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Review

Spider and wasp neurotoxins: pharmacological and biochemical aspects

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Abstract

Venoms from several arthropods are recognized as useful sources of bioactive substances, such as peptides, acylpolyamines, and alkaloids, which show a wide range of pharmacological effects on synaptic transmission. In this work, we summarize and compile several biochemical and pharmacological aspects related to spider and wasp neurotoxins. Their inhibitory and stimulatory actions on ion channels, receptors, and transporters involved in mammalian and insect neurotransmission are considered.

Keywords: Spider; Wasp; Neurotoxin; Ion channel; Receptor; Transporter and Neurotransmitter release

1. Introduction

Insects (Arthropoda, Insecta) are extraordinarily prevalent and widespread over the Earth; their adaptability and variety assure their continuous existence. They have been surviving for at least 300 millions years, in part due to their evolutionary characteristics, which include the acquisition of wings, an exoskeleton, adaptability to different niches, chemical means of communication, capacity for metamorphosis, and production of toxins (Meinwald and Eisner, 1995).

Venoms are considered a rich source of compounds showing a wide range of pharmacological effects on several biological systems, including the nervous system. Spider and wasp toxins characterized to this date include proteins, peptides, enzymes, amino acids, inorganic salts, and a range of amines, which, acting together, produce their biological effects (Blum, 1981; Schmidt, 1986; Jackson and Parks, 1989).

Acylpolyamines, proteins, and peptides are the most relevant and studied compounds representing the main toxic arsenal of spider and wasp venoms (McCormick and Meinwald, 1993; Olivera et al., 1994; Blagbrough et al., 1994).

Acylpolyamines, a natural link between spider and wasp venoms, are potent small molecules, often specific blockers of ion channels or receptors. Their targets are receptors that recognize excitatory amino acids in the mammalian and insect nervous systems. These compounds can be subdivided into two groups: the amino acid-containing acylpolyamines (Fig. 1A), and the nonamino acid-containing acylpolyamines (Fig. 1B). Both types act similarly as glutamate receptor blockers, and both incorporate the same family of aromatic end groups. Briefly, the amino acid-containing acylpolyamines, the first to be discovered, possess one, two, or three basic amino acids between the acyl end group and the polyamines chain (for review, see McCormick and Meinwald, 1993; Blagbrough et al., 1994).

A great number of polypeptides and peptides found in spider and wasp venoms are highly reticulated by several disulfide bridges and possess modified amino or carboxy termini or both, which increase their in vivo stability (Norton and Pallaghy, 1998). A fold, presumably largely

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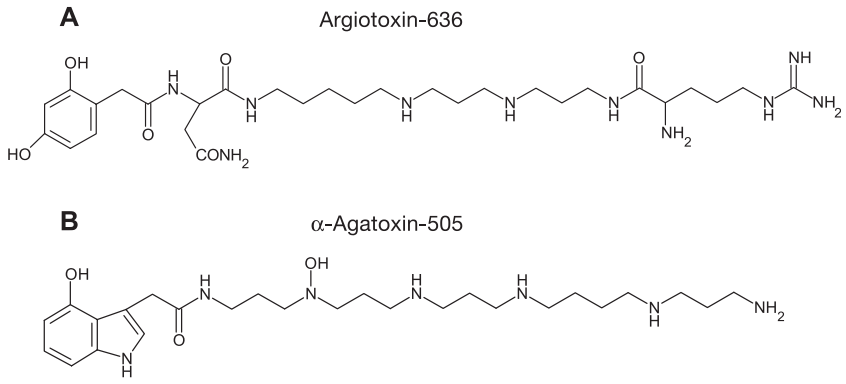

Fig. 1. Chemical structures of amino acid-containing (A) and nonamino acid-containing (B) acylpolyamines from spider venoms.

dictated by the gaps between the half-cystine residues and their pairing pattern, is crucial for the toxin's activities and appears to be an ideal compact globular scaffold for the presentation of functional groups, generating a range of peptides acting on diverse biological targets. As a matter of fact, many ion channel toxins display highly basic surfaces and a consensual sequence known as the cystine knot. This structural motif is characterized by a triple-stranded, antiparallel β-sheet stabilized by disulfide bridges (Fig. 2). It is suspected that these groups play a role in interactions between the toxin and the vestibule and the pore of the targeted ion channel. The robust nature of this motif makes it an excellent scaffold for the design and bioengineering of novel polypeptides showing enhanced activity against existing targets, or activity against new ones (Omecinsky et al., 1996; Norton and Pallaghy, 1998).

