2$ , and more recently  $Na_v1.8$  and have proven to be invaluable tools to dissect the physiological roles and pharmacologies of  $Na_v1.1$  to  $Na_v1.9$ . The potential of  $Na_v$ -targeting conotoxins as drug leads is only now beginning to be explored.

## A. μ-Conotoxin Inhibitors of Voltage-Gated Sodium Channels

Of the conotoxins targeting  $Na_v$ , the  $\mu$ -conotoxins are the most numerous and best characterized, with 20 different  $\mu$ -conotoxins described to date.  $\mu$ -Conotoxins belong to the M superfamily of conopeptides and comprise 16 to 26 residues including 3 conserved disulfide bonds that stabilize their three-dimensional structure (see Table 5, Fig. 5). The  $\mu$ -conotoxins are generally characterized by an overall net positive charge, which contributes to their ability to bind through electrostatic interactions to the outer vestibule of  $Na_v$  to inhibit ionic conductance (Hui et al., 2002; Hui et al., 2003; Li et al., 2003a).

 $\mu$ -Conotoxins are the only venom peptides that bind to site 1 on Na<sub>v</sub>, a site originally defined by the guanidinium pore blockers tetrodotoxin (TTX) and saxitoxin (see Fozzard and Lipkind, 2010). Although TTX (and saxitoxin) only distinguishes between TTX-sensitive (Na., 1.1, -1.2, -1.3, -1.4, -1.6, and -1.7) and TTX-resistant (Na<sub>v</sub>1.5, -1.8, and -1.9) isoforms,  $\mu$ -conotoxins display further subtype selectivity among mammalian TTX-sensitive Na, subtypes (see Table 5). This difference in pharmacology arises because the binding sites of TTX and  $\mu$ -conotoxins only partially overlap and involve multiple Na, residues in the case of the larger  $\mu$ -conotoxins (Sato et al., 1991; Dudley et al., 1995; Li et al., 1997), whereas TTX binding is crucially defined by relatively few residues deep in the pore of the Na. (see Fozzard and Lipkind, 2010). Not surprisingly, μ-conotoxin binding to Na<sub>ν</sub> involves multiple interacting peptide side chains (see below). This larger surface area of interaction at the entrance to the channel opens up prospects to engineer further Na<sub>v</sub> subtype selectivity, which has been considered challenging to date because of the overall high homology between Na<sub>v</sub> subtypes in the pore region.

The first  $\mu$ -conotoxins discovered were GIIIA to GIIIC, which selectively target the skeletal muscle subtype Na, 1.4 (Cruz et al., 1985). In recent years, the Na, subtype selectivity of  $\mu$ -conotoxins has been studied with renewed interest; many have now shown subtype selectivity for neuronal subtypes, including PIIIA, KIIIA, MIIIA, CIIIA, CnIIIA, CnIIIB, SIIIA, SmIIIA, and TIIIA. To date, no  $\mu$ -conotoxins have been identified with affinity for the TTX-resistant mammalian subtypes Na<sub>v</sub>1.5, -1.9, and -1.8 (Lewis et al., 2007; Schroeder et al., 2008; Leipold et al., 2011; Wilson et al., 2011a) despite a number showing activity at amphibian TTX-resistant isoforms (Zhang et al., 2006b) (see Table 5). The least susceptible isoforms include Na, 1.3, -1.7, and -1.5, and no  $\mu$ -conotoxins have yet been found to block recombinantly expressed Na, 1.8 (Lewis et al., 2007; Schroeder et al., 2008; Leipold et al., 2011; Wilson et al., 2011a). Instead, the  $\mu$ -conotoxins seem to either target the mammalian skeletal muscle isoform Na, 1.4 and/or the mammalian brain isoform Na, 1.2 with highest affinity. However, the modest variations in potency at the other neuronal Na<sub>v</sub> subtypes (Table 5) may allow the development of analogs that target therapeutically relevant Na<sub>v</sub>s.

Coexpression of the pore-forming  $\alpha$  subunits with relevant  $\beta$  subunits does not seem to affect  $\mu$ -conotoxin selectivity or affinity, at least in the case of KIIIA (Wilson et al., 2011a), although comprehensive studies using other  $\mu$ -conotoxins are lacking. It is noteworthy that for many  $\mu$ -conotoxins, Na<sub>v</sub> subtype selectivity seems to critically depend on dissociation rate constants. For example, the high affinity of KIIIA for Na<sub>v</sub>1.2 relates to its extremely slow dissociation constant, resulting in essentially irreversible high-affinity block of Na<sub>v</sub>1.2 but lower affinity reversible block of other TTX-sensitive channels (McArthur et al., 2011).

The pharmacophore of the  $\mu$ -conotoxins GIIIA, PIIIA, and TIIIA with five residues in loop 1 has been mapped to a critical Arg13 or Arg14 in similar positions on a turn in loop 2 (Chahine et al., 1995; Shon et al., 1998a; Lewis et al., 2007). In contrast, the pharmacophore of  $\mu$ -conotoxins with one to three residues in loop 1 has been mapped to an  $\alpha$ -helical motif formed by loops 2 and 3, with the equivalent loop 2 arginine playing only a minor role in affinity (Zhang et al., 2007; Schroeder et al., 2008). Reflecting the more extended surface of interaction with Na., for these small  $\mu$ -conotoxins, several residues have been found to contribute critically to Na<sub>v</sub> affinity and/or selectivity of  $\mu$ -conotoxins, opening up perhaps the best opportunities for the development of subtype specific inhibitors. Lys7 in KIIIA and the equivalent Lys11 in SIIIA contribute mostly to Na, 1.4 affinity (Zhang et al., 2007; Schroeder et al., 2008); Lys7 substitutions also affected the extent of Na, inhibition by KIIIA, the decrease in efficacy correlating with the residue size (Zhang et al., 2010). The corresponding Arg14 in PIIIA and TIIIA also significantly decreased Na, affinity, again most prominently at Na, 1.4 (Shon et al., 1998a; Lewis et al., 2007; Schroeder et al., 2008), consistent with direct binding of this residue in the pore of Navs (McArthur et al., 2011). Not surprisingly, substitution of the analogous residue in GIIIA (Arg13) produced a striking loss of activity at Na, 1.4 in this peptide (Sato et al., 1991; Becker et al., 1992), with interaction with Glu758 in domain II and Glu403 in domain I identified in Na, 1.4 (Chang et al., 1998).

Another residue with important implications for Na, affinity and selectivity is Trp8 in KIIIA and the equivalent Trp12 in SIIIA (Lewis et al., 2007; Schroeder et al., 2008; McArthur et al., 2011; Van Der Haegen et al., 2011); substitution of this residue in KIIIA and SIIIA decreases affinity and favors Na<sub>v</sub>1.2 selectivity over Na<sub>v</sub>1.4. In contrast, the unusual negative charge (Glu15) in loop 2 of TIIIA seems to introduce an unfavorable interaction or clash, with [E15A]TIIIA having enhanced affinity, especially for Na<sub>v</sub>1.2, where it is the most potent  $\mu$ -conotoxin inhibitor of neuronal Na, s identified to date (Lewis et al., 2007). The smaller  $\mu$ -conotoxins KIIIA (SIIIA) are also affected by alanine substitutions at Arg10 (Arg14) and Asp11 (Asp15), which favor Na<sub>v</sub>1.2 selectivity over Na<sub>v</sub>1.4 (Zhang et al., 2007; Schroeder et al., 2008). It is noteworthy that the sequence of SIIIA and SIIIB differ only in residues at this position (Arg14 and Asp15 for SIIIA, and Lys14 and Gly15 for SIIIB); accordingly, the major difference between these toxins is the preferential inhibition of Na, 1.2 over Na<sub>v</sub>1.4 by SIIIA and the reversed selectivity for SIIIB (Schroeder et al., 2008).

Several  $\mu$ -conotoxin analogs have been identified that have striking effects on subtype selectivity beyond Na<sub>v</sub>1.2/1.4. Most notably, KIIIA[R14A] has been found to preferentially inhibit Na<sub>v</sub>1.7 over Na<sub>v</sub>1.2 and Na<sub>v</sub>1.4, albeit with decreased affinity overall (McArthur et al., 2011). Nonetheless, this finding indicates that it is possible to engineer smaller  $\mu$ -conotoxins with tailored subtype selectivity, es-

pecially as our understanding of the precise structural requirements for Na<sub>v</sub> subtype binding are improved after the report of the first voltage-gated sodium channel structure (see Fig. 3B).

### B. μO-Conotoxin Inhibitors of Voltage-Gated Sodium Channels

The  $\mu$ O-conotoxins belong to the O superfamily of conopeptides and possess an ICK motif with appropriate disulfide bond connectivity (Table 5). Despite the relatively few peptides identified in this class,  $\mu$ O-conotoxins MrVIA and MrVIB have received considerable attention, because their analgesic effects in animal models of pain were ascribed to their relative selectivity for Na<sub>v</sub>1.8 over other TTXsensitive Na., subtypes expressed in DRG neurons (Daly et al., 2004; Bulaj et al., 2006; Ekberg et al., 2006). Significant differences in their affinity at native Na, 1.8 expressed in DRG neurons and heterologously expressed Na, 1.8 have been reported. These presumably arise because of the presence of auxiliary  $\beta$  subunits, particularly  $\beta$ 2, which has recently been shown to significantly increase the rate of Na, 1.8 inhibition by MrVIB and to increase its potency accordingly (Wilson et al., 2011b). It is noteworthy that μO-conotoxins can also differentiate between the TTXresistant subtypes Na<sub>v</sub>1.8 and -1.9, because the persistent Na, 1.9 current in DRG neurons was unaffected by MrVIB (Ekberg et al., 2006). Although MrVIA and MrVIB have been found to affect molluscan Ca, (Fainzilber et al., 1995c), this mechanism does not seem to contribute to the analgesic effect, because mammalian Ca, in DRG neurons were unaffected (Daly et al., 2004).

Downloaded from pharmrev.aspetjournals.org at Thammasart University on December 3,

The  $\mu$ O-conotoxin binding site remains to be fully defined but seems to overlap at least partially with site 6, in that MrVIA was able to displace δ-conotoxin TxVIA binding (Ekberg et al., 2006). In addition, selectivity of MrVIA at Na, 1.4 over Na, 1.2 has been attributed to the SS2 loop of domain III (Zorn et al., 2006). It is noteworthy that this site is also involved in  $\beta$  scorpion toxin binding to site 4 (Leipold et al., 2006), and both Na, 1.4 and Na, 1.8 share a homologous residue in this domain that is distinct from the other Na<sub>v</sub> subtypes and may contribute to the relative selectivity of MrVIA and MrVIB (Ekberg et al., 2006). Accordingly, functional overlap with the  $\beta$  scorpion toxin Ts1 was recently described, suggesting  $\mu$ O conotoxins interact with the voltage sensor of Na<sub>v</sub>s (Leipold et al., 2007). Indeed, voltage-dependent relief of Na, 1.4 inhibition by MrVIB was attributed to the voltage sensor of domain II (Leipold et al., 2007), another site shared with  $\beta$  scorpion toxins. Similar effects were also reported for Na, 1.8, where the  $K_{\text{off}}$  of MrVIB was also accelerated by strong depolarizations (Wilson et al., 2011b). However, compared with  $Na_v 1.4$ , the  $K_{off}$  of MrVIB at  $Na_v 1.8$  was significantly slower and required repeated depolarizations to relieve block (Wilson et al., 2011b).

In contrast to the  $\mu$ -conotoxins, relatively little is known about the structure-activity relationships of  $\mu$ O-conotoxins, in part because only MrVIA and MrVIB, which differ

Downloaded from pharmrev.aspetjournals.org at Thammasart University on December 3, 2012

by only three residues (Ser3/Arg3; Leu14/Ile14; Val17/ Ile17), have been available for pharmacological evaluation, and their effects remain to be evaluated across all Na, subtypes in parallel. In addition, difficulties synthesizing, correctly folding, and purifying hydrophobic  $\mu$ O-conotoxins have thwarted attempts to identify their pharmacophore through alanine-scanning approaches. However, recent

form part of the δ-conotoxin binding pocket, site 6 (Leipold et al., 2005).

Little is known about Na<sub>v</sub> subtype selectivity of the δ-conotoxins. δ-Conotoxins isolated from molluscivorous cone snails, with the exception of Am2766 (Sudarslal et al., 2003; Sarma et al., 2005), target only molluscan Na, and are inactive at mammalian targets, whereas δ-conotoxins from piscivorous cone snails seem to also inhibit inactivation of mammalian Na<sub>v</sub>. Intriguingly, although δ-conotoxins from molluscivorous cone snails, such as TxVIA and GmVIA, showed no activity at mammalian Na<sub>v</sub>, they still bind to mammalian tissues, suggesting that subtle differences in the  $\delta$ -conotoxin binding site lead to profound effects on activity (Fainzilber et al., 1995a; Hasson et al., 1995; Shichor et al., 1996). A number of  $\delta$ -conotoxins with activity at mammalian Na, 1.2 and Na, 1.4 have been described previously (Bulaj et al., 2001; Sudarslal et al., 2003; Leipold et al., 2005; Sarma et al., 2005), although detailed selectivity studies have not been conducted for the majority of these peptides. A recent report that EVIA displayed selectivity for neuronal subtypes Na, 1.2, 1.3 and 1.6 over muscle and cardiac subtypes Na<sub>v</sub>1.4 and Na<sub>v</sub>1.5 promises that subtype selectivity may be found for other  $\delta$ -conotoxins (Barbier et al., 2004).

Reminiscent of the  $\mu$ O-conotoxins, difficulties relating to the synthesis and purification of the hydrophobic  $\delta$ -conotoxins and their analogs again results in low yields of correctly folded peptide that have limited progress on both Na<sub>v</sub> subtype-selectivity and structure-activity relationships. High sequence homology between  $\delta$ -conotoxins from piscivorous cone snails has been noted, particularly in the central hydrophobic region of these peptides (Bulaj et al., 2001). It is noteworthy that the PVIA[F9A] PVIA[I12A] analogs lost activity at mammalian Na<sub>v</sub>, consistent with the hypothesis that these hydrophobic residues interact with hydrophobic residues that form part of site 6, whereas analogs of the adjacent residues T8A and K13A retained activity (Bulaj et al., 2001). However, the substantial reduction in affinity upon replacing Ile12 with an alanine is surprising if only hydrophobic interactions are involved, suggesting the possibility that this change may have in fact altered the structure of PVIA. It remains to be determined to what degree, if any, these residues affect Na<sub>v</sub> subtype selectivity, or whether these findings apply to  $\delta$ -conotoxins from molluscivorous cone snails that have divergent sequences.

## E. ι-Conotoxin Activators of Voltage-Gated Sodium Channels

Two ι-conotoxins have recently been identified as Na, activators with a mechanism of action distinct from that of the  $\delta$ -conotoxins.  $\iota$ -RXIA is a large, 46-residue peptide from the I superfamily that forms an ICK motif, whereas LtIIIA is a small, 17-residue peptide belonging to the M superfamily of conotoxins (Jimenez et al., 2003; Buczek et al., 2007; Wang et al., 2009). Despite these sequence and structural differences, both ι-conotoxins activate Na<sub>v</sub> without significantly affecting inactivation, in contrast to the δ-conotoxins. Specifically, LtIIIA enhanced the amplitude of TTX-sensitive whole-cell Na<sup>+</sup> currents in DRG without any apparent effects on inactivation (Wang et al., 2009), whereas RXIA shifted the voltage dependence of activation of Na<sub>v</sub>1.6 and Na<sub>v</sub>1.2 to more hyperpolarized potentials without affecting inactivation (Fiedler et al., 2008). It is noteworthy that the post-translationally modified Phe44 (D-Phe) was important for activity particularly at Na<sub>v</sub>1.2, suggesting participation of the disordered C-terminal tail

Aspet

in binding to a yet to be defined Na<sub>v</sub> site (Fiedler et al., 2008). RXIA was also active at Na<sub>v</sub>1.7 but did not affect other Na<sub>v</sub> subtypes (Fiedler et al., 2008), suggesting that detailed structure-activity relationship and subtype selectivity studies of the 17 *v*-conotoxins identified in *Conus radiatus* (Jimenez et al., 2003) might shed further light on the mode of action of this potentially interesting family of excitatory conopeptides.

#### F. Sodium Channel Inhibition in Pain Management

Sodium channel ( $Na_v$ ) inhibitors are used as local anesthetics, analgesics, antiarrhythmics, and anticonvulsants. They are most widely used for regional anesthesia and analgesia, as well as systemically for chronic pain management. Until very recently, the utility of small-molecule  $Na_v$  inhibitors, particularly systemic local anesthetics, has been limited both by unwanted actions on nontarget sodium channel types and difficulty in restricting distribution to target body compartments. For example, mexile-

tine, an orally administered lidocaine analog, is a third-line therapy for chronic pain management but has limited efficacy largely because of serious dose-limiting side effects (Dworkin et al., 2007). The severe side-effect profile of currently used  $\rm Na_v$  inhibitors is largely due to very poor selectivity among  $\rm Na_v$  subtypes (England and de Groot, 2009). Growth in knowledge of the diversity of  $\rm Na_v$  types, implication of specific  $\rm Na_v$  types in pain pathogenesis, together with highly selective expression in different tissues has provided a strong impetus to identify subtype selective  $\rm Na_v$  antagonists for pain management.

The selectivity of conotoxins for different channel types may provide novel drugs for clinical use in regional anesthesia and for systemic or intrathecal administration in chronic pain states. The potential restriction of peptide distribution to systemic tissue compartments rather than central nervous or local application in restricted tissue compartments could provide advantages for these selective peptide Na<sub>v</sub> antagonists. Among the conotoxin classes,

TABLE 6
Distribution of Na, subtypes and efficacy of inhibitors

Isoform	Distribution	Potential Effects/Side Effects	Selective Toxin Inhibitors	References
$\mathrm{Na_v}1.1~(\mathrm{SCN1A})$	Located primarily in neuronal soma. Found widely in CNS and peripheral neurons (including DRG) cardiac myocytes (T-tubules) (cortex, hippocampus, cerebellum), PNS: DRG, motor neurons.	Widespread effects on the nervous and cardiovascular systems.	No selective conotoxins	Catterall et al., 2005a Catterall, 2010
$\mathrm{Na_v} 1.2~(\mathrm{SCN2A})$	Located primarily in axons, node of Ranvier. Found in CNS low expression in adult DRG.	Widespread effects on the nervous and cardiovascular systems.	$\mu ext{-Conotoxins}$	Catterall et al., 2005a Schroeder et al., 2008; Catterall, 2010; Wilson et al., 2011a
$Na_v 1.3$ (SCN3A)	Widely distributed in embryonic nervous system but declines postnatally; adult rat CNS, particularly dorsal spinal cord; also upregulated after injury; adult human CNS.	Limited evidence for analgesia. If restricted to periphery, side effects should be minimal; intrathecal CNS side effects possible.	No selective conotoxins	Felts et al., 1997; Hains et al., 2003; Catterall et al., 2005a; Catterall, 2010
Na <sub>v</sub> 1.4 (SCN4A)	Skeletal muscle	Muscle dysfunction	$\mu ext{-Conotoxins}$	Catterall et al., 2005a Schroeder et al., 2008; Catterall, 2010; Wilson et al., 2011a
$Na_v 1.5 (SCN5A)$	Cardiac muscle, embryonic DRG	Cardiac side effects	No selective conotoxins	Catterall et al., 2005a Catterall, 2010
Na <sub>v</sub> 1.6 (SCN8A)	Highly localized in nodes of Ranvier in CNS and PNS, including DRG.	Widespread effects on the nervous and cardiovascular systems.	No selective conotoxins	Catterall et al., 2005a Catterall, 2010
Na <sub>v</sub> 1.7 (SCN9A)	High expression in small sensory (DRG) neurons and autonomic neurons, also Schwann cells, CD1 + dendritic cells, and some endocrine cells (adrenal medulla).	Analgesic effects. Possible autonomic side effects but autonomic function seems normal with loss-of-function mutations; olfactory side effects.	No selective conotoxins (ProTx-II, huwentoxin-IV)	Catterall et al., 2005a; Cummins and Rush 2007; Schmalhofer et al., 2008; Xiao et al., 2008a; Xiao et al., 2008b
$Na_v 1.8 (SCN10A)$	Small- to medium-sized (presumably nociceptive) DRG neurons, also found in retina and cardiac myocytes.	Analgesic effects. Visual and cardiac side-effects likely from distributional and human mutation studies.	MrVIA, MrVIB	Ekberg et al., 2006; Cummins and Rush 2007; O'Brien et al., 2008; Chambers et al., 2010
$\mathrm{Na_v}1.9~(\mathrm{SCN11A})$	Small-diameter, mostly unmyelinated DRG neurons, also found in retina.	Potential analgesic effects. Potential visual effects.	None known	Catterall et al., 2005a; Cummins and Rush 2007; O'Brien et al., 2008

some  $\mu$ - and  $\mu$ O-conotoxins show promise as selective Na<sub>v</sub> subtype inhibitors. A large number of  $\mu$ -conotoxins display considerable channel type selectivity. Most of those isolated inhibit Na<sub>v</sub>1.2 and Na<sub>v</sub>1.4 most potently (Schroeder et al., 2008; Wilson et al., 2011a). Although some  $\mu$ -conotoxins (e.g., KIIIA) have analgesic activity after systemic administration (Zhang et al., 2007), none to date have shown a clear Na<sub>v</sub> selectivity profile suitable for development as pain therapeutics. By contrast, several  $\mu$ O-conotoxins have potentially useful selectivity profiles (see below) for the major Na<sub>v</sub> types implicated in pain.

As summarized in Table 6, Na, 1.7, -1.8, -1.9, and perhaps -1.3 are potential candidate targets for pain therapeutics because of their established role in pain, selective expression in nociceptive neurons and nerve terminals, and/or their regulation in pain states (see reviews by Catterall et al., 2005a; Cummins and Rush, 2007; Dib-Hajj et al., 2009; England and de Groot, 2009; Catterall, 2010; Dib-Hajj et al., 2010). Other Na<sub>v</sub> types are also expressed in sensory neurons, including Na, 1.1, -1.6, and (at low levels) -1.5 (Catterall et al., 2005a; Catterall, 2010; Dib-Hajj et al., 2010), but inhibition studies with  $\mu$ -conotoxins have suggested that Na, 1.6 and Na, 1.7 are the major contributors to peripheral nerve action potentials (Wilson et al., 2011a). Widespread expression of these subtypes (except Na<sub>v</sub>1.7, see below) in other excitable tissues is likely to preclude the application of inhibitors of these channels as pain therapeutics. The role of Na, subtypes in pain pathways and their potential for selective targeting by conotoxins is outlined below.

Of the potential Na<sub>v</sub> pain targets, Na<sub>v</sub>1.7 is one of the most promising for pain management. Na, 1.7 is expressed almost exclusively in peripheral neurons (Catterall et al., 2005a) and selectively in nociceptive primary afferents (Raymond et al., 2004). Na, 1.7 gene deletion targeted to nociceptive sensory neurons (Na, 1.8 expressing) or knockdown reduces inflammatory pain and thermal hyperalgesia (Nassar et al., 2004) but not neuropathic pain (Nassar et al., 2005). The reason for this lack of effect in neuropathic pain is still unknown. Na, 1.7 expression is upregulated in injured nerves and in sensory neuron cell bodies in inflammatory pain and may contribute to ectopic action potential discharge in painful neuromas (Dib-Hajj et al., 2010). In humans, rare loss-of-function mutations of Na<sub>v</sub>1.7 produce a complete absence of pain sensation (Cox et al., 2006; Goldberg et al., 2007) and, conversely, gain-offunction mutations are associated with painful neuropathies (Fertleman et al., 2006; Han et al., 2006). Polymorphisms in Na, 1.7 in normal humans are also significantly correlated with pain sensitivity (Reimann et al., 2010). The mechanism responsible for the profound loss of function for Na. 1.7 mutations in human pain remain uncertain, but these channels are concentrated in nerve endings and amplify small depolarizations; they therefore have the potential to play a major role in coupling generator potentials in nociceptive afferent nerves with action potential generation (Cummins et al., 1998).

There are already several relatively selective smallmolecule inhibitors of Na, 1.7 that have shown efficacy in acute, inflammatory, and neuropathic pain models (Williams et al., 2007; London et al., 2008; Bregman et al., 2011; Chowdhury et al. 2011). Although many conotoxins can also inhibit Na<sub>v</sub>1.7 (see above), no highly selective antagonists have been identified to date, although analogs of the smaller  $\mu$ -conotoxins have potential (see above). At the moment, spider venoms are more promising sources of selective Na., 1.7 inhibitors. For example, ProTx-II from the tarantula Thrixopelma pruriens is a potent and selective inhibitor of Na<sub>v</sub>1.7 (~100-fold over other types) but shows limited efficacy in inflammatory pain models after intravenous or intrathecal administration (Schmalhofer et al., 2008). This lack of activity in pain models might suggest that selective Na, 1.7 inhibitors are not as useful as previously thought, but the possibility that the peptide distributes poorly from the circulation to nociceptive nerve endings cannot be excluded. Huwentoxin-IV, from the tarantula Ornithoctonus huwena, is also modestly selective for Na, 1.7 (it also inhibits Na, 1.2 and Na, 1.3), but actions in animal pain models have not been reported in detail (Xiao et al., 2008a,b).

Na<sub>v</sub> 1.7 is also expressed in several other tissues. A major off-target location of Na<sub>v</sub>1.7 expression are olfactory neurons (Ahn et al., 2011), and loss-of-function mutations can cause anosmia (Weiss et al., 2011). However, such side effects may not be of major concern to persons suffering severe pain, but loss of smell would be expected to reduce quality of life during prolonged treatment. More recently Na<sub>v</sub>1.7 expression in monocyte-derived dendritic cells has been shown to modify responses to cytokines (Kis-Toth et al., 2011), suggesting potential for immune modulation and potentially immunological side effects of Na<sub>v</sub>1.7 inhibitors.

The TTX-resistant channel Na<sub>v</sub>1.8 is also a promising target for pain management. It is expressed predominantly in small nociceptive primary afferent nerves (Catterall et al., 2005a; Cummins and Rush, 2007; Catterall, 2010; Dib-Hajj et al., 2010). Na, 1.8 contributes to the action potential and repetitive firing in small, nociceptive sensory neurons (Catterall et al., 2005a; Cummins and Rush, 2007; Catterall, 2010; Dib-Hajj et al., 2010). The activity of Na, 1.8 in sensory neurons is strongly enhanced by inflammatory mediators, both via up-regulation and/or modulation of channel kinetics by protein kinase A and C (see Dib-Hajj et al., 2010). Both protein and mRNA for Na, 1.8 are up-regulated locally in injured sciatic nerves in nerve injury models (Gold et al., 2003; Thakor et al., 2009). Na, 1.8 knockdown (Lai et al., 2002; Gold et al., 2003) and gene deletion (Akopian et al., 1999) studies have suggested an important role in neuropathic and inflammatory pain. Perhaps the most significant phenotype in Na, 1.8 knockout mice is a nearly complete loss of ectopic action potential activity in experimental neuromas (Roza et al., 2003). Together with the established redistribution of Na, 1.8 to sites of nerve injury and neuromas in animal models and

humans (e.g., Black et al., 2008), this finding may suggest a specific therapeutic use for  $Na_v1.8$  inhibitors in nerve injury-induced pain associated with neuromas.

μO-conotoxins MrVIA and MrVIB inhibit Na, 1.8 in sensory neurons (Daly et al., 2004; Ekberg et al., 2006) and significantly reduce mechanical and thermal pain in both neuropathic and inflammatory pain models; selectivity over motor side effects is more than 30-fold greater than found for lidocaine (Ekberg et al., 2006). It is noteworthy that potentially more stable and easier to synthesize selenocysteine analogs produced similar beneficial effects (de Araujo et al., 2011). These findings suggest that direct spinal administration of Na<sub>v</sub>1.8-selective conotoxins may have a place in pain management. MrVIB has also been shown to display local anesthetic actions after intraplantar administration (Bulaj et al., 2006). Although Na, inhibitory toxins from other species have yet to show preferential Na<sub>v</sub>1.8 targeting, a chimeric scorpion toxin (drosotoxin) shows quite low potency but high selectivity for TTXresistant (Na, 1.8) versus TTX-sensitive Na, currents in rat sensory neurons (Zhu et al., 2010). Consistent with results obtained using µO-conotoxins, small-molecule antagonists preferentially targeting Na, 1.8 (Jarvis et al., 2007; Scanio et al., 2010) have since been reported to show efficacy in neuropathic pain models.

