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Review

Pharmacologically active spider peptide toxins

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Abstract. Advances in mass spectrometry and peptide biochemistry coupled to modern methods in electrophysiology have permitted the isolation and identification of numerous novel peptide toxins from animal venoms in recent years. These advances have also opened up the field of spider venom research, previously unexplored due to methodological limitations. Many peptide toxins from spider venoms share structural features, amino acid composition and consensus sequences that allow them to interact with related classes of cellular receptors. They have become increasingly useful agents for the study of voltage-sensitive and ligand-gated ion channels and the discrimination of their cellular subtypes. Spider peptide toxins have also been recognized as useful agents for their antimicrobial properties and the study of pore formation in cell membranes. Spider peptide toxins with nanomolar affinities for their receptors are thus promising pharmacological tools for understanding the physiological role of ion channels and as leads for the development of novel therapeutic agents and strategies for ion channel-related diseases. Their high insecticidal potency can also make them useful probes for the discovery of novel insecticide targets in the insect nervous system or for the development of genetically engineered microbial pesticides.

Key words. Spider; peptide; toxin; insecticide; structure; pharmacology; ion channel.

Introduction

Toxins from animal venoms have been invaluable tools in pharmacological studies of voltage-sensitive and ligand-gated ion channels [1]. Cone snail and sea anemone toxins are examples of useful, high-affinity ligands for sodium and calcium ion channels [2, 3]. Arachnid (spiders and scorpions) venoms contain toxic peptides with a large range (2–12 kDa) of molecular masses, but spider venoms apparently possess a much higher diversity of ion channel and other cell receptor antagonists than scorpion venoms [4]. Spider peptides, like other natural ligands from sea anemone, cone snail and scorpion venoms are fast becoming recognized as essential tools for the study of cellular receptors.

Small peptide toxins from spiders represent good biochemical probes because they are relatively easy to produce by chemical synthesis or recombinant means, avoiding the need for extraction from large quantities of venom, which is often not accessible. Moreover, peptide ligands of strong affinity require much lower amounts of protein target for functional assays, thus minimizing the efforts for obtaining suitable receptor preparations. Peptide toxins from spiders have proved to be useful agents for discriminating between different components of native ion channel currents, and for the molecular isolation and designation of cellular receptors. A recent boom in the study of spider venoms has resulted in the identification of many novel spider toxins for voltage- and ligand-

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gated ion channels, and of peptides acting on phospholipid membranes [5–10].

In this review, we summarize some of the most recent and important findings on the spider peptide toxins (SpPTxs) that interact with ion channel receptors or lipid membranes.

Taxonomy and constituents of venom glands

The biological and ecological diversity of spiders is immense: 37,972 spider species are grouped into 3,526 genera and 109 families [11], but few species are truly dangerous to humans and therefore the study of their venom has lagged behind that of other venomous creatures. Spiders are divided into labidognaths (araneomorphs) and orthognaths (mygalomorphs) according to the position of their chelicera. The largest spiders (tarantulas, family Theraphosidae) belong to the mygalomorphs and, with the exception of the genus *Atrax,* are far from being the most dangerous species. These are found in the araneomorphs and include black widows (*Latrodectus* spp., Theridiidae), violin or gaucho spiders (*Loxosceles* spp., Loxoscelidae) and banana spiders (*Phoneutria* spp., Ctenidae), which are responsible for many severe envenomation cases, and recorded mortality [12].

All spiders except one family (Uloboridae) possess a venom apparatus and produce neurotoxins designed to paralyze and kill their prey. Spider venoms are complex mixtures, comprising both low molecular-weight components and polypeptide toxins. These may include inorganic ions and salts, free acids, glucose, free amino acids, biogenic amines and neurotransmitters. They also comprise the acylpolyamines, composed of a hydrophobic moiety (an aromatic carboxylic acid) linked to a lateral chain which comprises one to nine aminopropyl, aminobutyl or aminopentyl units. Acylpolyamines act as voltage-dependent open-channel blockers, and perhaps as pore blockers of the ion channel associated with the glutamate receptor. They also act on voltage-sensitive calcium channels and nicotinic acetylcholine receptors [13, 14]. Acylpolyamines appear to possess essentially insecticidal activity and induce fast insect paralysis via a reversible block of the insect neuromuscular junction. Polypeptide toxins include mainly ion channel blockers, pore-forming peptides, and enzymes, and are the main components in the venom of spiders. Table 1 introduces the suborder and family

Table 1. Spider species studied and SpPTxs in their venom.

Spider	Peptide	Target*	Spider	Peptide	Target*
Suborder Mygalomorphae			**Suborder Araneomorphae**		
Family Theraphosidae			**Family Agelenidae**		
Grammostola spatula	HaTx1, 2	K^+	*Agelenopsis aperta*	ω-AgaI–IVA	Ca^{2+}
	VSTx1			μ-agatoxin 1–6	Na^+
	GsMTx2, 4	MS	*Hololena curta*	curtatoxin I–III	Na^+
	GSTxSIA	Ca^{2+}	*Paracoelotes luctuosus*	δ-PaluIT1–4	Na^+
		K^+	*Tegenaria agrestis*	TaITx1–3	?
Phrixotrichus auratus	PaTx1, 2	K^+	**Family Segestriidae**		
Stromatopelma calceata	ScTx1	K^+	*Segestria florentina*	SNX-325	Ca^{2+}
Heteroscodra maculata	HmTx1, 2	K^+	**Family Diguetidae**		
*Scodra griseipes (*now *Stromatopelma calceata)*	SGTx1	K^+	*Diguetia canities*	DTX9.2	?
Thrixopelma pruriens	ProTxI, II	Na^+	**Family Filistatidae**		
			Filistata hibernalis	DW13.3	Ca^{2+}
Psalmopoeus cambridgei	PcTx1	ASIC	**Family Ctenidae**		
Selenocosmia huwena (now	huwentoxin I	Ca^{2+}	*Phoneutria nigriventer*	PhTx4(6-1)	Na^+
Ornithoctonus huwena)	huwentoxin IV	Na^+			
Hysterocrates gigas	SNX-482	Ca^{2+}		PnTX3-4	GU
Acanthoscurria gomesiana	gomesin	PLM		PhTx3-1	K^+
				ω-PTxIIA	Ca^{2+}
Family Hexathelidae			*Cupiennus salei*	cupiennin 1–4	PLM
Hadronyche versuta	ω-ACTX-Hv1a	Ca^{2+}	**Family Plectreuridae**		
	ω-ACTX-Hv2a	Ca^{2+}	*Plectreurys tristis*	PlTxI–VIII	Ca^{2+}
	δ-ACTX-Hv1	Na^+	**Family Lycosidae**		
	J-ACTX-Hv1c	?	*Lycosa carolinensis*	lycotoxin I, II	PLM
Atrax robustus	δ-ACTX-Ar1	Na^+	**Family Oxyopidae**		
			Oxyopes kitabensis	oxyopinin 1, 2a, b	PLM
Family Cyrtaucheniidae			**Family Sparassidae**		
Aptostichus schlingeri	ApTxs	?	*Heteropoda venatoria*	HpTx1–3	K^+

* Na^+, Ca^{2+}, and K^+, sodium, calcium and potassium ion channels; PLM, phospholipid membranes; MS, mechano-sensitive ion channels; ASIC, acid-sensing ion channels; ?, unknown; GU, glutamate uptake. Reference to the toxins are given throughout the text and in table 2.

names of some spiders studied to date and the SpPTxs isolated from their venoms.

Advanced techniques for the study of spider venoms and toxins

Obtaining venoms from living spiders requires highly specialized skills to avoid contamination with other biological fluids when 'milking' or dissecting venom glands. This fact, added to the low amounts of biological material, the paucity of venom supply resources and the difficulties inherent in purifying very small amounts of the biologically active peptides, represents a daunting challenge to the study of spider venom components compared to those from other venomous animals such as snakes or scorpions. On the pharmacological side, the capability to use cDNA clones of the cellular receptors expressed in cell lines has facilitated the screening of a greater number of samples by electrophysiological techniques, and provided tools to follow the purification of active peptides. An increase in the study and discovery of novel peptide toxins from spider venoms has been due mainly to the application of new methods of high-performance liquid chromatography (HPLC) separation, and to the extensive use of sensitive mass spectrometric techniques such as matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) and electrospray tandem mass spectrometry (ESI-MS/MS) coupled to separation techniques. As an example of the combination of modern separation and spectrometric techniques in recent studies, figure 1A presents the mass spectra of the peptide toxins found in the venom of male and female *Macrothele gigas* (Hexathelidae) and figure 1B presents the HPLC chromatographic separation of the major components of the female venom.