Acylpolyamines and polypeptides have been used as tools to extend our knowledge of molecular mechanisms of neurotransmission (Usherwood and Blagbrough, 1991; Olivera and Cruz, 2001). Research on such neurotoxins could also aim at the development of natural insecticides and drugs potentially useful for the treatment of neuropathologies (Quistad et al., 1991; Bloomquist et al., 1996; Harvey et al., 1998; Heading, 2002).

This review will focus on current knowledge of neurotoxins from spider and wasp venoms, reporting information on the biochemistry and pharmacology of these extraordinary molecules. For didactic simplicity, the toxins were grouped according to their pharmacological actions.

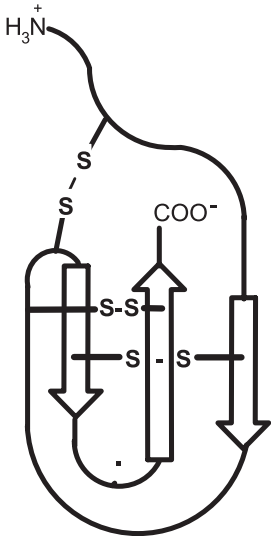

Fig. 2. Schematic diagram illustrating the cystine knot motif for a three-disulfide-bridge (–S–S–) toxin. Modified from Norton and Pallaghy (1998).

2. Toxins acting on Na^+ channels

Voltage-gated Na^+ channels are present in most excitable cell membranes, and are responsible for the generation and propagation of action potentials in these cells. They represent transmembrane proteins composed of a major α-subunit of approximately 260 kDa (sufficient for functional expression) and of at least three different auxiliary β-subunits of 30–40 kDa, which modify the kinetics and voltage dependence of channel gating. α-Subunit consists of four homologous domains (I–IV), each showing six transmembrane segments referred to as S1–S6 as well as a pore loop located between S5 and S6 segments. Under the influence of an electrical field, the S4 segment acts as an electric sensor, participating in the channel activation mechanism; the intracellular region between domains III and IV is important for inactivation (for reviews, see Catterral, 2000; Ogata and Ohishi, 2002; Yu and Catterall, 2003).

A variety of different isoforms of voltage-gated Na^+ channels have now been identified by electrophysiological and biochemical techniques and cloning (Goldin, 2001). These channels can be classified into two different categories based on their amino acid sequence: Na_v1 isoforms (comprising nine homologous clones: $Na_v1.1$–$Na_v1.9$) and Na_x, structurally different from Na_v1 isoforms in critical functional motifs such as S4 voltage sensors, and inactivation gate and pore region (Goldin et al., 2000). The relationships of Na_v1 isoforms and subthreshold electrical activity, and their pivotal roles in pain production, alongside with their well-known functions in action potential upstroke in

excitable cells, have been recently demonstrated. Tetrodotoxin (TTX)-sensitive $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, and $Na_v1.7$ channels are expressed in neurons. $Na_v1.5$, $Na_v1.8$, and $Na_v1.9$ are highly expressed in heart and dorsal root ganglion neurons. The latter present differing tetrodotoxin resistances evoked by changes at a single position in domain I. $Na_v1.4$ and $Na_v1.6$ are tetrodotoxin-sensitive, primarily expressed in skeletal muscles and the central nervous system, respectively (Goldin et al., 2000; Ogata and Ohishi, 2002). Na_x isoforms are tetrodotoxin-resistant and may be related to transport or absorption of Na^+ ions by sensing the Na^+ concentration gradient across the membrane (Watanabe et al., 2000).

A number of toxins and chemicals that block or modulate the function of voltage-gated Na^+ channels have been discovered; some of these are presently being used as chemical tools to study these channels (Table 1). At least six neurotoxin receptor sites have been identified on the mammalian Na^+ channel (Strichartz et al., 1987; Catterall, 1995). The binding sites at which neurotoxins affect gating were found to be allosterically coupled, suggesting that conformational changes induced by neurotoxin binding modify the equilibrium between the open and the closed/inactivated states and also modify conformation and toxin binding affinity at other neurotoxin receptor sites (Catterall and Beneski, 1980). Some spider and wasp toxins acting on Na^+ channels, like δ-atracotoxin, δ-palutoxins, and pompilidotoxins (all described below), bind at site 3, which involves the extracellular loops IS5–S6, IVS3–S4, and IVS5–S6 of the ionic channel (Rogers et al., 1996; Cestele and Catterall, 2000). Neurotoxins from scorpions and sea anemone also act on Na^+ channel site 3, which slow the coupling of sodium channel activation to inactivation (Richard Benzinger et al., 1999; Leipold et al., 2004).