Although small-molecule and conotoxin inhibitors of Na<sub>v</sub>1.8 have produced efficacy in pain models, there are concerns about adverse side effects. For example, in addition to their selective expression in nociceptive sensory nerves, Na<sub>v</sub>1.8 (and Na<sub>v</sub>1.9) is expressed in retina (O'Brien et al., 2008), where small-molecule antagonists could produce retinal side effects, although peptides are potentially excluded from this compartment. Of more concern is the recent finding that Na<sub>v</sub>1.8 is expressed in cardiac myocytes and that polymorphisms in the Na<sub>v</sub>1.8 gene influence QT interval, electrocardiogram conduction block, and ventricular fibrillation (Chambers et al., 2010), raising the potential for severe cardiac side effects from systemic use of Na<sub>v</sub>1.8 inhibitors that would complicate long-term use.

The tetrodotoxin-resistant channel, Na, 1.9 is also a promising target for pain management but has been less thoroughly studied than Na, 1.7 or Na, 1.8, largely because attempts to express the channel in heterologous expression systems have been unsuccessful. It is expressed predominantly in small sensory neurons, particularly in a subclass of C-fiber nociceptors (Fang et al., 2006) and visceral sensory nerves (Rugiero et al., 2003). It has also been detected in retina (O'Brien et al., 2008). Na, 1.9 produces a persistent sodium current that modulates resting membrane potential and amplifies small depolarizations, contributing to excitability of small nociceptive nerves. The activity of Na. 1.9 is strongly enhanced by inflammatory mediators (e.g., Maingret et al., 2008; Ostman et al., 2008), although a number of Na<sub>v</sub>1.9 gene deletion studies have consistently found little or no effect on neuropathic pain, except for inhibition of cold allodynia (Leo et al., 2010). In contrast, thermal pain sensitivity is consistently blunted in inflammatory pain models in  $\rm Na_v 1.9$  knockout animals (Priest et al., 2005; Amaya et al., 2006).  $\rm Na_v 1.9$  inhibitors may therefore be useful for inflammatory and visceral pain. Because of the difficulties in heterologously expressing  $\rm Na_v 1.9$ , no selective pharmacological tools, including conotoxins, have been identified to study its function in normal neurons and pain states. However, some spider and scorpion toxins including ProTxI and TsVII were recently found to interact with  $\rm Na_v 1.9$  endogenously expressed in DRG neurons in a nonselective manner (Bosmans et al., 2011).

Na<sub>v</sub>1.3 is also a potential target for pain therapeutics, although its role in the maintenance of pain remains controversial. It is highly expressed in embryonic DRG neurons but declines to very low levels in adult sensory neurons (Felts et al., 1997). Expression of Na, 1.3 in sensory neurons is strongly up-regulated by nerve injury (Black et al., 1999; Lindia and Abbadie, 2003; Fukuoka et al., 2008) and is up-regulated in humans in painful neuromas (Black et al., 2008) and the spinal cord (Hains et al., 2003). Antisense knockdown of Na<sub>v</sub>1.3 in nerve-injured rats has been shown to attenuate signs of neuropathic pain (Hains et al., 2003), but in other studies, no effects of knockdown were found (Lindia et al., 2005). Moreover, both global and nociceptor-specific (Na<sub>v</sub>1.8 Cre mice) deletion of Na<sub>v</sub>1.3 in mice had no effect on neuropathic pain behavior or ectopic discharges from damaged nerves, suggesting Na, 1.3 is neither necessary nor sufficient for development of nerveinjury related pain (Nassar et al., 2006). The latter findings have strongly questioned the importance of Na, 1.3 in nerve injury-related pain and have reduced the impetus to develop Na<sub>v</sub>1.3 specific inhibitors. Although several μ-conotoxins can inhibit Na, 1.3, they all interact more potently with Na<sub>v</sub>1.2 or Na<sub>v</sub>1.4 (Wilson et al., 2011a), and more selective analogs are required before chemical dissection of the role of Na<sub>v</sub>1.3 in different pain states can be investigated.

Downloaded from pharmrev.aspetjournals.org at Thammasart University on December 3, 2012

In conclusion, the potential for identification and development of Na<sub>v</sub> subtype-selective conotoxins remains largely untapped. Conotoxins that selectively interact with Na<sub>v</sub>1.7, Na<sub>v</sub>1.9, and/or Na<sub>v</sub>1.3 need to identified; mixed block of these subtypes potentially produces greater efficacy than the selective block of a single subtype. It should also be noted that the potency of conotoxins may differ in injured versus uninjured neurons. As observed for  $\omega$ -conotoxins (see section II.C), interactions of  $\mu$ O-conotoxins with Na, channel types are also influenced by auxiliary subunit expression. Na<sub>ν</sub>β2 subunits are up-regulated in sensory neurons in neuropathic pain models (Pertin et al., 2005) and interact with Na<sub>v</sub>s to increase the on-rate of MrVIB in heterologous systems (Wilson et al., 2011b), providing the μO-conotoxins with a window of functional selectivity. It is noteworthy that Na, \$3 subunits are also up-regulated in pain states (Shah et al., 2000), and their expression levels influence gating of Na<sub>v</sub>1.3 (Cusdin et al., 2010) to potentially influence µO-conotoxin pharmacology in a diseasedependent manner.

TABLE 7  $\kappa$ -Conotoxins inhibiting  $K_n$  potassium channels ( $\kappa$ A/M-conotoxin loop formula C0C6-7CC2-4C0-3C)

Lower-case serine or threonine residues are glycosylated, and the lower case tryptophan is a D-amino acid. Cysteines are bolded and aligned separately for the different  $\kappa$ -conotoxin classes, with residues affecting potassium channel affinity underlined.

~ .			Sequence (Disulfide Bonding Likely 1–4/2–5/3–6)		
Species	Name	Diet	12 3 4 5 6	Selectivity $(K_v x)$	References
κM- and κA- Conotoxir	ns				
C. radiatus	RIIIJ	P	LOOCCTOOKKH-COAOACKYKOCCKS	1.2	Chen et al., 2010
	RIIIK	Р	<u>L</u> PS <b>CC</b> SLNL <u>R</u> L- <b>C</b> PVPA <b>C</b> <u>KR</u> NP <b>CC</b> T*	$ ext{TSha1} \sim Shaker \sim \\ 1.2 > 1.5 \sim 1.6 \sim \\ 1.1 \sim 1.3 \sim \\ 1.4$	Chen et al., 2010; Ferber et al., 2003 2004; Al-Sabi et al., 2004
C. consors	CcTx	P	AOWLVPsQITTCCGYNOGTMCOSCMCTNT-C		Favreau et al., 1999
C. magus	MIVA	P	AOyLVVtAtTNCCGYNOMTICOOCMCTYSCOOKRKO*	?	Santos et al., 2004
C. purpurascens	PIVE	P	DCCGVKLEM-CHPCLCDNSCKNYGK*	Frog, fish	Teichert et al., 2007a
	PIVF	P	DCCGVKLEM-CHPCLCDNSCKKSGK*	?	Teichert et al., 2007a
C. striatus	SIVA	P	ZKSLVPsVITTCCGYDOGTMCOOCRCTNSC*	Frog, fish, Shaker	Craig et al., 1998; Santos et al., 2004
	SIVB	P	ZKELVPsVITTCCGYDOGTMCOOCRCTNSCOTKOKKO*	?	Santos et al., 2004
C. stercusmuscarum	SmIVA	P	ZTWLVPstITTCCGYDOGTMCOTCMCDNTCKOKOKKS*	?	Santos et al., 2004
	SmIVB	P	ZPWLVPstITTCCGYDOGSMCOOCMCNNTCKOKOKKS	?	Santos et al., 2004
Other κ-conotoxins					,
C. planorbis	pl14a	V	FPRPRI <b>C</b> NLA <b>C</b> RAGIGHKYPF <b>C</b> H <b>C</b> R*	$\begin{array}{c} 1.6 > 1.1 > 1.2 \sim \\ 1.3 \sim 1.4 \sim 1.5 \sim \\ 2.1 \sim 3.4 \end{array}$	Imperial et al., 2006
C. virgo	ViTx	V	SRCFPPGIYC-TSYLPCCWGICC-STCRNVCHLRIGK	$1.1 \sim 1.3 > 1.2$	Kauferstein et al., 2003
C. spurius	sr11a	V	$\mathbf{c}\mathtt{RTEGMS}\mathbf{c}\gamma\gamma\mathtt{NQQ}-\mathbf{c}\mathbf{c}\mathtt{wRS}\mathbf{c}\mathbf{c}\mathtt{rge}\mathbf{c}\mathtt{e}\mathtt{a}\mathtt{p}\mathbf{c}\mathtt{rfgp}*$	$1.2 \sim 1.6 > 1.3$	Aguilar et al., 2007, 2010
C. betulinus	BeTX	V	CRAYGTYC-YNDSQCCLNYCCWGGCGHOCRHP*	BK	Fan et al., 2003
C. purpurascens	PVIIA	P	CRIPNOKCFOHLDDCCSRKCNRFNKCV*	Shaker K.	Huang et al., 2005
C. striatus	Conkunitzin-S1	P	KDRPSLCDLPADSGSGTKAEKRIYYNSARKQCLRFDYTGQG GNENNFRRTYDCQRTCL	Shaker Kv	Bayrhuber et al., 2005
C. ventricosus	Contryphan-Vn	P	GDCPwKPWC*	Voltage-gated and Ca <sup>2+</sup> -dependent K <sup>+</sup> channels	Massilia et al., 2003

 $P, fish; M, molluscs; V, worms; *, C-terminal amidation; TSha1, from trout, frog K_{v}, the skeletal muscle form; BK, Ca^{2+}/voltage-dependent potassium channel. The skeletal muscle form; Ca^{2+}/v$ 

# III. κ-Conotoxins Interacting with Potassium Channels

Potassium channels comprise a vastly diverse family of ion channels, with a large number of genes encoding for the pore-forming  $\alpha$ -subunits, which are divided into channels with six, four, and two transmembrane domains. Accordingly, multiple families of  $\kappa$ -conotoxins targeting several different potassium channels have been isolated from cone snail venom (Table 7). Activity at potassium channels appears to constitute an important component of the "lightning-strike" cabal of piscivorous cone snails (Terlau et al., 1996); however, relatively little is known about subtype selectivity and structure-activity relationship of conotoxins targeting potassium channels, and potential therapeutic uses have not yet been determined.

#### A. κA-Conotoxins

The  $\kappa$ A-conotoxins MIVA, SIVA, SIVB, SmIVA, SmIVB, PIVE, CcTx, and PIVF (Teichert et al., 2007a) are relatively short excitatory peptides that elicit potent effects after intramuscular injection in fish and direct exposure to amphibian neuromuscular preparations, but not in mammals. It remains unclear whether these observed differences are due to pharmacodynamic effects (such as restricted access to mammalian  $K_{\nu}$  channels expressed in

juxtaparanodal regions of myelinated motor axons) or they reflect strong differences in subtype affinity (Teichert et al., 2007a). Although some of these peptides inhibit Shaker  $K_{\rm v}$  channels, the precise target and structure activity relationship for many of the  $\kappa A$ -conotoxins remains unclear.

#### B. κO-Conotoxins

The κO-conotoxin PVIIA has received particular attention because it was shown to exhibit cardioprotective properties in several animal models of ischemia-reperfusion injury (Lubbers et al., 2005). Arg2, Lys7, Phe9, and Phe23 were shown to contribute to voltage-dependent interactions with the Shaker potassium channel (Scanlon et al., 1997), which was identified as the molecular target of PVIIA and is highly homologous with mammalian K<sub>v</sub> isoforms (Terlau et al., 1996; Shon et al., 1998b; Huang et al., 2005). These residues form part of a lysine-hydrophobic residue dyad, which is evolutionally conserved and contributes to activity at K, channels in several other toxins, including charybdotoxin from scorpion and BgK from sea anemone (Savarin et al., 1998; García et al., 1999). Figure 6 shows a model of the interaction between PVIIA and the shaker potassium channel, revealing a likely model of interaction of the dyad comprising Lys7 and Phe9. It is surprising that the rat K<sub>v</sub>1.1 isoform was resistant to

PHARMACOLOGICAL REVIEWS

## A (PVIIA - Shaker Potassium channel)

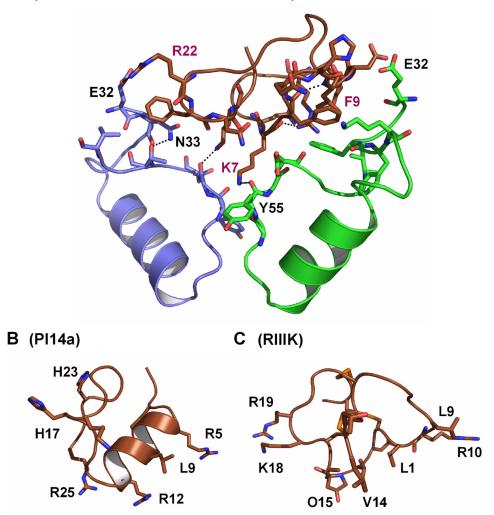


Fig. 6. Structure activity relationship of conotoxins interacting with potassium channels. A, model of the interaction between PVIIA and the shaker potassium channel, revealing a likely model of interaction of the dyad comprising Lys7 and Phe9. B, structure of Pl14a, a  $\kappa$ J-conotoxin. C, structure of RIIIK, a  $\kappa$ M-conotoxin.

PVIIA (Shon et al., 1998b), indicating that other presently unidentified mammalian  $K_{\rm v}$  isoforms contribute to the cardioprotective effects of PVIIA.

### C. кM-Conotoxins

Like PVIIA, the  $\kappa$ M-conotoxin RIIIJ was found to exert cardioprotective effects, presumably due to the high affinity of this peptide for mammalian K<sub>v</sub>1.2 and K<sub>v</sub>1.2-containing K<sub>v</sub>1.1, K<sub>v</sub>1.5, and K<sub>v</sub>1.6 heteromultimers (Chen et al., 2010). Lys9, in conjunction with other less clearly identified residues, contributes to the high affinity of RIIIJ for K<sub>v</sub>1.2. It is noteworthy that RIIIJ was less potent at K<sub>v</sub>1.2/K<sub>v</sub>1.7-heteromultimers than the related  $\kappa$ M-conotoxin RIIIK, demonstrating that different K<sub>v</sub> channel heteromultimers are pharmacologically distinct. Several residues, including Leu1, Arg10, Lys18, and Arg19, were found to contribute critically to activity of RIIIK at the trout TSha1 K<sup>+</sup> channel, the K<sub>v</sub> isoform most sensitive to block by RIIIK (Al-Sabi et al., 2004). It is surprising that

these residues do not seem to form part of a functional dyad but interact with the channel by means of electrostatic interactions (Al-Sabi et al., 2004; Verdier et al., 2005). Based on docking interactions at the Shaker potassium channel, residues potentially involved in the pharmacophore are indicated in Fig. 6C.

#### D. кJ-Conotoxins

pl14a, a member of the J superfamily, is an unusual conotoxin in that it inhibits both  $K_v1.6$  and several isoforms of the nicotinic acetylcholine receptor (nAChR) (Imperial et al., 2006). Homology modeling suggests that this peptide possesses both the putative functional dyad, formed by residues Lys18 and Tyr19, and a ring of basic residues comprising Arg3, Arg5, Arg12, and Arg25, reminiscent of the pharmacophore found in the  $\kappa$ M-conotoxin RIIIK (Mondal et al., 2007). However, it remains to be determined whether these residues are involved in interactions with  $K_v$  channels as predicted. Based on docking

interactions at the Shaker potassium channel, residues potentially involved in the pharmacophore are indicated in Fig. 6B.

#### $E. \kappa I(2)$ -Conotoxins

ViTx, sr11a, and BeTX belong to the  $\kappa I(2)$  class of conotoxins and constitute an interesting class of structurally related potassium channel modulators. ViTx inhibits K, 1.1 and K, 1.3 but not K, 1.2 (Kauferstein et al., 2003), whereas the recently identified sr11a inhibits K, 1.2 and K, 1.6 but not K, 1.3 (Aguilar et al., 2010). In contrast, BeTX displays an unusual potentiation of Ca<sup>2+</sup>- and voltage-dependent BK channel current by increasing the channel open probability (Fan et al., 2003). This effect was independent of channel inactivation and was readily reversible, suggesting an interaction with an extracellular binding site (Fan et al., 2003). The molecular basis of the unique subtype selectivity of the  $\kappa I(2)$  conotoxins remains unknown to date. The only lysine in ViTx occurs at the C terminus, suggesting that the conserved dyad motif is not involved and implicates alternate mechanisms of interaction with K<sub>v</sub> channels (Kauferstein et al., 2003). Likewise, sr11a seems to lack a functional dyad motif; however, homology modeling identified Arg17 and Arg29 as residues possibly involved in the biological activity of this novel peptide (Aguilar et al., 2010).

#### F. Contryphan-Vn

Contryphan-Vn was recently identified as a D-trypto-phan-containing disulfide-constrained nonapeptide with activity at both voltage-gated and Ca<sup>2+</sup>-dependent K<sup>+</sup> channels, perhaps owing to the presence of a unique Lys-Trp dyad. However, the structural requirements for activity of Contryphan-Vn at these molecular targets, as well as its subtype-selectivity, remain to be determined (Massilia et al., 2003).

#### G. Conkunitzin-S1

The potassium channel-targeted toxin conkunitzin-S1 is a 60-amino acid peptide that, despite belonging to the Kunitz domain family of proteins, is stabilized by only two disulfide bonds (Bayrhuber et al., 2005). Conkunitzin-S1 was shown to inhibit the Shaker potassium channel by interacting with the ion channel pore through K427 (Bayrhuber et al., 2005).

# IV. Conotoxins Interacting with Ligand-Gated Ion Channels

Cone snails have evolved multiple classes of conopeptides to target ligand-gated ion channels, including nAChR, 5-hydroxytryptamine<sub>3</sub> (5-HT<sub>3</sub>), and N-methyl-D-aspartate (NMDA) antagonists as well as  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) enhancers. Of these, antagonists of nAChRs are the largest and most diverse and, along with the NMDA antagonists,

show most potential as leads to new ligand-gated ion channel therapeutics (Table 8).

## A. Conotoxin Inhibitors of Nicotinic Acetylcholine Receptors

All Conus spp. venoms investigated to date contain at least one conotoxin that inhibits the nAChR (McIntosh et al., 1999; Dutertre et al., 2007). Overall, seven different families of conotoxins are known to target the nAChR:  $\alpha$ -conotoxins,  $\alpha$ C-conotoxins,  $\alpha$ D-conotoxins,  $\psi$ -conotoxins,  $\alpha$ S-conotoxins,  $\alpha$ L-conotoxins, and  $\alpha$ J-conotoxins (Table 8). The  $\alpha$ -conotoxins are selective antagonists of the muscletype (3/5) and neuronal-type (4/7, 4/4, and 4/3) nAChRs and arguably represent the largest group of characterized Conus spp. peptides (McIntosh et al., 1999) (Table 8). α-Conotoxins selective for neuronal subtype of nAChR have significantly contributed to their characterization both in vivo and in vitro, and some of these peptides may have the rapeutic potential (Livett et al., 2006). The pharmacophore of these  $\alpha$ -conotoxins has been thoroughly investigated and has been shown to be composed of a conserved hydrophobic patch in the first loop that determines binding and a more variable second loop that confers selectivity through pairwise interactions with different nAChR subunits (Dutertre et al., 2005).

One of the first structure-activity relationship studies on α-conotoxin was carried out on the closely related PnIA and PnIB (Fainzilber et al., 1994a). The sequences differ by only two amino acids at position 10 and 11, yet PnIA is selective for  $\alpha 3\beta 2$ , whereas PnIB binds preferentially to  $\alpha 7$ nAChR. It was later determined that position 10 alone was responsible for the shift in nAChR selectivity (Hogg et al., 1999; Luo et al., 1999). In addition, sequential truncation of the second loop was shown to influence potency and ultimately significantly alter the structure of PnIA (Jin et al., 2008). On the receptor side, mutation of three residues on the α3 nAChR subunit (Pro182, Ile188, and Gly198) affected the high affinity of PnIA (Everhart et al., 2003). These structure-activity relationship studies allowed the construction of a model of the interaction between PnIA and nAChR (Dutertre et al., 2004), which was consistent with the subsequent cocrystal structure of the acetylcholine binding protein (AChBP) and a variant of PnIA (Celie et al., 2005).

ImI is another  $\alpha$ -conotoxin for which structure-activity relationship studies have identified determinants that influence potency. Modification of Asp5, Pro6, Arg7, and Trp10 altered the potency of ImI at the  $\alpha$ 7 nAChR (Quiram and Sine, 1998a, b). Later, thermodynamic mutant cycle analysis identified pairwise interactions between ImI and  $\alpha$ 7 nAChR (Quiram et al., 1999). The results revealed a major interaction between Arg7 of ImI and Tyr195 in  $\alpha$ 7, accompanied by smaller contributions between Asp5 of ImI and Trp149, Tyr151, and Gly153 of  $\alpha$ 7, and between Trp10 of ImI and Thr77 and Asn111 of  $\alpha$ 7. Again, most of these pairwise interactions were confirmed in the struc-



PHARM

TABLE 8  $\alpha \text{-}Conotoxins \ inhibiting \ nAChRs \ (loop \ formula \ \textbf{C0C3-4C3-7C}, \ excluding \ 3 \ + \ disulfide \ bonded \ and \ framework \ XIV \ \alpha \text{-}conotoxins)$  Cysteines are bolded and aligned separately for the different  $\alpha \text{-}conotoxin$  classes.

Species	Diet	Name	Sequence (Disulfide Bonded 1–3/2–4)	nAChR select	References
opecies	Diet	raille	12 3 4	naont select	references
C. achatinus	P	α-Ac1.1a	-NGRCC-HPACGKHFNC*	Muscle nAChR	Liu et al., 2007b
	_	$\alpha$ -Ac1.1b	-NGRCC-HPACGKHFSC*	Muscle nAChR	Liu et al., 2007b
C. anemone	V	α-AnIA	CCSHPACAANNQDyC*	$\alpha 3 \beta 2$	Loughnan et al., 2004
		α-AnIB	GGCCSHPACAANNQDyC*	$\alpha 3\beta 2 > \alpha 7$	Loughnan et al., 2004
C. arenatus	V	$\alpha$ -ArIA	IRDECCSNPACRVNNPHVCRRR	$lpha7^{'}\simlpha3eta2$	Whiteaker et al., 2007
		$\alpha$ -ArIB	DECCSNPACRVNNPHVCRRR	lpha 7 > lpha 3 eta 2	Whiteaker et al., 2007
C. aulicus	M	α-AuIA	GCCSYPPCFATNSDYC*	$\alpha 3 \beta 4$	Luo et al., 1998
		α-AuIB	GCCSYPPC-FATNPDC*	$\alpha 3\beta 4 > \alpha 7$	Luo et al., 1998
		$\alpha$ -AuIC	GCCSYPPCFATNSGYC*	$\alpha 3 \beta 4$	Luo et al., 1998
C. bullatus	P	α-BuIA	GCCSTPPCAVLYC*	$\alpha 6\alpha 3\beta 2 > \alpha 6\alpha 3\beta 4 > \alpha 3\beta 2$	Azam et al., 2005
C. consors	P	$\alpha$ -CnIA	GRCC-HPACGKYYSC*	Muscle nAChR	Favreau et al., 1999
C. ermineus	P	$\alpha$ -EI	-RDOCCYHPTCNMSNPQIC*	Muscle nAChR $(\alpha/\delta > \alpha/\gamma)$	Martinez et al., 1995
C. episcopatus	M	$\alpha ext{-EpI}$	GCCSDPRCNMNNPDyC*	$\alpha 7$	Loughnan et al., 1998 Nicke et al., 2003b
C. geographus	P	$\alpha$ -GI	ECC-NPACGRHYSC*	Muscle nAChR	Gray et al., 1981
		$\alpha$ -GIA	ECC-NPACGRHYSCGK	Muscle nAChR	Gray et al., 1981
		$\alpha$ -GIC	GCCSHPACAGNNQHIC*	$\alpha 3\beta 2 > \alpha 4\beta 2$	McIntosh et al., 2002
		$\alpha$ -GID	IRD YCC SNPACRVNNOHVC	$lpha7\simlpha3eta2>lpha4eta2$	Nicke et al., 2003a
		$\alpha$ -GII	ECC-HPACGKHFSC*	Muscle nAChR	Gray et al., 1981
C. imperialis	V	$\alpha$ -ImI	GCCSDPRC-AWRC*	lpha 3eta 2 > lpha 7 > lpha 9	McIntosh et al., 1994;
•				*	Ellison et al., 2004
		$\alpha$ -ImII	ACCSDRRC-RWRC*	$\alpha$ 7, noncompetitive	Ellison et al., 2004
C. leopardus	V	$\alpha$ -Lp1.1	GCCARAACAGIHQELC*	$\alpha 3\beta 2 > \alpha 6\alpha 3\beta 2$	Peng et al., 2008
C. litteratus	V	$\alpha$ -LtIA	G <b>cc</b> araa <b>c</b> agihqel <b>c</b> *	$\alpha 3\beta 2 > \alpha 6\alpha 3\beta 2\beta 3$	Luo et al., 2010
C. magus	P	α-MI	GRCC-HPACGKNYSC*	Muscle nAChR	McIntosh et al., 1982
	_	α-MII	GCCSNPVCHLEHSNLC*	$\alpha 6\alpha 3\beta 2\beta 3 > \alpha 3\beta 2 > \alpha 7$	Cartier et al., 1996
C. omaria	M	α-OmIA	GCCSHPACNVNNPHICG*	$\alpha 3\beta 2 > \alpha 7$	Talley et al., 2006
C. pergrandis	V	$\alpha$ -PeIA	GCCSHPACSVNHPELC*	$\alpha 9/\alpha 10 > \alpha 3\beta 2 > \alpha 3\beta 4$	McIntosh et al., 2005
C. purpurascens	P	α-PIA	-RDPCCSNPVCTVHNPQIC*	α6α3β2	Dowell et al., 2003
o. parparaccente	-	α-PIB	ZSOGCCWNPACVKNRC*	Muscle	López-Vera et al., 200
C. pennaceus	$\mathbf{M}$	α-PnIA	GCCSLPPCAANNPDyC*	$\alpha 3\beta 2 > \alpha 7$	Fainzilber et al., 1994
c. pennaccas	111	α-PnIB	GCCSLPPCALSNPDyC*	$\alpha 7 > \alpha 3 \beta 2$	Fainzilber et al., 1994
C. quercinus	V	$\alpha$ -Qc1.2	QCCANPPCKHVNC*	$\alpha 3\beta 2, \alpha 3\beta 4$	Peng et al., 2009
C. regius	v	α-Reg1b/c	GCCSDORCKHQC*	N.D.	Franco et al., 2006
C. regius	v	α-Reg1d	GCCSDPRCKHEC*	N.D.	Franco et al., 2006
		α-Reg1e	GCCSDORCRYRC*	N.D.	Franco et al., 2006
		α-Reg1f	DYCCRROOCTLIC*	N.D.	Franco et al., 2006
		α-Reg11 α-Reg2a	GCCSHPACNVNNPHIC*	N.D.	Franco et al., 2006
		α-RgIA	GCCSDPRCRYRCR	$\alpha 9/\alpha 10$	Ellison et al., 2006
C. striatus	P	α-NgIA α-SI	ICC-NPAC-GPKYS-C*	Muscle nAChR	Zafaralla et al., 1988
C. striutus	1	$\alpha$ -SIA		Muscle nAChR	Myers et al., 1991
C. spurius	V	$\alpha$ -SIA $\alpha$ -SrIA	YCC-HPAC-GKNFD-C*	Muscle/ $\alpha 4\beta 2$	López-Vera et al., 200'
C. spurius	V	$\alpha$ -SrIA $\alpha$ -SrIB	RTCCSROTCRMYYPYLCG*	Muscle/ $\alpha$ 4 $\beta$ 2	-
C. tinianus	V		RTCCSROTCRMEYPγLCG*		López-Vera et al., 200
		α-TiIA α-TxIA	GGCCSHPAC-QNNPDyC*	Neuronal nAChR	Kauferstein et al., 201
C. textile	M		GCCSRPPCIANNPDLC	$\alpha 3 \beta 2 > \alpha 7$	Dutertre et al., 2007
C. victoriae	$\mathbf{M}$	$\alpha$ -VcIA	GCCSDPRCNYDHPEIC*		Sandall et al., 2003;
Q	D	CIT	CARANTO ACDIVIC ACTUAR	Managara A Cla D	Vincler et al., 2006
C. striatus	P	α-SII	GCCCNPACGPNYGCGTSCS	Muscle nAChR	Ramilo et al., 1992
C. ermineus	P	αA-EIVA	GCCGPYONAACHOCGCKVGROOYCDROSGG*	Muscle nAChR	Jacobsen et al., 1997
a 1	D	αA-EIVB	GCCGKYONAACHOCGCTVGROOYCDROSGG*	Muscle nAChR	Jacobsen et al., 1997
C. obscurus	P	αA-OIVA	CCG-VONAACHOCVCKNTC*	Muscle nAChR	Teichert et al., 2004
a 1:	**	αA-OIVB	CCG-VONAACPOCVCNKTCG*	Fetal > adult muscle nAChR	Teichert et al., 2005b
C. pergrandis	V	αA-PeIVA	CCG-VONAACHOCVCTGKC	Fetal > adult muscle	Teichert et al., 2006
~	_	αA-PeIVB	CCG-IONAACHOCVCTGKC	Fetal > adult muscle	Teichert et al., 2006
C. purpurascens	P	αA-PIVA	GCCGSYONAACHOCSCKDROS-YCGQ*	Muscle nAChR	Hopkins et al., 1995
		$\psi$ -PIIIE	-HOOCCLYGKCRRYOGCSSASCCQR*	Muscle, noncompetitive	Shon et al., 1997
		$\psi ext{-PIIIF}$	-GOOCCLYGSCROFOGCYNALCCRK*	Muscle, noncompetitive	Van Wagoner et al., 2003
C. litteratus	V	$\alpha$ L-LtXIVA	MCPPLCKPSCTNC*	Neuronal nAChR	Peng et al., 2006
C. planorbis	V	$\alpha$ J-PlXIVA	FPRPRICNLACRAGIGHKYPFCHCR*	$Muscle > \alpha 3\beta 2$	Imperial et al., 2006
C. radiatus	P	$\alpha$ S-RVIIIA	KCNFDKCKGTGVYNCG\grCT\gLHS CRCTYNIGSMKSGCACICTYY	Muscle and neuronal nAChR	Teichert et al., 2005a
C. parius	P	$\psi ext{-PrIIIE}$	-AARCCTYHGSCLKEKCRRKYCC*	Adult > fetal muscle nAChR	Lluisma et al., 2008
C. vexillum	V	$\alpha$ D-VxXXA	DVQDCQVSTOGSKWGRCCLNRVCGPMCCPASH	N.D.	Loughnan et al., 2006
		αD-VxXXB	CYCVYHRGRGHGCSC (dimer)	$\alpha 7 > \alpha 3 \beta 2$	Loughnan et al., 2006
		αυ-νχλλΩ	DDysyciintrdspwgrccrtrmcgsmccprngct cvyhwrrghgcscpg (dimer)	αι / αυρΔ	Louginian et al., 2000
		$\alpha \text{D-VxXXC}$	DLRQCTRNAPGSTWGRCCLNPMCGNFCCPRSG CTCAYNWRRGIYCSC (dimer)	N.D.	Loughnan et al., 2006