Both MALDI and electrospray ionization techniques coupled with various mass analyzers have gained wide acceptance for the study of animal venoms [15]. Protein and peptide toxin studies have greatly benefited from the introduction of MALDI-TOF MS and the technology has been applied to various problems: crude venom characterization, peptide purification and sequencing, monitoring of biochemical modification reactions, production and refolding of synthetic and recombinant toxins, study of post-translational modifications, and disulfide bridge determination. Venom peptide mass fingerprinting has been used for spider species determination and molecular evolution studies [16, 17]. In conjunction with HPLC fractionation, MALDI-TOF profiling permits venom fingerprinting as well as fast repurification of known toxins for pharmacological studies. Exact mass measurements of peptides permits confirmation of primary sequence, determination of oxidation state, confirmation of disulfide bridge pairing and identification of post-translational modifications such as C-terminal amidation. Disulfide bridge pairing has also be determined through a combination of partial reduction/alkylation and chemical digestion for disulfide-rich spider venom toxins [6].

Nomenclature of SpPTxs

The nomenclature of SpPTxs has mainly followed the nomenclature proposed according to the receptor targets of cone snail toxins [18]. This nomenclature has been useful for classifying the SpPTxs with a known mode of action. The prefix delta (δ-) is for SpPTxs that slow inactivation of the voltage-gated sodium channels. Mu (μ-) is for SpPTxs that block voltage-gated sodium channels similarly to the sodium channel pore blockers saxitoxin and tetrodotoxin. Omega (ω-) is for SpPTxs that block voltage-gated calcium channels associated with nerve impulse transmission at the neuromuscular junction. Kappa (κ-) is for toxins that affect potassium channels and induce tremors because they block the *shaker* class of potassium channels. However the κ-prefix has not received general acceptance, since many SpPTxs would not induce those particular symptoms in mice. SpPTxs with unknown mode of action should follow the nomenclature of abbreviating the genus and species initial and arabic numeral indicating order of discovery. A useful toxin nomenclature has been used for the SpPTxs from Australian funnel-web spiders [19], and could be the starting point for the organization of the dozens of spider peptide sequences already described.

Structure of SpPTxs

Two main types of SpPTx are considered in this review: large polypeptide chains with the presence of a variable number of cysteine residues engaged in disulfide bridges, and polypeptide chains without cysteine residues.

SpPTxs with a variable number of cysteine residues appear to contain some consensus features: almost all peptide toxins from spiders have six amino acid residues between the first and second cysteine from their N terminus (underlined in table 2), which seems more conserved than the C-terminal region of the SpPTxs. Moreover, the particular amino acid composition of these polypeptides confers on SpPTxs a distinct secondary structure independent of the number of disulfide bridges. SpPTxs are mainly composed of β sheets and unordered chains. Each SpPTx seems to have a particular secondary structure conformation. As an example, both δ-palutoxin 1 and μ-agatoxin 1 share 83% similarity in their amino acid composition and are specific neuropeptides that target insect voltage-gated sodium channels. The circular dichroism (CD) spectra of δ-palutoxin 1 and μ-agatoxin 1 differ

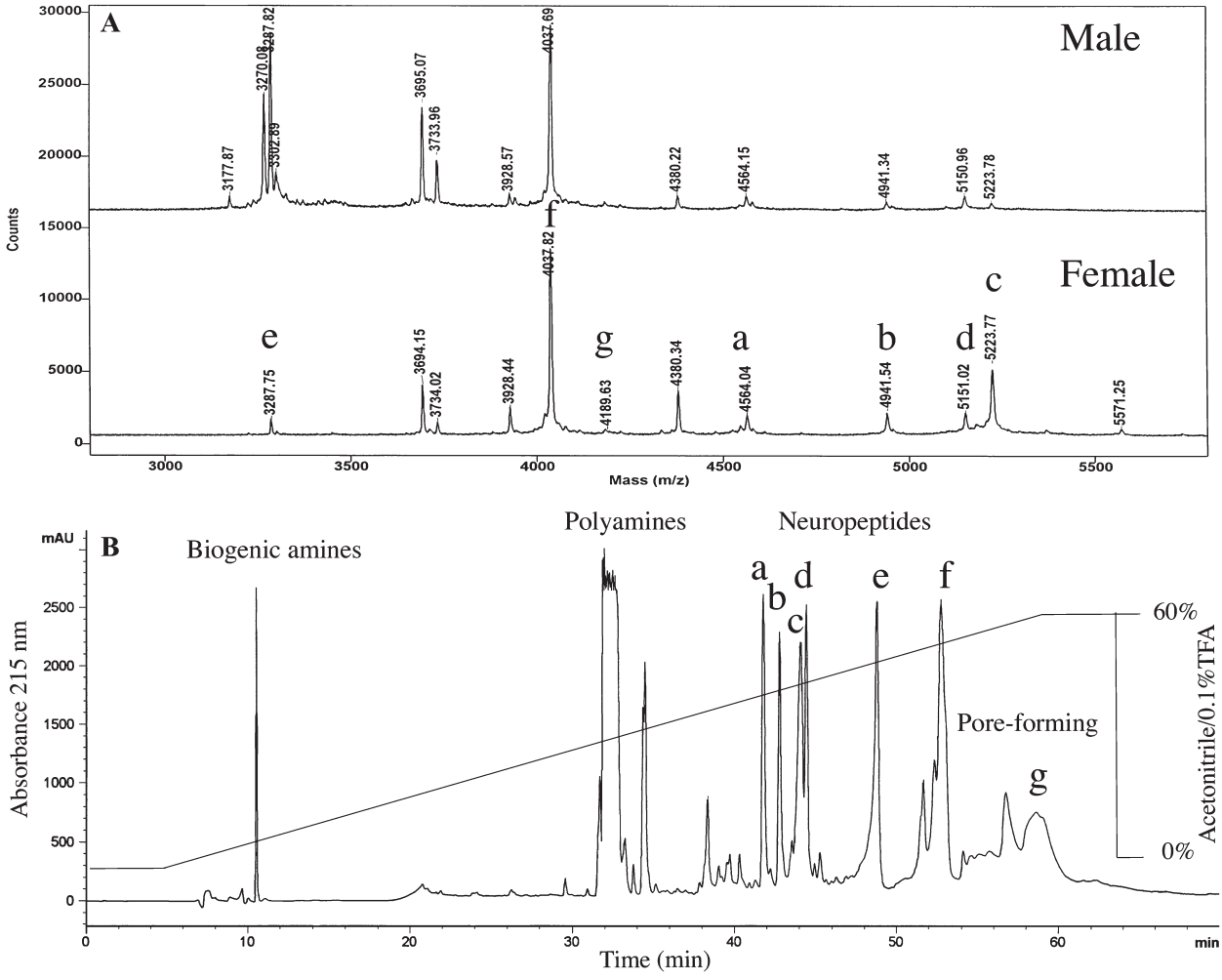

Figure 1. Mass spectrum of the crude venom of male and female *Macrothele gigas* venom (*A*) and reverse phase HPLC chromatogram of the crude female venom of *M. gigas* showing the major groups of components in the spider venom. The letters a, b, c, d, e, f and g represent the same peptide toxins in the mass/charge ratio (*A*) and retention times under RP-HPLC conditions (*B*).

substantially from each other. However, both resemble mixtures of β_I and β_{II} type proteins with negative bands in the 200–220 nm region (fig. 2A). ω-PlTxII and ω-AgaIV, two spider toxins that affect calcium channels also differ in their CD spectra. ω-PlTxII contains a significant fraction of unordered chains in the 190–200 nm region, and ω-AgaIV resembles β_I proteins (fig. 2B). Neither the amino acid sequence nor the overall secondary structure seem to be indicative of the SpPTx mode of action.

Polypeptide chains without cysteine residues in SpPTxs are represented by peptides acting on lipid membranes. They represent another structural class of peptide toxins and possess substantially more cationic residues and consequently less anionic residues than the ion channel toxins. Structural features of such peptides are different, and instead of forming complex contact surfaces, they adopt amphipathic structures in solution, allowing them to interact efficiently with cellular membranes, resulting in the formation of pores. Pore-forming polypeptides display an unordered secondary structure under aqueous solutions but they exhibit a typical α helix secondary structure under hydrophobic conditions (fig. 2C).

Most of the cysteine-containing SpPTx structures elucidated to date appear to revolve around a common structural motif consisting of a basic cystine knot that characterizes the three-dimensional structures of SpPTxs and other toxins from the venom of cone snails, scorpions and insects, as well as several protease inhibitors. The inhibitor cystine knot motif (ICK) consists of a ring formed by two disulfide bridges and the intervening polypeptide backbone, through which a third disulfide bridge passes [20]. Another component of this motif is a double- or triple-stranded, antiparallel β sheet stabilized by the cystine knot (fig. 2D). However, as pointed out by King et al. [21], to include 'a triple-stranded, anti-parallel β sheet stabilized by the cystine knot' in the definition of the ICK motif would exclude many other cystine knot SpPTxs,

Table 2. Amino acid sequences of SpPTxs affecting ion channels, glutamate uptake and phospholipid membranes.