Most of the research works on spider toxins interfering with Na^+ channels deal with molecules isolated from *Agelenopsis aperta*, *Phoneutria nigriventer*, *Atrax robustus*, and *Hadronyche versuta* spider venoms. *A. aperta* venom was the first to be characterized as possessing Na^+ channel toxins, peptides rich in cysteine residues referred to as μ-agatoxins I–IV. They cause irreversible paralysis and repetitive firing in presynaptic terminals of houseflies, and evoke massive release of neurotransmitters by increasing Na^+ influx through tetrodotoxin-sensitive channels, shifting the activation curve to more negative potentials (Omecinsky et al., 1996; Skinner et al., 1989). Other venom neurotoxins showing structural homology with μ-agatoxins have been isolated and characterized; they include curtatoxins (I–III) and huwentoxin-I, from the venom of *Hololena curta* and *Selenocosmia huwena*, respectively (Stapleton et al., 1990; Liang et al., 2000).

Versutoxin (VTX or δ-atracotoxin-Ar1) and robustoxin (RTX or δ-atracotoxin-Hv1a), isolated from the venom of the Australian spiders *H. versuta* and *A. robustus*, respectively, produce intense effects on autonomic system and skeletal neuromuscular junctions. These toxins are peptides with 42 amino acid residues, four disulfide bridges, several basic amino acids, and a high sequence homology (Nicholson et al., 1996; Pallaghy et al., 1997; Fletcher et al., 1997). Both versutoxin and robustoxin decrease batracho-

Table 1
Toxins acting on Na^+ channels

Toxin	Source	Chemistry	Main action(s)	References
μ-Agatoxins I–IV (μ-Aga-I to μ-Aga-IV)	*A. aperta*	Peptides, 35–36 amino acids	Increase Na^+ influx by tetrodotoxin-sensitive channel, shifting the activation curve to more negative potentials	Adams et al. (1989), Skinner et al. (1989), Omecinsky et al. (1996)
δ-Palutoxins IT1–IT4 (δ-paluIT1 to δ-paluIT4)	*P. luctuosus*	Peptides, 36–37 amino acids	IT1 acts by affecting insect Na^+ channel inactivation, resulting in the appearance of a late-maintained Na^+ current	Corzo et al. (2000)
α-Pompilidotoxin (α-PMTX)	*A. samariensis*	Peptide, 13 amino acids	Might slow or block conformational changes required for fast inactivation of the Na^+ channels on the extracellular surface	Sahara et al. (2000)
β-Pompilidotoxin (β-PMTX)	*B. maculifrons*	Peptide, 13 amino acids	Slows the inactivation of Na^+ channels on rat hippocampal CA1 interneurons	Miyawaki et al. (2002)
Huwentoxin-IV (HWTX-IV)	*S. huwena*	Peptides, 35 amino acids	It has been proposed that this toxin affects the Na^+ channel through a mechanism quite similar to that of tetrodotoxin	Peng et al. (2002)
P. nigriventer peptide toxin 2–6 (PnTx2–6)	*P. nigriventer*	Peptide, 48 amino acids	Increases the time of channel inactivation and alters the threshold of activation for a hyperpolarizing direction	Matavel et al. (2002)
Robustoxin (RTX or δ-atracotoxin-Hv1a)	*A. robustus*	Peptide, 42 amino acids	Inhibits the conversion of the open state to the inactivated state of tetrodotoxin-sensitive sodium channels in rat dorsal root ganglion	Nicholson et al. (1996), Pallaghy et al. (1997)
Versutoxin (VTX or δ-atracotoxin-Ar1)	*H. versuta*	Peptide, 42 amino acids	Decreases the Na^+ current in tetrodotoxin-sensible channels by increasing the threshold of activation and the rate of recovery from inactivation, in whole-cell patch-clamp experiments in the rat dorsal root ganglion	Brown et al. (1988), Nicholson et al. (1996), Fletcher et al. (1997)

toxin-activated Na^+ flux (EC_{50} = 10 and 14 nM, respectively) and increase veratridine-activated $^{22}Na^+$ flux in rat cortical synaptosomes. Versutoxin also decreases the Na^+ current in tetrodotoxin-sensitive channels by increasing the threshold of activation and the rate of recovery from inactivation, as shown by whole cell patch clamp experiments in rat dorsal root ganglion (Nicholson et al., 1994).