ture of ImI bound to AChBP (Hansen et al., 2005; Ulens et

A third  $\alpha$ -conotoxin of particular interest is MII, which blocks  $\alpha 3\beta 2$ - and  $\alpha 6$ -containing nAChRs (Cartier et al., 1996). A series of analogs were designed to selectively target the  $\alpha 6\alpha 3\beta 2\beta 3$  combination (McIntosh et al., 2004) and later used to determine the contribution of  $\alpha$ 6containing nAChR in dopamine release in the striatum and to reveal a down-regulation of this subtype upon longterm nicotine exposure (McCallum et al., 2005; Perry et al., 2007). Radiolabeled and flurorescently labeled MII/ analogs have also been used to demonstrate downregulation of these  $\alpha$ -conotoxin binding sites in nigrostriatal damaged animal models as well as in post mortem brain from humans with Parkinson's disease (Quik et al., 2004). Residues involved in MII binding on the  $\alpha 3\beta 2$ nAChR have been identified, and these include Lys185 and Ile188 on  $\alpha$ 3, and Thr59, Val109, Phe117, and Leu119 on the  $\beta$  subunit, allowing a model of the interaction to be built (Harvey et al., 1997; Dutertre et al., 2005). More recently, ArIB, which blocks both  $\alpha$ 7 and  $\alpha$ 3 $\beta$ 2 nAChR, was compared with other  $\alpha$ -conotoxin sequences and rationally modified to increase α7 nAChR selectivity (Whiteaker et al., 2007). This structurefunction analysis yielded two analogs, V11L,V16A-ArIB and V11L, V16D-ArIB, which had low affinity for  $\alpha 3\beta 2$  but retained  $\alpha 7$  nAChR activity as predicted, with an iodinated form developed as a pharmacological tool (Whiteaker et al., 2008).

GID is the only  $\alpha$ -conotoxin with relatively high affinity for the  $\alpha 4\beta 2$  nAChR subtype (Nicke et al., 2003a). This  $\alpha$ -conotoxin is unusual in that it possesses an extended N terminus of four residues, as well as two post-translational modifications. A complete alanine scan of all noncysteine residues revealed that most analogs had at least a 10-fold reduced activity at the  $\alpha 4\beta 2$  subtype, implying a highly specific interaction (Millard et al., 2009). Docking of GID to  $\alpha 4\beta 2$  or its corrystallization with AChBP could reveal the specific interactions responsible for its  $\alpha 4\beta 2$  affinity and provide a rationale basis for the design of more selective analogs.

Newly discovered  $\alpha$ -conotoxins Vc1.1, RgIA, and PeIA target the  $\alpha 9\alpha 10$  nAChR subtype (McIntosh et al., 2005; Clark et al., 2006; Ellison et al., 2006), which may potentially be involved in immune responses and pain (Satkunanathan et al., 2005; McIntosh et al., 2009). Scanning mutagenesis of  $\alpha$ -conotoxin Vc1.1 revealed that Ser4 and Asn9 are important for  $\alpha 9\alpha 10$  nAChR potency (Halai et al., 2009), whereas for RgIA, Asp5, Pro6, and Arg7 were shown to be critical for both  $\alpha 9\alpha 10$  and  $\alpha 7$  nAChR blockade (Ellison et al., 2008). It is noteworthy that these  $\alpha 9\alpha 10$ selective  $\alpha$ -conotoxins are analgesic in different animal models of chronic pain, but there is an uncertainty about the true molecular target responsible for this effect. Indeed, whereas  $\alpha 9\alpha 10$  nAChR-selective small molecules were recently shown to also provide pain relief, N-type calcium channel inactivation through a novel interaction with GABA<sub>B</sub> receptor has also been proposed (Callaghan and Adams, 2010; Zheng et al., 2011).

The determination of several X-ray crystal structures of AChBP/α-conotoxin complexes significantly advanced our understanding of the structural basis for the nAChR subtype selectivity of  $\alpha$ -conotoxins (Celie et al., 2005; Hansen et al., 2005; Ulens et al., 2006; Dutertre et al., 2007). We were surprised to find that the three conotoxins ( $\alpha$ -ImI, and variants of  $\alpha$ -PnIA and  $\alpha$ -TxIA) that have been cocrystallized with AChBP showed a similar orientation within the ACh binding pocket despite divergent primary sequences. All three structures show a strong contribution of hydrophobic contacts between a conserved proline and several hydrophobic residues of the  $\alpha$ -conotoxins and several residues in the aromatic cage of AChBP (Fig. 7). Therefore, the different nAChR selectivity profiles arise from specific electrostatic interactions and hydrogen bonds formed between  $\alpha$ -conotoxin and nAChR subunits. For instance,  $\alpha$ -conotoxin TxIA uses a unique electrostatic pairing between Arg5 and AChBP-Asp195 to achieve its high-affinity binding (Dutertre et al., 2007). In addition, a tilt in the orientation of the  $\alpha$ -conotoxin TxIA structure within the ACh binding pocket was found to correlate with nAChR subtype selectivity. Clearly, additional  $\alpha$ -conotoxin-AChBP crystal structures and new models of  $\alpha$ -conotoxinnAChR interactions are expected to emerge, which should further expand our understanding of subtype-selectivity at nAChR. The structures of the 4/7  $\alpha$ -conotoxin A10L-TxIA and the smaller 4/3  $\alpha$ -conotoxin ImI and their interactions with the AChBP are shown in Fig. 7.

Compared with the  $\alpha$ -conotoxins, the other conotoxin families targeting nAChRs are less well understood, both in their mode of action and pharmacophore. These nontraditional  $\alpha$ -conotoxins are often named with a letter to designate their superfamily origins. For example, the first member of the  $\alpha$ C-conotoxin family, PrXA, was isolated from the venom of *Conus spurius* (Jimenez et al., 2007). This 32-amino acid peptide induced paralysis when injected into mice and fish and was shown to potently inhibit the muscle nAChR in vitro in a competitive manner. The primary structure of this peptide seems very different from that of  $\alpha$ -conotoxins, with only one disulfide bond and three hydroxyprolines. A second family comprises  $\alpha$ S-Conotoxin RVIIIA, which is also unique, with a broad selectivity profile against nAChR subtypes and the presence of γ-carboxyl glutamates, a post-translational modification not otherwise found in conotoxins targeting nAChRs (Teichert et al., 2005a). RVIIIA is also paralytic to mice and fish but elicits seizures in mice upon intracranial injection. Electrophysiological recordings of recombinantly expressed nAChR in oocytes revealed it competitively inhibited several neuronal subtypes in addition to the muscle nAChR. Within the same family is  $\sigma$ -GVIIIA, a peptide that targets another ligand-gated ion channel, the serotonin receptor (see section IV.B). Conotoxin lt14a belongs to yet another family αL-conotoxin and was discovered using a cDNA library from the venom gland of *C. litteratus* (Peng et al.,



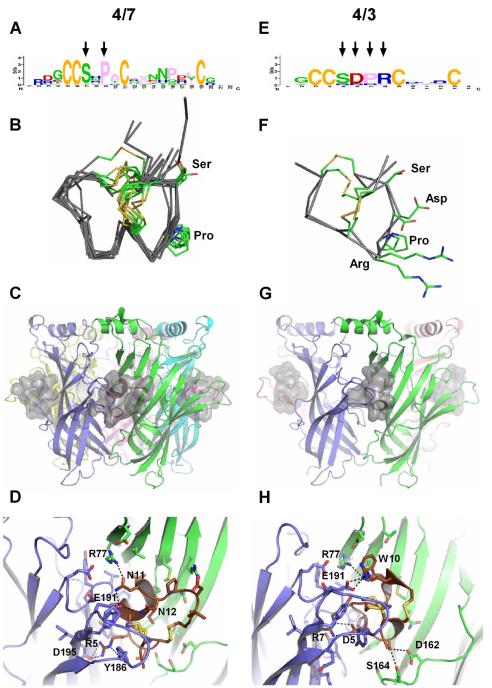


FIG. 7. Structure activity relationship of 4/7 (A, B, C, and D) and 4/3 (E, F, G, and H)  $\alpha$ -conotoxins. A, the consensus sequence for 4/7  $\alpha$ -conotoxins. B, overlay of the structures of  $\alpha$ -conotoxins OmIA (2GCZ), EI (1K64), GIC (1UL2), GID (1MTQ), MII (1MII), PIA (1ZLC), PnIA (1PEN), PnIB (1AKG), and Vc1.1 (2H8S). C, crystal structure of  $\alpha$ -A10L-TxIA bound to AChBP. D, details of the interactions of  $\alpha$ -A10L-TxIA with AChBP. E, consensus sequence for 4/3  $\alpha$ -conotoxins. F, overlay of the structures of  $\alpha$ -ImI (1CNL) and RgIA (2JUT). G, crystal structure of ImI bound to AChBP. H, details of the interactions of  $\alpha$ -ImI with AChBP.

2006). The sequence showed no homology to other families, but the cysteine arrangement is reminiscent of that of the  $\alpha$ -conotoxins. A synthetic version was tested on frog nerve preparations and PC12 cells, assuming the  $\alpha$ -conotoxin cysteine connectivity (1–3; 2–4), and it was found to block the response of AChevoked currents. It is noteworthy that although this peptide has not been tested across the various subtypes of nAChRs, lt14a produced dose-dependent an-

algesia in a hot-plate assay in mice, suggesting that it may target the rapeutically relevant nAChRs. Further atypical  $\alpha$ -conotox ins include the  $\alpha$ J-pl14a, which was recently identified with an unusual dual activity at two different classes of ion channels (Imperial et al., 2006). Indeed, this 25-amino acid peptide inhibits both  $K_{\rm v}1.6$  and muscle and  $\alpha3\beta2$  nAChRs (see section III.D). Its well defined structure, comprising an  $\alpha$ -helix and two short  $3_{10}$ -helices, may allow future struc-



An unusually large family of conopeptides found only in one clade of worm-hunting cone snails also inhibit nAChRs (Loughnan et al., 2006, 2009; Kauferstein et al., 2009). These  $\alpha$ D-conotoxins have a molecular mass of  $\sim$ 11 kDa, with Edman degradation, mass spectrometric, and NMR data revealing they assemble as a pseudo-homodimer comprising two 47- to 50-residue peptides. So far,  $\alpha$ Dconotoxins have been isolated only from worm-hunting species of cone snails where traditional  $\alpha$ -conotoxins seem to be absent. Therefore, they may represent another example of convergent evolution to a common prey target. Binding assays and two-electrode voltage-clamp analyses showed that  $\alpha$ D-conotoxins are potent inhibitors of several nAChRs, including  $\alpha$ 7,  $\alpha$ 3 $\beta$ 2, and  $\alpha$ 4 $\beta$ 2 subtypes. Finally, several noncompetitive conotoxins have also been discovered (Shon et al., 1997). These  $\psi$ -conotoxins show homology to the  $\mu$ -conotoxins, which are Na<sub>v</sub> channel pore blockers. PIIIE was originally shown to block muscle nAChR but targets a different binding site compared with  $\alpha$ -bungarotoxin. PrIIIE was shown to also potently inhibit muscle nAChR, with a preference for the adult over the fetal subtype (Lluisma et al., 2008). Thus, it seems that Conus spp. have devised many original solutions to target this important physiological target, providing us with as many templates for nAChR subtypeselective tools and drugs.

## B. σ-Conotoxin Inhibitors of Serotonin 5-HT<sub>3</sub> Receptors

One family of conotoxins targets the 5-HT $_3$  receptor, pentameric, cation-selective channels that open in response to the binding of the neurotransmitter serotonin (Hoyer et al., 1994) and share overall topology with nicotinic receptors. Five human subtypes (5-HT $_{3A}$ , 5-HT $_{3B}$ , 5-HT $_{3C}$ , 5-HT $_{3D}$ , and 5-HT $_{3E}$ ) have been cloned (Karnovsky et al., 2003; Niesler et al., 2003) but only homomeric 5-HT $_{3A}$  and heteromeric combinations of 5-HT $_{3A}$  and 5-HT $_{3B}$  have been pharmacologically characterized in

detail. To date, only the large  $\sigma$ -conotoxin GVIIIA has been identified to target 5-HT $_3$  receptors (England et al., 1998). GVIIIA inhibits 5-HT $_3$  receptors expressed in *Xenopus laevis* oocytes in a competitive manner. This 41-amino acid peptide contains 10 cysteines (five disulfide bonds), an amidated C terminus, and the unusual post-translational modification 6-bromoptryptophan. Unfortunately, this complicated structure precluded chemical synthesis and any structure-activity relationship studies at the receptor.

## C. Ikot-Ikot Conopeptide Enhancers of α-Amino-3hydroxy-5-methyl-4-isoxazolepropionic acid Receptors

A novel polypeptide isolated from *C. striatus* venom has been shown to selectively target AMPA receptors (Walker et al., 2009). This toxin, named con-ikot-ikot (referring to the Filipino word for "spinning around" in reference to the swimming phenotype observed in fish injected with this toxin), is 86 amino acids long with 13 cysteines, corresponding to a molecular mass of 9432 Da. It is noteworthy that the active form of con-ikot-ikot is a dimer of dimers, as evidenced by a band corresponding to a molecular mass of 38 kDa on an electrophoresis gel. This peptide achieves its biological effect through block of AMPA desensitization, greatly increasing the current magnitude. AMPA receptors belong to the ionotropic glutamate receptors, and together with NMDA and kainate receptors, mediate excitatory neurotransmission in the central nervous system (Dingledine et al., 1999). Native AMPA receptors assemble as heterotetramers (dimer of dimers) to form a functional channel. Each subunit (GluA1-4) consists of four domains: the N-terminal domain, the ligand binding domain, the pore forming transmembrane domain, and finally a small C-terminal domain (Chen and Wyllie, 2006). The structure of intact AMPA receptors was solved by Sobolevsky et al. (2009), revealing a dimer of dimers arrangement of four subunits. This structure is expected to help identify the binding site of con-ikot-ikot and guide further structureactivity studies for this novel class of conotoxin.

TABLE 9
Conantokins inhibiting NMDA glutamate receptors

Species	Diet	Name	Sequence	NMDA Selectivity	References
C. caracteristicus	V	Con-Ca2	GYyyRyIAyTVRyLEEA	N.D.	Franco et al., 2006
C. ermineus	P	Con-E	GEγγHSKYQγCLRγIRVNNVQQγC	N.D.	Gowd et al., 2008
C. geographus	P	Con-G	GEγγLQγNQγLIRγKSN*	NR2B > NR2C, NR2D	McIntosh et al., 1984; Gowd et al., 2008
C. gloriamaris	$\mathbf{M}$	Con-Gm	GAKYRNNAYAVRYRLEEI	N.D.	Franco et al., 2006
C. lynceus	V	Con-L	GEγγVAKMAAγLARγDAVN*	N.D.	Jimenez et al., 2002
C. purpurascens	P	Con-P	GEYYHSKYQYCLRYIRVNKVQQYC	NR2B, NR2A	Gowd et al., 2008
C. orchroleucus	V	Con-Oc	GEγγRKAMAγLEAKKAQγALKA	N.D.	Franco et al., 2006
C. parius	P	Con-Pr1	GEDγYΑγGIRγYQLIHGKI	NR2B, NR2D	Teichert et al., 2007b
		Con-Pr2	DEPγYAγAIRγYQLKYGKI	NR2B > NR2D	Teichert et al., 2007b
		Con-Pr3	GEPYVAKWAYGLRYKAASN*	NR2B, NR2D	Teichert et al., 2007b
C. quercinus	V	Con-Qu	GΥγγRγVAγTVRγLDAA	N.D.	Franco et al., 2006
C. radiatus	P	Con-R	GEγγVAKMAAγLARγNIAKGCKVNCYP	NR2B, NR2A	White et al., 2000
C. sulcatus (brettinghami)	P	Con-Br	GDyyYSKFIyRERyAGRLDLSKFP	NR2B, NR2D, NR2A $>$ NR2C	Twede et al., 2009
C. tulipa	P	Con-T	GEγγΥQKMLγNLRγAEVKKNA*	NR2B, NR2A	Haack et al., 1990; Klein et al., 2001





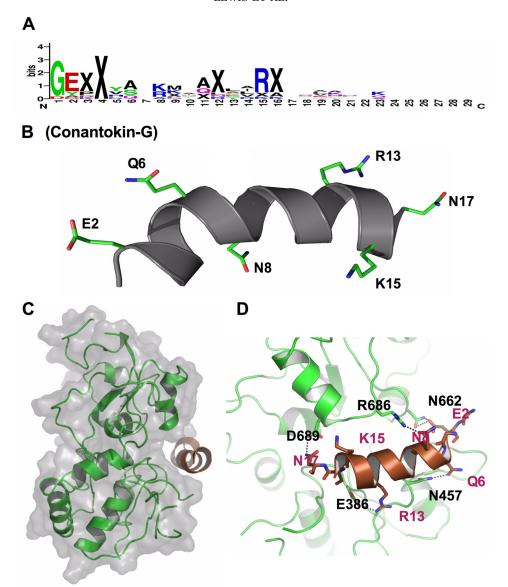


FIG. 8. Structure activity relationship of conantokins. A, consensus sequence for conantokins. B, structure of conantokin-G. C, model of likely interactions between conantokin-G and extracellular domain of the NMDA receptor NR2 subunit. D, details of the interactions between conantokin-G and NR2.

## D. Conantokin Inhibitors of N-Methyl-D-aspartate Receptors

Conantokins are small peptides (17–22 amino acids) isolated from the venom of various Conus species that selectively and potently inhibit NMDA receptors. The first conantokin isolated was Con-G; more recent members have been isolated from another 11 species (see Table 9). Conantokins have therapeutic potential as analgesics (Malmberg et al., 2003) and anticonvulsants (Jimenez et al., 2002). As opposed to the vast majority of conopeptides, conantokins do not contain cysteines but instead have a high content of  $\gamma$ -carboxyglutamic acid residues that when correctly spaced, induce  $\alpha$ -helicity in the presence of divalent cations. Structure-activity studies of conantokins (Nielsen et al., 1999b) have revealed a number of important residues for binding, and a docking model showed that Con-G can fit into the agonist binding cleft of the NR2 subunit (see

Fig. 8) (Wittekindt et al., 2001), revealing interactions between Glu386/Asp689 and Asn662 from NR2 subunit and Asn17/Lys15 and Glu2 of Con-G, respectively.

Downloaded from pharmrev.aspetjournals.org at Thammasart University on December 3, 2012

NMDA receptors are tetrameric ligand-gated ion channels with high Ca<sup>2+</sup> permeability that mediate fast excitatory neurotransmission in the CNS (Dingledine et al., 1999). They are composed mainly of two NR1 and two NR2 subunits, with an NR3 subunit potentially coassembling with NR1 and NR2 to modify NMDA receptor-mediated responses, reducing calcium permeability and single-channel conductance (Ciabarra et al., 1995). The topology of each subunit comprises an extracellular N-terminal domain, an agonist binding core, three transmembrane domains (domains 1 and 2 are separated by a "P-loop"), and a cytoplasmic tail of a variable length (Mayer and Armstrong, 2004). For the proper functioning and gating of NMDA receptors, the binding of both the agonist gluta-



mate (on the NR2 subunit) and the coagonist glycine (on the NR1 subunit) is required at the same time. Differential expression of isoforms (i.e., NR2A–D) occurs in the central nervous system, where they mediate fast excitatory neurotransmission. NMDA receptors have also been implicated in a number of chronic and acute neurological disorders, and significant research efforts are currently directed toward the development of specific drugs that can inhibit these targets (Loftis and Janowsky, 2003). Atomic resolution structures of the NMDA extracellular binding domains bound to agonists, partial agonists, and antagonists have been determined that might open the way to the rational development of NMDA receptor antagonists (Furukawa and Gouaux, 2003; Furukawa et al., 2005). Based on the docking model shown in Fig. 8, it is conceivable that conantokin analogs might be rationally designed to have selectivity for subunit combinations of clinical interest.

### E. Ligand-Gated Ion Channels Inhibitors in Pain Management

A number of ligand-gated ion channels have been implicated in pain modulation and chronic pain states but this discussion will focus on the two families of channels with evidence for application of conopeptides. Conopeptides targeting subtypes of nAChR subtypes (α-conotoxins) and NMDA receptors (several conantokins) have shown good efficacy in preclinical pain models and have reached different stages of development for use in humans. Their pharmacological characteristics underlying their analgesic effects are discussed below.

The involvement of nAChR subtypes in pain modulation is complex, and the mechanisms of action of  $\alpha$ -conotoxins in pain relief remains controversial. Much of the difficulty in resolving the actual targets of these toxins arises from the complexity of physiologically and pathologically relevant nAChR subtypes found in vivo, together with limited knowledge of expression and role of subunits in pain pathways (Olivera et al., 2008). For example, nicotinic agonists selective for  $\alpha 4\beta 2$  subtypes of nAChRs produce analysis actions in neuropathic pain models (Bannon et al., 1998; Rashid and Ueda, 2002), probably as a result of stimulation  $\alpha 4\beta 2$  nAChRs on inhibitory interneurons in the dorsal horn of the spinal cord (Genzen and McGehee, 2005). By contrast, analgesic  $\alpha$ -conotoxins are selective antagonists of different neuronal nAChR subtypes (and agonists at GABA<sub>b</sub>; see below) but avoid muscle or  $\alpha 4\beta 2$  nAChRs.

Synthetic Vc1.1 (ACV1) identified from cDNA was the first  $\alpha$ -conotoxin shown to exhibit efficacy in pain models (Sandall et al., 2003; Satkunanathan et al., 2005). The peptide was potent and peripherally active after intramuscular injection, producing partial reversal of mechanical allodynia in several neuropathic pain models. The findings that the effectiveness of the peptide escalated with repeated daily injections and seemed to allow functional recovery of injured nerves (Satkunanathan et al., 2005) suggested that it had clinical potential in treatment of neuropathic pain. Vc1.1 was initially selected for testing in pain models on the basis of its potent block of neuronal (bovine adrenal chromaffin cells) versus muscle nAChRs (Livett et al., 2006; see Table 10). Bovine adrenal chromaffin cells express  $\alpha 3$ -,  $\alpha 5$ -,  $\alpha 7$ -, and  $\beta 4$ -nAChR subunits (Sala et al., 2008). Accordingly, the main nAChR subtypes expressed on nociceptive peripheral fibers and their central terminations are composed of  $\alpha 3$ ,  $\alpha 5$ ,  $\beta 4$ , and  $\beta 2$  subunits (Khan et al., 2003; Lang et al., 2003; Rau et al., 2005). Vc1.1 also inhibited peripheral nerve-mediated vascular responses in rats (Satkunanathan et al., 2005) and, at low concentrations (100 nM), inhibited excitatory responses of

TABLE 10 Target selectivity and efficacy of analgesic  $\alpha$ -conotoxins administered systemically

$\alpha\text{-Conotoxins}$	Efficacy	nAChR selectivity	$\begin{array}{c} {\rm Nanomolar~GABA_{B}\text{-}Ca_{v}} \\ {\rm Activity} \end{array}$	References
Vc1.1	Partially reverses mechanical allodynia in several neuropathic models after intramuscular dosing. Escalating actions and recovery of nerve function.	$lpha 9lpha 10 \gg lpha 6lpha 3eta 2eta > lpha 6lpha 3eta 4 > lpha 3eta 4 \sim lpha 3eta 2$	Yes	Sandall et al., 2003; Satkunanathan et al., 2005; Vincler et al., 2006; Klimis et al., 2011
CyclizedVc1.1	Partially reverses mechanical allodynia in several neuropathic models after oral dosing.	Very weak $\alpha 9\alpha 10$	Yes	Clark et al., 2010
Vc1a	No reversal of allodynia in a neuropathic pain model. Recovery of nerve function retained	$lpha 9lpha 10 \gg lpha 3eta 4 \sim lpha 3eta 2$	No	Nevin et al., 2007
Rg1A	Partially reverses mechanical allodynia in several neuropathic models after intramuscular dosing.	$lpha 9lpha 10\gglpha 7\gglpha 3eta 4\ \simlpha 3eta 2$	Yes	Vincler et al., 2006
AuIB	Partially reverses mechanical allodynia in several neuropathic models after intramuscular dosing.	$\alpha 3\beta 4 > \alpha 3\beta 2 \gg \alpha 9\alpha 10$	Yes	Klimis et al., 2011
MII	Partially reverses mechanical allodynia in several neuropathic models after intramuscular dosing.	$\alpha 3\beta 2 > \alpha 3\beta 4 \gg \alpha 9\alpha 10$	No	Klimis et al., 2011
Lt14a	Acute thermal antinociception (hotplate test) after intraperitoneal administration.	Unkown but inhibits PC12 cell nAChR ( $\alpha$ 3- $\alpha$ 5-, $\alpha$ 7-, $\beta$ 2-, $\beta$ 3-, and $\beta$ 4-subunits)	Unknown	Sun et al., 2011



human sural nerve to nicotine (Lang et al., 2005). Vc1.1 has relatively low affinity for  $\alpha 3\beta 2$  and  $\alpha 3\beta 4$  nAChRs (Clark et al., 2006; Vincler and McIntosh, 2007) but may have higher affinity for more complex subunit combinations; e.g., its affinity for  $\alpha 6/\alpha 3\alpha 2\beta 2\beta 3$  nAChRs is 140 nM (Vincler and McIntosh, 2007). It was therefore suggested that Vc1.1 might produce pain relief via inhibition of  $\alpha$ 3 and/or  $\alpha$ 5 nAChR channels on sensory nerves. It is noteworthy that the analgesic  $\alpha$ -AuIB has relatively low affinity for  $\alpha 3\beta 4$  but MII has very high affinity for  $\alpha 3\beta 2$ nAChRs ( $\sim 1 nM$ ). It therefore remains possible that pain relief may be achieved in the periphery by antagonism of nAChRs comprising  $\alpha 3$ ,  $\alpha 5$ ,  $\beta 4$ , and  $\beta 2$  subunits, perhaps in combination with other less common subunits. The novel  $\alpha$ -conotoxin, lt14a, potently inhibits nAChR responses in rat PC12 cells and produces analgesia in a hotplate assay after systemic administration (Peng et al., 2006). Because PC12 cells express  $\alpha 3$ -  $\alpha 5$ -,  $\alpha 7$ -,  $\beta 2$ -,  $\beta 3$ -, and  $\beta$ 4-nAChR subunits (Henderson et al., 1994), the analgesic target likely involves subunit combinations that comprise one or more of these subunits. Other potential targets of Vc1.1 and other analgesic  $\alpha$ -conotoxins are summarized in Table 10 and discussed below.