Name	Amino acid sequences	aa	db	pI	Toxin activity	Reference
Voltage-gated						
K⁺ blockers						
Hanatoxin 1	E**C**RYLFGG**C**KTTSD**CC**KHLG**C**KFRDKY**C**AWDFTFS	35	3	8.3	Kv2.1 (shab-related) and shab1-related channels expressed in *Xenophus laevis* oocytes	24
Heteropodatoxin 1	D**C**GTIWHY**C**GTDQSE**CC**EGWK**C**SRQL**C**KYVIDW	33	3	4.8	Kv4.2 expressed in *X. laevis* oocytes	26
Phrixotoxin 1	Y**C**QKWMWT**C**DSARK**CC**EGLV**C**RLW**C**KKII	29	3	8.9	Kv4.2 and Kv4.3 in COS transfected cells and in *X. laevis* oocytes	27
ScTx1	D**C**TRMFGA**C**RRDSD**CC**PHLG**C**KPTSKY**C**AWDGTI	33	3	7.8	Kv2.1 and Kv2.2 in COS transfected cells	9
HmTx1	E**C**RYLFGG**C**SSTSD**CC**KHLS**C**RSDWKY**C**AWDGTFS	35	3	6.8	Kv4 and Kv4.1 in COS transfected cells	9
SGTx1	T**C**RYLFGG**C**KTTAD**CC**KHLA**C**RSDGKY**C**AWDGTF	34	3	8.3	Fast transient and delayed rectifier currents in rat cerebellum granular cells	31
PhTx3-1	AE**C**AAVYER**C**GKGYKR**CC**EERP**C**K**C**NIVMDN**C**T **C**KKFISE	40	4	8.2	A-type currents in GH3 cells	32
Ca²⁺ blockers						
ω-AgaIA	AKALPPGSV**C**DGNESD**C**K**C**YGKWHK**C**R**C**PWKWH FTGEGP**C**T**C**EKGMKHT**C**ITKLH**C**PNKAEW GLDW-SP**C**	69	5	8.4	L-type in rat dorsal root ganglion neurons	36, 116, 117
ω-AgaIIA	G**C**IEIGGD**C**DGYQEKSY**C**Q**CC**RNNGF**C**S… incomplete	–	–	–	N-type in chick synaptosomes	36, 38
ω-AgaIIIA	S**C**IDIGGD**C**DGEKDD**C**Q**CC**RRNGY**C**S**C**YSLFGY LKSG**C**K**C**VVGTSAEFQGI**C**RRKARQ**C**YNSDP DK**C**ESHNKPKRR	76	6	8.5	L-, P/Q-, R-, N-type in rat brain synaptosomes and HEK293 transfected cells	38, 118, 119
ω-AgaIVA	KKK**C**IAKDYGR**C**KWGGTP**CC**RGRG**C**I**C**SIMGTN **C**E**C**KPRLIMEGLGLA	48	4	8.5	P/Q-type and P-type currents in cerebellar Purkinje neurons	39, 40
SNX-482	GVDKAG**C**RYMFGG**C**SVNDD**CC**PRLG**C**HSLFSY**C** AWDLTFSD	41	3	4.8	R-type current in rat neurohypophyseal nerve terminals and L-type calcium channel in transfected HEK cells	7
SNX-325	GS**C**IESGKS**C**THSRSMKNGL**CC**PKSR**C**N**C**RQIQ HRHDYLGKRKYS**C**R**C**S	49	4	9.5	N-type expressed in *X. laevis* oocytes	51
GSTxSIA	D**C**VRFWGK**C**SQTSD**CC**PHLA**C**KSKWPRNI **C**VWDGSV	36	3	8.3	N- and P/Q-type in rat hippocampal neurons	49
Huwentoxin I	A**C**KGVFDA**C**TPGKNE**CC**PNRV**C**SDKHKW**C**KWKL	33	3	8.9	N-type expressed in prostaglandin E1-differentiated NG108-15 cells	52, 53
DW13.3	AE**C**LMIGDTS**C**VPRLGRR**CC**YGAW**C**Y**C**DQQLS**C** RRVGRKRE**C**GWVEVN**C**K**C**GWSWSQRIDDWRAD YS**C**K**C**PEDQ	74	6	8.0	P/Q-, N-, L-, and R-type in *X. laevis* oocytes	55
ω-ACTX-Hv1a	SPT**C**IPSGQP**C**PYNEN**CC**SQS**C**TFKENE NGNTVKR**C**D	37	4	5.0	VSCC in abdominal ganglia neurons of cockroach *Periplaneta americana*	19
ω-ACTX-Hv2a	LLA**C**LFGNGR**C**SSNRD**CC**ELTPV**C**KRGS**C**VSSG PGLVGGILGGIL	45	4	8.4	VSCC in neurons of honeybee *Apis mellifera*	61
ω-phonetoxin IIA	S**C**INVGDF**C**DGKKDD**C**Q**CC**RDNAF**C**S**C**SVIFGY KTN**C**R**C**EVGTTATSYGI**C**MAKHK**C**GRQTT**C** TKP**C**LSKR**C**KKNH	76	7	8.8	P/Q-, N-, and R-type in BHK cell lines	57
ω-PlTxII	AD**C**SATGDT**C**DHTKK**CC**DD**C**YT**C**R**C**GTPWGAN**C** R**C**DYYKAR**C**DT-**Pa**	44	5	5.5	blocks insect pre-synaptic Ca^{2+}	43
Na⁺ blockers						
μ-Agatoxin 1	E**C**VPENGH**C**RDWY-DE**CC**EGFY**C**S**C**RQP PK**C**I**C**RNNN	36	4	5.0	induces repetitive firing of action potentials in ventrolateral muscles of *Musca domestica*	75, 120
Curtatoxin 1	S**C**VGEYGR**C**RSAY-ED**CC**DGYY**C**N**C**SQP PY**C**L**C**RNNN	36	4	4.8	induces repetitive firing of action 76 potentials in ventrolateral muscles of *Musca domestica*	
δ-Palutoxin 1	G**C**LGEGEK**C**ADWSGPS**CC**DGFY**C**S**C**RSM PY**C**R**C**RNNS	37	4	6.2	blocks inactivation of Na^+ currents in cockroach *P. Americana* axon	6

Table 2 (continued)

Name	Amino acid sequences	aa	db	pI	Toxin activity	Reference
Robustoxin	**C**AKKRNW**C**GKNED**CCC**PMK**C**IYAWYNQQGS**C**QT TITGLFKK**C**	42	4	8.9	blocks inactivation of Na^+ currents in adult rat dorsal root ganglion neurons	66, 70
Versutoxin	**C**AKKRNW**C**GKTED**CCC**PMK**C**VYAWYNEQGS**C**QS TISALWKK**C**	42	4	8.7	blocks inactivation of Na^+ currents in adult rat dorsal root ganglion neurons	67, 69
PhTx2-6	AT**C**AGQDQP**C**KET**C**D**CC**GERGE**C**V**C**GGP**C**I**C**RQ GYFWIAWYKLAN**C**KK	48	5	7.7	modifies Na^+ currents in frog *Rana catesbiana* skeletal muscle	79, 81
PhTx4(6-1)	**C**GDINAA**C**KED**C**D**CC**GYTTA**C**D**C**YWSKS**C**K**C**RE AAIVIYTAPKKKLT**C**	48	5	7.6	blocks inactivation of Na^+ currents in cockroach *P. americana* axon	82, 83
Huwentoxin IV	E**C**LEIFKA**C**NPSNDQ**CC**KSSKLV**C**SRKTRW**C**KYQI	35	3	8.9	blocks the pore of Na^+ channels in adult rat dorsal root ganglion neurons	84
ProTxI	E**C**RYWLGG**C**SAGQT**CC**KHLV**C**SRRHGW**C**VWDGTFS	35	3	8.3	Nav 1.8 and Kv 2.1 expressed in *X. laevis* oocytes, and T-type in HEK293 cells	8
ProTxII	Y**C**QKWMWT**C**DSERK**CC**EGMV**C**RLW**C**KKKW	29	3	8.9	Nav 1.8 and Kv 2.1 expressed in *X. laevis* oocytes	8
Ligand-gated						
PcTx1	ED**C**IPKWKG**C**VNRHGD**CC**EGLE**C**WKRRRSFEV**C** VPKTPKT	40	3	8.7	ASIC in sensory neurons from rat and ASIC1a expressed in *X. laevis* oocytes and COS cells	10
Mechano sensitive ion channels						
GsMTx4	G**C**LEFWWK**C**NPNDDK**CC**RPKLK**C**SKLFKL**C**NFSSA	35	3	8.9	stretch-activated channels in adult rat astrocytes	92
Glutamate uptake						
PnTX3-4	S**C**INVGDF**C**DGKKD**CC**Q**C**DRDNAF**C**S**C**SVIFGY KTN**C**R**C**E	40	4	5.0	inhibits glutamate uptake in rat synaptosomes	87
Unknown mode of action						
J-ACTX-Hv1c	AI**C**TGADRP**C**AA**CC**P**CC**PGTS**C**KAESN GVSY**C**RKDEP	37	4	6.2	unknown	98
Pore-forming						
Lycotoxin I	IWLTALKFLGKHAAKHLAKQQLSKL	25	0	10.6	phospholipids, promotes efflux of Ca^{2+} from synaptosomes, red blood cells	105
Lycotoxin II	KIKWFKTMKSIAKFIAKEQMKKHLGGE	27	0	10.2	phospholipids, promotes efflux of Ca^{2+} from synaptosomes, red blood cells	105
Cupiennin 1	GFGALFKFLAKKVAKTVAKQAAKQGAKYVVNKQME	35	0	10.3	antimicrobial analysis, red blood cells	108
Oxyopinin 1	FRGLAKLLKIGLKSFARVLKKVLPKAAKAGKAL AKSMADENAIRQQNQ	48	0	11.3	phospholipids, reduction of cell membrane resistance by opening non-selective ion channels in Sf9 cells	5
Oxyopinin 2a	GKFSVFGKILRSIAKVFKGVGKVRKQF KTASDLDKNQ	37	0	10.8	phospholipids, reduction of cell membrane resistance by opening non-selective ion channels, red blood cells	5
Gomesin	Q**C**RRL**C**YKQR**C**VTY**C**RGR	18	2	9.9	antimicrobial	110

