Review

Novel Active Principles from Spider Venom

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Dedicated to the memory of Professor Franc Gubenšek

Abstract

Spiders are one of the most intriguing groups of venomous animals. Substances found in their venom vary from simple inorganic compounds to large multi-domain proteins. In this article, we review some of the latest work presenting active principles that add to the known spider toxin universe. Two aspects of novelty are addressed in particular, structural (novel types of molecules in terms of structure) and functional (novel types of biological targets hit by substances from spider venom and novel mechanisms of action).

Keywords: *Araneae*, toxin, venom gland, neurotoxin, cytotoxin, protein and peptide structure and function

1. Introduction

Some animals from distinct taxonomic groups can generate venoms that help them to subdue prey or survive in unfriendly environment. Venomous animals, such as sea anemones, cone snails, spiders, scorpions, and snakes, are able to produce in their venom glands combinatorial libraries of compounds, the most prominent being peptide neurotoxins. Spider venoms are probably the best example of such libraries.[1]

Spider venoms are complex mixtures of diverse components adapted for attack or defense (note that some components can be nontoxic but instead perform other important functions, for instance, they may stabilize the toxic components and facilitate their action). In venoms studied to date, three main groups of components have been found, differing by their chemical nature and molecular mass, *i.e.* diverse low molecular mass compounds, peptides (<10 kDa), and higher molecular mass proteins. Each of the three distinct groups contains homologous families or combinatorial libraries of substances. For example, venoms of the spiders *Argiope lobata* (Araneidae) and *Agelenopsis aperta* (Agelenidae) contain at least 9 and 33 low molecular mass polyamine toxins, respectively.[2,3] The total number of peptide components in single spider venom may run up to several hundred, this group of spider venom compounds seems the most numerous and diversified.[1,4] Currently, two major sub-groups may be noted, the disulfide-containing peptides (most usually neurotoxins, *i.e.* toxins primarily targeting the nervous system), and the linear peptides (typically cytotoxins, *i.e.* toxins affecting cells in general). In case of the disulfide-rich neurotoxins, the cysteine residues are usually strictly conserved, whereas residues lying in loops between the cysteines are hyper-variable. Based on similarity, peptides are assembled into families. For instance, over a dozen different families were found in the venom of *Chilobrachys guangxiensis* (Theraphosidae)[5]. Each family contains several closely related peptides; for example, 7 homologous β/δ-agatoxins were purified from the venom of *Agelena orientalis* (Agelenidae), and 6 more were predicted from cDNA cloning.[6,7] Toxic proteins from spider venom include neurotoxins and enzymes. For instance, venom of the spider *Latrodectus tredecimguttatus* (Theridiidae) contains a family of at least 7 related high molecular mass neurotoxins (>100 kDa), latrotoxins, presenting selective toxicity against various animals;[8] and a number of receptors have been identified in mammalian nervous system that mediate activity of α-latrotoxin on these animals.[9–13] The present paper deals with recent discoveries that widen our knowledge of spider venom molecular repertoire.

2. Novel Structures

As already mentioned, spider venom constituents are classified into three large groups based on molecular mass. Recent findings suggest high structural variability inside each of the groups.

2. 1. Low Molecular Mass Components

Low molecular mass components vary in their structure from inorganic substances such as salts to simple organic compounds like biogenic amines (for example, histamine; Fig. 1F) to more elaborate molecules like acylpolyamines (for instance, argiopin from *A. lobata*, Fig. 1A). The latter, discovered in 1986,[14] are probably the best characterized and are among "classical" spider venom constituents. They represent a major fraction in certain spider venoms (for example, in members of the Araneidae family) and inhibit insect glutamate receptors but also some other targets.[15] Other examples of structurally complex organic molecules identified in spider venoms include a bis(agmatine)oxalamide (Fig. 1B) from *Plectreurys tristis* (Plectreuridae),[16] sulfated nucleosides like HF-7 (Fig. 1C) from *Hololena curta* (Agelenidae),[17] tetrahydro-β-carboline toxins, such as PwTX-I (Fig. 1E) from *Parawixia bistriata* (Araneidae),[18] and a hydroxylhydrazyl-dioxopiperidine (nigriventrine, Fig. 1D) from *Phoneutria nigriventer* (Ctenidae).[19] Function and mechanism of action of these compounds are poorly understood. No activity was reported for the bis(agmatine)oxalamide. HF-7 was reported to target glutamate receptors,[17] and it is interesting to note that sulfated nucleosides have recently been detected in venoms of spiders from many families, suggesting some functional role for these compounds, which may interfere with processes that involve "usual" phosphorylated nucleosides.[20] Tetrahydro-β-carbolines were found to exhibit strong insect toxicity,[18] recently suggested to reside in inhibition of monoamine oxidase, which catalyzes deamination of endogenous amines.[21] Finally, nigriventrine was shown to cause convulsions in rats, but its molecular target is unclear. To summarize, the versatility of low molecular mass components in spider venoms may be wider than currently believed, and further research into structure-function relationships of these chemicals is anticipated.