Vc1.1 was also shown to be a potent antagonist at  $\alpha 9\alpha 10$ nAChRs, potentially contributing to its analysesic effects (Vincler et al., 2006). Another  $\alpha$ -conotoxin, RgIA, displayed similar activity at  $\alpha 9\alpha 10$  nAChRs and was also efficacious in neuropathic pain models (Vincler et al., 2006). However,  $\alpha 9\alpha 10$  nAChRs show very limited tissue distribution, being expressed predominantly in the olivocochlear system (Vetter et al., 2007), and their role in sensory nerve function is unclear. A more plausible hypothesis was forwarded by Vincler and McIntosh (2007), who suggested the analgesic efficacy of  $\alpha$ -conotoxins that inhibit  $\alpha 9\alpha 10$  nAChRs was due to inhibition of immune cell migration to injured nerves, although it remains to be determined whether this process is modulated by  $\alpha 9\alpha 10$  nAChRs. However, two analogs of Vc1.1, Vc1a and [P60]Vc1.1, retain full activity  $\alpha 9\alpha 10$  nAChRs but lose activity at other targets (see below) and fail to reverse allodynia in a neuropathic pain model (Nevin et al., 2007). Conversely,  $\alpha$ -AuIB and  $\alpha$ -MII are efficacious in a neuropathic pain model but do not inhibit  $\alpha 9\alpha 10$  nAChRs (Klimis et al., 2011). These findings cast doubt on the role of  $\alpha 9\alpha 10$  nAChRs in acute analysis in neuropathic pain models, although immune cell interactions may contribute to the longer term functional recovery of nerves. In support, Livett et al. (2008) claimed that Vc1a retained activity at  $\alpha 9\alpha 10$  nAChRs and lost analysis activity as reported by Nevin et al. (2007) but retained an apparent ability to induce functional recovery in injured nerves as measured by the peripheral vascular response to substance P.

Another potential target for the pain-relieving actions of  $\alpha\text{-conotoxins}$  was identified by Callaghan et al. (2008). This group reported that the analgesic  $\alpha\text{-conotoxins}$  Vc.1.1, RgIA, and AuIB, but not MII, potently inhibit calcium currents mediated by N-type (Ca<sub>v</sub>2.2) channels in mouse

sensory neurons (Callaghan et al., 2008; Klimis et al., 2011). After extensive pharmacological characterization, they identified that this effect was blocked by GABA<sub>B</sub> receptor antagonists and mediated by nonclassical G-protein-coupled modulation of Ca. 2.2 channels (Callaghan et al., 2008; Klimis et al., 2011). To date, all  $\alpha$ -conotoxins tested that potently inhibit Ca, 2.2 are also analgesic when administered systemically (Callaghan et al., 2008; Clark et al., 2010; Klimis et al., 2011) and analogs of Vc1.1 (Vc1a and [P60]Vc1.1) that retain activity at  $\alpha 9\alpha 10$  nAChRs but lose analgesic activity (Nevin et al., 2007) also lose their ability to modulate N-type currents in sensory neurons (Callaghan et al., 2008). Furthermore, modulation of Ntype calcium channels persists in DRG neurons from  $\alpha 9\alpha 10$  knockout mice (Callaghan and Adams, 2010). However, the mechanism of GABA<sub>B</sub> receptor-dependent α-conotoxin modulation of N-type calcium channels remains controversial. Ca<sub>v</sub>2.2 inhibition by classic GABA<sub>B</sub> agonists is due to direct association of G-protein  $\beta \gamma$  subunits with the  $Ca_v 2.2$   $\alpha$ -subunit. This effect is rapid, involves slowing of the activation of the channel, and is relieved by strong depolarizations (Herlitze et al., 1996; Ikeda, 1996). None of these properties were observed for inhibition by Vc1.1, strongly suggesting another mechanism is involved. Another form of G-protein-coupled inhibition of N-type channels in sensory neurons is voltageindependent and mediated by tyrosine phosphorylation of the e37a splice variant (Raingo et al., 2007). However, this mechanism is unlikely to account for the actions of  $\alpha$ -conotoxins on N-type calcium channels, because direct  $\beta\gamma$ subunit-mediated inhibition also occurs on both the e37a and 37b variants expressed by sensory neurons (Raingo et al., 2007). This interpretation is consistent with the failure of Vc1.1 and RgIA to modulate GIRK channels via coexpressed GABA<sub>B</sub> receptors, which is also G-protein  $\beta\gamma$ mediated (McIntosh et al., 2009).

In summary, several  $\alpha$ -conotoxins seem to be potential candidates for development of treatments for neuropathic pain. Although the mechanism(s) of action remain controversial, they produce partial relief from the signs of neuropathic pain and seem to accelerate functional recovery of sensory nerve function. The  $\alpha$ -conotoxins are also relatively small peptides, and the recent development of a cyclized analog of Vc1.1 (Clark et al., 2010) revealed that the peptide could relieve signs of neuropathic pain when administered orally. ACV1 (Vc1.1) was taken through a phase I clinical trials by Metabolic Pharmaceuticals (Melbourne, VIC, Australia) but subsequently dropped after completion of a phase 2A trial over potential concerns over efficacy and its reduced affinity at human versus rat  $\alpha 9/\alpha 10$  nAChRs.

Finally, NMDA receptors, particularly those in the dorsal spinal cord that include the NR2B subunit, have long been recognized as candidates for development of pain therapeutics (Parsons, 2001), with small-molecule NR2B-selective antagonists selectively inhibiting signs of chronic pain in animal models (Boyce et al., 1999).

Downloaded from pharmrev.aspetjournals.org at Thammasart University on December 3, 2012

 ${\footnotesize \begin{array}{c} {\footnotesize TABLE \ 11} \\ {\footnotesize Conopeptides \ targeting \ GPCRs \ and \ monoamine \ transporters} \end{array}}$ 

Species	Diet	Name	Sequence	Target	Reference
			12 3 4		
C. tulipa	F	ρ-TIA	FNWRCCLIPACRRNHKKFC*	α1-Adrenoceptor	Sharpe et al., 2001
C. marmoreus	$\mathbf{M}$	$\chi$ -MrIA	-NGVCCGYKLCHOC	NET	Sharpe et al., 2001
		$\chi$ -MrIB	-VGVCCGYKLCHOC	NET	Sharpe et al., 2001
		$\chi$ -CMrVIA	VCCGYKLCHOC	N.D.	Balaji et al., 2000
		χ-CMrX	GICCGVSFCYOC	N.D.	Balaji et al., 2000
C. geographus	P	Conopressin G	CFIRNCPKG*	Vasopressin R	Cruz et al., 1987; Nielsen et al., 1994
C. imperialis	V	•	CIIRNCPRG*	-	
C. striatus	P	Conopressin S	<b>C</b> FIRN <b>C</b> OP	$V_{1b}R$ , OTR $> V_{1a}R$	Cruz et al., 1987; Dutertre et al., 2008
C. textile	$\mathbf{M}$	Conopressin Tx	CYIQNCLRV*	N.D.	Ueberheide et al., 2009
C. tulipa	P	Conopressin-T	CLIQDCPγG*	OT, $V_{1a}R$	Dutertre et al., 2008
C. villepini	V	Conopressin-vil	ZSEEGGSNAtKKPYIIL	N.Ď.	Möller and Marí, 2007
C. geographus	$\mathbf{F}$	Contulakin-G	ZSEEGGSNAtKKPYIIL	Neurotensin R	Craig et al., 1999

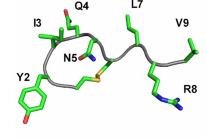
P, fish; M, molluscs; V, worms; γ, γ-carboxyglutamate; \*, C-terminal amidation; N.D., not determined.

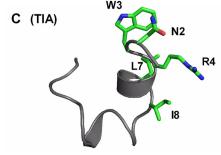
Several conantokins, including conantokin-G, selectively inhibit NMDA receptors composed of NR1 and NR2B subunits (Layer et al., 2004) and inhibit signs of neuropathic pain intrathecally. Unfortunately, Con-G

produced motor side effects in a similar dose range in nerve injury models (Malmberg et al., 2003), and the utility of intrathecal conantokins targeting NR2B for pain management remains to be established.



#### B (Conopressin-T)





(MrIA)

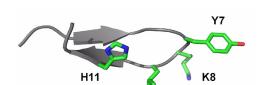


Fig. 9. Structure activity relationship of conopeptides interacting with GPCRs and transporters. A, consensus sequence for conopressins. B, structure of conopressin-T, a  $V_{1\Lambda}R$  selective antagonist. C, structure of  $\rho\text{-TIA}$ , a selective, noncompetitive antagonist of the  $\alpha\text{1B-adrenergic}$  receptor. D, structure of  $\chi\text{-MrIA}$ , a selective and noncompetitive antagonist of the noradrenaline transporter.

## V. Conotoxins Interacting with G Protein-Coupled Receptors and Neurotransmitter Transporters

Two novel classes of conopeptide ( $\rho$ - and  $\chi$ -conopeptides) were discovered that inhibit noradrenaline activation of  $\alpha$ 1-adrenoceptors or its transport via the noradrenaline transporter (Sharpe et al., 2001). It is noteworthy that both noncompetitively (allosterically) inhibited noradrenaline, making them useful new probes of allosteric sites on these physiologically and pathologically important membrane proteins. In contrast, competitive (isosteric) agonists of the vasopressin and neurotensin receptors have also been discovered that are closely related to the endogenous peptides for these targets. Sequences of known conopeptides belonging to these four classes are shown in Table 11, and their structures are compared in Fig. 9.

#### A. $\rho$ -Conopeptide Inhibitors of $\alpha 1$ -Adrenoceptors

The conopeptide  $\rho$ -TIA is a 19-amino acid peptide with two disulfide bonds isolated from the venom of Conus tulipa (Sharpe et al., 2001). This peptide was originally discovered after reduction of the noradrenergic component of the electrically evoked contraction of the rat vas deferens. Competition experiments with radiolabeled ligands revealed TIA was a selective and negative allosteric modulator of hamster  $\alpha_{1B}$  (Sharpe et al., 2003b). Additional radioligand displacement studies showed that TIA is also 10-fold selective for human  $\alpha_{1B}$  over  $\alpha_{1A}$  and  $\alpha_{1D}$  (Chen et al., 2004). An alanine walk identified Asn2, Trp3, Arg4, Leu7, and Ile8 as the major binding determinants on TIA (Sharpe et al., 2003b). It is noteworthy that the first four residues in the N-terminal "tail" are key determinants of binding, despite being outside the disulfide bonds and relatively unconstrained in solution (Sharpe et al., 2003b). F18A (and F18N) analogs of TIA had increased selectivity for  $\alpha_{1B}$ , whereas the I8A analog had slightly reduced affinity for  $\alpha_{1B}$ , and its mode of action was switched to become a competitive antagonist (Chen et al., 2004).

High-resolution crystal structures of the closely related β-ARs have been determined in both the resting and activated states, as well as in complex with various ligands (Cherezov et al., 2007; Rasmussen et al., 2007, 2011a, 2011b; Warne et al., 2008; Rosenbaum et al., 2011; Warne et al., 2011). Although these structures confirmed the location of the orthosteric binding site for competitive ligands that had been mapped previously using affinity labeling, site-directed mutagenesis, and substituted-cysteine accessibility methods, the site of allosteric compounds remains rather elusive. Allosteric ligands are of particular interest to both fundamental science and the pharmaceutical industry, because they can achieve greater selectivity and act only where the endogenous agonist exerts its physiological effect (Conn et al., 2009). Using the recent crystal structure of  $\beta$ -adrenoceptors, modeling of the interaction between TIA and  $\alpha_{1B}$ -AR should provide a clearer view of this allosteric binding site and potentially allow the development of more selective inhibitors.

It is worthwhile to consider the therapeutic potential of peptide inhibitors of  $\alpha$ 1-adrenoceptors, a traditional smallmolecule GPCR drug target. These receptors belong to the family A of GPCRs, and therefore share the common topology comprising a core composed of seven transmembrane helices connected by three extracellular and three intracellular loops. Adrenoceptors regulate critical aspects of blood pressure control, myocardial contractility, pulmonary function, metabolism, and activities in the CNS (Koch et al., 1995). They are broadly divided in  $\alpha_1$ -,  $\alpha_2$ -, and  $\beta$ -adrenoceptors based on anatomical localization; then, each group is further separated into three subtypes based on pharmacological and molecular strategies ( $\alpha_{1A}$ ,  $\alpha_{1B}$ , and  $\alpha_{1D}$ ;  $\alpha_{2A}$ ,  $\alpha_{2B}$ , and  $\alpha_{2C}$ ;  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$ ) (Bylund et al., 1994; Graham et al., 1996). The  $\alpha_1$ -ARs are located on postsynaptic membranes, whereas  $\alpha_2$ -ARs are found on presynaptic nerve terminals (Langer, 1974). In particular,  $\alpha_1$ -ARs are crucial for the stimulation of smooth muscle contraction (Bylund et al., 1994). Agonists at the  $\alpha_1$ -ARs may be associated with an alerting or antidepressant action, whereas antagonists are being used and refined for the treatment of benign prostatic hypertrophy (Hieble and Ruffolo, 1996). Peptides with improved selectivity and restricted to peripheral compartments have the potential to treat diseases such as benign prostatic hypertrophy.

# B. $\chi$ -Conopeptide Inhibitors of the Noradrenaline Transporter

 $\chi$ -Conopeptides are small hydrophobic peptides that selectively inhibit the transport of noradrenaline by NET, an important target for drugs treating a range of neurological diseases, including depression, anxiety, obsessive-compulsive disorder, and attention deficit hyperactivity disorder (Goddard et al., 2010).  $\chi$ -MrIA and -MrIB noncompetitively inhibit NET (Sharpe et al., 2001), providing opportunities to regulate NET efficiently, irrespective of the

concentration of noradrenaline present.  $\chi$ -MrIA seems to be specific for NET, with no effect on the function of a diverse range of targets (Sharpe et al., 2001), including other monoamine neurotransmitter transporters (Sharpe et al., 2001). The solution structure of MrIA revealed that  $\chi$ -conopeptides have a rigid framework stabilized by two disulfide bonds and two antiparallel strands connected by an inverse  $\gamma$ -turn (Sharpe et al., 2001; Nilsson et al., 2005). Although the cysteine spacings in the linear sequence are reminiscent of small  $\alpha$ -conotoxins,  $\gamma$ -conopeptides have an alternative cysteine-stabilized scaffold, with cysteines bonded 1 to 4 and 2 to 3 (see Table 11), that allows a  $\gamma$ -turn to form in an optimized conformation for high-affinity interactions with NET. Residues in this  $\gamma$  turn (Gly6, Tyr7, Lys8, and Leu9), plus His11 in loop 2, have been identified as critical for high-affinity interactions at NET through alanine-scanning mutagenesis, but only mutations to Gly6 affect structure (Sharpe et al., 2003a). To define the  $\chi$ -conopeptide pharmacophore, an expanded series of analogs was synthesized, revealing that  $\chi$ -conopeptides interact with NET mainly through interaction of Tyr7 with NET via aromatic and/or H-bond acceptors, Lys8 contributing an essential basic residue for NET binding, Leu9 interacting with a large hydrophobic patch on NET, and His11 and hydroxyproline 12 contributing weaker interactions through aromatic and basic interactions (Brust et al., 2009).

Based on its mode of action to inhibit NET, intrathecal administration of MrIA was evaluated in rodent models of acute, inflammatory, and neuropathic pain, where it was able to produce significant reversal of the signs of acute and neuropathic pain in mice and rats, respectively, without significant side effects (McIntosh et al., 2000; Nielsen et al., 2005). To consider  $\chi$ -MrIA as a therapeutic lead, its chemically unstable N-terminal asparagine needed to be replaced or removed to allow long-term human use in implanted pumps. Extensive replacements and truncations/extensions at the N-termini revealed that replacing asparagine with a pyroglutamate significantly improved chemical stability without compromising the sideeffect profile observed for MrIA (Brust et al., 2009). Pyroglutamate1-MrIA (Xen2174), produced few side effects even at high doses, and an extended duration of action after a single bolus intrathecal dose in the rat model of neuropathic pain (Nielsen et al., 2005). Xen2174 was also found to accelerate recovery from pain when given pre-emptively in a rat-paw-incision model of postsurgical pain (Obata et al., 2005). Xen2174 was progressed to a phase I intravenous study, where it was determined to be safe and well tolerated in healthy volunteers, and most recently a phase IIa intrathecal study in patients with cancer pain. This open label, dose-escalating study was conducted in 37 cancer patients suffering severe chronic pain that was poorly controlled using conventional therapies. It is noteworthy that Xen2174 reportedly provided pain relief rapidly and for a sustained period across a wide range of tolerated intrathecal bolus doses from 0.1 to 30



mg. Xen2174 is presently being prepared for a phase IIb double-blind study to establish whether Xen2174 is safe and efficacious in a bunionectomy setting of postsurgical pain. Further details on the discovery and development of Xen2174 are reported in a review by Lewis (2011).

## C. Conopeptides Modulating Vasopressin/Oxytocin Receptors

Conopressins were first discovered in the venom of fishhunting cone snails based on the "scratching effect" induced in mice upon intracerebral injection (Cruz et al., 1987). The sequences of both conopressin-G and conopressin-T were very similar to vasopressin itself, with only an additional positive charge in position 4. It is noteworthy that conopressin-G was later isolated from a wormhunting cone snail species, Conus imperialis, as well as from the nonvenomous snails *Lymnea stagnalis* and *Aply*sia californica and the leech Erpobdella octoculata (Mc-Master et al., 1992; Salzet et al., 1993; Nielsen et al., 1994; Van Kesteren et al., 1995). Therefore, conopressin-G was proposed to represent the endogenous vasopressin homolog in these invertebrates. However, novel conopressins have since been discovered from other cone snail species, supporting a role in envenomation (Möller and Marí, 2007; Dutertre et al., 2008; Ueberheide et al., 2009). We were surprised that conopressin-T acted as a V<sub>1</sub>-vasopressin receptor-selective antagonist, with partial agonist activity at the oxytocin receptor and replacing Gly9 with Val9 also switched the activity of vasopressin and oxytocin from agonist to antagonist (Dutertre et al., 2008). Therefore, new conopressins may serve as templates for the design of novel ligands with tailored pharmacological profiles.

Vasopressin and oxytocin receptors belong to the GPCR family A and apparently bind vasopressins and conopressins in a pocket formed by the arrangement of the seven transmembrane domains (Mouillac et al., 1995; Dutertre et al., 2008). On the basis of extracellular domain exchanges between and oxytocin receptor, it has been proposed that the three extracellular loops are also important for agonist binding (Postina et al., 1996). Models of the interaction of vasopressin with  $V_1$  and  $V_3$  developed using a rhodopsin structural template were consistent with most experimental data and supported by follow-up sitedirected mutagenesis (Rodrigo et al., 2007). At least three different subpopulations of vasopressin receptors have been recognized:  $V_1$  (or  $V_{1a}$ ),  $V_2$ , and  $V_3$  (or  $V_{1b}$ ) (Birnbaumer, 2000). V<sub>1</sub> regulates blood pressure and vasoconstriction, whereas activation of V<sub>2</sub> produces an antidiuretic effect on the kidney, and stimulation of V<sub>3</sub> is responsible for corticotrophin release from the pituitary gland. Only one oxytocin receptor has been characterized, and its activation stimulates the contraction of uterine and mammary myocytes during parturition and lactation, respectively (Petersson, 2002). Using such models, it may be possible to rationally develop vasopressin/oxytocin receptor antagonists with tailored selectivity.

#### D. Neurotensin Receptor Agonists

Contulakin-G (also known as CGX-1160) was isolated from the venom of *Conus geographus* and was shown to possess agonist activity at neurotensin receptors 1 (NTSR1) and 2 (NTSR2) (Craig et al., 1999). The primary sequence of contulakin-G (ZSEEGGSNATKKPYIL) closely resembles neurotensin (ZLYENKPRRPYIL), yet contulakin-G is approximately 150-fold less potent at NTSR2 and NTSR1 receptors compared with the endogenous ligand, which contains a glycosylation at Thr10 (Craig et al., 1999, 2001). It is noteworthy that the native peptide was more biologically active than the synthetic nonglycosylated peptide (Craig et al., 1999).

Neurotensin plays important roles in neurotransmission and neuromodulation in the nervous system (Hermans and Maloteaux, 1998) by activating NTSR1 and NTSR2. which belong to G protein-coupled receptor family A (Tanaka et al., 1990; Vita et al., 1993). Both subtypes exhibit distinct pharmacological properties, NTSR1 showing high affinity for levocabastine, whereas NTSR2 has much lower affinity (Vita et al., 1998). Docking of neurotensin in the NTSR1 binding pocket was modeled based on rhodopsin structure (Härterich et al., 2008). Neurotensin has since been shown also to interact with D2 dopamine receptor (Fuxe et al., 1992); the recent crystal structure of D3 dopamine receptor may provide a more appropriate template to model these interactions (Chien et al., 2010). Because neurotensin is analgesic, contulakin-G was assessed for its antinociceptive activity in formalin models of acute and inflammatory pain in rats and dogs (Allen et al., 2007). Contulakin-G showed an antinociceptive effect greater than morphine, without motor deficits, prompting preclinical development and progression to phase I clinical trials. However, the mechanism by which contulakin-G induces analgesia remains unclear, because the antinociceptive effect is realized at a concentration 100-fold lower than its NTSR1 binding affinity.

## VI. Conclusions and Outlook

In addition to their well recognized value as pharmacological tools to dissect the physiological and pathophysiological roles of ion channels, receptors, and transporters in a vast range of disease pathways, cone snail venom peptides are attracting growing interest as potential therapeutic leads because of their high potency and specificity at mammalian targets. With only a fraction of the conopeptides identified and characterized to date, the natural product space of cone snail venom peptides represents a vast untapped biological resource. Given this potential, accelerated discovery platforms are becoming increasingly important to unravel conotoxin diversity. In particular, high-throughput and high-content approaches promise to accelerate the discovery of novel bioactives as well as the potential to provide unique insight into the effects and mechanisms of action of conopeptides even on heterogeneous cell populations such as DRG neurons. In addition,

recent technological advances and improved bioinformatic tools have led to the successful application of transcriptomic and proteomic approaches to *Conus* spp. venom peptide discovery. These approaches are likely to lead to the rapid identification of novel conotoxins belonging to known pharmacological classes and to accelerate the identification and characterization of conopeptides with new sequences and potential novel biological activities.

Naturally occurring conotoxins are ideal scaffolds for the interrogation of specific pharmacophores using alaninescanning mutagenesis approaches, and these structureactivity relationships can help guide molecular docking studies to define new allosteric sites on membrane proteins. Detailed studies of the interaction of conotoxins with their respective pharmacological targets have already provided invaluable insight into the pharmacology of ion channels, receptors and transporters. Exemplifying this are Na, interactions with naturally derived compounds, including  $\mu$ -,  $\mu$ O-, and  $\delta$ -conotoxins, which have contributed significantly to our understanding of this channel's structure and function. As crystal structures of these membrane protein targets become available, such as the recently reported sodium channel structure (Payandeh et al., 2011), further refinement of our understanding of toxintarget interactions will without doubt lead to improved conotoxin-derived compounds with exceptional affinity and subtype selectivity. It is noteworthy that non-native disulfide bond isomers of conopeptides can also provide useful membrane protein probes, further expanding on the pharmacology of conopeptides. For example, the two isomers of  $\alpha$ -AuIB differ in their inhibitory mechanisms, with the AuIB (ribbon) form competitively inhibiting only  $\alpha 3\beta 4$ nAChRs containing an  $\alpha$ 3 subunit in the fifth position, an effect distinct from the native AuIB (globular) form, which inhibited  $\alpha 3\beta 4$  nAChRs independent of the fifth subunit, primarily through a noncompetitive mechanism (Grishin et al., 2010). Furthermore, a structural isomer of  $\rho$ -TIA named NMB-1 was found to preferentially inhibit the sustained component of mechanically evoked current in DRG neurons, providing a novel diagnostic tool for the molecular definition of channels involved in hearing and pressureevoked pain (Drew et al., 2007).

In recent years, an increasing emphasis has been placed on genetic ablation or knockdown of pharmacological targets to dissect their role in physiology and the pathophysiology of disease. However, an inherent flaw of this approach is that heteromultimeric ion channels composed of several subunits, such as nAChR or  $K_v$  channels, cannot be targeted selectively. This has profound implications for our understanding of the composition and identity of receptor subtypes expressed in native tissues, as well as their physiological roles. The high selectivity and subtype specificity of conopeptides can yield novel insights especially for such targets and can help unravel the physiologic and pathophysiological roles of specific subunit combinations of these ion channels. Here, the  $\alpha$ -conotoxins have proved especially valuable for chemically dissecting the role of a wide

range of physiologically and pathologically relevant nAChR subunit combinations.