aa, number of amino acids; db, disulfide bridges; the protein pI was calculated using pK values of amino acids described at http://us.expasy.org/tools/pi_tool.html [121–123]; **Pa**, o-palmitoylated threonine amide at the carboxy terminus; VSCC, voltage-sensitive calcium channel; ASIC, acid-sensing ion channel.

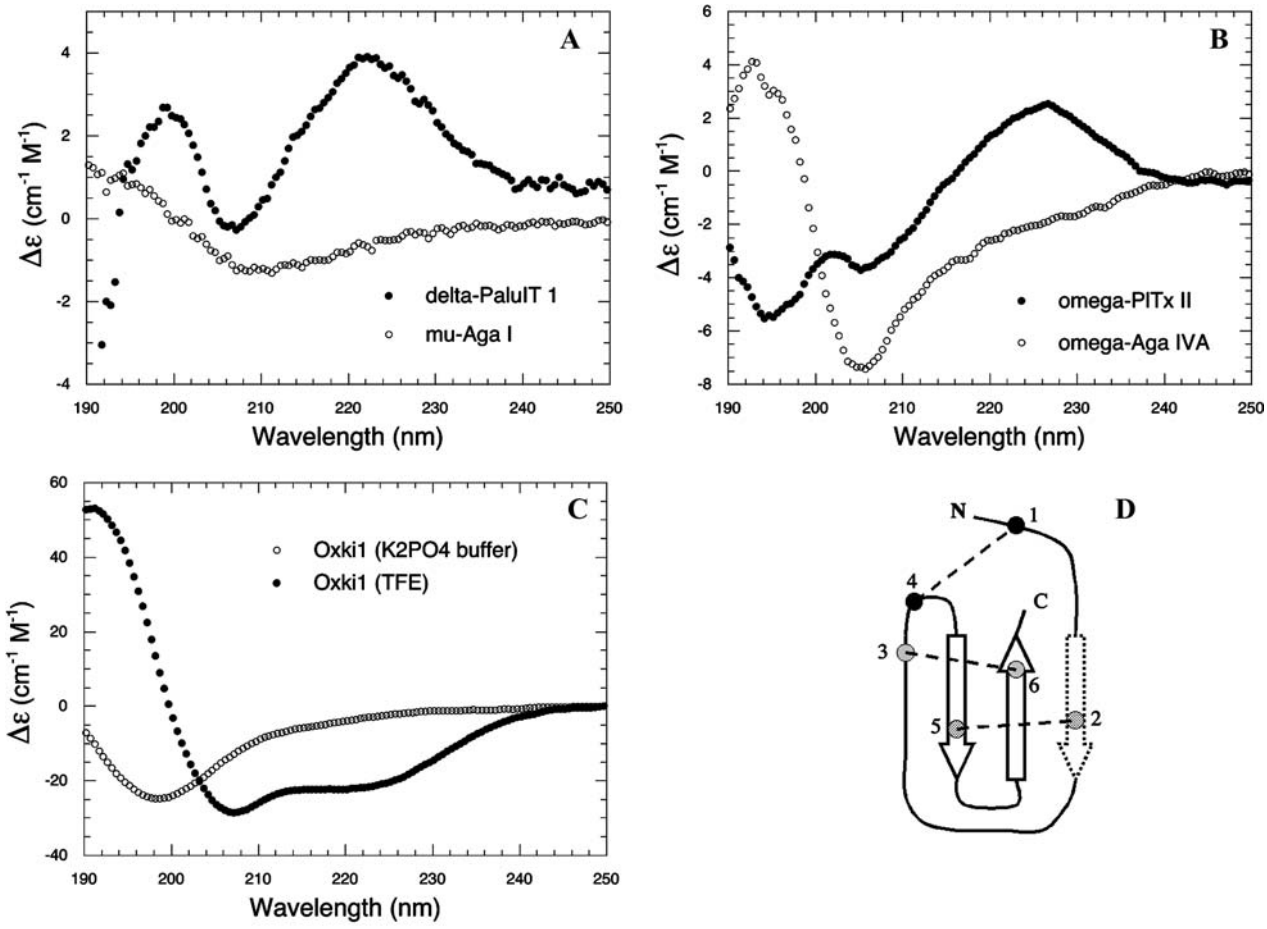

Figure 2. CD spectra of SpPTxs and the ICK motif. (*A*) CD spectra of sodium channel toxins μ-agatoxin I and δ-palutoxin 1. (*B*) CD spectra of calcium channel toxins ω-agatoxin IVA and ω-PltxII. (*C*) CD spectra of oxyopinin 1, a pore-forming toxin, in aqueous (phosphate buffer) and hydrophobic (60% trifluoroethanol) environments. (*D*) Schematic representation of the inhibitor cystine knot motif. The dotted arrow represents a β strand, which might be not found in some SpPTxs [21].

since a large number of them contain only a double-stranded β sheet lacking the first N-terminal β strand (Fig. 2D). The ICK motif appears to be one of the smallest stable globular domains found in proteins and is commonly used in nature to block the function of larger protein receptors such as ion channels or proteases [20]. Therefore, at least three disulfide bridges are one of the prerequisites for peptide toxins to belong to the ICK family. To date, SpPTxs acting on ion channel receptors have been found to contain from three to seven disulfide bridges (table 2). The presence of a variable number of disulfide bridges provides the SpPTxs with high chemical stability and resistance to both denaturation and proteolysis, but also helps in forming a contact surface in which most of the residues participate, around a generally hydrophobic core. A common feature observed in these tightly packed structures is their dipole moment, usually created by cationic and anionic amino acid residues (fig. 3A–D). The dipole moment of SpPTxs has been proposed as the major driving force permitting their diffusion toward their receptor. The dipole would orientate the toxin in the electrical field of the receptor, thus indicating the putative contact surface. However, this hypothesis remains a matter of some controversy.

Biological function of SpPTXs

SpPTxs acting on potassium channels

Potassium channels represent the largest and most diverse sub-group of ion channels and play a central role in regulating the membrane potential of cells. Members of this channel family play critical roles in cellular signaling processes regulating neurotransmitter release, heart rate, insulin secretion, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction and cell volume regulation [22]. There are several families of K^+ channels in animals, including: (i) Kv channels (voltage activated); (ii) inward rectifier channels (Kirs), which have a higher conductance for K^+ ions moving into the cell, and (iii) two-pore channels (K2P), a relatively new family of potassium channels that have two pore-lining

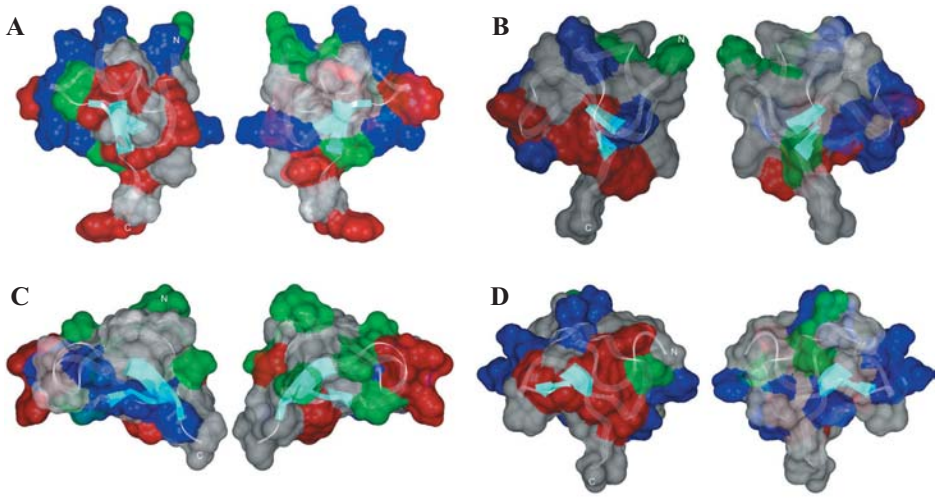

Figure 3. Three-dimensional (3D) structures of peptide toxins from spider venoms: Hanatoxin 1 (*A*), ω-grammotoxin-SIA (*B*), μ-agatoxin I (*C*), and GsMTx4 (*D*). 3D structural data were obtained from the protein data bank (http://www.rcsb.org/pdb/) and the structures were displayed with the software INSIGHT@. Charged and aromatic residues are represented in blue (basic), green (acidic) and aromatic (red). In each panel, the structure on the right represents a view after a 180° z-axis rotation.

domains, each flanked by two transmembrane domains [22]. More than 50 human genes encoding various K^+ channel subunits have been cloned during the past decade [23], and the biophysical properties of resulting channels and their modulation by ligands have been addressed.