2. 2. Polypeptide Components

As for polypeptides, 5 types of fold have been unambiguously assigned to spider venom constituents (Fig. 2).[1] Most cytolytic peptides are linear and therefore mainly disordered in solution, but adopt α-helical conformation when bound to target membranes[22] (for instance, structure of latarcin 1 from *Lachesana tarabaevi* (Zodariidae) in complex with dodecyl sulfate micelles[23] is shown in Fig. 2E). In peptide neurotoxins, the inhibitor cystine knot (ICK) fold is most common and may be regarded as "classical".[24] However, other structural types were also noted. For example, the

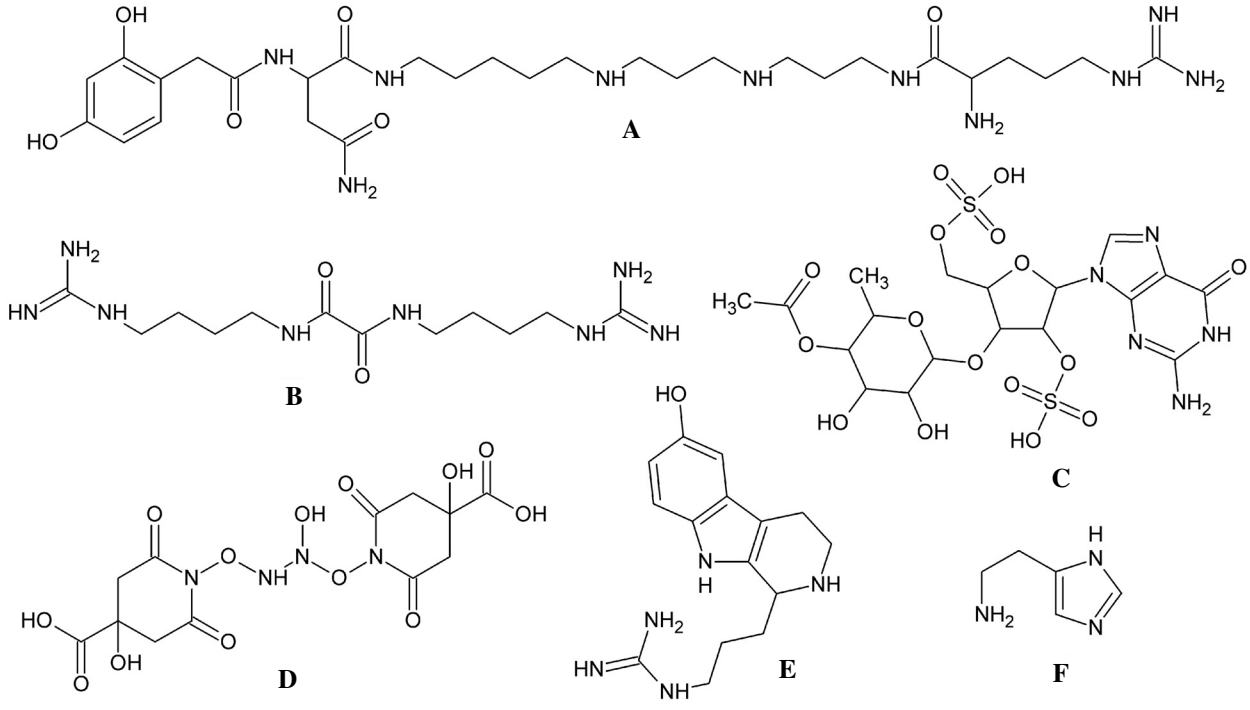

Figure 1. Low molecular mass components found in spider venom. Shown are: (A) argiopin from *A. lobata*; (B) bis(agmatine)oxalamide from *P. tristis*; (C) HF-7 from *H. curta*; (D) nigriventrine from *P. nigriventer*; (E) PwTX-I from *P. bistriata*; (F) histamine.