In addition, the importance of conotoxins as therapeutics in their own right is becoming increasingly apparent. Both naturally occurring conotoxins such as synthetic  $\omega$ -MVIIA (ziconotide) or the sequence-optimized  $\chi$ -conopeptide Xen2174 have become either novel pharmaceutics or promising drug leads. Although conotoxins acting at peripheral targets can be delivered by systemic routes, central targets still require direct delivery to the site of action (e.g., intrathecal injection) because of the limited permeability of the blood-brain barrier to peptides. However, our understanding of conotoxin structure-activity relationships is now being applied to the design of smaller, orally active conotoxin peptidomimetics, with the hope of engineering novel therapeutic molecules based on the unique pharmacological properties of conotoxins. The growing realization of the many limitations inherent to small molecules, as well as improved peptide dosage form design and drug delivery strategies, such as liposomal packaging, pro-drug approaches, conjugation to carrier molecules, and microinjection devices (Malik et al., 2007), promise to refocus drug development efforts beyond traditional approaches to more fully embrace the potential of peptide therapeutics. The favorable characteristics of conotoxins as peptide therapeutics in particular arise from their small size, relative ease of synthesis and high structural stability as a result of their disulfide connectivity and compact, relatively rigid structures. These properties of conotoxins also impart a low immunogenic potential and perhaps advantageous pharmacodynamic and pharmacokinetic behavior allowing compartmentalized delivery that can reduce side effects. For example, delivery of conotoxins to the intrathecal space could, in principle (on the basis of limited redistribution because of their peptidic nature), avoid side effects resulting from on-target effects at different sites. In addition, once delivered to these compartments, it seems that many conotoxins are surprisingly stable, with half-lives of hours or more, rather than minutes (Wermeling et al., 2003; Kern et al., 2007). For example, a relatively long half-life in cerebrospinal fluid may also account for the extended duration of action observed after intrathecal dosing of Xen2174 (Lewis, 2011). In addition, it is now possible to engineer potentially more stable N-C-cyclized forms as exemplified by using  $\alpha$ -conotoxin Vc1.1, which retained analgesic activity even after oral dosing (Clark et al., 2010). Thus, it is now possible to extend the broadly evolved bioactivity of cone snail venomderived peptides to novel uses that will further expand the potential and unique source of new research tools and potential therapeutic agents.

#### Acknowledgments

Dr. Anderson Wang prepared the homology models of Na<sub>v</sub>1.2 and Ca<sub>v</sub>2.2 and performed the docking simulations shown in Fig. 3.



Downloaded from pharmrev.aspetjournals.org at Thammasart University on December

2012

#### **Authorship Contributions**

Wrote or contributed to the writing of the manuscript: Lewis, Dutertre, Vetter, and Christie.

#### References

- Abbott JR and Litzinger MJ (1994) Different ω-conotoxins mark the development of Swiss Webster mouse cortex suggesting N-type voltage sensitive calcium channel subtypes. *Int J Dev Neurosci* 12:43–47.
- Abe T and Saisu H (1987) Identification of the receptor for  $\omega$ -conotoxin in brain. Probable components of the calcium channel. *J Biol Chem* **262**:9877–9882.
- Adams DJ, Alewood PF, Craik DJ, Drinkwater RD, and Lewis RJ (1999) Conotoxins and their potential pharmaceutical applications. *Drug Dev Res* **46:**219–234.
- Aguilar MB, López-Vera E, Heimer de la Cotera EP, Falcón A, Olivera BM, and Maillo M (2007) I-conotoxins in vermivorous species of the West Atlantic: peptide sr11a from Conus spurius. Peptides 28:18–23.
- Aguilar MB, Pérez-Reyes LI, López Z, de la Cotera EP, Falcón A, Ayala C, Galván M, Salvador C, and Escobar LI (2010) Peptide sr11a from *Conus spurius* is a novel peptide blocker for  $K_{\rm v}1$  potassium channels. *Peptides* **31:**1287–1291. Ahn HS, Black JA, Zhao P, Tyrrell L, Waxman SG, and Dib-Hajj SD (2011)  $Na_{\rm v}1.7$
- Ahn HS, Black JA, Zhao P, Tyrrell L, Waxman SG, and Dib-Hajj SD (2011) Na<sub>v</sub>1.7 is the predominant sodium channel in rodent olfactory sensory neurons. *Mol Pain* 7:32.
- Akopian AN, Souslova V, England S, Okuse K, Ogata N, Ure J, Smith A, Kerr BJ, McMahon SB, Boyce S, et al. (1999) The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. *Nat Neurosci* **2**:541–548.
- Al-Sabi A, Lennartz D, Ferber M, Gulyas J, Rivier JE, Olivera BM, Carlomagno T, and Terlau H (2004)  $\kappa$ M-conotoxin RIIIK, structural and functional novelty in a K<sup>+</sup> channel antagonist. *Biochemistry* **43**:8625–8635.
- Allen JW, Hofer K, McCumber D, Wagstaff JD, Layer RT, McCabe RT, and Yaksh TL (2007) An assessment of the antinociceptive efficacy of intrathecal and epidural contulakin-G in rats and dogs. *Anesth Analg* 104:1505–1513.
- Altier C, Dale CS, Kisilevsky ÅE, Chapman K, Castiglioni AJ, Matthews EA, Evans RM, Dickenson AH, Lipscombe D, Vergnolle N, et al. (2007) Differential role of N-type calcium channel splice isoforms in pain. *J Neurosci* 27:6363–6373.
- Amaya F, Wang H, Costigan M, Allchorne AJ, Hatcher JP, Egerton J, Stean T, Morisset V, Grose D, Gunthorpe MJ, et al. (2006) The voltage-gated sodium channel  $\mathrm{Na_v}1.9$  is an effector of peripheral inflammatory pain hypersensitivity. J Neurosci **26**:12852–12860.
- Andersson A, Baell JB, Duggan PJ, Graham JE, Lewis RJ, Lumsden NG, Tranberg CE, Tuck KL, and Yang A (2009) ω-Conotoxin GVIA mimetics based on an anthranilamide core: effect of variation in ammonium side chain lengths and incorporation of fluorine. Bioorg Med Chem 17:6659-6670.
- Andrade A, Denome S, Jiang YQ, Marangoudakis S, and Lipscombe D (2010) Opioid inhibition of N-type Ca<sup>2+</sup> channels and spinal analgesia couple to alternative splicing. *Nat Neurosci* 13:1249–1256.
- Azam L, Dowell C, Watkins M, Stitzel JA, Olivera BM, and McIntosh JM (2005) α-Conotoxin BuIA, a novel peptide from Conus bullatus, distinguishes among neuronal nicotinic acetylcholine receptors. J Biol Chem 280:80–87.
- Balaji RA, Ohtake A, Sato K, Gopalakrishnakone P, Kini RM, Seow KT, and Bay BH (2000) lambda-conotoxins, a new family of conotoxins with unique disulfide pattern and protein folding. Isolation and characterization from the venom of Conus marmoreus. J Biol Chem 275:39516-39522.
- Bannon AW, Decker MW, Kim DJ, Campbell JE, and Arneric SP (1998) ABT-594, a novel cholinergic channel modulator, is efficacious in nerve ligation and diabetic neuropathy models of neuropathic pain. *Brain Res* 801:158–163.
- Barbier J, Lamthanh H, Le Gall F, Favreau P, Benoit E, Chen H, Gilles N, Ilan N, Heinemann SH, Gordon D, et al. (2004) A  $\delta$ -conotoxin from *Conus ermineus* venom inhibits inactivation in vertebrate neuronal Na $^+$  channels but not in skeletal and cardiac muscles. *J Biol Chem* **279**:4680–4685.
- Bauer CS, Rahman W, Tran-van-Minh A, Lujan R, Dickenson AH, and Dolphin AC (2010) The anti-allodynic α2δ ligand pregabalin inhibits the trafficking of the calcium channel α2δ-1 subunit to presynaptic terminals in vivo. Biochem Soc Trans 38:525–528.
- Bayrhuber M, Vijayan V, Ferber M, Graf R, Korukottu J, Imperial J, Garrett JE, Olivera BM, Terlau H, Zweckstetter M, et al. (2005) Conkunitzin-S1 is the first member of a new Kunitz-type neurotoxin family. Structural and functional characterization. J Biol Chem 280:23766–23770.
- Becker S, Prusak-Sochaczewski E, Zamponi G, Beck-Sickinger AG, Gordon RD, and French RJ (1992) Action of derivatives of  $\mu$ -conotoxin GIIIA on sodium channels. Single amino acid substitutions in the toxin separately affect association and dissociation rates. *Biochemistry* **31**:8229–8238.
- Bell TJ, Thaler C, Castiglioni AJ, Helton TD, and Lipscombe D (2004) Cell-specific alternative splicing increases calcium channel current density in the pain pathway. *Neuron* 41:127–138.
- Berecki G, Motin L, Haythornthwaite A, Vink S, Bansal P, Drinkwater R, Wang CI, Moretta M, Lewis RJ, Alewood PF, et al. (2010) Analgesic ω-conotoxins CVIE and CVIF selectively and voltage-dependently block recombinant and native N-type calcium channels. *Mol Pharmacol* 77:139–148.
- Birnbaumer M (2000) Vasopressin receptors. Trends Endocrinol Metab 11:406–410. Black JA, Cummins TR, Plumpton C, Chen YH, Hormuzdiar W, Clare JJ, and Waxman SG (1999) Upregulation of a silent sodium channel after peripheral, but not central, nerve injury in DRG neurons. J Neurophysiol 82:2776–2785.
- Black JA, Nikolajsen L, Kroner K, Jensen TS, and Waxman SG (2008) Multiple sodium channel isoforms and mitogen-activated protein kinases are present in painful human neuromas. *Ann Neurol* **64**:644–653.
- Bourinet E, Alloui A, Monteil A, Barrère C, Couette B, Poirot O, Pages A, McRory J, Snutch TP, Eschalier A, et al. (2005) Silencing of the Ca,3.2 T-type calcium channel gene in sensory neurons demonstrates its major role in nociception. EMBO J 24:315–324.
- Bourinet E, Soong TW, Sutton K, Slaymaker S, Mathews E, Monteil A, Zamponi GW,

- Nargeot J, and Snutch TP (1999) Splicing of α1A subunit gene generates phenotypic variants of P- and Q-type calcium channels. *Nat Neurosci* **2:**407–415. Bosmans F, Puopolo M, Martin-Eauclaire MF, Bean BP, and Swartz KJ (2011)
- Bosmans F, Puopolo M, Martin-Eauclaire MF, Bean BP, and Swartz KJ (2011) Functional properties and toxin pharmacology of a dorsal root ganglion sodium channel viewed through its voltage sensors. J Gen Physiol 138:59–72.
- Bowersox SS, Gadbois T, Singh T, Pettus M, Wang YX, and Luther RR (1996) Selective N-type neuronal voltage-sensitive calcium channel blocker, SNX-111, produces spinal antinociception in rat models of acute, persistent and neuropathic pain. J Pharmacol Exp Ther 279:1243–1249.
- Boyce S, Wyatt A, Webb JK, O'Donnell R, Mason G, Rigby M, Sirinathsinghji D, Hill RG, and Rupniak NM (1999) Selective NMDA NR2B antagonists induce antinociception without motor dysfunction: correlation with restricted localisation of NR2B subunit in dorsal horn. Neuropharmacology 38:611–623.
- Bregman H, Berry L, Buchanan JL, Chen A, Du B, Feric E, Hierl M, Huang L, Immke D, Janosky B, et al. (2011) Identification of a potent, state-dependent inhibitor of Na<sub>v</sub>1.7 with oral efficacy in the formalin model of persistent pain. J Med Chem 54:4427—4445.
- Brust A, Palant E, Croker DE, Colless B, Drinkwater R, Patterson B, Schroeder CI, Wilson D, Nielsen CK, Smith MT, et al. (2009)  $\chi$ -Conopeptide pharmacophore development: toward a novel class of norepinephrine transporter inhibitor (Xen2174) for pain. *J Med Chem* **52**:6991–7002.
- Buczek O, Wei D, Babon JJ, Yang X, Fiedler B, Chen P, Yoshikami D, Olivera BM, Bulaj G, and Norton RS (2007) Structure and sodium channel activity of an excitatory I1-superfamily conotoxin. *Biochemistry* 46:9929–9940.
- Bulaj G, DeLaCruz R, Azimi-Zonooz A, West P, Watkins M, Yoshikami D, and Olivera BM (2001) δ-Conotoxin structure/function through a cladistic analysis. *Biochemistry* **40:**13201–13208.
- Bulaj G, West PJ, Garrett JE, Watkins M, Marsh M, Zhang MM, Norton RS, Smith BJ, Yoshikami D, and Olivera BM (2005) Novel conotoxins from Conus striatus and Conus kinoshitai selectively block TTX-resistant sodium channels. Biochemistry 44:7259–7265.
- Bulaj G, Zhang MM, Green BR, Fiedler B, Layer RT, Wei S, Nielsen JS, Low SJ, Klein BD, Wagstaff JD, et al. (2006) Synthetic  $\mu$ O-conotoxin MrVIB blocks TTX-resistant sodium channel Na<sub>v</sub>1.8 and has a long-lasting analgesic activity. *Biochemistry* 45:7404–7414.
- Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR Jr, and Trendelenburg U (1994) International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol Rev* **46**:121–136.
- Callaghan B and Adams DJ (2010) Analgesic  $\alpha$ -conotoxins Vc1.1 and RgIA inhibit N-type calcium channels in sensory neurons of  $\alpha 9$  nicotinic receptor knockout mice. *Channels (Austin)* 4:51–54.
- Callaghan B, Haythornthwaite A, Berecki G, Clark RJ, Craik DJ, and Adams DJ (2008) Analgesic α-conotoxins Vc1.1 and Rg1A inhibit N-type calcium channels in rat sensory neurons via GABA<sub>B</sub> receptor activation. *J Neurosci* **28:**10943–10951.
- Cartier GE, Yoshikami D, Gray WR, Luo S, Olivera BM, and McIntosh JM (1996) A new  $\alpha$ -conotoxin which targets  $\alpha 3\beta 2$  nicotinic acetylcholine receptors. J Biol Chem 271:7522–7528.
- Catterall WA (2010) Signaling complexes of voltage-gated sodium and calcium channels. Neurosci Lett 486:107–116.
- Catterall WA, Cestèle S, Yarov-Yarovoy V, Yu FH, Konoki K, and Scheuer T (2007) Voltage-gated ion channels and gating modifier toxins. *Toxicon* **49:**124–141.
- Catterall WA, Goldin AL, and Waxman SG (2005a) International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. *Pharmacol Rev* 57:397–409.
- Catterall WA, Perez-Reyes E, Snutch TP, and Striessnig J (2005b) International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. *Pharmacol Rev* 57:411–425.
- Celie PH, Kasheverov IE, Mordvintsev DY, Hogg RC, van Nierop P, van Elk R, van Rossum-Fikkert SE, Zhmak MN, Bertrand D, Tsetlin V, et al. (2005) Crystal structure of nicotinic acetylcholine receptor homolog AChBP in complex with an  $\alpha$ -conotoxin PnIA variant. *Nat Struct Mol Biol* 12:582–588.
- Chahine M, Chen LQ, Fotouhi N, Walsky R, Fry D, Santarelli V, Horn R, and Kallen RG (1995) Characterizing the  $\mu$ -conotoxin binding site on voltage-sensitive sodium channels with toxin analogs and channel mutations. *Receptors Channels* **3:**161–174.
- Chambers JC, Zhao J, Terracciano CM, Bezzina CR, Zhang W, Kaba R, Navaratnarajah M, Lotlikar A, Sehmi JS, Kooner MK, et al. (2010) Genetic variation in SCN10A influences cardiac conduction. *Nat Genet* **42**:149–152.
- Chan AW, Owens S, Tung C, and Stanley EF (2007) Resistance of presynaptic  $Ca_v2.2$  channels to voltage-dependent inactivation: dynamic palmitoylation and voltage sensitivity. *Cell Calcium* **42:**419–425.
- Chang NS, French RJ, Lipkind GM, Fozzard HA, and Dudley S Jr (1998) Predominant interactions between  $\mu$ -conotoxin Arg-13 and the skeletal muscle Na<sup>+</sup> channel localized by mutant cycle analysis. *Biochemistry* **37**:4407–4419.
- Chen P, Dendorfer A, Finol-Urdaneta RK, Terlau H, and Olivera BM (2010) Biochemical characterization of κM-RIIIJ, a K<sub>ν</sub>1.2 channel blocker: evaluation of cardioprotective effects of κM-conotoxins. *J Biol Chem* **285**:14882–14889.
- Chen PÉ and Wyllie DJ (2006) Pharmacological insights obtained from structurefunction studies of ionotropic glutamate receptors. Br J Pharmacol 147:839–853.
- Chen Z, Rogge G, Hague C, Alewood D, Colless B, Lewis RJ, and Minneman KP (2004) Subtype-selective noncompetitive or competitive inhibition of human  $\alpha$ 1-adrenergic receptors by  $\rho$ -TIA. J Biol Chem 279:35326–35333.
- Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK, et al. (2007) High-resolution crystal structure of an engineered human  $\beta$ 2-adrenergic G protein-coupled receptor. Science 318:1258–1265.
- Chien EY, Liu W, Zhao Q, Katritch V, Han GW, Hanson MA, Shi L, Newman AH, Javitch JA, Cherezov V, et al. (2010) Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. Science 330:1091–1095.
- in complex with a D2/D3 selective antagonist. Science 330:1091–1095. Choe W, Messinger RB, Leach E, Eckle VS, Obradovic A, Salajegheh R, Jevtovic-Todorovic V, and Todorovic SM (2011) TTA-P2 is a potent and selective blocker of

- T-type calcium channels in rat sensory neurons and a novel antinociceptive agent. *Mol Pharmacol* **80:**900–910.
- Choi JY, Seo HN, Lee MJ, Park SJ, Park SJ, Jeon JY, Kang JH, Pae AN, Rhim H, and Lee JY (2007) Synthesis and biological evaluation of novel T-type calcium channel blockers. *Bioorg Med Chem Lett* 17:471–475.
- Chowdhury S, Chafeev M, Liu S, Sun J, Raina V, Chui R, Young W, Kwan R, Fu J, and Cadieux JA (2011) Discovery of XEN907, a spirooxindole blocker of Na<sub>v</sub>1.7 for the treatment of pain. *Bioorg Med Chem Lett* 21:3676–3681.
- Chung D, Gaur S, Bell JR, Ramachandran J, and Nadasdi L (1995) Determination of disulfide bridge pattern in  $\omega$ -conopeptides. Int J Pept Protein Res 46:320–325.
- Ciabarra AM, Sullivan JM, Gahn LG, Pecht G, Heinemann S, and Sevarino KA (1995) Cloning and characterization of chi-1: a developmentally regulated member of a novel class of the ionotropic glutamate receptor family. J Neurosci 15:6498– 6508
- Clark RJ, Fischer H, Nevin ST, Adams DJ, and Craik DJ (2006) The synthesis, structural characterization, and receptor specificity of the α-conotoxin Vc1.1. J Biol Chem 281:23254-23263.
- Clark RJ, Jensen J, Nevin ST, Callaghan BP, Adams DJ, and Craik DJ (2010) The engineering of an orally active conotoxin for the treatment of neuropathic pain. Angew Chem Int Ed Engl 49:6545-6548.
- Conn PJ, Christopoulos A, and Lindsley CW (2009) Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat Rev Drug Discov* 8:41-54
- Copeland RA, Pompliano DL, and Meek TD (2006) Drug-target residence time and its implications for lead optimization. Nat Rev Drug Discov 5:730-739.
- Cox JJ, Reimann F, Nicholas AK, Thornton G, Roberts E, Springell K, Karbani G, Jafri H, Mannan J, Raashid Y, et al. (2006) An SCN9A channelopathy causes congenital inability to experience pain. Nature 444:894–898.
- Craig AG, Norberg T, Griffin D, Hoeger C, Akhtar M, Schmidt K, Low W, Dykert J, Richelson E, Navarro V, et al. (1999) Contulakin-G, an O-glycosylated invertebrate neurotensin. *J Biol Chem* **274**:13752–13759.
- Craig AG, Park M, Fischer WH, Kang J, Compain P, and Piller F (2001) Enzymatic glycosylation of contulakin-G, a glycopeptide isolated from *Conus* venom, with a mammalian ppGalNAc-transferase. *Toxicon* **39:**809–815.
- Craig AG, Zafaralla G, Cruz LJ, Santos AD, Hillyard DR, Dykert J, Rivier JE, Gray WR, Imperial J, DelaCruz RG, et al. (1998) An O-glycosylated neuroexcitatory Conus peptide. Biochemistry 37:16019-16025.
- Cruz LJ, de Santos V, Zafaralla GC, Ramilo CA, Zeikus R, Gray WR, and Olivera BM (1987) Invertebrate vasopressin/oxytocin homologs. Characterization of peptides from Conus geographus and Conus straitus venoms. J Biol Chem 262:15821– 15894
- Cruz LJ, Gray WR, Olivera BM, Zeikus RD, Kerr L, Yoshikami D, and Moczydlowski E (1985) Conus geographus toxins that discriminate between neuronal and muscle sodium channels. J Biol Chem 260:9280–9288.
- Cummins TR, Howe JR, and Waxman SG (1998) Slow closed-state inactivation: a novel mechanism underlying ramp currents in cells expressing the hNE/PN1 sodium channel. *J Neurosci* 18:9607–9619.
- Cummins TR and Rush AM (2007) Voltage-gated sodium channel blockers for the treatment of neuropathic pain. Expert Rev Neurother 7:1597–1612.
- Cusdin FS, Nietlispach D, Maman J, Dale TJ, Powell AJ, Clare JJ, and Jackson AP (2010) The sodium channel {beta}3-subunit induces multiphasic gating in Na<sub>v</sub>1.3 and affects fast inactivation via distinct intracellular regions. J Biol Chem 285: 33404–33412.
- Daly NL, Ekberg JA, Thomas L, Adams DJ, Lewis RJ, and Craik DJ (2004) Structures of μO-conotoxins from Conus marmoreus. Inhibitors of tetrodotoxin (TTX)-sensitive and TTX-resistant sodium channels in mammalian sensory neurons. J Biol Chem 279:25774-25782.
- Davis J, Jones A, and Lewis RJ (2009) Remarkable inter- and intra-species complexity of conotoxins revealed by LC/MS. *Peptides* **30**:1222–1227.
- de Araujo AD, Callaghan B, Nevin ST, Daly NL, Craik DJ, Moretta M, Hopping G, Christie MJ, Adams DJ, and Alewood PF (2011) Total synthesis of the analgesic conotoxin MrVIB through selenocysteine-assisted folding. Angew Chem Int Ed Engl 50:6527-6529.
- Dib-Hajj SD, Black JA, and Waxman SG (2009) Voltage-gated sodium channels: therapeutic targets for pain. *Pain Med* 10:1260–1269.
- Dib-Hajj SD, Cummins TR, Black JA, and Waxman SG (2010) Sodium channels in normal and pathological pain. *Annu Rev Neurosci* 33:325–347.
- Dingledine R, Borges K, Bowie D, and Traynelis SF (1999) The glutamate receptor ion channels. Pharmacol Rev 51:7-61.
- Dogrul A, Gardell LR, Ossipov MH, Tulunay FC, Lai J, and Porreca F (2003) Reversal of experimental neuropathic pain by T-type calcium channel blockers. Pain 105:159–168.
- Dowell C, Olivera BM, Garrett JE, Staheli ST, Watkins M, Kuryatov A, Yoshikami D, Lindstrom JM, and McIntosh JM (2003)  $\alpha$ -Conotoxin PIA is selective for  $\alpha$ 6subunit-containing nicotinic acetylcholine receptors. *J Neurosci* **23**:8445–8452.
- Drew LJ, Rugiero F, Cesare P, Gale JE, Abrahamsen B, Bowden S, Heinzmann S, Robinson M, Brust A, Colless B, et al. (2007) High-threshold mechanosensitive ion channels blocked by a novel conopeptide mediate pressure-evoked pain. *PLoS One* 2:e515.
- Dudley SC, Jr., Todt H, Lipkind G, and Fozzard HA (1995) A  $\mu$ -conotoxin-insensitive Na $^+$  channel mutant: possible localization of a binding site at the outer vestibule. Biophys J **69**:1657–1665.
- Dutertre S, Croker D, Daly NL, Andersson A, Muttenthaler M, Lumsden NG, Craik DJ, Alewood PF, Guillon G, and Lewis RJ (2008) Conopressin-T from Conus tulipa reveals an antagonist switch in vasopressin-like peptides. J Biol Chem 283:7100–7108.
- Dutertre S, Nicke A, and Lewis RJ (2005)  $\beta$ 2 subunit contribution to 4/7  $\alpha$ -conotoxin binding to the nicotinic acetylcholine receptor. J Biol Chem 280:30460–30468.
- Dutertre S, Nicke A, Tyndall JD, and Lewis RJ (2004) Determination of  $\alpha$ -conotoxin binding modes on neuronal nicotinic acetylcholine receptors. J Mol Recognit 17: 339–347.