SpPTxs have permitted, in particular, the study of new voltage-dependent potassium channels (Kv). The first SpPTxs found to block Kv channels were hanatoxins 1 and 2 (HaTx1 and 2) from the Chilean tarantula *Grammostola spatulata* [24]. HaTxs block the Kv2.1 voltage-activated K^+ channel (*shab*-related K^+ channel) with a K_d of 42 nM. HaTxs are unrelated in their primary sequence to other K^+ channel inhibitors such as Kv peptide toxins from scorpion venoms. Other Kv channels such as the *shaker*-related (Kv1-like), *shaw*-related (Kv3-like), and *eag* K^+ channels are insensitive to HaTxs, whereas a *shal*-related (Kv4-like) K^+ channel is sensitive [24]. Recently, semaphorins, which contribute to axon guidance during development of the nervous system, were found to share some amino acid similarities to hanatoxins [25]. Based on this finding, semaphorins were hypothesized to affect neuronal growth by an interaction with voltage-gated ion channels. Similar to the HaTxs, the heteropodatoxins (HpTx1 and 2) were isolated from the venom of the Malayan spider *Heteropoda venatoria* [26]. The HpTxs prolonged the action potential duration of isolated rat ventricular myocytes, suggesting that the peptides blocked K^+ currents. The toxins block the Kv4.2 in a voltage-dependent manner, with less block at more positive potentials. In addition, the HpTxs slow the time course of current activation and inactivation and shift the voltage dependence of current inactivation to more positive potentials [26]. Following that study, the phrixotoxins (PaTxs) were purified from the venom of the Chilean tarantula *Phrixotrichus auratus* [27]. Phrixotoxins specifically block Kv4.3 and Kv4.2 currents with IC_{50}s of 5–70 nM, by altering the gating properties of the channels, and also block the cardiac current *Ito*1 in isolated murine cardiomyocytes. The *shaker* (Kv1), *shab* (Kv2) and *shaw* (Kv3) sub-families of currents were not inhibited by phrixotoxins [27]. The hanatoxins, heteropodatoxins and phrixotoxins are structurally similar peptides of 28–32 amino acids and share substantial sequence homologies (table 2). More recently, other SpPTxs that inhibit voltage-dependent potassium channels in the *shab* (Kv2) and *shal* (Kv4) sub-families were isolated from the venom of the African tarantulas *Stromatopelma calceata* (ScTx1) and *Heteroscodra maculata* (HmTx1, HmTx2) [9]. ScTx1 is the first high-affinity inhibitor of the Kv2.2 channel sub-type to be described, with an IC_{50} of 21.4 nM. ScTx1 also inhibits the Kv2.1 channels, with an IC_{50} of 12.7 nM, as well as Kv2.1/Kv9.3 heteromultimers that have been proposed to be involved in O_2 sensing in pulmonary artery myocytes. HmTxs share sequence similarities with HaTxs, HpTxs and PaTxs. They inhibit potassium currents associated with Kv2 subtypes in the 100–300 nM concentration range. HmTx2 seems to be a specific inhibitor of Kv2 channels (IC_{50} of 1.2 nM), whereas HmTx1 also inhibits Kv4 channels, including Kv4.1, with the same potency [9]. The most recent addition to that family of peptides is VSTx1, the voltage-sensor toxin isolated from the venom of *G. spatulata* [28], which inhibits the archaebacterial potassium channel KvAP, demonstrating the very high conservation of voltage-sensing domains in potassium channels through molecular evolution, from prokaryotic to eukaryotic organisms. The three-dimensional structures of HaTx1 and HpTx2 have been determined by nuclear

magnetic resonance (NMR) spectroscopy (fig. 3A). HaTx1 and HpTx2 adopt an ICK motif and are composed of two β-strands connected by four loops. A comparison of the surface features of HaTx1 and HpTx2 with those of other gating modifier toxins of voltage-gated Ca^{2+} and Na^{+} channels suggests that the combination of a hydrophobic patch and surrounding charged residues may represent the structural basis of the specificity for the binding of gating modifier toxins to voltage-gated ion channels [29, 30].

Since the discovery of hanatoxins, several other toxin peptides blocking Kv channels have been isolated. However, the Kv subtypes targeted by the toxins have not been precisely determined. A toxin, named SGTx1, was purified from the venom of the tarantula *Scodra griseipes* [31]. SGTx1 reversibly inhibits more than 40% of outward potassium currents. This result is reminiscent of the effect described for hanatoxins on Kv2.1 and Kv4.2 channels. Another potassium channel blocker (PnTx3-1) has also been isolated from the venom of the South American spider *Phoneutria nigriventer* [32]. PnTx3-1 reversibly inhibits the outward-rectifying A-type K^{+} current (I_A) but does not block other K^{+} currents (delayed rectifying, inward rectifying, and large conductance Ca^{2+} sensitive) or Ca^{2+} channels. An NH_2-extended mutant of PnTx3-1 (ISEF-PnTx3-1) [33] blocks A-type K^{+} currents, possibly mediated by Kv1.4 subunits, in a manner similar to that observed with the wild-type toxin. These peptide toxins may therefore represent new ligands that target as yet unknown potassium channels or other heteromultimeric combinations of Kv subunits.

SpPTxs acting on vertebrate calcium channels

Voltage-sensitive calcium channels (VSCCs) play a crucial role in coupling the electrical activity of neurons to a variety of cellular processes such as neurotransmitter and hormone secretion, cell proliferation and gene expression [34]. To date, at least five different types of VSCC have been identified. The L-type Ca^{2+} channels are present mainly in the cardiovascular system, and are highly sensitive to non-peptide drugs such as dihydropyridines (DHPs), phenylalkylamines and benzodiazepines. The non-L-type Ca^{2+} channels (T-, N-, P/Q- and R-type) are insensitive to dihydropyridines; however, they can be antagonized by many peptide toxins from animal venoms. Such peptide drugs may therefore represent a novel generation of Ca^{2+} channel modulators with neuroprotective and analgesic properties [35].

The venom of the North American funnel-web spider *Agelenopsis aperta*, was the source of the first peptide blockers of non-L VSCCs. The peptide toxins were categorized based on their neuromuscular effects on insects and the displacement of previously identified calcium channel blockers such as the conotoxins, in chick synaptosomes [34]. Four types of polypeptide presynaptic antagonists of VSCCs (ω-agatoxins) were described. The first type, named ω-AgaIA, blocks insect neuromuscular transmission pre-synaptically, but does not inhibit the specific binding of ^{125}I-labeled ω-conotoxin GVIA to chick synaptosomal membranes. The second type, ω-AgaIIA, blocks insect neuromuscular transmission pre-synaptically as well as inhibiting the specific binding of ^{125}I-labeled ω-conotoxin GVIA to chick synaptosomal membranes [36]. The third type, ω-AgaIIIA, does not block insect neuromuscular transmission pre-synaptically [37], but inhibits the specific binding of ^{125}I-labeled ω-conotoxin GVIA to chick synaptosomal membranes [38]. The fourth type represented by ω-AgaIVA, does not block insect neuromuscular transmission pre-synaptically, and does not inhibit the specific binding of ^{125}I-labeled ω-conotoxin GVIA to chick synaptosomal membranes. However, ω-AgaIVA is a potent inhibitor of ^{45}Ca influx into rat brain synaptosomes and also blocks P-type currents in cerebellar Purkinje neurons [34, 39, 40]. Although ω-AgaIVA is a rather long peptide (>5 kDa), it has been chemically synthesized [41] and is commercially available. Therefore, because of its availability, specificity and high affinity, ω-AgaIVA has been the most important of all four types of ω-agatoxins for pharmacological investigations. The structure of ω-AgaIVA has been determined by two-dimensional 1H NMR spectroscopy, combined with simulated annealing calculations. The molecular structure of ω-agatoxin IVA is composed of a short triple-stranded antiparallel β-sheet, three loops and disordered N- and C-terminal segments. Irrespective of differences in the number of disulfide bonds and low primary sequence homology, ω-AgaIVA and ω-conotoxin GVIA, a cone snail VSCC blocker, show significant similarity in their spatial structures. Voltage-clamp recordings using rat cerebellar cells suggest that the hydrophobic C-terminal segment of ω-agatoxin IVA plays a crucial role in its blocking of the P-type calcium channel. This hydrophobic C-terminal segment does not exist in ω-conotoxin GVIA that is, conversely, a specific blocker of N-type calcium channels [42]. Prior to the discovery of ω-agatoxins, spider neurotoxins from the venom of another North American spider *Plectreurys tristis* were shown to antagonize insect and vertebrate calcium channels [43–45]. However, the primary structures of the peptide toxins were not described at that time. Four years later, the toxin PlTxII from *P. tristis* was shown to possess a peculiar post-translational modification, palmitoylation of its C-terminal threonine residue [46]. PlTxII has also been chemically synthesized and the palmitoylated peptide was proven to be identical to the natural one [47]. PlTxII is also commercially available; however, it blocks insect and vertebrate VSCCs with low affinity, and it has no particular specificity for the various types of VSCC. SNX-482, a selective peptide antagonist of R-