insecticidal peptide huwentoxin-II from *Haplopelma schmidti* (Theraphosidae), for which the molecular target is unknown, does not conform to the ICK, but assumes the disulfide-directed β-hairpin (DDH) fold (Fig. 2D).[25] Interestingly, the ICK is regarded an evolutionary elaboration on the ancestral and more general DDH, and molecules like huwentoxin-II may represent molecular fossils testifying in favor of fold evolution. More recently, the serine protease inhibitor huwentoxin-XI from the same spider has been shown to adopt the Kunitz-type fold (Fig. 2B), common to molecules from sources as diverse as sea anemones, snake venom, and mammalian pancreas, and raising important questions of fold recruitment into different biological systems and protein structure evolution.[26] For larger proteins from spider venoms, the 3D structure is known only for the necrotic toxin sphingomyelinase D from *Loxosceles laeta* (Sicariidae), assuming the TIM barrel fold most commonly met in enzymes (Fig. 2C).[27] To summarize, we should note that the known fold variability in polypeptides from spider venoms is rather scarce compared to that of snakes[28] or *Conus* snails.[29] However, a number of sequences are available that do not contain the characteristic signatures of any fold described above, for instance, the heterodimeric ω-agatoxin IA from *A. aperta*[30] and similar peptides. Those molecules are presumed to assume novel folds yet to be described.

Another recent addition to the diversity of spider venom components are the so-called modular toxins. They contain two modules, or domains, each corresponding to a "usual" spider toxin. For example, CpTx 1 from *Cheiracanthium punctorium* (Miturgidae)[31] and DkTx ("double-knot" toxin) from *C. guangxiensis*[32] feature two ICK modules, whereas cyto-insectotoxins from *L. tarabaevi*[33] contain two linear modules. Combination of the usual toxin domains into larger polypeptide assemblies may be a common strategy in venomous animals, since similar examples are known, for instance, in scorpions[34] and snakes.[35]

Comparison of novel structures with the known homologues or unrelated polypeptides that share common target of action may yield important conclusions on the functionally important residues and structure of the "pharmacophores".[6,36] Many novel primary structures of polypeptides from spider venom are published each year, yet most allocate to the well-established ICK-type toxins. However, some recent publications break off this trend. For instance, a whole new family of astacin-like metalloproteases was discovered in venoms of *Loxosceles* spp. that may be directly involved in digestion of prey, other toxins maturation and spreading, and deleterious symptoms such as hemorrhage.[37,38] Huwentoxin-XI homologues also seem to represent a family of Kunitz-type toxins found in venoms of several spiders.[26]

3. Novel Activities

Spider venoms can roughly be classified according to the produced symptoms as necrotic (cytolytic)

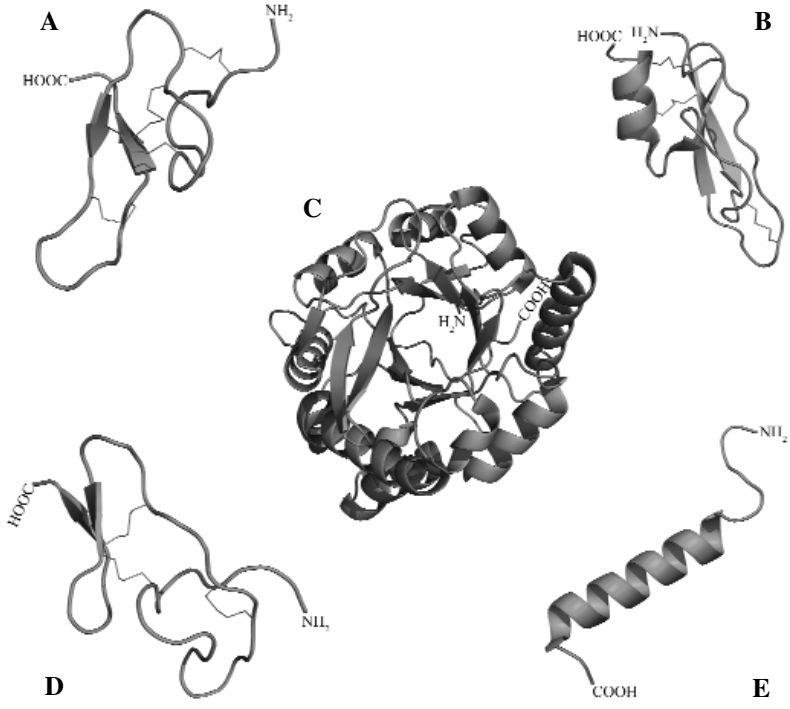

Figure 2. Polypeptide components found in spider venom. Shown are: (A) purotoxin from *Geolycosa* sp. (ICK fold; PDB accession no. 2KGU); (B) huwentoxin-XI from *H. schmidti* (Kunitz; 2JOT); (C) sphingomyelinase D from *L. laeta* (TIM barrel; 1XX1); (D) huwentoxin-II from *H. schmidti* (DDH; 1I25); (E) latarcin 1 from *L. tarabaevi* (2PCO). Cartoons were generated using PyMOL (http://www.pymol.org/). S-S-bonds in peptides are shown with thin lines.