- Dutertre S, Ulens C, Büttner R, Fish A, van Elk R, Kendel Y, Hopping G, Alewood PF, Schroeder C, Nicke A, et al. (2007) AChBP-targeted  $\alpha$ -conotoxin correlates distinct binding orientations with nAChR subtype selectivity. *EMBO J* **26**:3858–3867
- Dworkin RH, O'Connor AB, Backonja M, Farrar JT, Finnerup NB, Jensen TS, Kalso EA, Loeser JD, Miaskowski C, Nurmikko TJ, et al. (2007) Pharmacologic management of neuropathic pain: evidence-based recommendations. *Pain* 132:237–251.
- Ekberg J, Jayamanne Á, Vaughan CW, Aslan S, Thomas L, Mould J, Drinkwater R, Baker MD, Abrahamsen B, Wood JN, et al. (2006)  $\mu$ O-conotoxin MrVIB selectively blocks Na<sub>v</sub>1.8 sensory neuron specific sodium channels and chronic pain behavior without motor deficits. *Proc Natl Acad Sci USA* 103:17030–17035.
- Ellinor PT, Zhang JF, Horne WA, and Tsien RW (1994) Structural determinants of the blockade of N-type calcium channels by a peptide neurotoxin. Nature 372:272– 275
- Ellison M, Feng ZP, Park AJ, Zhang X, Olivera BM, McIntosh JM, and Norton RS (2008)  $\alpha$ -RgIA, a novel conotoxin that blocks the  $\alpha 9\alpha 10$  nAChR: structure and identification of key receptor-binding residues. *J Mol Biol* 377:1216–1227.
- Ellison M, Gao F, Wang HL, Sine SM, McIntosh JM, and Olivera BM (2004)  $\alpha$ -Conotoxins ImI and ImII target distinct regions of the human  $\alpha$ 7 nicotinic acetylcholine receptor and distinguish human nicotinic receptor subtypes. *Biochemistry* 43:16019–16026.
- Ellison M, Haberlandt C, Gomez-Casati ME, Watkins M, Elgoyhen AB, McIntosh JM, and Olivera BM (2006)  $\alpha$ -RgIA: a novel conotoxin that specifically and potently blocks the  $\alpha 9\alpha 10$  nAChR. *Biochemistry* **45**:1511–1517.
- England LJ, Imperial J, Jacobsen R, Craig AG, Gulyas J, Akhtar M, Rivier J, Julius D, and Olivera BM (1998) Inactivation of a serotonin-gated ion channel by a polypeptide toxin from marine snails. Science 281:575–578.
- England S and de Groot MJ (2009) Subtype-selective targeting of voltage-gated sodium channels. Br J Pharmacol 158:1413–1425.
- Everhart D, Reiller E, Mirzoian A, McIntosh JM, Malhotra A, and Luetje CW (2003) Identification of residues that confer  $\alpha$ -conotoxin-PnIA sensitivity on the  $\alpha 3$  subunit of neuronal nicotinic acetylcholine receptors. *J Pharmacol Exp Ther* **306**:664–670.
- Fainzilber M, Hasson A, Oren R, Burlingame AL, Gordon D, Spira ME, and Zlotkin E (1994a) New mollusc-specific α-conotoxins block Aplysia neuronal acetylcholine receptors. Biochemistry 33:9523–9529.

at Thammasart University on December 3,

- Fainzilber M, Kofman O, Zlotkin E, and Gordon D (1994b) 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* **269:**2574–2580.
- Fainzilber M, Lodder JC, Kits KS, Kofman O, Vinnitsky I, Van Rietschoten J, Zlotkin E, and Gordon D (1995a) A new conotoxin affecting sodium current inactivation interacts with the  $\delta$ -conotoxin receptor site. J Biol Chem 270:1123–1129.
- Fainzilber M, Lodder JC, van der Schors RC, Li KW, Yu Z, Burlingame AL, Geraerts WP, and Kits KS (1996) A novel hydrophobic ω-conotoxin blocks molluscan dihydropyridine-sensitive calcium channels. Biochemistry 35:8748–8752.
- Fainzilber M, Nakamura T, Gaathon A, Lodder JC, Kits KS, Burlingame AL, and Zlotkin E (1995b) A new cysteine framework in sodium channel blocking conotoxins. Biochemistry 34:8649–8656.
- Fainzilber M, van der Schors R, Lodder JC, Li KW, Geraerts WP, and Kits KS (1995c) New sodium channel-blocking conotoxins also affect calcium currents in *Lymnaea* neurons. *Biochemistry* **34**:5364–5371.
- Fan CX, Chen XK, Zhang C, Wang LX, Duan KL, He LL, Cao Y, Liu SY, Zhong MN, Ulens C, et al. (2003) A novel conotoxin from *Conus betulinus*, κ-BtX, unique in cysteine pattern and in function as a specific BK channel modulator. *J Biol Chem* **278**:12624–12633.
- Fang X, Djouhri L, McMullan S, Berry C, Waxman SG, Okuse K, and Lawson SN (2006) Intense isolectin-B4 binding in rat dorsal root ganglion neurons distinguishes C-fiber nociceptors with broad action potentials and high  $\rm Na_v 1.9$  expression. *J Neurosci* 26:7281–7292.
- Favreau P, Gilles N, Lamthanh H, Bournaud R, Shimahara T, Bouet F, Laboute P, Letourneux Y, Ménez A, Molgó J, et al. (2001) A new  $\omega$ -conotoxin that targets N-type voltage-sensitive calcium channels with unusual specificity. *Biochemistry* **40**:14567–14575.
- Favreau P, Krimm I, Le Gall F, Bobenrieth MJ, Lamthanh H, Bouet F, Servent D, Molgo J, Ménez A, Letourneux Y, et al. (1999) Biochemical characterization and nuclear magnetic resonance structure of novel α-conotoxins isolated from the venom of Conus consors. Biochemistry 38:6317–6326.
  Felts PA, Yokoyama S, Dib-Hajj S, Black JA, and Waxman SG (1997) Sodium
- channel  $\alpha$ -subunit mRNAs I, II, III, NaG, Na6 and hNE (PN1): different expression patterns in developing rat nervous system. Brain Res Mol Brain Res 45: 71–82.
- Feng ZP, Hamid J, Doering C, Bosey GM, Snutch TP, and Zamponi GW (2001) Residue Gly1326 of the N-type calcium channel  $\alpha 1B$  subunit controls reversibility of  $\omega$ -conotoxin GVIA and MVIIA block. *J Biol Chem* **276**:15728–15735.
- Ferber M, Al-Sabi A, Stocker M, Olivera BM, and Terlau H (2004) Identification of a mammalian target of  $\kappa$ M-conotoxin RIIIK. Toxicon 43:915–921.
- Ferber M, Sporning A, Jeserich G, DeLaCruz R, Watkins M, Olivera BM, and Terlau H (2003) A novel conus peptide ligand for K<sup>+</sup> channels. *J Biol Chem* **278**:2177–2183.
- Fertleman CR, Baker MD, Parker KA, Moffatt S, Elmslie FV, Abrahamsen B, Ostman J, Klugbauer N, Wood JN, Gardiner RM, et al. (2006) SCN9A mutations in paroxysmal extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes. Neuron 52:767–774.
- Fiedler B, Zhang MM, Buczek O, Azam L, Bulaj G, Norton RS, Olivera BM, and Yoshikami D (2008) Specificity, affinity and efficacy of t-conotoxin RXIA, an agonist of voltage-gated sodium channels Na<sub>v</sub>1.2, 1.6 and 1.7. Biochem Pharmacol 75:2334-2344.
- Flinn JP, Pallaghy PK, Lew MJ, Murphy R, Angus JA, and Norton RS (1999) Roles of key functional groups in  $\omega$ -conotoxin GVIA synthesis, structure and functional assay of selected peptide analogues. Eur J Biochem **262**:447–455.

at Thammasart University on December

ώ

- Fossat P, Dobremez E, Bouali-Benazzouz R, Favereaux A, Bertrand SS, Kilk K, Léger C, Cazalets JR, Langel U, Landry M, et al. (2010) Knockdown of L calcium channel subtypes: differential effects in neuropathic pain. J Neurosci 30:1073-
- Fozzard HA and Lipkind GM (2010) The tetrodotoxin binding site is within the outer vestibule of the sodium channel. Mar Drugs 8:219-234.
- Franco A, Pisarewicz K, Moller C, Mora D, Fields GB, and Marì F (2006) Hyperhydroxylation: a new strategy for neuronal targeting by venomous marine molluscs. Prog Mol Subcell Biol 43:83-103.
- Fry BG, Roelants K, Champagne DE, Scheib H, Tyndall JD, King GF, Nevalainen TJ, Norman JA, Lewis RJ, Norton RS, et al. (2009) The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. Annu Rev Genomics Hum
- Fukuoka T, Kobayashi K, Yamanaka H, Obata K, Dai Y, and Noguchi K (2008) Comparative study of the distribution of the  $\alpha$ -subunits of voltage-gated sodium channels in normal and axotomized rat dorsal root ganglion neurons. J Comp Neurol 510:188-206
- Furukawa H and Gouaux E (2003) Mechanisms of activation, inhibition and specificity: crystal structures of the NMDA receptor NR1 ligand-binding core. EMBO J **22:**2873-2885.
- Furukawa H, Singh SK, Mancusso R, and Gouaux E (2005) Subunit arrangement and function in NMDA receptors. Nature 438:185-192.
- Fuxe K, Von Euler G, Agnati LF, Merlo Pich E, O'Connor WT, Tanganelli S, Li XM, Tinner B, Cintra A, and Carani C (1992) Intramembrane interactions between neurotensin receptors and dopamine D2 receptors as a major mechanism for the neuroleptic-like action of neurotensin. Ann NY Acad Sci 668:186-204.
- Gandía L, Lara B, Imperial JS, Villarroya M, Albillos A, Maroto R, García AG, and Olivera BM (1997) Analogies and differences between ω-conotoxins MVIIC and MVIID: binding sites and functions in bovine chromaffin cells. Pflugers Arch 435:55-64.
- García E, Scanlon M, and Naranjo D (1999) A marine snail neurotoxin shares with scorpion toxins a convergent mechanism of blockade on the pore of voltage-gated K channels. J Gen Physiol 114:141-157.
- Gardezi SR, Taylor P, and Stanley EF (2010) Long C terminal splice variant  $\mathrm{Ca_v}2.2$ identified in presynaptic membrane by mass spectrometric analysis. Channels (Austin) 4:58-62.
- Genzen JR and McGehee DS (2005) Nicotinic modulation of GABAergic synaptic transmission in the spinal cord dorsal horn. Brain Res 1031:229-237.
- Goddard AW, Ball SG, Martinez J, Robinson MJ, Yang CR, Russell JM, and Shekhar A (2010) Current perspectives of the roles of the central norepinephrine system in anxiety and depression. Depress Anxiety 27:339-350.
- Gold MS, Weinreich D, Kim CS, Wang R, Treanor J, Porreca F, and Lai J (2003) Redistribution of Na<sub>v</sub>1.8 in uninjured axons enables neuropathic pain. J Neurosci **23:**158-166.
- Goldberg YP, MacFarlane J, MacDonald ML, Thompson J, Dube MP, Mattice M, Fraser R, Young C, Hossain S, Pape T, et al. (2007) Loss-of-function mutations in the Na<sub>v</sub>1.7 gene underlie congenital indifference to pain in multiple human populations. Clin Genet 71:311-319.
- Gowd KH, Twede V, Watkins M, Krishnan KS, Teichert RW, Bulaj G, and Olivera BM (2008) Conantokin-P, an unusual conantokin with a long disulfide loop. Toxicon 52:203-213.
- Graham RM, Perez DM, Hwa J, and Piascik MT (1996) α1-Adrenergic receptor subtypes. Molecular structure, function, and signaling. Circ Res 78:737–749.
- Gray WR, Luque A, Olivera BM, Barrett J, and Cruz LJ (1981) Peptide toxins from Conus geographus venom. J Biol Chem 256:4734-4740.
- Grishin AA, Wang CI, Muttenthaler M, Alewood PF, Lewis RJ, and Adams DJ (2010) Alpha-conotoxin AuIB isomers exhibit distinct inhibitory mechanisms and differential sensitivity to stoichiometry of alpha3beta4 nicotinic acetylcholine receptors. J Biol Chem 285:22254-22263.
- Haack JA, Kinser P, Yoshikami D, and Olivera BM (1993) Biotinylated derivatives of ω-conotoxins GVIA and MVIID: probes for neuronal calcium channels. Neuropharmacology 32:1151-1159.
- Haack JA, Rivier J, Parks TN, Mena EE, Cruz LJ, and Olivera BM (1990) Conantokin-T. A gamma-carboxyglutamate containing peptide with N-methyl-d-
- aspartate antagonist activity. J Biol Chem 265:6025–6029. Hains BC, Klein JP, Saab CY, Craner MJ, Black JA, and Waxman SG (2003) Upregulation of sodium channel Na<sub>v</sub>1.3 and functional involvement in neuronal hyperexcitability associated with central neuropathic pain after spinal cord injury. J Neurosci 23:8881-8892.
- Halai R, Clark RJ, Nevin ST, Jensen JE, Adams DJ, and Craik DJ (2009) Scanning mutagenesis of  $\alpha$ -conotoxin Vc1.1 reveals residues crucial for activity at the  $\alpha 9\alpha 10$ nicotinic acetylcholine receptor. J Biol Chem 284:20275-20284.
- Han C, Rush AM, Dib-Hajj SD, Li S, Xu Z, Wang Y, Tyrrell L, Wang X, Yang Y, and Waxman SG (2006) Sporadic onset of erythermalgia: a gain-of-function mutation in Na.1.7. Ann Neurol 59:553-558.
- Hansen SB, Sulzenbacher G, Huxford T, Marchot P, Taylor P, and Bourne Y (2005) Structures of Aplysia AChBP complexes with nicotinic agonists and antagonists reveal distinctive binding interfaces and conformations. EMBO J 24:3635-3646.
- Härterich S, Koschatzky S, Einsiedel J, and Gmeiner P (2008) Novel insights into GPCR-peptide interactions: mutations in extracellular loop 1, ligand backbone methylations and molecular modeling of neurotensin receptor 1. Bioorg Med Chem 16:9359-9368.
- Harvey SC, McIntosh JM, Cartier GE, Maddox FN, and Luetje CW (1997) Determinants of specificity for  $\alpha$ -conotoxin MII on  $\alpha 3\beta 2$  neuronal nicotinic receptors. Mol Pharmacol 51:336-342.
- Hasson A, Shon KJ, Olivera BM, and Spira ME (1995) Alterations of voltageactivated sodium current by a novel conotoxin from the venom of Conus gloriamaris. J Neurophysiol 73:1295-1301.
- Heinke B, Balzer E, and Sandkühler J (2004) Pre- and postsynaptic contributions of voltage-dependent  ${\rm Ca^{2+}}$  channels to nociceptive transmission in rat spinal lamina I neurons. Eur J Neurosci 19:103-111.

- Heinke B, Gingl E, and Sandkühler J (2011) Multiple targets of  $\mu$ -opioid receptormediated presynaptic inhibition at primary afferent Aδ- and C-fibers. J Neurosci 31:1313-1322.
- Henderson LP, Gdovin MJ, Liu C, Gardner PD, and Maue RA (1994) Nerve growth factor increases nicotinic ACh receptor gene expression and current density in wild-type and protein kinase A-deficient PC12 cells. J Neurosci 14:1153-1163.
- Henriques ST and Craik DJ (2010) Cyclotides as templates in drug design. Drug Discov Today 15:57-64.
- Herlitze S, Garcia DE, Mackie K, Hille B, Scheuer T, and Catterall WA (1996) Modulation of Ca<sup>2+</sup> channels by G-protein beta gamma subunits. Nature 380:258-
- Hermans E and Maloteaux JM (1998) Mechanisms of regulation of neurotensin receptors. Pharmacol Ther 79:89-104.
- Hieble JP and Ruffolo RR, Jr. (1996) The use of  $\alpha$ -adrenoceptor antagonists in the pharmacological management of benign prostatic hypertrophy: an overview. Pharmacol Res 33:145-160.
- Hildebrand ME, Smith PL, Bladen C, Eduljee C, Xie JY, Chen L, Fee-Maki M, Doering CJ, Mezeyova J, Zhu Y, et al. (2011) A novel slow-inactivation-specific ion channel modulator attenuates neuropathic pain. Pain 152:833-843.
- Hill JM, Alewood PF, and Craik DJ (1997) Solution structure of the sodium channel antagonist conotoxin GS: a new molecular caliper for probing sodium channel geometry. Structure 5:571-583.
- Hillyard DR, Monje VD, Mintz IM, Bean BP, Nadasdi L, Ramachandran J, Miljanich G, Azimi-Zoonooz A, McIntosh JM, and Cruz LJ (1992) A new *Conus* peptide ligand for mammalian presynaptic Ca<sup>2+</sup> channels. *Neuron* **9:**69–77.

  Hogg RC, Miranda LP, Craik DJ, Lewis RJ, Alewood PF, and Adams DJ (1999)
- Single amino acid substitutions in α-conotoxin PnIA shift selectivity for subtypes of the mammalian neuronal nicotinic acetylcholine receptor. J Biol Chem 274: 36559-36564.
- Holford M, Zhang MM, Gowd KH, Azam L, Green BR, Watkins M, Ownby JP, Yoshikami D, Bulaj G, and Olivera BM (2009) Pruning nature: Biodiversityderived discovery of novel sodium channel blocking conotoxins from Conus bullatus. Toxicon 53:90-98.
- Hopkins C, Grilley M, Miller C, Shon KJ, Cruz LJ, Gray WR, Dykert J, Rivier J, Yoshikami D, and Olivera BM (1995) A new family of Conus peptides targeted to the nicotinic acetylcholine receptor. J Biol Chem 270:22361-22367.
- Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, and Humphrey PP (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol Rev 46:157–203.
- Huang X, Dong F, and Zhou HX (2005) Electrostatic recognition and induced fit in the  $\kappa$ -PVIIA toxin binding to Shaker potassium channel. J Am Chem Soc 127: 6836 - 6849.
- Hui K, Lipkind G, Fozzard HA, and French RJ (2002) Electrostatic and steric contributions to block of the skeletal muscle sodium channel by  $\mu$ -conotoxin. J Gen Physiol 119:45-54.
- Hui K, McIntyre D, and French RJ (2003) Conotoxins as sensors of local pH and electrostatic potential in the outer vestibule of the sodium channel. J Gen Physiol 122:63-79.
- Ikeda H, Heinke B, Ruscheweyh R, and Sandkühler J (2003) Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia. Science 299:1237-1240.
- Ikeda H, Stark J, Fischer H, Wagner M, Drdla R, Jäger T, and Sandkühler J (2006) Synaptic amplifier of inflammatory pain in the spinal dorsal horn. Science 312:
- Ikeda SR (1996) Voltage-dependent modulation of N-type calcium channels by Gprotein  $\beta \gamma$  subunits. Nature **380:**255–258.
- Imperial JS, Bansal PS, Alewood PF, Daly NL, Craik DJ, Sporning A, Terlau H, López-Vera E, Bandyopadhyay PK, and Olivera BM (2006) A novel conotoxin inhibitor of Kv1.6 channel and nAChR subtypes defines a new superfamily of conotoxins. Biochemistry 45:8331-8340.
- Jacobsen R, Yoshikami D, Ellison M, Martinez J, Gray WR, Cartier GE, Shon KJ, Groebe DR, Abramson SN, Olivera BM, et al. (1997) Differential targeting of nicotinic acetylcholine receptors by novel  $\alpha$ A-conotoxins. J Biol Chem 272:22531-
- Jagodic MM, Pathirathna S, Joksovic PM, Lee W, Nelson MT, Naik AK, Su P, Jevtovic-Todorovic V, and Todorovic SM (2008) Upregulation of the T-type calcium current in small rat sensory neurons after chronic constrictive injury of the sciatic nerve. J Neurophysiol 99:3151-3156.
- Jagodic MM, Pathirathna S, Nelson MT, Mancuso S, Joksovic PM, Rosenberg ER, Bayliss DA, Jevtovic-Todorovic V, and Todorovic SM (2007) Cell-specific alterations of T-type calcium current in painful diabetic neuropathy enhance excitability of sensory neurons. J Neurosci 27:3305-3316.
- Jarvis MF, Honore P, Shieh CC, Chapman M, Joshi S, Zhang XF, Kort M, Carroll W, Marron B, Atkinson R, et al. (2007) A-803467, a potent and selective  $\rm Na_v 1.8$  sodium channel blocker, attenuates neuropathic and inflammatory pain in the rat. Proc Natl Acad Sci USA 104:8520-8525.
- Jimenez EC, Donevan S, Walker C, Zhou LM, Nielsen J, Cruz LJ, Armstrong H, White HS, and Olivera BM (2002) Conantokin-L, a new NMDA receptor antagonist: determinants for anticonvulsant potency. Epilepsy Res 51:73-80.
- Jimenez EC, Olivera BM, and Teichert RW (2007) αC-conotoxin PrXA: a new family of nicotinic acetylcholine receptor antagonists. Biochemistry 46:8717-8724.
- Jimenez EC, Shetty RP, Lirazan M, Rivier J, Walker C, Abogadie FC, Yoshikami D, Cruz LJ, and Olivera BM (2003) Novel excitatory *Conus* peptides define a new conotoxin superfamily. J Neurochem 85:610-621.
- Jin AH, Daly NL, Nevin ST, Wang CI, Dutertre S, Lewis RJ, Adams DJ, Craik DJ, and Alewood PF (2008) Molecular engineering of conotoxins: the importance of loop size to  $\alpha$ -conotoxin structure and function. J Med Chem 51:5575-5584.
- Jobling P, Gibbins IL, Lewis RJ, and Morris JL (2004) Differential expression of calcium channels in sympathetic and parasympathetic preganglionic inputs to neurons in paracervical ganglia of guinea-pigs. *Neuroscience* **127**:455–466. Kaneko S, Cooper CB, Nishioka N, Yamasaki H, Suzuki A, Jarvis SE, Akaike A,

- Satoh M, and Zamponi GW (2002) Identification and characterization of novel human  $\mathrm{Ca_v2.2}$  ( $\alpha \mathrm{1B}$ ) calcium channel variants lacking the synaptic protein interaction site. J Neurosci 22:82–92.
- Karnovsky AM, Gotow LF, McKinley DD, Piechan JL, Ruble CL, Mills CJ, Schellin KA, Slightom JL, Fitzgerald LR, Benjamin CW, et al. (2003) A cluster of novel serotonin receptor 3-like genes on human chromosome 3. Gene 319:137–148.
- Kauferstein S, Huys I, Lamthanh H, Stöcklin R, Sotto F, Menez A, Tytgat J, and Mebs D (2003) A novel conotoxin inhibiting vertebrate voltage-sensitive potassium channels. *Toxicon* 42:43–52.
- Kauferstein S, Kendel Y, Nicke A, Coronas FI, Possani LD, Favreau P, Krizaj I, Wunder C, Kauert G, and Mebs D (2009) New conopeptides of the D-superfamily selectively inhibiting neuronal nicotinic acetylcholine receptors. *Toxicon* 54:295–301.
- Kauferstein S, Porth C, Kendel Y, Wunder C, Nicke A, Kordis D, Favreau P, Koua D, Stöcklin R, and Mebs D (2011) Venomic study on cone snails (Conus spp.) from South Africa. Toxicon 57:28–34.
- Kern SE, Allen J, Wagstaff J, Shafer SL, and Yaksh T (2007) The pharmacokinetics of the conopeptide contulakin-G (CGX-1160) after intrathecal administration: an analysis of data from studies in beagles. *Anesth Analg* 104:1514–1520.
- Khan I, Osaka H, Stanislaus S, Calvo RM, Deerinck T, Yaksh TL, and Taylor P (2003) Nicotinic acetylcholine receptor distribution in relation to spinal neurotransmission pathways. J Comp Neurol 467:44–59.
- Kim DS, Yoon CH, Lee SJ, Park SY, Yoo HJ, and Cho HJ (2001) Changes in voltage-gated calcium channel α1 gene expression in rat dorsal root ganglia following peripheral nerve injury. Brain Res Mol Brain Res 96:151–156.
- Kim JJ, Takahashi M, Ogura A, Kohno T, Kudo Y, and Sato K (1994) Hydroxyl group of Tyr13 is essential for the activity of ω-conotoxin GVIA, a peptide toxin for N-type calcium channel. J Biol Chem 269:23876–23878.
- Kim JI, Takahashi M, Ohtake A, Wakamiya A, and Sato K (1995) Tyr13 is essential for the activity of ω-conotoxin MVIIA and GVIA, specific N-type calcium channel blockers. Biochem Biophys Res Commun 206:449–454.
- Kis-Toth K, Hajdu P, Bacskai I, Szilagyi O, Papp F, Szanto A, Posta E, Gogolak P, Panyi G, and Rajnavolgyi E (2011) Voltage-gated sodium channel Na,1.7 maintains the membrane potential and regulates the activation and chemokine-induced migration of a monocyte-derived dendritic cell subset. J Immunol 187:1273-1280.
- Kits KS, Lodder JC, van der Schors RC, Li KW, Geraerts WP, and Fainzilber M (1996) Novel ω-conotoxins block dihydropyridine-insensitive high voltage-activated calcium channels in molluscan neurons. J Neurochem 67:2155–2163.
- Klein RC, Prorok M, Galdzicki Z, and Castellino FJ (2001) The amino acid residue at sequence position 5 in the conantokin peptides partially governs subunit-selective antagonism of recombinant N-methyl-D-aspartate receptors. J Biol Chem 276: 26860–26867.
- Klimis H, Adams DJ, Callaghan B, Nevin S, Alewood PF, Vaughan CW, Mozar CA, and Christie MJ (2011) A novel mechanism of inhibition of high-voltage activated calcium channels by  $\alpha$ -conotoxins contributes to relief of nerve injury-induced neuropathic pain. Pain 152:259–266.
- Koch WJ, Rockman HA, Samama P, Hamilton RA, Bond RA, Milano CA, and Lefkowitz RJ (1995) Cardiac function in mice overexpressing the β-adrenergic receptor kinase or a β ARK inhibitor. Science 268:1350-1353.
- Kolosov A, Aurini L, Williams ED, Cooke I, and Goodchild CS (2011) Intravenous injection of leconotide, an  $\omega$  conotoxin: synergistic antihyperalgesic effects with morphine in a rat model of bone cancer pain. Pain Med 12:923–941.
- Kolosov A, Goodchild CS, and Cooke I (2010) CNSB004 (Leconotide) causes antihyperalgesia without side effects when given intravenously: a comparison with ziconotide in a rat model of diabetic neuropathic pain. Pain Med 11:262–273.
- Lai J, Gold MS, Kim CS, Bian D, Ossipov MH, Hunter JC, and Porreca F (2002) Inhibition of neuropathic pain by decreased expression of the tetrodotoxinresistant sodium channel, Na<sub>v</sub>1.8. Pain 95:143–152.
- Lang PM, Burgstahler R, Haberberger RV, Sippel W, and Grafe P (2005) A Conus peptide blocks nicotinic receptors of unmyelinated axons in human nerves. Neuroreport 16:479–483.
- Lang PM, Burgstahler R, Sippel W, Irnich D, Schlotter-Weigel B, and Grafe P (2003) Characterization of neuronal nicotinic acetylcholine receptors in the membrane of unmyelinated human C-fiber axons by in vitro studies. J Neurophysiol 90:3295– 3303.
- Langer SZ (1974) Presynaptic regulation of catecholamine release. Biochem Pharmacol 23:1793–1800.
- Layer RT, Wagstaff JD, and White HS (2004) Conantokins: peptide antagonists of NMDA receptors. Curr Med Chem 11:3073–3084.
- Lee S, Kim Y, Back SK, Choi HW, Lee JY, Jung HH, Ryu JH, Suh HW, Na HS, Kim HJ, et al. (2010) Analgesic effect of highly reversible  $\omega$ -conotoxin FVIA on N type Ca<sup>2+</sup> channels. *Mol Pain* **6:**97.
- Leipold E, DeBie H, Zorn S, Borges A, Olivera BM, Terlau H, and Heinemann SH (2007)  $\mu$ O conotoxins inhibit Na $_{\rm v}$  channels by interfering with their voltage sensors in domain-2. *Channels (Austin)* 1:253–262.
- Leipold E, Hansel A, Borges A, and Heinemann SH (2006) Subtype specificity of scorpion beta-toxin Tz1 interaction with voltage-gated sodium channels is determined by the pore loop of domain 3. *Mol Pharmacol* 70:340–347.
- Leipold E, Hansel A, Olivera BM, Terlau H, and Heinemann SH (2005) Molecular interaction of δ-conotoxins with voltage-gated sodium channels. FEBS Lett 579: 3881–3884.
- Leipold E, Markgraf R, Miloslavina A, Kijas M, Schirmeyer J, Imhof D, and Heinemann SH (2011) Molecular determinants for the subtype specificity of  $\mu$ -conotoxin SIIIA targeting neuronal voltage-gated sodium channels. Neuropharmacology **61**: 105–111.
- Leo S, D'Hooge R, and Meert T (2010) Exploring the role of nociceptor-specific sodium channels in pain transmission using  $Na_v1.8$  and  $Na_v1.9$  knockout mice. Behav Brain Res 208:149–157.
- Lew MJ, Flinn JP, Pallaghy PK, Murphy R, Whorlow SL, Wright CE, Norton RS, and Angus JA (1997) Structure-function relationships of  $\omega$ -conotoxin GVIA. Synthesis,

- structure, calcium channel binding, and functional assay of alanine-substituted analogues.  $J \ Biol \ Chem \ 272:12014-12023.$
- Lewis RJ (2011) Discovery and development of the χ-conopeptide class of analgesic peptides. *Toxicon* http://dx.doi.org/10.1016/j.toxicon.2011.07.012.
- Lewis RJ and Garcia ML (2003) Therapeutic potential of venom peptides. Nat Rev Drug Discov 2:790-802.
- Lewis RJ, Nielsen KJ, Craik DJ, Loughnan ML, Adams DA, Sharpe IA, Luchian T, Adams DJ, Bond T, Thomas L, et al. (2000) Novel ω-conotoxins from Conus catus discriminate among neuronal calcium channel subtypes. J Biol Chem 275:35335–35344.
- Lewis RJ, Schroeder CI, Ekberg J, Nielsen KJ, Loughnan M, Thomas L, Adams DA, Drinkwater R, Adams DJ, and Alewood PF (2007) Isolation and structure-activity of  $\mu$ -conotoxin TIIIA, a potent inhibitor of tetrodotoxin-sensitive voltage-gated sodium channels. *Mol Pharmacol* **71**:676–685.
- Li RA, Hui K, French RJ, Sato K, Henrikson CA, Tomaselli GF, and Marbán E (2003a) Dependence of mu-conotoxin block of sodium channels on ionic strength but not on the permeating [Na $^+$ ]: implications for the distinctive mechanistic interactions between Na $^+$  and K $^+$  channel pore-blocking toxins and their molecular targets. *J Biol Chem* **278**:30912 $^-$ 30919.
- Li RA, Tsushima RG, Kallen RG, and Backx PH (1997) Pore residues critical for  $\mu$ -CTX binding to rat skeletal muscle Na $^+$  channels revealed by cysteine mutagenesis. Biophys J **73:**1874–1884.
- Li Z, He XP, Xie ZP, Dai QY, and Huang PT (2003b) Effect of new O-superfamily conotoxin SO3 on sodium and potassium currents of cultured hippocampal neurons. Brain Res 965:155–158.
- Lindia JA and Abbadie C (2003) Distribution of the voltage gated sodium channel  $\rm Na_v 1.3$ -like immunoreactivity in the adult rat central nervous system. Brain Res 960:132–141.
- Lindia JA, Köhler MG, Martin WJ, and Abbadie C (2005) Relationship between sodium channel  $\mathrm{Na_v}1.3$  expression and neuropathic pain behavior in rats. Pain
- Lipscombe D, Pan JQ, and Gray AC (2002) Functional diversity in neuronal voltage-gated calcium channels by alternative splicing of  $\text{Ca}_{\nu}\alpha 1$ . Mol Neurobiol **26:**21–44.