type and L-type VSCCs was isolated from the African tarantula *Hysterocrates gigas* [7]. At low nanomolar concentrations (~40 nM), SNX-482 blocked a native resistant (R-type) Ca^{2+} current in rat neurohypophyseal nerve terminals, but concentrations of 200–500 nM had no effect on R-type Ca^{2+} currents in several types of rat central neurons. Experiments involving chimeric channels combining structural features of R-type (alpha1E) and L-type (alpha1C) calcium channel subunits indicated that the presence of the domain III and IV of R-type is a prerequisite for a strong gating inhibition. SNX482 also has small but pronounced effects on L-type calcium channel gating [48]. Another peptide, named ω-GsTxSIA, was isolated from the venom of the tarantula *G. spatulata* [49], and blocks N-, P- and Q-type VSCCs. The three-dimensional structure of this toxin was determined by NMR spectroscopy (fig. 3B) [50]. Although GsTxSIA was originally identified as an inhibitor of voltage-gated Ca^{2+} channels, it also binds to K^+ channels although with much lower affinity. Similar binding was observed for HaTx1, which binds to the voltage-sensing domains of both K^+ and Ca^{2+} channels but with very different affinities. A comparison between HaTx1 and GsTxSIA structures (fig. 3A, B) identifies a conserved face containing a large hydrophobic patch surrounded by positively charged residues which appears to be important in binding to the receptor site on the channel, possibly located on or near the voltage-sensor. This also suggests a conserved binding domain relating to the voltage-sensing capability of ion channels of different ion selectivity as revealed by toxin binding. Another interesting N-type VSCC peptide toxin, SNX-325, was isolated from the venom of the spider *Segestria florentina* [51]. At nanomolar concentrations (IC_{50}s 3–30 nM), SNX-325 is a selective blocker of the N-type but not cardiac L-, P/Q-, or R-type calcium channels. Similarly, huwentoxin I (HWTX-I) from the venom of the Chinese bird spider *Selenocosmia huwena* [52] inhibited N-type Ca^{2+} channels and had only very weak effects on L-type Ca^{2+} channels [53]. The three-dimensional structure in aqueous solution of native huwentoxin I was determined from two-dimensional H1 NMR data recorded at 500 and 600 MHz. The molecule adopts a compact structure consisting of a small triple-stranded antiparallel β-sheet and five β-turns. A small hydrophobic patch consisting of Phe6, Trp28, and Trp31 is located on one side of the molecule [54].

Larger peptides (>8 kDa) with VSCC specificity have also been found in spider venoms. The SpPTx DW13.3 isolated from the venom of the spider *Filistata hibernalis* causes a potent but transient block of native calcium channels [55]. DW13.3 had the highest affinity for P/Q-type, followed by N- > L- > R-type with no effect on T-type currents recorded from GH3 cells. Even though blocking occurred with a dose-response with a Hill coefficient of 1.0 for all calcium channel sub-types, suggesting a single molecular target, DW13.3 produced a partial block of both alpha1A (60%) currents and P-type (76%) currents in cerebellar Purkinje cells. Selective exclusion of the P/Q-type channel ligand ω-conotoxin MVIIC (but not ω-agatoxin IVA) from its binding site in Purkinje neurons suggests that DW13.3 binds to a site close to the pore of the channel. Another large peptide, ω-phonetoxin IIA purified from the venom of the spider *P. nigriventer* [56], blocked almost irreversibly P/Q-type and N-type calcium currents at 3 nM, whereas R-type currents showed partial and readily reversible inhibition. Binding and displacement assays with mono ^{125}I-ω-PTxIIA, demonstrated that rat brain synaptosomes displayed multiple classes of binding sites. High-affinity binding of ^{125}I-ω-PTxIIA was totally displaced by ω-PTxIIA (Ki = 100 pM), but only partially by ω-conotoxin GVIA (25% inhibition) and ω-conotoxin MVIIC (50% inhibition at 0.3 μM) [57].

SpPTxs acting on insect calcium channels

The classification of vertebrate VSSC channels based on electrophysiological and pharmacological criteria is not entirely applicable to invertebrate VSCCs. For example, L-type invertebrate channels often lack the DHP sensitivity that defines an L-type vertebrate channel, and none of the invertebrate VSCCs strongly resemble their mammalian counterpart [58, 59]. To date, only a few peptide toxins have been isolated from spiders with selectivity for insect VSCCs. The ω-atracotoxins, Hv1a–f, are a family of neurotoxins that block insect but not mammalian voltage-gated calcium channels. This family of insect calcium channel blockers was isolated from the Australian funnel-web spider, *Hadronyche versuta* [19, 60, 61]. A peculiarity of this series of toxins is that their N-terminal residues are structurally disordered in solution but highly conserved in several peptide analogs. By a progression of N-terminal truncations, the N-terminal residues were proven to contribute significantly to insecticidal potency, and loss of activity did not correlate with deletion of highly conserved residues. Therefore, the nature of the N-terminal charge, rather than the chemical properties of the N-terminal residues themselves, was suggested to be critical for the insecticidal activity of ω-atracotoxins [19, 60]. Several other blockers of insect voltage-gated calcium channels have also been isolated from the Australian hexathelids *H. versuta*, *Hadronyche infensa* and *Atrax* sp. [61]. The ω-atracotoxins Hv2, Hi2a–b and As2a–b are a family of peptides that display exceptional phylogenetic specificity, with at least a 10,000-fold preference for insect versus vertebrate calcium channels. Contrary to the structure of the Hv1a ω-atracotoxin, Hv2a ω-atracotoxins have a structurally disordered C-terminal extension that may be essential for channel-blocking activity.

SpPTxs acting on sodium channels

Voltage-activated Na^+ channels (Nav), together with K^+ channels represent the physiological basis of signal transmission in the nervous system. Their coordinated function is essential for efficient impulse generation and propagation in the central and peripheral nervous system. There are at least ten different genes encoding distinct sodium channels in mammals and one or two genes in invertebrate species [62]. The structural diversity of sodium channels appears to be paralleled by their diversity in physiological and pharmacological properties [63]. Sodium channel organization in the neuron might affect nerve activity, leading to sensory and motor dysfunction. In humans, this could contribute to the pathophysiological mechanism of several neurological diseases, such as multiple sclerosis, epilepsy, stroke, peripheral neuropathies and neuropathic pain [64]. Scorpion, sea anemone and cone snail peptide toxins have significantly contributed to the understanding of the topology and pharmacology of sodium channels [65]. SpPTxs as sodium channel ligands and current modifiers could also substantially contribute to the understanding of sodium channel complexity and physiological role in the nervous system.

The first sodium channel SpPTxs isolated were δ-atracotoxin Ar1 (robustoxin) and δ-atracotoxin Hv1 (versutoxin) from the venoms of the hexathelid spiders *Atrax robustus* and *H. versuta,* respectively [66, 67]. The Australian funnel-web spiders *A. robustus* and *H. versuta* are dangerous species, with respiratory failure observed after human envenomation by *A. robustus* [68]. δ-Atracotoxin Ar1 and Hv1 have the unique characteristic of a cysteine triplet at residues 14–16 (table 2). They consist of a small, triple-stranded, antiparallel β-sheet and several reverse turns. δ-Atracotoxins Ar1 and Hv1 slow the inactivation of tetrodotoxin (TTX)-sensitive (TTX-S) sodium channels [69, 70], and bind to site 3 of rat and insect sodium channels, displacing with nanomolar affinity the scorpion insecticidal alpha-toxins [71]. They show no structural homology with either sea anemone or alpha-scorpion toxins that modify the inactivation kinetics of voltage-gated sodium channels also by interacting with the site 3 of sodium channels. Nevertheless, δ-atracotoxin Ar1 and Hv1 contain charged residues that are topologically related to those implicated in the binding of sea anemone and alpha-scorpion toxins to mammalian voltage-gated sodium channels, suggesting similarities in their mode of binding with these channels [72–74]. A similar group of SpPTxs that affect only insect sodium channels has been found in the venom of the North American spiders *A. aperta* (μ-agatoxins 1–6, fig. 3C) and *Hololena curta* (curtatoxins), and in the venom of the Asian spider *Paracoelotes luctuosus* (δ-palutoxins 1-4) [6, 75, 76]. These agelenid toxins increase the sodium influx through insect sodium channels by slowing down their inactivation. The highly specific toxicity of these peptides for insects has led to the proposal of using them as biopesticides, using transgenic baculoviruses as delivery vectors [77]. The structural data shows that the μ-agatoxins (fig. 3C) share common secondary and tertiary structural motifs with phylogenetically diverse peptide toxins such as cone snail and scorpion toxins targeting calcium and potassium channels [78].