and neurotoxic. Whereas necrosis is usually associated with enzymes such as sphingomyelinase D from *Loxosceles* spp., or non-specific cytolytic peptides, neurotoxicity is often caused by components that specifically target protein receptors in the neurons or myocytes of organisms bitten by spiders. Since 1980–90s, when several seminal works were published describing acylpolyamines targeting glutamate receptors,[14] and peptides μ- and ω-agatoxins from *A. aperta* affecting voltage-gated Na^+ and Ca^{2+} channels,[42–44] and hanatoxins from *Grammostola rosea* (Theraphosidae) active against voltage-gated K^+ channels,[45] a plethora of compounds have been isolated from spider venoms that modulate activity of different receptors in the nervous system of insects and mammals (see Table 1 for examples). Those targets mentioned above may be considered "classical"; they are vital components of the nervous signaling mechanisms, and a great diversity of spider venom components have been described affecting those receptors. More recent work has shown, however, that other "non-classical" targets are hit by spider venoms.

Table 1. Examples of molecular targets hit by spider venom constituents.

Target	Compound	Effect	Reference
K^+ channels	hanatoxin 1	inhibition	45
Na^+ channels	μ-agatoxin I	activation	42
Ca^{2+} channels	ω-agatoxin IVA	inhibition	43
Glutamate receptors	argiopin	inhibition	14
Mechanoreceptors	GsMTx-4	inhibition	57
ASIC	psalmotoxin 1	inhibition	52
TRPV	vanillotoxin 3	activation	56
P2X	purotoxin 1	inhibition	53

3. 1. Novel Targets

The "classical" targets of spider venoms are generally indispensible and vital parts of the prey organisms. Thus, neurotoxins target receptors implicated in the generation (glutamate receptors) or propagation (Na^+, K^+ channels) of action potentials, or neurotransmitter release (Ca^{2+} channels). Toxins affecting voltage-gated Ca^{2+} channels, for example, have received increasing interest, since they may differentiate between channel isoforms[43,46] and exhibit high taxon specificity.[47–49] As such, these compounds represent leads for drug[50] and pesticide[51] development.

A more recently discovered and growing group of spider venom components target the so-called sensory receptors of the nervous system, associated with reception of diverse stimuli (Table 1). For example, psalmotoxin 1 from *Psalmopoeus cambridgei* (Theraphosidae) was found to specifically block ASIC1a acid-sensing ion channels that detect lowering of pH.[52] Similarly, purotoxin 1 from *Geolycosa* sp. (Lycosidae) was recently found to block P2X3 purinergic receptors of sensory neurons that are activated by extracellular ATP.[53] Both types of receptors are involved in pain perception and accordingly their blockers, *i.e.* psalmotoxin and purotoxin, present potent analgesic properties (for instance, purotoxin produced comparable effects with previously known blockers of P2X receptors at almost 3 orders of magnitude lower doses).[53,54] These molecules may therefore be regarded as lead structures for development of novel generation of painkillers. Most interestingly, both peptides affect cognate receptors state-dependently, and seem to achieve the blocking effect *via* stabilization of their desensitized states.[53,55] A contrasting example is presented by peptide toxins from *P. cambridgei* (vanillotoxins)[56] and *C. guangxiensis* (DkTx)[32] that affect another principal receptor involved in pain sensation, the vanilloid receptor TRPV1. These toxins are not blockers, but instead activators of the receptor; thus, they cause intensive pain, and their biological role might be to drive off aggressors. Still another type of sensory receptors, the mechanosensitive channels, was found to be targeted by a spider venom peptide GsMTx-4 ("mechanotoxin 4") from *G. rosea*.[57] The exact role of this peptide is unclear; however, it presents a variety of activities, including modulation of a number of eukaryotic and prokaryotic channels. The peptide possesses analgesic activity probably due to its ability to inhibit mammalian stretch-activated channels,[58] but also antimicrobial activity attributed to either its membrane-active properties *per se*,[59] or activation of bacterial mechanosensitive channels.[60,61]

Other "non-classical" targets of spider venom components include monoamine oxidase (see above), and carbohydrates, as in case of the mini-lectin SHL-1 (huwenlectin-I) from *H. schmidti*.[62,63] Psalmopeotoxins from *P. cambridgei* show anti-malarial activity, and although their molecular target is unknown, they may interfere with some vital processes of the intracellular parasite lifecycle.[64,65] All these new compounds may provide important clues to structure-function relationships in substances with diverse functions but also find application in biotechnology and medicine.