at Thammasart University on December

- Liu J, Wu Q, Pi C, Zhao Y, Zhou M, Wang L, Chen S, and Xu A (2007a) Isolation and characterization of a T-superfamily conotoxin from *Conus litteratus* with targeting tetrodotoxin-sensitive sodium channels. *Peptides* 28:2313–2319.
- Liu L, Chew G, Hawrot E, Chi C, and Wang C (2007b) Two potent  $\alpha 3/5$  conotoxins from piscivorous Conus achatinus. Acta Biochim Biophys Sin (Shanghai) **39:**438–444.
- Livett BG, Khalil Z, Gayler KR, Down JG, Sandall DW, and Keays DA (2008), inventors; Livett BG, assignee. Alpha conotoxin peptides with analgesic properties. U.S. patent 7,348,400. 2008 Mar 25.
- Livett BG, Sandall DW, Keays D, Down J, Gayler KR, Satkunanathan N, and Khalil Z (2006) Therapeutic applications of conotoxins that target the neuronal nicotinic acetylcholine receptor. *Toxicon* 48:810–829.
- Lluisma AO, López-Vera E, Bulaj G, Watkins M, and Olivera BM (2008) Characterization of a novel  $\psi$ -conotoxin from Conus parius Reeve. Toxicon 51:174–180.
- Loftis JM and Janowsky A (2003) The N-methyl-D-aspartate receptor subunit NR2B: localization, functional properties, regulation, and clinical implications. *Pharma-col Ther* 97:55–85.
- $\begin{array}{l} London\ C,\ Hoyt\ SB,\ Parsons\ WH,\ Williams\ BS,\ Warren\ VA,\ Tschirret-Guth\ R,\ Smith\ MM,\ Priest\ BT,\ McGowan\ E,\ Martin\ WJ,\ et\ al.\ (2008)\ Imidazopyridines:\ a\ novel class\ of\ hNa_v1.7\ channel\ blockers.\ Bioorg\ Med\ Chem\ Lett\ 18:1696-1701. \end{array}$
- López-Vera E, Aguilar MB, Schiavon E, Marinzi C, Ortiz E, Restano Cassulini R, Batista CV, Possani LD, Heimer de la Cotera EP, Peri F, et al. (2007a) Novel  $\alpha$ -conotoxins from  $Conus\ spurius$  and the  $\alpha$ -conotoxin EI share high-affinity potentiation and low-affinity inhibition of nicotinic acetylcholine receptors.  $FEBS\ J$  274:3972–3985.
- López-Vera E, Jacobsen RB, Ellison M, Olivera BM, and Teichert RW (2007b) A novel  $\alpha$  conotoxin ( $\alpha$ -PIB) isolated from *C. purpurascens* is selective for skeletal muscle nicotinic acetylcholine receptors. *Toxicon* **49:**1193–1199.
- Loughnan M, Bond T, Atkins A, Cuevas J, Adams DJ, Broxton NM, Livett BG, Down JG, Jones A, Alewood PF, et al. (1998)  $\alpha$ -Conotoxin EpI, a novel sulfated peptide from *Conus episcopatus* that selectively targets neuronal nicotinic acetylcholine receptors. *J Biol Chem* **273**:15667–15674.
- Loughnan M, Nicke A, Jones A, Schroeder CI, Nevin ST, Adams DJ, Alewood PF, and Lewis RJ (2006) Identification of a novel class of nicotinic receptor antagonists: dimeric conotoxins VxXIIA, VxXIIB, and VxXIIC from Conus vexillum. J Biol Chem 281:24745–24755.
- Loughnan ML, Nicke A, Jones A, Adams DJ, Alewood PF, and Lewis RJ (2004) Chemical and functional identification and characterization of novel sulfated  $\alpha$ -conotoxins from the cone snail *Conus anemone*. J Med Chem 47:1234–1241.
- Loughnan ML, Nicke A, Lawrence N, and Lewis RJ (2009) Novel  $\alpha$ D-conopeptides and their precursors identified by cDNA cloning define the D-conotoxin superfamily. Biochemistry 48:3717–3729.
- Lu BS, Yu F, Zhao D, Huang PT, and Huang CF (1999) Conopeptides from Conus striatus and Conus textile by cDNA cloning. Peptides 20:1139–1144.
- Lubbers NL, Campbell TJ, Polakowski JS, Bulaj G, Layer RT, Moore J, Gross GJ, and Cox BF (2005) Postischemic administration of CGX-1051, a peptide from cone snail venom, reduces infarct size in both rat and dog models of myocardial ischemia and reperfusion. J Cardiovasc Pharmacol 46:141–146.
- Luo S, Akondi KB, Zhangsun D, Wu Y, Zhu X, Hu Y, Christensen S, Dowell C, Daly NL, Craik DJ, et al. (2010) Atypical alpha-conotoxin LtIA from Conus litteratus targets a novel microsite of the alpha3beta2 nicotinic receptor. J Biol Chem 285:12355–12366.
- Luo S, Kulak JM, Cartier GE, Jacobsen RB, Yoshikami D, Olivera BM, and McIntosh JM (1998)  $\alpha$ -Conotoxin AuIB selectively blocks  $\alpha 3 \beta 4$  nicotinic acetylcholine receptors and nicotine-evoked norepinephrine release. *J Neurosci* 18:8571–8579.
- Luo S, Nguyen TA, Cartier GE, Olivera BM, Yoshikami D, and McIntosh JM (1999) Single-residue alteration in  $\alpha$ -conotoxin PnIA switches its nAChR subtype selectivity. *Biochemistry* **38**:14542–14548.

at Thammasart University on December 3,

- Maingret F, Coste B, Padilla F, Clerc N, Crest M, Korogod SM, and Delmas P (2008) Inflammatory mediators increase Na<sub>v</sub>1.9 current and excitability in nociceptors through a coincident detection mechanism. *J Gen Physiol* **131:**211–225.
- Malik DK, Baboota S, Ahuja A, Hasan S, and Ali J (2007) Recent advances in protein and peptide drug delivery systems. Curr Drug Deliv 4:141–151.
- Malmberg AB, Gilbert H, McCabe RT, and Basbaum AI (2003) Powerful antinociceptive effects of the cone snail venom-derived subtype-selective NMDA receptor antagonists conantokins G and T. Pain 101:109–116.
- Malmberg AB and Yaksh TL (1994) Voltage-sensitive calcium channels in spinal nociceptive processing: blockade of N- and P-type channels inhibits formalininduced nociception. J Neurosci 14:4882–4890.
- Malmberg AB and Yaksh TL (1995) Effect of continuous intrathecal infusion of ω-conopeptides, N-type calcium-channel blockers, on behavior and antinociception in the formalin and hot-plate tests in rats. *Pain* **60**:83–90.
- Martinez JS, Olivera BM, Gray WR, Craig AG, Groebe DR, Abramson SN, and McIntosh JM (1995)  $\alpha$ -Conotoxin EI, a new nicotinic acetylcholine receptor antagonist with novel selectivity. *Biochemistry* **34**:14519–14526.
- Massilia GR, Eliseo T, Grolleau F, Lapied B, Barbier J, Bournaud R, Molgó J, Cicero DO, Paci M, Schininà ME, et al. (2003) Contryphan-Vn: a modulator of Ca<sup>2+</sup>-dependent K<sup>+</sup> channels. *Biochem Biophys Res Commun* **303**:238–246.
- Matthews EA, Bee LA, Stephens GJ, and Dickenson AH (2007) The  $\rm Ca_v 2.3$  calcium channel antagonist SNX-482 reduces dorsal horn neuronal responses in a rat model of chronic neuropathic pain. Eur J Neurosci 25:3561–3569.
- Mayer ML and Armstrong N (2004) Structure and function of glutamate receptor ion channels. Annu Rev Physiol **66:**161–181.
- McArthur JR, Singh G, McMaster D, Winkfein R, Tieleman DP, and French RJ (2011) Interactions of key charged residues contributing to selective block of neuronal sodium channels by  $\mu$ -conotoxin KIIIA. *Mol Pharmacol* **80:**573–584. McCallum SE, Parameswaran N, Bordia T, McIntosh JM, Grady SR, and Quik M
- McCallum SE, Parameswaran N, Bordia T, McIntosh JM, Grady SR, and Quik M (2005) Decrease in  $\alpha 3^*/\alpha 6^*$  nicotinic receptors but not nicotine-evoked dopamine release in monkey brain after nigrostriatal damage. *Mol Pharmacol* **68**:737–746.
- McDonough SI, Boland LM, Mintz IM, and Bean BP (2002) Interactions among toxins that inhibit N-type and P-type calcium channels. *J Gen Physiol* 119:313–328
- McGuire D, Bowersox S, Fellmann JD, and Luther RR (1997) Sympatholysis after neuron-specific, N-type, voltage-sensitive calcium channel blockade: first demonstration of N-channel function in humans. *J Cardiovasc Pharmacol* **30**:400–403.
- McIntosh JM, Absalom N, Chebib M, Elgoyhen AB, and Vincler M (2009) α9 nicotinic acetylcholine receptors and the treatment of pain. *Biochem Pharmacol* **78:**693–702.
- McIntosh JM, Azam L, Staheli S, Dowell C, Lindstrom JM, Kuryatov A, Garrett JE, Marks MJ, and Whiteaker P (2004) Analogs of  $\alpha$ -conotoxin MII are selective for  $\alpha$ 6-containing nicotinic acetylcholine receptors. *Mol Pharmacol* **65**:944–952.
- McIntosh JM, Corpuz GO, Layer RT, Garrett JE, Wagstaff JD, Bulaj G, Vyazovkina A, Yoshikami D, Cruz LJ, and Olivera BM (2000) Isolation and characterization of a novel Conus peptide with apparent antinociceptive activity. J Biol Chem 275: 32391–32397.
- McIntosh JM, Dowell C, Watkins M, Garrett JE, Yoshikami D, and Olivera BM (2002) α-Conotoxin GIC from Conus geographus, a novel peptide antagonist of nicotinic acetylcholine receptors. J Biol Chem 277:33610–33615.
- McIntosh JM, Hasson A, Spira ME, Gray WR, Li W, Marsh M, Hillyard DR, and Olivera BM (1995) A new family of conotoxins that blocks voltage-gated sodium channels. *J Biol Chem* **270**:16796–16802.
- McIntosh JM, Olivera BM, Cruz LJ, and Gray WR (1984)  $\gamma$ -Carboxyglutamate in a neuroactive toxin. J Biol Chem 259:14343–14346.
- McIntosh JM, Plazas PV, Watkins M, Gomez-Casati ME, Olivera BM, and Elgoyhen AB (2005) A novel  $\alpha$ -conotoxin, PeIA, cloned from *Conus pergrandis*, discriminates between rat  $\alpha 9\alpha 10$  and  $\alpha 7$  nicotinic cholinergic receptors. *J Biol Chem* **280**:30107–30112.
- McIntosh JM, Santos AD, and Olivera BM (1999) Conus peptides targeted to specific nicotinic acetylcholine receptor subtypes. Annu Rev Biochem 68:59–88.
- McIntosh JM, Yoshikami D, Mahe E, Nielsen DB, Rivier JE, Gray WR, and Olivera BM (1994) A nicotinic acetylcholine receptor ligand of unique specificity, α-conotoxin ImI. J Biol Chem 269:16733–16739.
- McIntosh M, Cruz LJ, Hunkapiller MW, Gray WR, and Olivera BM (1982) Isolation and structure of a peptide toxin from the marine snail *Conus magus*. *Arch Biochem Biophys* **218**:329–334.
- McMaster D, Kobayashi Y, and Lederis K (1992) A vasotocin-like peptide in *Aplysia kurodai* ganglia: HPLC and RIA evidence for its identity with Lys-conopressin G. *Peptides* 13:413–421.
- Messinger RB, Naik AK, Jagodic MM, Nelson MT, Lee WY, Choe WJ, Orestes P, Latham JR, Todorovic SM, and Jevtovic-Todorovic V (2009) In vivo silencing of the Ca, 3.2 T-type calcium channels in sensory neurons alleviates hyperalgesia in rats with streptozocin-induced diabetic neuropathy. Pain 145:184-195.
- Miljanich GP (2001) Ziconotide: neuronal calcium channel blocker for treating severe chronic pain. Curr Med Chem 11:3029–3040.
- Millard EL, Nevin ST, Loughnan ML, Nicke A, Clark RJ, Alewood PF, Lewis RJ, Adams DJ, Craik DJ, and Daly NL (2009) Inhibition of neuronal nicotinic acetylcholine receptor subtypes by  $\alpha$ -Conotoxin GID and analogues. *J Biol Chem* **284**: 4944–4951.
- Milne TJ, Abbenante G, Tyndall JD, Halliday J, and Lewis RJ (2003) Isolation and characterization of a cone snail protease with homology to CRISP proteins of the pathogenesis-related protein superfamily. *J Biol Chem* **278**:31105–31110.
- Möller C and Marí F (2007) A vasopressin/oxytocin-related conopeptide with γ-carboxyglutamate at position 8. Biochem J 404:413–419.
- Mondal S, Babu RM, Bhavna R, and Ramakumar S (2007) In silico detection of binding mode of J-superfamily conotoxin pl14a with Kv1.6 channel. In Silico Biol 7:175–186.
- Monje VD, Haack JA, Naisbitt SR, Miljanich G, Ramachandran J, Nasdasdi L, Olivera BM, Hillyard DR, and Gray WR (1993) A new Conus peptide ligand for Ca channel subtypes. Neuropharmacology 32:1141-1149.

- Morris JL, Ozols DI, Lewis RJ, Gibbins IL, and Jobling P (2004) Differential involvement of N-type calcium channels in transmitter release from vasoconstrictor and vasodilator neurons. *Br J Pharmacol* **141**:961–970.
- Motin L and Adams DJ (2008) ω-Conotoxin inhibition of excitatory synaptic transmission evoked by dorsal root stimulation in rat superficial dorsal horn. Neuropharmacology 55:860–864.
- Motin L, Yasuda T, Schroeder CI, Lewis RJ, and Adams DJ (2007) ω-conotoxin CVIB differentially inhibits native and recombinant N- and P/Q-type calcium channels. Eur J Neurosci 25:435–444.
- Mouillac B, Chini B, Balestre MN, Jard S, Barberis C, Manning M, Tribollet E, Trumpp-Kallmeyer S, Hoflack J, and Elands J (1995) Identification of agonist binding sites of vasopressin and oxytocin receptors. Adv Exp Med Biol 395:301– 310
- Mould J, Yasuda T, Schroeder CI, Beedle AM, Doering CJ, Zamponi GW, Adams DJ, and Lewis RJ (2004) The  $\alpha$ 2 $\delta$  auxiliary subunit reduces affinity of  $\omega$ -conotoxins for recombinant N-type (Ca $_{\alpha}$ 2.2) calcium channels. J Biol Chem 279:34705–34714.
- Murakami M, Nakagawasai O, Suzuki T, Mobarakeh II, Sakurada Y, Murata A, Yamadera F, Miyoshi I, Yanai K, Tan-No K, et al. (2004) Antinociceptive effect of different types of calcium channel inhibitors and the distribution of various calcium channel  $\alpha 1$  subunits in the dorsal horn of spinal cord in mice. Brain Res 1024:122–129.
- Myers RA, Zafaralla GC, Gray WR, Abbott J, Cruz LJ, and Olivera BM (1991) α-Conotoxins, small peptide probes of nicotinic acetylcholine receptors. Biochemistry 30:9370–9377.
- Nadasdi L, Yamashiro D, Chung D, Tarczy-Hornoch K, Adriaenssens P, and Ramachandran J (1995) Structure-activity analysis of a *Conus* peptide blocker of N-type neuronal calcium channels. *Biochemistry* **34**:8076–8081.
- Nassar MA, Baker MD, Levato A, Ingram R, Mallucci G, McMahon SB, and Wood JN (2006) Nerve injury induces robust allodynia and ectopic discharges in  $\rm Na_v 1.3~null~mutant~mice.~\it Mol~Pain~2:33.$
- Nassar MA, Levato A, Stirling LC, and Wood JN (2005) Neuropathic pain develops normally in mice lacking both  $Na_v1.7$  and  $Na_v1.8$ . Mol Pain 1:24.
- Nassar MA, Stirling LC, Forlani G, Baker MD, Matthews EA, Dickenson AH, and Wood JN (2004) Nociceptor-specific gene deletion reveals a major role for Na $_{\rm v}$ 1.7 (PN1) in acute and inflammatory pain. *Proc Natl Acad Sci USA* **101**:12706–12711.
- Nevin ST, Clark RJ, Klimis H, Christie MJ, Craik DJ, and Adams DJ (2007) Are  $\alpha 9 \alpha 10$  nicotinic acetylcholine receptors a pain target for  $\alpha$ -conotoxins? Mol Pharmacol 72:1406–1410.
- Nicke A, Loughnan ML, Millard EL, Alewood PF, Adams DJ, Daly NL, Craik DJ, and Lewis RJ (2003a) Isolation, structure, and activity of GID, a novel  $\alpha 4$ /7-conotoxin with an extended N-terminal sequence. *J Biol Chem* **278**:3137–3144.
- Nicke A, Samochocki M, Loughnan ML, Bansal PS, Maelicke A, and Lewis RJ (2003b) α-Conotoxins EpI and AuIB switch subtype selectivity and activity in native versus recombinant nicotinic acetylcholine receptors. FEBS Lett 554:219– 223.
- Nielsen CK, Lewis RJ, Alewood D, Drinkwater R, Palant E, Patterson M, Yaksh TL, McCumber D, and Smith MT (2005) Anti-allodynic efficacy of the  $\chi$ -conopeptide, Xen2174, in rats with neuropathic pain. Pain 118:112–124.
- Nielsen DB, Dykert J, Rivier JE, and McIntosh JM (1994) Isolation of Lysconopressin-G from the venom of the worm-hunting snail, Conus imperialis. Toxicon 32:845–848.
- Nielsen KJ, Adams D, Thomas L, Bond T, Alewood PF, Craik DJ, and Lewis RJ (1999a) Structure-activity relationships of  $\omega$ -conotoxins MVIIA, MVIIC and 14 loop splice hybrids at N and P/Q-type calcium channels. J Mol Biol 289:1405–1421.
- Nielsen KJ, Skjaerbaek N, Dooley M, Adams DA, Mortensen M, Dodd PR, Craik DJ, Alewood PF, and Lewis RJ (1999b) Structure-activity studies of conantokins as human N-methyl-D-aspartate receptor modulators. J Med Chem 42:415–426.
- Nielsen KJ, Thomas L, Lewis RJ, Alewood PF, and Craik DJ (1996) A consensus structure for  $\omega$ -conotoxins with different selectivities for voltage-sensitive calcium channel subtypes: comparison of MVIIA, SVIB and SNX-202. *J Mol Biol* **263**:297–310.
- Niesler B, Frank B, Kapeller J, and Rappold GA (2003) Cloning, physical mapping and expression analysis of the human 5-HT3 serotonin receptor-like genes HTR3C, HTR3D and HTR3E. *Gene* 310:101–111.
- Nilsson KP, Lovelace ES, Caesar CE, Tynngård N, Alewood PF, Johansson HM, Sharpe IA, Lewis RJ, Daly NL, and Craik DJ (2005) Solution structure of  $\chi$ -conopeptide MrIA, a modulator of the human norepinephrine transporter. *Biopolymers* 80:815–823.
- Nudler S, Piriz J, Urbano FJ, Rosato-Siri MD, Renteria ES, and Uchitel OD (2003) Ca2+ channels and synaptic transmission at the adult, neonatal, and P/Q-type deficient neuromuscular junction. Ann NY Acad Sci 998:11-17.
- O'Brien BJ, Caldwell JH, Ehring GR, Bumsted O'Brien KM, Luo S, and Levinson SR (2008) Tetrodotoxin-resistant voltage-gated sodium channels Na<sub>v</sub>1.8 and Na<sub>v</sub>1.9 are expressed in the retina. *J Comp Neurol* **508**:940–951.

  Obata H, Conklin D, and Eisenach JC (2005) Spinal noradrenaline transporter
- Obata H, Conklin D, and Eisenach JC (2005) Spinal noradrenaline transporter inhibition by reboxetine and Xen2174 reduces tactile hypersensitivity after surgery in rats. Pain 113:271–276.
- Olivera BM, Cruz LJ, de Santos V, LeCheminant GW, Griffin D, Zeikus R, McIntosh JM, Galyean R, Varga J, and Gray WR (1987) Neuronal calcium channel antagonists. Discrimination between calcium channel subtypes using  $\omega$ -conotoxin from Conus magus venom. Biochemistry **26**:2086–2090.
- Olivera BM, Gray WR, Zeikus R, McIntosh JM, Varga J, Rivier J, de Santos V, and Cruz LJ (1985) Peptide neurotoxins from fish-hunting cone snails. *Science* 230: 1338–1343.
- Olivera BM, McIntosh JM, Cruz LJ, Luque FA, and Gray WR (1984) Purification and sequence of a presynaptic peptide toxin from *Conus geographus* venom. *Biochemistry* 23:5087–5090.
- Olivera BM, Miljanich GP, Ramachandran J, and Adams ME (1994) Calcium channel diversity and neurotransmitter release: the ω-conotoxins and ω-agatoxins. Annu Rev Biochem **63:**823–867.
- Olivera BM, Quik M, Vincler M, and McIntosh JM (2008) Subtype-selective cono-

- peptides targeted to nicotinic receptors: Concerted discovery and biomedical applications, Channels (Austin) 2:143-152.
- Ostman JA, Nassar MA, Wood JN, and Baker MD (2008) GTP up-regulated persistent  $\mathrm{Na}^+$  current and enhanced no ciceptor excitability require  $\mathrm{Na_v} 1.9.~J~Physiol~\bf 586:1077-1087.$
- Pande J, Szewczyk MM, and Grover AK (2010) Phage display: concept, innovations, applications and future. Biotechnol Adv 28:849-858.
- Parsons CG (2001) NMDA receptors as targets for drug action in neuropathic pain. Eur J Pharmacol 429:71-78.
- Payandeh J, Scheuer T, Zheng N, and Catterall WA (2011) The crystal structure of a voltage-gated sodium channel. Nature 475:353-358.
- Peng C, Chen W, Han Y, Sanders T, Chew G, Liu J, Hawrot E, Chi C, and Wang C (2009) Characterization of a novel  $\alpha$ 4/4-conotoxin, Qc1.2, from vermivorous Conus quercinus. Acta Biochim Biophys Sin (Shanghai) 41:858–864.
- Peng C, Liu L, Shao X, Chi C, and Wang C (2008) Identification of a novel class of conotoxins defined as V-conotoxins with a unique cysteine pattern and signal peptide sequence. Peptides 29:985-991.
- Peng C, Tang S, Pi C, Liu J, Wang F, Wang L, Zhou W, and Xu A (2006) Discovery of a novel class of conotoxin from Conus litteratus, lt14a, with a unique cysteine pattern. Peptides 27:2174-2181.
- Penn RD and Paice JA (2000) Adverse effects associated with the intrathecal administration of ziconotide. Pain 85:291-296.
- Perry DC, Mao D, Gold AB, McIntosh JM, Pezzullo JC, and Kellar KJ (2007) Chronic nicotine differentially regulates  $\alpha$ 6- and  $\beta$ 3-containing nicotinic cholinergic receptors in rat brain. J Pharmacol Exp Ther 322:306-315.
- Pertin M, Ji RR, Berta T, Powell AJ, Karchewski L, Tate SN, Isom LL, Woolf CJ, Gilliard N, Spahn DR, et al. (2005) Upregulation of the voltage-gated sodium channel  $\beta$ 2 subunit in neuropathic pain models: characterization of expression in injured and non-injured primary sensory neurons. J Neurosci 25:10970-10980.
- Petersson M (2002) Cardiovascular effects of oxytocin. Prog Brain Res 139:281–288. Pi C, Liu J, Wang L, Jiang X, Liu Y, Peng C, Chen S, and Xu A (2007) Soluble expression, purification and functional identification of a disulfide-rich conotoxin derived from Conus litteratus. J Biotechnol 128:184-193.
- Postina R, Kojro E, and Fahrenholz F (1996) Separate agonist and peptide antagonist binding sites of the oxytocin receptor defined by their transfer into the V2 vasopressin receptor. J Biol Chem 271:31593-31601.
- Priest BT, Murphy BA, Lindia JA, Diaz C, Abbadie C, Ritter AM, Liberator P, Iver LM, Kash SF, Kohler MG, et al. (2005) Contribution of the tetrodotoxin-resistant voltage-gated sodium channel Na<sub>v</sub>1.9 to sensory transmission and nociceptive behavior. Proc Natl Acad Sci USA 102:9382-9387.
- Quik M, Bordia T, Forno L, and McIntosh JM (2004) Loss of α-conotoxinMII- and A85380-sensitive nicotinic receptors in Parkinson's disease striatum. J Neurochem 88:668-679
- Quiram PA, Jones JJ, and Sine SM (1999) Pairwise interactions between neuronal  $\alpha7$  acetylcholine receptors and  $\alpha\text{-conotoxin ImI.}$  J Biol Chem 274:19517–19524.
- Quiram PA and Sine SM (1998a) Identification of residues in the neuronal  $\alpha$ 7 acetylcholine receptor that confer selectivity for conotoxin ImI. J Biol Chem **273:**11001-11006.
- Quiram PA and Sine SM (1998b) Structural elements in α-conotoxin ImI essential for binding to neuronal  $\alpha$ 7 receptors. J Biol Chem 273:11007–11011.
- Raingo J, Castiglioni AJ, and Lipscombe D (2007) Alternative splicing controls G protein-dependent inhibition of N-type calcium channels in nociceptors. Nat Neurosci 10:285-292.
- Ramilo CA, Zafaralla GC, Nadasdi L, Hammerland LG, Yoshikami D, Gray WR, Kristipati R, Ramachandran J, Miljanich G, and Olivera BM (1992) Novel  $\alpha$ - and ω-conotoxins from Conus striatus venom. Biochemistry 31:9919–9926.
- Rashid MH and Ueda H (2002) Neuropathy-specific analgesic action of intrathecal nicotinic agonists and its spinal GABA-mediated mechanism. Brain Res 953:
- Rasmussen SG, Choi HJ, Fung JJ, Pardon E, Casarosa P, Chae PS, Devree BT, Rosenbaum DM, Thian FS, Kobilka TS, et al. (2011a) Structure of a nanobodystabilized active state of the  $\beta2$  adrenoceptor. Nature 469:175–180.
- Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR, Sanishvili R, Fischetti RF, et al. (2007) Crystal structure of the human  $\beta 2$  adrenergic G-protein-coupled receptor. Nature 450: 383-387.
- Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, et al. (2011b) Crystal structure of the β2 adrenergic receptor-Gs protein complex. Nature 477:549-555.
- Rau KK, Johnson RD, and Cooper BY (2005) Nicotinic AChR in subclassified capsaicin-sensitive and -insensitive nociceptors of the rat DRG. J Neurophysiol 93:
- Raymond CK, Castle J, Garrett-Engele P, Armour CD, Kan Z, Tsinoremas N, and Johnson JM (2004) Expression of alternatively spliced sodium channel α-subunit genes. Unique splicing patterns are observed in dorsal root ganglia. J Biol Chem **279**:46234-46241.
- Reimann F, Cox JJ, Belfer I, Diatchenko L, Zaykin DV, McHale DP, Drenth JP, Dai F, Wheeler J, Sanders F, et al. (2010) Pain perception is altered by a nucleotide polymorphism in SCN9A. Proc Natl Acad Sci USA 107:5148-5153.
- Rodrigo J, Pena A, Murat B, Trueba M, Durroux T, Guillon G, and Rognan D (2007) Mapping the binding site of arginine vasopressin to V1a and V1b vasopressin receptors. Mol Endocrinol 21:512-523.
- Rosenbaum DM, Zhang C, Lyons JA, Holl R, Aragao D, Arlow DH, Rasmussen SG, Choi HJ, Devree BT, Sunahara RK, et al. (2011) Structure and function of an irreversible agonist- $\beta$ 2 adrenoceptor complex. Nature 469:236-240.
- Roza C, Laird JM, Souslova V, Wood JN, and Cervero F (2003) The tetrodotoxinresistant Na+ channel Nav1.8 is essential for the expression of spontaneous activity in damaged sensory axons of mice. J Physiol 550:921-926.
- Rugiero F, Mistry M, Sage D, Black JA, Waxman SG, Crest M, Clerc N, Delmas P, and Gola M (2003) Selective expression of a persistent tetrodotoxin-resistant Na