Another structurally distinct group of SpPTxs, from the Brazilian spider *P. nigriventer,* also targets sodium channels [79]. This series of peptides from the toxic fraction PhTx2 of the venom of *P. nigriventer* prolongs the inactivation and deactivation processes of sodium ion channels in vertebrate muscles [80, 81]. A toxin named Tx4(6-1), from the PhTx4 toxic fraction of venom from the same spider has also been described [82]. Tx4(6-1) affects the peripheral nervous system of insects by stimulating glutamate release at the neuromuscular junction. It competes with the scorpion alpha-like toxin ^{125}I-BomIV for binding to site 3 of insect voltage-activated sodium channels (Nav) with an IC_{50} value of 25 nM. Tx4(6-1) prolongs evoked axonal action potentials due to the slowing down of sodium current inactivation. The effects of Tx4(6-1) on the sodium current were compared with those of a typical scorpion alpha-toxin and the spider δ-palutoxins and δ-atracotoxins on insect Nav channels. Tx4(6-1) appears to be insect selective, being ineffective on mammalian Nav channels [83]. Recently, another sodium channel neurotoxin from the venom of the Chinese bird spider *S. huwena*, which has been named huwentoxin IV, was described [84]. It specifically inhibits the neuronal tetrodotoxin-sensitive (TTX-S) voltage-gated sodium channel in dorsal root ganglia (DRG) neurons (IC_{50} = 30 nM), while having no significant effect on the TTX-resistant (TTX-R) sodium channels. According to these results, and since it suppresses the peak sodium current without altering the activation or inactivation, huwentoxin IV is proposed to bind at site 1 of the sodium channel through a mechanism quite similar to that of TTX. Two more peptides, ProTxI and ProTxII, from the venom of the tarantula *Thrixopelma pruriens*, have been recently isolated and characterized [8]. These peptides inhibit the TTX-R Na^+ channel, Nav 1.8. The peptides showed promiscuity across ionic channel families. ProTxI also shifts the voltage dependence of activation of T-type VSCCs with an IC_{50} of 50 nM without affecting the voltage-dependence of inactivation, and also inhibits Kv 2.1 channels with ten-fold less potency than its potency on Nav channels.

SpPTxs acting on glutamate receptors

Glutamate receptors are classified on the basis of agonists that selectively activate them, and include the NMDA, AMPA and kainate receptors [85]. Glutamate receptors mediate fast excitatory transmission at synapses in the brain and spinal cord [86]. The toxin phoneutria-

toxin 3-4 (PhTx3-4) from the spider *P. nigriventer* has been reported to inhibit the uptake of glutamate in a time-dependent manner and to lead to a decrease in the Ca^{2+}-independent release of glutamate [87]. Based on results obtained with manipulation of the redox state of cysteine residues in rat synaptosomes and in PhTx3-4, the effect is suggested as being due to interactions that involve cysteines both in the toxin and in the glutamate transporters. However, the specificity of PhTx3-4 for glutamate transporters is unknown. The toxin PhTx4-7 from the same spider seems to produce similar effects [88, 89].

SpPTxs acting on acid-sensing ion channels and mechano-sensitive ion channels

Acid-sensing ion channels (ASICs) are associated with nociception, taste transduction and perception of extracellular pH fluctuations in the brain [90]. A novel toxin (PcTx1) from the venom of the South American tarantula *Psalmopoeus cambridgei*, blocks with an IC_{50} of 0.9 nM a particular sub-class of ASICs that are expressed in both central nervous system neurons and sensory neurons in DRG [10]. As the first high-affinity and highly selective pharmacological agent for this novel class of ionic channels, PcTx1 will be instrumental in fully characterizing the physiological role of these channels.

Compared to voltage-dependent or ligand-gated ion channels that have been extensively studied over the last 20 years, there is little knowledge available on the structure and function of mechano-sensitive channels that constitute the third major group of ion channels classified according to their gating mechanism [91]. Mechano-sensitive channels, also called stretch-activated ion channels, open and close in response to mechanical stimuli. GsMTx4, a SpPTx from *G. spatulata,* produced a complete block of stretch-activated ion channels in outside-out patches in neurons, and appeared specific since it had no effect on whole-cell voltage-sensitive currents [92]. Moreover, in isolated ventricular cells from a rabbit dilated cardiomyopathy model, GsMTx4 produced a nearly complete block of the volume-sensitive cation-selective current, but did not affect the anion current. GsMTx4 is the first peptide toxin that specifically blocks stretch-activated currents, although with a rather low affinity (650 nM). Using two-dimensional NMR spectroscopy, the solution structures of the mechano-sensitive ion channel toxin GsMTx4 and another peptide (GsMTx2) have been determined (fig. 3D). As was noted with the potassium channel toxins HaTx1 and HpTx2, the dominant feature of the two structures was a hydrophobic patch surrounded by charged residues [93].

Insecticidal SpPTxs without a defined mode of action

All spiders are predators, and spider venoms are therefore potentially rich sources of insecticidal compounds. Several reports have described the toxic activity of SpPTxs toward insect cells, but their mode of action remains unknown. Nevertheless, because of their capacity to paralyze and kill insects at low doses, they appear to target the nervous system of insects with high affinity. Some of these insecticidal peptides have been isolated from venom of the spider *Tegenaria agrestis* (TaITx1–3) and their full sequences have been determined from cloned cDNA [94]. These toxins consist of 50 amino acid residues, 6 of which are cysteines. In lepidopteran larvae and corn rootworm beetles, *T. agrestis* toxins caused an unusual excitatory symptomatology with 50% paralytic doses ranging from 0.23 to 2.6 nmol/g of insect. In electrophysiological experiments using housefly larvae, these toxins caused an elevated rate of firing from central nervous system neurons. However, no significant effects were found when peripheral sensory or motor systems were examined. Similarly, insecticidal peptide toxins have been purified from the venom of the primitive weaving spider *Diguetia canities* [95]. In lepidopteran larvae, *D. canities* toxins caused a progressive spastic paralysis, with 50% paralytic doses ranging from 0.38 to 3.18 nmol/g. Other insecticidal peptides were isolated from the venom of the trap-door spider, *Aptostichus schlingeri* [96]. Some of these toxins cause flaccid paralysis of insect larvae within 10 min of injection, and all were lethal within 24 h. The complete amino acid sequences (32–76 residues) of six peptides were determined. In addition, SpPTxs from *P. tristis* displayed insecticidal properties when injected into insect pests such as the larvae of the lepidoptera *Heliothis virescens*, *Spodoptera exigua* (beet armyworm) and *Manduca sexta* (tobacco hornworm) [97]. Due to their high potency and effects on lepidoptera, these toxins were hypothesized to affect sodium channels.

The Janus-faced atracotoxins (J-ACTXs) represent an interesting family of novel peptide toxins [98]. They are a family of insect-specific excitatory toxins isolated from the venom of Australian funnel-web spiders (genera *Atrax* and *Hadronyche*). These toxins contain a rare vicinal disulfide bond thought to be critical for insecticidal activity. An alanine scan of 24 mutants has provided a map of the bioactive surface of this peptide [99]. Based on insecticidal activity, the most important residues of J-ACTX-Hv1c have been found to form a bipartite surface patch on one face of the toxin. The 'hot spot' of this toxin is formed by the residues Arg8, Pro9, Tyr31 and the Cys13-Cys14 (vicinal disulfide). The Arg8-Tyr31 diad in J-ACTX-Hv1c superimposes closely to the Lys-(Tyr/Phe) diad that is spatially conserved across a range of K^+ channel blockers, leading the authors to speculate that the J-ACTXs might target an invertebrate K^+ channel [100].

SpPTxs might be useful for defining new insecticidal targets or for pioneering novel biopesticides [21]. One approach has been the creation of insect-specific baculoviruses [101] that have been proposed as an attractive

alternative for pest control. Recombinant viruses expressing scorpion and spider toxins could lead to the reduction of crop damage [77, 102, 103]. The need for high insect specificity will naturally lead the search toward venomous arthropods such as spiders that have evolved highly selective toxins for insect prey.