A new δ-atracotoxin 1b (Hv1b) from *H. versuta* spider venom has been isolated and characterized. This peptide, inactive in insects, has 67% homology with δ-atracotoxin-Hv1a and is approximately 15–30-fold less potent than the latter (Szeto et al., 2000). Whole cell patch clamp experiments in rat ganglion dorsal neurons show that this toxin has activity similar to that of δ-atracotoxins and scorpion δ-toxins. Other toxins with δ-atracotoxin-like activity have been characterized, such as that isolated from the *Missulena bradleyi* spider venom, which induces neurotransmitter release in smooth and skeletal muscle preparations (Rash et al., 2000).

Four insecticidal peptides were purified from the venom of the spider *Paracoelotes luctuosus* and referred to as δ-palutoxins IT1–IT4, with LD_{50} values in 9–50 μg/g of insect range (*Spodoptera litura*). They show 36–37 amino acid residues, four disulfide bridges, and high homology between them and μ-agatoxins and curtatoxins. δ-Palutoxin IT1 is the most potent and insect-selective, and acts by affecting Na^+ channel inactivation, resulting in the appearance of a late-maintained Na^+ current. It is distinguished from scorpion and sea anemone toxins by its lack of effect on peak Na^+ conductance on the early phase of Na^+ current inactivation and by the lack of a shift in the Na^+ channel activation voltage (Corzo et al., 2000).

Hainantoxin-IV (HNTX-IV), isolated from the venom of the spider *Seleconosmia hainana*, inhibits mammalian neural tetrodotoxin-sensitive Na^+ currents, causing a hyperpolarizing shift of the voltage midpoint of steady-state Na^+ channel inactivation in adult rat dorsal root ganglion neurons (EC_{50} = 44.6 nM). However, it is ineffective in modifying the kinetics of the Na^+ currents. It has been proposed that this neurotoxin acts in a distinct way from versutoxin and μ-agatoxins (Xiao and Liang, 2003).

Huwentoxin-IV, a 4.1-kDa peptide from *S. huwena* spider, specifically inhibits the tetrodotoxin-sensitive voltage-gated Na^+ channel in adult rat dorsal root ganglion neurons (EC_{50} = 30 nM). It has been proposed that this toxin affects Na^+ channels through a mechanism quite similar to that of tetrodotoxin, suppressing the peak Na^+ current without altering the activation or inactivation kinetics (Peng et al., 2002).

The *P. nigriventer* peptide toxin 2–6 (PnTx2–6), at micromolar concentration, increases the time of channel inactivation and alters the threshold of activation in a hyperpolarizing direction; it also reduces current amplitude in frog skeletal muscle. The mature toxin has 48 amino acid residues, 10 of them cysteines. *P. nigriventer* peptide toxin 2–6 activity is proposed to be similar to site 3 toxins (Matavel et al., 2002).

Three important insecticidal polypeptides (LD_{50} = 2.42–22.5 μg/g on *Heliothis virescens*) with molecular weight ranging from 6371 to 7080 Da were isolated from the venom of the spider *Diguetia canities*. The peptide referred to as DTX9.2 induces spastic paralysis in Lepidoptera larvae, blocked by tetrodotoxin, suggesting that DTX9.2 acts upon the voltage-dependent sodium channels of insect nerve membrane (Bloomquist et al., 1996).

Regarding wasp toxins, mandaratoxin (molecular weight 20 kDa) is a polypeptide isolated from the venom of the hornet *Vespa mandarinia*, which reduces the Na^+ current in the neuromuscular junctions of a lobster walking leg (Abe et al., 1982).

α-Pompilidotoxin and β-pompilidotoxin are two small peptides with 13 amino acid residues isolated from the venom of the solitary wasps *Anoplius samariensis* and *Batozonellus maculifrons*, respectively. Electrophysiological studies in lobster leg muscles and in rat trigeminal neurons demonstrated that α-pompilidotoxin, at micromolar concentration, might slow or block conformational changes required for fast inactivation of Na^+ channels on the extracellular surface (Sahara et al., 2000; Yokota et al., 2001). Synthesized analogs of α-pompilidotoxin reveal that the substitutions of Arg1, Lys3, and Lys12 by others markedly change the toxic potency of this molecule (Konno et al., 2000). Studies using the Na^+ channel α-subunit expressed in human embryonic kidney cells reveal that β-pompilidotoxin modifies rat brain type II Na^+ channel, but fails to act on the rat heart α-subunit at similar concentrations (Kinoshita et al., 2001). Finally, it was also demonstrated that α-pompilidotoxin and β-pompilidotoxin slow the inactivation of Na^+ channels on rat hippocampal CA1 oriens, radiatum, and lacunosum molecular interneurons (Miyawaki et al., 2002).