- current and Na<sub>v</sub>1.9 subunit in myenteric sensory neurons. J Neurosci 23:2715-
- Rycroft BK, Vikman KS, and Christie MJ (2007) Inflammation reduces the contribution of N-type calcium channels to primary afferent synaptic transmission onto NK1 receptor-positive lamina I neurons in the rat dorsal horn. J Physiol 580:883-
- Safo P, Rosenbaum T, Shcherbatko A, Choi DY, Han E, Toledo-Aral JJ, Olivera BM, Brehm P, and Mandel G (2000) Distinction among neuronal subtypes of voltageactivated sodium channels by  $\mu$ -conotoxin PIIIA. J Neurosci 20:76–80. Sala F, Nistri A, and Criado M (2008) Nicotinic acetylcholine receptors of adrenal
- chromaffin cells. Acta Physiol (Oxf) 192:203-212.
- Salzet M, Bulet P, Van Dorsselaer A, and Malecha J (1993) Isolation, structural characterization and biological function of a lysine-conopressin in the central nervous system of the pharyngobdellid leech Erpobdella octoculata. Eur J Biochem **217**:897–903
- Sandall DW, Satkunanathan N, Keays DA, Polidano MA, Liping X, Pham V, Down JG, Khalil Z, Livett BG, and Gayler KR (2003) A novel α-conotoxin identified by gene sequencing is active in suppressing the vascular response to selective stim-
- ulation of sensory nerves in vivo. Biochemistry 42:6904–6911.
  Santos AD, McIntosh JM, Hillyard DR, Cruz LJ, and Olivera BM (2004) The A-superfamily of conotoxins: structural and functional divergence. J Biol Chem 279:17596-17606.
- Sarma SP, Kumar GS, Sudarslal S, Iengar P, Ramasamy P, Sikdar SK, Krishnan KS, and Balaram P (2005) Solution structure of δ-Am2766: a highly hydrophobic δ-conotoxin from Conus amadis that inhibits inactivation of neuronal voltagegated sodium channels. Chem Biodivers 2:535-556.
- Sasaki T, Feng ZP, Scott R, Grigoriev N, Syed NI, Fainzilber M, and Sato K (1999) Synthesis, bioactivity, and cloning of the L-type calcium channel blocker  $\omega$ -conotoxin TxVII. Biochemistry 38:12876-12884.
- Satkunanathan N, Livett B, Gayler K, Sandall D, Down J, and Khalil Z (2005)  $\alpha$ -Conotoxin Vc1.1 alleviates neuropathic pain and accelerates functional recovery of injured neurones. Brain Res 1059:149-158.
- Sato K, Ishida Y, Wakamatsu K, Kato R, Honda H, Ohizumi Y, Nakamura H, Ohya M, Lancelin JM, and Kohda D (1991) Active site of  $\mu$ -conotoxin GIIIA, a peptide blocker of muscle sodium channels. J Biol Chem **266**:16989–16991.

at Thammasart University on December

- Sato K, Park NG, Kohno T, Maeda T, Kim JI, Kato R, and Takahashi M (1993) Role of basic residues for the binding of  $\omega$ -conotoxin GVIA to N-type calcium channels. Biochem Biophys Res Commun 194:1292-1296.
- Savarin P, Guenneugues M, Gilquin B, Lamthanh H, Gasparini S, Zinn-Justin S, and Ménez A (1998) Three-dimensional structure of  $\kappa$ -conotoxin PVIIA, a novel potassium channel-blocking toxin from cone snails. Biochemistry 37:5407-5416.
- Scanio MJ, Shi L, Drizin I, Gregg RJ, Atkinson RN, Thomas JB, Johnson MS, Chapman ML, Liu D, Krambis MJ, et al. (2010) Discovery and biological evaluation of potent, selective, or ally bioavailable, pyrazine-based blockers of the Na. 1.8 sodium channel with efficacy in a model of neuropathic pain. Bioorg Med Chem 18:7816-7825.
- Scanlon MJ, Naranjo D, Thomas L, Alewood PF, Lewis RJ, and Craik DJ (1997) Solution structure and proposed binding mechanism of a novel potassium channel toxin  $\kappa$ -conotoxin PVIIA. Structure 5:1585–1597.
- Schmalhofer WA, Calhoun J, Burrows R, Bailey T, Kohler MG, Weinglass AB, Kaczorowski GJ, Garcia ML, Koltzenburg M, and Priest BT (2008) ProTx-II, a selective inhibitor of Na<sub>v</sub>1.7 sodium channels, blocks action potential propagation in nociceptors. Mol Pharmacol 74:1476-1484.
- Schmidtko A, Lötsch J, Freynhagen R, and Geisslinger G (2010) Ziconotide for treatment of severe chronic pain. Lancet 375:1569-1577.
- Schroeder CI, Ekberg J, Nielsen KJ, Adams D, Loughnan ML, Thomas L, Adams DJ, Alewood PF, and Lewis RJ (2008) Neuronally  $\mu$ -conotoxins from Conus striatus utilize an α-helical motif to target mammalian sodium channels. J Biol Chem 283:21621-21628.
- Schroeder CI, Smythe ML, and Lewis RJ (2004) Development of small molecules that mimic the binding of ω-conotoxins at the N-type voltage-gated calcium channel. Mol Divers 8:127-134.
- Scott DA, Wright CE, and Angus JA (2002) Actions of intrathecal ω-conotoxins CVID, GVIA, MVIIA, and morphine in acute and neuropathic pain in the rat. Eur J Pharmacol 451:279-286.
- Shah BS, Stevens EB, Gonzalez MI, Bramwell S, Pinnock RD, Lee K, and Dixon AK (2000)  $\beta 3$ , a novel auxiliary subunit for the voltage-gated sodium channel, is expressed preferentially in sensory neurons and is upregulated in the chronic constriction injury model of neuropathic pain. Eur J Neurosci 12:3985–3990.
- Sharpe IA, Gehrmann J, Loughnan ML, Thomas L, Adams DA, Atkins A, Palant E, Craik DJ, Adams DJ, Alewood PF, et al. (2001) Two new classes of conopeptides inhibit the α1-adrenoceptor and noradrenaline transporter. Nat Neurosci 4:902-
- Sharpe IA, Palant E, Schroeder CI, Kaye DM, Adams DJ, Alewood PF, and Lewis RJ (2003a) Inhibition of the norepine phrine transporter by the venom peptide  $\chi\textsc{-MrIA}.$ Site of action, Na+ dependence, and structure-activity relationship. J Biol Chem **278:**40317-40323.
- Sharpe IA, Thomas L, Loughnan M, Motin L, Palant E, Croker DE, Alewood D, Chen S, Graham RM, Alewood PF, et al. (2003b) Allosteric α1-adrenoreceptor antagonism by the conopeptide  $\rho$ -TIA. J Biol Chem 278:34451-34457.
- Shichor I, Fainzilber M, Pelhate M, Malecot CO, Zlotkin E, and Gordon D (1996) Interactions of  $\delta$ -conotoxins with alkaloid neurotoxins reveal differences between the silent and effective binding sites on voltage-sensitive sodium channels. J Neurochem 67:2451-2460.
- Shon KJ, Grilley M, Jacobsen R, Cartier GE, Hopkins C, Gray WR, Watkins M, Hillyard DR, Rivier J, Torres J, et al. (1997) A noncompetitive peptide inhibitor of the nicotinic acetylcholine receptor from Conus purpurascens venom. Biochemistry 36:9581-9587.
- Shon KJ, Hasson A, Spira ME, Cruz LJ, Gray WR, and Olivera BM (1994) δ-Conotoxin GmVIA, a novel peptide from the venom of Conus gloriamaris. Biochemistry 33:11420-11425.

at Thammasart University on December

- Shon KJ, Olivera BM, Watkins M, Jacobsen RB, Gray WR, Floresca CZ, Cruz LJ, Hillyard DR, Brink A, Terlau H, et al. (1998a)  $\mu$ -Conotoxin PIIIA, a new peptide for discriminating among tetrodotoxin-sensitive Na channel subtypes. *J Neurosci* 18:4473–4481.
- Shon KJ, Stocker M, Terlau H, Stühmer W, Jacobsen R, Walker C, Grilley M, Watkins M, Hillyard DR, Gray WR, et al. (1998b) κ-Conotoxin PVIIA is a peptide inhibiting the shaker K<sup>+</sup> channel. *J Biol Chem* **273**:33–38.
- Smith MT, Cabot PJ, Ross FB, Robertson AD, and Lewis RJ (2002) The novel N-type calcium channel blocker, AM336, produces potent dose-dependent antinociception after intrathecal dosing in rats and inhibits substance P release in rat spinal cord slices. Pain 96:119–127.
- Sobolevsky AI, Rosconi MP, and Gouaux E (2009) X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. *Nature* 462:745–756.
- Stocker JW, Nadasdi L, Aldrich RW, and Tsien RW (1997) Preferential interaction of ω-conotoxins with inactivated N-type Ca<sup>2+</sup> channels. *J Neurosci* 17:3002–3013.
- Sudarslal S, Majumdar S, Ramasamy P, Dhawan R, Pal PP, Ramaswami M, Lala AK, Sikdar SK, Sarma SP, Krishnan KS, et al. (2003) Sodium channel modulating
- activity in a δ-conotoxin from an Indian marine snail. FEBS Lett 553:209–212. Sun D, Ren Z, Zeng X, You Y, Pan W, Zhou M, Wang L, and Xu A (2011) Structure-function relationship of conotoxin lt14a, a potential analgesic with low cytotoxicity. Peptides 32:300–305.
- Takasusuki T and Yaksh TL (2011) The effects of intrathecal and systemic gabapentin on spinal substance P release. Anesth Analg 112:971–976.
- Talley TT, Olivera BM, Han KH, Christensen SB, Dowell C, Tsigelny I, Ho KY, Taylor P, and McIntosh JM (2006)  $\alpha$ -Conotoxin OmIA is a potent ligand for the acetylcholine-binding protein as well as  $\alpha 3\beta 2$  and  $\alpha 7$  nicotinic acetylcholine receptors. *J Biol Chem* **281**:24678–24686.
- Tanaka K, Masu M, and Nakanishi S (1990) Structure and functional expression of the cloned rat neurotensin receptor. *Neuron* 4:847–854.
- Teichert RW, Jacobsen R, Terlau  $\hat{H}$ , Yoshikami D, and Olivera BM (2007a) Discovery and characterization of the short  $\kappa$ A-conotoxins: a novel subfamily of excitatory conotoxins. *Toxicon* **49:**318–328.
- Teichert RW, Jimenez EC, and Olivera BM (2005a) αS-conotoxin RVIIIA: a structurally unique conotoxin that broadly targets nicotinic acetylcholine receptors. Biochemistry 44:7897–7902.
- Teichert RW, Jimenez EC, Twede V, Watkins M, Hollmann M, Bulaj G, and Olivera BM (2007b) Novel conantokins from *Conus parius* venom are specific antagonists of N-methyl-D-aspartate receptors. *J Biol Chem* **282**:36905–36913.
- Teichert RW, López-Vera E, Gulyas J, Watkins M, Rivier J, and Olivera BM (2006) Definition and characterization of the short αA-conotoxins: a single residue determines dissociation kinetics from the fetal muscle nicotinic acetylcholine receptor. Biochemistry 45:1304-1312.
- Teichert RW, Rivier J, Dykert J, Cervini L, Gulyas J, Bulaj G, Ellison M, and Olivera BM (2004)  $\alpha$ A-Conotoxin OIVA defines a new  $\alpha$ A-conotoxin subfamily of nicotinic acetylcholine receptor inhibitors. *Toxicon* 44:207–214.
- Teichert RW, Rivier J, Torres J, Dykert J, Miller C, and Olivera BM (2005b) A uniquely selective inhibitor of the mammalian fetal neuromuscular nicotinic acetylcholine receptor. J Neurosci 25:732–736.
- Terlau H and Olivera BM (2004) Conus venoms: a rich source of novel ion channel-targeted peptides. Physiol Rev 84:41-68.
   Terlau H, Shon KJ, Grilley M, Stocker M, Stühmer W, and Olivera BM (1996)
- Terlau H, Shon KJ, Grilley M, Stocker M, Stühmer W, and Olivera BM (1996) Strategy for rapid immobilization of prey by a fish-hunting marine snail. *Nature* **381**:148–151.
- Thakor DK, Lin A, Matsuka Y, Meyer EM, Ruangsri S, Nishimura I, and Spigelman I (2009) Increased peripheral nerve excitability and local Na<sub>v</sub>1.8 mRNA upregulation in painful neuropathy. *Mol Pain* 5:14.
- Todorovic SM and Jevtovic-Todorovic V (2011) T-type voltage-gated calcium channels as targets for the development of novel pain therapies. Br J Pharmacol 163:484-495.
- Triggle DJ (2007) Calcium channel antagonists: clinical uses—past, present and future. Biochem Pharmacol 74:1–9.
- Twede VD, Teichert RW, Walker CS, Gruszczyński P, Kaźmierkiewicz R, Bulaj G, and Olivera BM (2009) Conantokin-Br from Conus brettinghami and selectivity determinants for the NR2D subunit of the NMDA receptor. Biochemistry 48:4063–4073.
- Uchitel OD, Protti DA, Sanchez V, Cherksey BD, Sugimori M, and Llinás R (1992) P-type voltage-dependent calcium channel mediates presynaptic calcium influx and transmitter release in mammalian synapses. *Proc Natl Acad Sci USA* 89: 3330–3333.
- Ueberheide BM, Fenyö D, Alewood PF, and Chait BT (2009) Rapid sensitive analysis of cysteine rich peptide venom components. *Proc Natl Acad Sci USA* **106**:6910–6915.
- Ulens C, Hogg RC, Celie PH, Bertrand D, Tsetlin V, Smit AB, and Sixma TK (2006) Structural determinants of selective  $\alpha$ -conotoxin binding to a nicotinic acetylcholine receptor homolog AChBP. *Proc Natl Acad Sci USA* **103**:3615–3620.
- Van Der Haegen A, Peigneur S, and Tytgat J (2011) Importance of position 8 in  $\mu$ -conotoxin KIIIA for voltage-gated sodium channel selectivity. FEBS J 278: 3408-3418
- Van Kesteren RE, Smit AB, De Lange RP, Kits KS, Van Golen FA, Van Der Schors RC, De With ND, Burke JF, and Geraerts WP (1995) Structural and functional evolution of the vasopressin/oxytocin superfamily: vasopressin-related conopressin is the only member present in *Lymnaea*, and is involved in the control of sexual behavior. *J Neurosci* 15:5989–5998.
- Van Wagoner RM, Jacobsen RB, Olivera BM, and Ireland CM (2003) Characterization and three-dimensional structure determination of ψ-conotoxin PIIIf, a novel noncompetitive antagonist of nicotinic acetylcholine receptors. *Biochemistry* 42: 6353-6362
- Vanegas H and Schaible H (2000) Effects of antagonists to high-threshold calcium channels upon spinal mechanisms of pain, hyperalgesia and allodynia. *Pain* 85: 9–18.
- Verdier L, Al-Sabi A, Rivier JE, Olivera BM, Terlau H, and Carlomagno T (2005)

- Identification of a novel pharmacophore for peptide toxins interacting with K<sup>+</sup> channels, J Biol Chem **280**:21246–21255.
- Vetter DE, Katz E, Maison SF, Taranda J, Turcan S, Ballestero J, Liberman MC, Elgoyhen AB, and Boulter J (2007) The al0 nicotinic acetylcholine receptor subunit is required for normal synaptic function and integrity of the olivocochlear system. *Proc Natl Acad Sci USA* 104:20594–20599.
- Vincler M and McIntosh JM (2007) Targeting the  $\alpha 9 \alpha 10$  nicotinic acetylcholine receptor to treat severe pain. Expert Opin Ther Targets 11:891–897.
- Vincler M, Wittenauer S, Parker R, Ellison M, Olivera BM, and McIntosh JM (2006) Molecular mechanism for analgesia involving specific antagonism of  $\alpha 9\alpha 10$  nicotinic acetylcholine receptors. *Proc Natl Acad Sci USA* 103:17880–17884.
- Vita N, Laurent P, Lefort S, Chalon P, Dumont X, Kaghad M, Gully D, Le Fur G, Ferrara P, and Caput D (1993) Cloning and expression of a complementary DNA encoding a high affinity human neurotensin receptor. FEBS Lett 317:139-142.
- Vita N, Oury-Donat F, Chalon P, Guillemot M, Kaghad M, Bachy A, Thurneyssen O, Garcia S, Poinot-Chazel C, Casellas P, et al. (1998) Neurotensin is an antagonist of the human neurotensin NT2 receptor expressed in Chinese hamster ovary cells. Eur J Pharmacol 360:265–272.
- Walewska A, Skalicky JJ, Davis DR, Zhang MM, Lopez-Vera E, Watkins M, Han TS, Yoshikami D, Olivera BM, and Bulaj G (2008) NMR-based mapping of disulfide bridges in cysteine-rich peptides: application to the  $\mu$ -conotoxin SxIIIA. J Am Chem Soc 130:14280–14286.
- Walker CS, Jensen S, Ellison M, Matta JA, Lee WY, Imperial JS, Duclos N, Brockie PJ, Madsen DM, Isaac JT, et al. (2009) A novel Conus snail polypeptide causes excitotoxicity by blocking desensitization of AMPA receptors. Curr Biol 19:900– 908.
- Wang L, Liu J, Pi C, Zeng X, Zhou M, Jiang X, Chen S, Ren Z, and Xu A (2009) Identification of a novel M-superfamily conotoxin with the ability to enhance tetrodotoxin sensitive sodium currents. *Arch Toxicol* 83:925–932.
- Wang L, Pi C, Liu J, Chen S, Peng C, Sun D, Zhou M, Xiang H, Ren Z, and Xu A (2008) Identification and characterization of a novel O-superfamily conotoxin from Conus litteratus. J Pept Sci 14:1077–1083.
- Wang YX, Bezprozvannaya S, Bowersox SS, Nadasdi L, Miljanich G, Mezo G, Silva D, Tarczy-Hornoch K, and Luther RR (1998) Peripheral versus central potencies of N-type voltage-sensitive calcium channel blockers. *Naunyn Schmiedebergs Arch Pharmacol* 357:159–168.
- Warne T, Moukhametzianov R, Baker JG, Nehmé R, Edwards PC, Leslie AG, Schertler GF, and Tate CG (2011) The structural basis for agonist and partial agonist action on a β1-adrenergic receptor. Nature **469**:241–244.
- Warne T, Serrano-Vega MJ, Baker JG, Moukhametzianov R, Edwards PC, Henderson R, Leslie AG, Tate CG, and Schertler GF (2008) Structure of a  $\beta$ 1-adrenergic G-protein-coupled receptor. *Nature* **454**:486–491.
- Weiss J, Pyrski M, Jacobi E, Bufe B, Willnecker V, Schick B, Zizzari P, Gossage SJ, Greer CA, Leinders-Zufall T, et al. (2011) Loss-of-function mutations in sodium channel Na<sub>v</sub>1.7 cause anosmia. *Nature* **472**:186–190.
- Wen L, Yang S, Qiao H, Liu Z, Zhou W, Zhang Y, and Huang P (2005) SO-3, a new O-superfamily conopeptide derived from *Conus striatus*, selectively inhibits N-type calcium currents in cultured hippocampal neurons. *Br J Pharmacol* **145**:728–739.
- Wermeling D, Drass M, Ellis D, Mayo M, McGuire D, O'Connell D, Hale V, and Chao S (2003) Pharmacokinetics and pharmacodynamics of intrathecal Ziconotide in chronic pain patients. *J Clin Pharmacol* **43:**624–636.
- West PJ, Bulaj G, Garrett JE, Olivera BM, and Yoshikami D (2002) μ-Conotoxin SmIIIA, a potent inhibitor of tetrodotoxin-resistant sodium channels in amphibian sympathetic and sensory neurons. *Biochemistry* 41:15388–15393.
- Westenbroek RE, Hoskins L, and Catterall WA (1998) Localization of Ca<sup>2+</sup> channel subtypes on rat spinal motor neurons, interneurons, and nerve terminals. *J Neurosci* 18:6319–6330.
- White HS, McCabe RT, Armstrong H, Donevan SD, Cruz LJ, Abogadie FC, Torres J, Rivier JE, Paarmann I, Hollmann M, et al. (2000) In vitro and in vivo characterization of conantokin-R, a selective NMDA receptor antagonist isolated from the venom of the fish-hunting snail Conus radiatus. J Pharmacol Exp Ther 292:425–432.
- Whiteaker P, Christensen S, Yoshikami D, Dowell C, Watkins M, Gulyas J, Rivier J, Olivera BM, and McIntosh JM (2007) Discovery, synthesis, and structure activity of a highly selective  $\alpha 7$  nicotinic acetylcholine receptor antagonist. *Biochemistry* 46:6628-6638.
- Whiteaker P, Marks MJ, Christensen S, Dowell C, Collins AC, and McIntosh JM (2008) Synthesis and characterization of 1251- $\alpha$ -conotoxin ArIB[V11L;V16A], a selective  $\alpha$ 7 nicotinic acetylcholine receptor antagonist. *J Pharmacol Exp Ther* 325:910–919.
- Williams BS, Felix JP, Priest BT, Brochu RM, Dai K, Hoyt SB, London C, Tang YS, Duffy JL, Parsons WH, et al. (2007) Characterization of a new class of potent inhibitors of the voltage-gated sodium channel Na<sub>v</sub>1.7. *Biochemistry* **46**:14693–14703.
- Wilson MJ, Yoshikami D, Azam L, Gajewiak J, Olivera BM, Bulaj G, and Zhang MM (2011a)  $\mu$ -Conotoxins that differentially block sodium channels Na<sub>4</sub>.1.1 through 1.8 identify those responsible for action potentials in sciatic nerve. *Proc Natl Acad Sci USA* **108**:10302–10307.
- Wilson MJ, Zhang MM, Azam L, Olivera BM, Bulaj G, and Yoshikami D (2011b)  $Na_v\beta$  subunits modulate the inhibition of  $Na_v1.8$  by the analgesic gating modifier  $\mu$ O-conotoxin MrVIB. *J Pharmacol Exp Ther* **338**:687–693.
- Winquist RJ, Pan JQ, and Gribkoff VK (2005) Use-dependent blockade of Ca<sub>v</sub>2.2 voltage-gated calcium channels for neuropathic pain. *Biochem Pharmacol* **70:**489–499.
- Wittekindt B, Malany S, Schemm R, Otvos L, Maccecchini ML, Laube B, and Betz H (2001) Point mutations identify the glutamate binding pocket of the N-methyl-daspartate receptor as major site of conantokin-G inhibition. *Neuropharmacology* 41:753–761.
- Woppmann A, Ramachandran J, and Miljanich GP (1994) Calcium channel subtypes in rat brain: biochemical characterization of the high-affinity receptors for  $\omega$ -cono-

- peptides SNX-230 (synthetic MVIIC), SNX-183 (SVIB), and SNX-111 (MVIIA). Mol Cell Neurosci 5:350-357.
- Wright CE, Hawkes AL, and Angus JA (2000) Postural hypotension following N-type Ca<sup>2+</sup> channel blockade is amplified in experimental hypertension. *J Hypertens* **18**:65–73.
- Xiao Y, Bingham JP, Zhu W, Moczydlowski E, Liang S, and Cummins TR (2008a) Tarantula huwentoxin-IV inhibits neuronal sodium channels by binding to receptor site 4 and trapping the domain ii voltage sensor in the closed configuration. J Biol Chem 283:27300-27313.
- Xiao Y, Luo X, Kuang F, Deng M, Wang M, Zeng X, and Liang S (2008b) Synthesis and characterization of huwentoxin-IV, a neurotoxin inhibiting central neuronal sodium channels. *Toxicon* 51:230–239.
- Yaksh TL (2006) Calcium channels as the rapeutic targets in neuropathic pain. J Pain 7:S13-30.
- Yanagawa Y, Abe T, Satake M, Odani S, Suzuki J, and Ishikawa K (1988) A novel sodium channel inhibitor from Conus geographus: purification, structure, and pharmacological properties. Biochemistry 27:6256-6262.
- Yao S, Zhang MM, Yoshikami D, Azam L, Olivera BM, Bulaj G, and Norton RS (2008) Structure, dynamics, and selectivity of the sodium channel blocker  $\mu$ -conotoxin SIIIA. *Biochemistry* 47:10940–10949.
- Zafaralla GC, Ramilo C, Ğray WR, Karlstrom R, Olivera BM, and Cruz LJ (1988) Phylogenetic specificity of cholinergic ligands:  $\alpha$ -conotoxin SI. *Biochemistry* 27: 7102–7105.
- Zamponi GW, Lewis RJ, Todorovic SM, Arneric SP, and Snutch TP (2009) Role of voltage-gated calcium channels in ascending pain pathways. Brain Res Rev 60: 84-89.

- Zhang H, Fu Y, Altier C, Platzer J, Surmeier DJ, and Bezprozvanny I (2006a)  $\rm Ca_v 1.2$  and  $\rm Ca_v 1.3$  neuronal L-type calcium channels: differential targeting and signaling to pCREB. Eur J Neurosci 23:2297–2310.
- Zhang MM, Fiedler B, Green BR, Catlin P, Watkins M, Garrett JE, Smith BJ, Yoshikami D, Olivera BM, and Bulaj G (2006b) Structural and functional diversities among \(\mu\)-conotoxins targeting TTX-resistant sodium channels. \(Bicchemistry \) 45:3723-3732.
- Zhang MM, Green BR, Catlin P, Fiedler B, Azam L, Chadwick A, Terlau H, McArthur JR, French RJ, Gulyas J, et al. (2007) Structure/function characterization of µ-conotoxin KIIIA, an analgesic, nearly irreversible blocker of mammalian neuronal sodium channels. J Biol Chem 282:30699–30706.
- Zhang MM, Han TS, Olivera BM, Bulaj G, and Yoshikami D (2010)  $\mu$ -Conotoxin KIIIA derivatives with divergent affinities versus efficacies in blocking voltage-gated sodium channels. *Biochemistry* **49**:4804–4812.
- Zhao D and Huang P (2000) Cloning of novel conotoxin precursor sequences from Conus pulicarius. Prog Nat Sci 10:33–36.
- Zheng G, Zhang Z, Dowell C, Wala E, Dwoskin LP, Holtman JR, McIntosh JM, and Crooks PA (2011) Discovery of non-peptide, small molecule antagonists of  $\alpha 9 \alpha 10$  nicotinic acetylcholine receptors as novel analgesics for the treatment of neuropathic and tonic inflammatory pain. Bioorg Med Chem Lett 21:2476–2479.
- Zhu S, Gao B, Deng M, Yuan Y, Luo L, Peigneur S, Xiao Y, Liang S, and Tytgat J (2010) Drosotoxin, a selective inhibitor of tetrodotoxin-resistant sodium channels. Biochem Pharmacol 80:1296-1302.
- Zorn S, Leipold E, Hansel A, Bulaj G, Olivera BM, Terlau H, and Heinemann SH (2006) The  $\mu$ O-conotoxin MrVIA inhibits voltage-gated sodium channels by associating with domain-3. FEBS Lett 580:1360–1364.