Spider peptides acting on lipid bilayers

Amphipathic and cationic helical peptides are broadly found in animals as part of their biological defense system [104]. Linear cationic peptides with α-helical conformation share some common characteristics such as antimicrobial activities at low micromolar concentrations and α-helix formation in hydrophobic environments. Such peptides cause the disruption of cell membranes by molecular mechanisms depending principally on the charge distribution of the peptide and on the phospholipid composition of the cell membranes. Amphipathic and α-helical peptides are also found in the venom of spiders. Two peptide toxins with antimicrobial activity, lycotoxins I and II, were identified from the venom of the wolf spider *Lycosa carolinensis* [105]. Lycotoxins are linear antimicrobial peptides that display the amphipathic α-helix character typical of pore-forming peptides [106]. Following the lycotoxins, cupiennins and oxyopinins, from the venom of the wolf spiders *Cupiennus salei* and *Oxyopes kitabensis,* respectively, were also reported to have antimicrobial properties [5, 107, 108]. Cupiennins and oxyopinins are the largest pore-forming peptides isolated from the venom of a spider thus far. A hydrophobic N-terminal chain region and a C terminus composed preferentially of polar and charged residues characterize cupiennins and oxyopinins. The pore-forming activity of pore-forming peptides reduces ion and voltage gradients across cell membranes, promotes efflux of ions from synaptosomes and causes hemolysis of erythrocytes [5, 105, 108]. Cupiennins and oxyopinins have shown pronounced insecticidal activity; therefore, pore-forming peptides may have a synergistic action with neurotoxins, thereby facilitating prey capture [5]. Peptides acting on lipid bilayers were also isolated from the venom of *L. singoriensis* [109] although no primary structures are available yet.

Gomesin is a cysteine-rich, cationic antimicrobial peptide with homology to tachyplesin and polyphemusin from horseshoe crabs. Gomesin was isolated from the hemolymph of the tarantula *Acanthoscurria gomesiana* [110]. The solution structure of gomesin has been determined and consists of a two-stranded antiparallel β sheet connected by a non-canonical β turn. Since gomesin contains only two cysteines, it does not belong to the ICK family. A comparison between the structures of gomesin and other cysteine-rich antimicrobial peptides from arthopods outlines several common features in the distribution of hydrophobic and hydrophilic residues, where one face of the β sheet is completely hydrophilic, while the other is hydrophobic [111].

Toxin selectivity and promiscuous toxins

Although spider venom toxins have proven to be invaluable tools in the study of a variety of cell receptors and ion channels, particularly those of higher vertebrates, such systems and potential targets of pharmaceutical interest are often only remotely relevant to the true targets of those toxins under natural conditions. Indeed, the natural prey of spiders are often taxonomically very different from the physiological systems studied in in vitro neurobiological studies performed on mammalian cells. Although some of the larger spiders can prey on vertebrates such as small reptiles, frogs, rodents, fish and birds, by far the most widespread predation behavior in spiders revolves around a diet of small invertebrates, and mainly insects. As a consequence, the molecular evolution of venom components has probably selected toxins that will efficiently target excitable cells in the insect nervous system. These tightly folded molecules are resistant to enzymatic breakdown and travel rapidly, via diffusion, in the hemolymph, quickly and efficiently reaching the central nervous system.

Despite evolving to target primarily insect ion channels, some peptide toxins from spider venoms exhibit remarkable specificity and selectivity for mammalian ion channels. This is likely due to the high evolutionary conservation of ion channel proteins through phylogenetically diverse zoological groups. δ-Atracotoxins are a good example of such cross-phylum structural recognition, binding to sodium channels in both insects and vertebrates. In contrast, other toxins such as the δ-palutoxins recognize only sodium channels in insects, highlighting evolutionary differences. Indeed, the high and specific affinity of δ-atracotoxin Ar1 for primate sodium channels has resulted in human deaths while other mammalian vertebrates such as dogs or rodents are relatively immune to the toxicity of this peptide. Conversely, other toxins from this group display stronger toxicity in rodents than in humans. This illustrates perfectly the variability of toxin affinity for particular receptors and at the same time opens the possibility of discovering extremely potent pharmacological tools in almost any spider venom, independent of the ecology or behavior of the spider species.

Another key feature of toxin activity is selectivity across a wide range of related receptors. Clearly, for biochemical tools, the narrowest possible selectivity (i.e. high affinity for a single receptor subtype) is highly desirable. Many of the SpPTxs studied to date have been assayed against a limited range of receptors so their true selectivity remains unknown. In addition, SpPTxs can show promiscuity

across ionic channel families, that is they display affinities of various levels on ion channels with different ion selectivity such as potassium and calcium channels [49, 112]. As a recent example, ProTxI and ProTxII from the venom of the tarantula *T. pruriens* inhibit the TTX-R Na channel, Nav 1.8, but in addition, ProTxI also modifies the T-type VSCC currents and can also act on Kv 2.1 channels [8]. Moreover, the authors found that the amino acid sequence of ProTxI is similar to that of the Kv2.1 potassium channel blocking hanatoxins, but did not mention that ProTxII is also similar (82 % identity) to the phrixotoxins which act on Kv4 [27]. So the potential of ProTxII to inhibit the Kv4 ion channels in a similar fashion to phrixotoxins should be further investigated. Another example is ω-GsTxSIA which inhibits several types of calcium channel (N-type, P/Q-type), and also inhibits potassium channels (*drk1*), albeit with lower affinity [49, 112].

These results suggest that spiders have evolved a generalist toxin model that allows for sufficient flexibility to target a whole family of ion channels that are important in central nervous system function, and therefore represent potential targets for prey-paralyzing venoms. The multiple possibilities offered by the spider toxin scaffold thus enable the diversification of venom composition based on directed mutagenesis, allowing for more efficient venoms and the exploration of additional ecological niches and prey types.

Molecular determinants of interaction

The rapid accumulation of experimental data generated by the increased interest in these venoms is leading us toward a better understanding of the physical parameters linking structure and biological activity. On the other hand, continued work on ion channel structure and pharmacology may enable the elucidation of the localization and structure of channel 'hot spots' that may represent common toxin binding sites, as proposed in previous reports [113]. Mutagenesis of voltage-gated K^+ channels suggests that hanatoxins and ω-GsTxSIA could recognize the same structural motif, possibly conserved (or at least partially) in both calcium and potassium channels. Hanatoxins, ω-GsTxSIA and most likely the other Kv2 toxins such as ScTx1 appear to recognize a voltage-sensing domain in voltage-gated ion channels that has a conserved three-dimensional structure [112].

Based on multiple substitutions at identified positions in the C terminus of segment 3 of the *drk1* channel domain, the HaTx1 receptor site was found to be restricted within the voltage-sensing domain of the channel [114]. In particular, HaTx1 binds to an α-helical section of segment 3 of the *drk1* channel that interfaces with water. This structural characteristic may be common with other voltage-gated ion channels [115]. The possible conservation of a structurally homologous site across all voltage-dependent channels would be a seductive hypothesis to explain the cross-reactivity of many SpPTxs, although sufficient experimental data are still lacking to demonstrate common features for toxin binding.

Perspectives

Mass spectrometry and pharmacological analysis of venoms have revealed the great peptide diversity found in individual species such as *A. aperta* or *P. nigriventer* or more generally in families such as the Theraphosidae [15, 16]. Although about 38,000 spider species have been described, probably upward of 100,000 have not been described yet, thus indicating a discovery potential of literally hundreds of thousands of novel toxic peptides. In addition, very few of the widely diverse spider families have been explored, suggesting that even broader pharmacological or structural diversity could be found. This in turn could lead to broader applications of SpPTxs in the fields of pharmacology, physiology and drug discovery. To follow this path, and increase the success rate of discovery, the emergence of more standardized bioassay methods is probably desirable, in order to catalog and compare biological activities obtained in different studies. The comparison of results is too often difficult due to the variety of excitable cells and molecular targets used in functional assays (table 2). The even greater diversity of possible ion channel targets in excitable cells renders the task even more daunting. The use of a large library of cloned channels representing all identified sub-types, in standardized assays that could be done in a highly parallel way and in miniaturized form would permit great strides in generating large libraries of potentially useful peptides. In this respect, the potential of the natural combinatorial libraries developed by the spiders under evolutionary pressure would be put to use for the discovery of a treasure trove of novel ligands of pharmacological interest, similar to the exploitation of large-scale chemical libraries now being generated for high-throughput screening in the search for new drugs.

Finally, detailed studies of the molecular recognition processes involved in ligand-receptor interaction will permit a better understanding of the spatial geometry of receptor sites, of the biophysical constraints involved at the atomic level and therefore lead the evolution in the design and optimization of novel therapeutic agents.

With help from the humble spider, the path to novel discoveries and drug development is lined with almost infinite possibilities.

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