3. 2. Novel Modes to Affect "Classical" Targets

Based on the mechanism, spider neurotoxins targeting ionotropic receptors may be allocated into two broad groups, pore blockers and various gating modulators,[1] although it might turn out that some compounds exhibit both types of activity.[66]

Many acylpolyamine toxins are known to present moderate specificities, targeting a number of ionotropic receptors and ion channels.[15] One of the general trends in spider venom research that has gathered more evidence recently is multi-functionality, or even "promiscuity", of spider peptide toxins. For example, the well-studied scorpion α- and β-toxins are known to target the so-called receptor sites 3 and 4 on voltage-gated Na^+ channels and affect inactivation and activation processes, respectively.[67] Some recent studies on spider toxins bring chaos into this clear-cut picture. δ-Palutoxins-IT1 and 2 from *Pireneitega luctuosa* (Agelenidae) were found to induce effects reminiscent of scorpion α-toxins but instead compete with β-toxins for binding to insect Na^+ channels.[68] β/δ-Agatoxins from *A. orientalis* exhibited effects on both activation and inactivation of the same channels, resembling effects of both α- and β-toxins.[6] It seems that the "classical" concept of receptor sites 3 and 4 of sodium channels becomes rather "blurred", and that no strict correspondence exists between the toxin binding site and its effect on the channel function.

Plenty of examples have been accumulating that illustrate spider toxin multi-functionality. The classical hanatoxin 1 is known to affect subtypes of voltage-gated K^+ but also Ca^{2+} channels, albeit with much lower affinity.[45,69] Related protoxin I from *Thrixopelma pruriens* (Theraphosidae) targets several Na^+, K^+, and Ca^{2+} channels,[70] and jingzhaotoxins-IX and XI from *C. guangxiensis* target subtypes of both Na^+ and K^+ channels, producing diverse effects on activation, inactivation and deactivation.[71,72] Even more so, several well-studied toxins from *G. rosea*, such as VSTx 1 ("voltage sensor toxin 1")[73] and GsMTx-4,[57] have recently been shown strikingly promiscuous, targeting a wide number of Na^+ and K^+ channels with similar potencies.[66] To top that off, spider toxins may recognize selectively a particular binding site within the target channel, but may also bind to a number of sites with similar affinity.[74,75] Such diversity of spider toxin action profiles renders the task of function prediction from primary structure especially challenging.

Essentially two modes of interaction between spider neurotoxins and cognate ionotropic receptors may be considered, one being "direct" and the other "membrane-mediated". The latter was proposed for certain gating modifiers that also possess the ability to bind to lipid membranes.[76] Spider toxin and target channel pharmacology has recently experienced broadening due to recognition of the fact that both the mechanical state and composition of membranes markedly influence the toxin-channel interactions. Thus, the activity of VSTx 1 and protoxin I on K^+ and Na^+ channels was found to depend on the surrounding membrane.[77,78] It is becoming obvious, that at least for some toxins, one should consider not only the protein-ligand interactions, but regard the tri-party complex "lipids-protein-ligand" as unity.

4. Conclusion

The molecular diversity of spider venom components seems to be underestimated. Recently, we have seen an impressive increase in both the number of novel molecular structures solved and the number of targets hit by spider venoms, but also in the versatility of ways in which the well-known targets are affected. Further insight into spider venom composition and function of the diverse substances purified from this source is highly anticipated.

5. References

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Povzetek

Pajki so ena od najbolj zanimivih skupin strupenih živali. Snovi, ki jih zasledimo v njihovih strupih, segajo od preprostih anorganskih spojin do velikih proteinov, sestavljenih iz več domen. V predstavljenem članku podajamo pregled nekaterih najnovejših raziskav na področju učinkovin, izoliranih iz pajkovih strupov. Obravnavamo jih predvsem z dveh vidikov novih spoznanj: strukturnega (nove vrste molekulskih struktur) in funkcionalnega (nove biološke tarče in novi mehanizmi delovanja).