3. Toxins acting on K^+ channels

K^+ channels are the most abundant among ion channels and play important roles in neurons and muscle cells signaling processes, such as membrane potential, neuronal excitability, and the release of neurotransmitters. K^+ channels are typical tetramers, comprising four pore-forming α-subunits and, in some case, additional β-subunits that regulate channel function. Many K^+ channels subtypes, with a wide range of localizations, have been described so far, but their physiological functions are still far from being fully understood (Coetzee et al., 1990; Yi et al., 2001).

Although several physiologically important K^+ channels remain to be cloned, two families of K^+ channel genes have been described to date. One includes the outward-rectifying, voltage-gated K^+ (Kv) channels and the calcium-activated K^+ (K_{Ca}) channels. Voltage-gated K^+ channels regulate resting membrane potential, action potential duration and frequency, and neurotransmitter release. Mammalian voltage-gated K^+ channels are classified into four groups Kv1,

Kv2, Kv3, and Kv4, based on their relation to the *Shaker*, *Shab*, *Shaw*, and *Shal* channels cloned from *Drosophila* (Terlau and Stuhmer, 1998). Structurally, calcium-activated K^+ channels can be subdivided into two groups: the small- or intermediate-conductance K^+ channel, and the α high-conductance K^+ channels (Vergara et al., 1998; Coetzee et al., 1990).

The other family, distantly related to voltage-sensitive K^+ channels, is composed of inwardly rectifying K^+ (Kir) channels. They stabilize the resting membrane potential near the K^+ equilibrium potential. The various types of inwardly rectifying K^+ channels (Kir1.x–Kir7.x) are distinguished by their effector functions (Nichols and Lopatin, 1997; Yamada et al., 1998).

Spider toxins were especially important for the structural and pharmacological characterization of two subtypes of outward-rectifying channels: Kv2 and Kv4 (Table 2) (Dolly and Parcej, 1996; Kukuljan et al., 1995).

Two peptides referred to as phrixotoxins 1 and 2 (PaTx1 and PaTx2), purified from the venom of the spider *Phrixotrichus auratus* and composed by 29–31 amino acid residues, specifically block Kv4.3 and Kv4.2 subtypes elicited currents, by alteration of the channel gating properties (EC_{50} = 5–70 nM) (Diochot et al., 1999).

Argiotoxin-636 (Arg-636), isolated from the venom of the spider *Argiope* sp., is an acylpolyamine that inhibits ligand-gated Na^+ channels and has been characterized as an inhibitor of voltage-gated K^+ channels, blocking the inward-rectifying Kir2.1 subtype (Lee et al., 1999). Further information about this toxin can be found in the Toxins Acting on Glutamate Receptors section.

Hanatoxins (HaTx1 and HaTx2) from *Grammostola spatulata* spider venom, unrelated in their primary sequence to other K^+ channel inhibitors, are 35 amino acid residue peptides with three disulfide bridges. In Kv2.1 K^+ channels, the blockage occurs as a consequence of a depolarizing shift in the voltage dependence of activation (K_d = 42 nM for both) and not by occlusion of the channel pore (Swartz and MacKinnon, 1997; Takahashi et al., 2000).

The heteropodatoxins (HpTX1–HpTX3) from spider *Heteropoda venatoria* are 29–32 amino acid residue peptides that prolong action potentials in rat ventricular myocytes. This action has been attributed to blockage of voltage-dependent K^+ currents, as demonstrated to occur in *Xenopus laevis* oocytes expressing Kv4.2. The toxins have been show to slow the time course of current activation and inactivation, and shift the voltage dependence of current inactivation to more positive potentials (EC_{50} = 100 nM for heteropodatoxins 1 and 2, and 67 nM for heteropodatoxin 3) (Sanguinetti et al., 1997; Bernard et al., 2000).

Recently, the peptide stromatoxin (ScTx1) was isolated from the venom of the African tarantula *Stromatopelma calceata* and characterized as a strong inhibitor of Kv4.2 and Kv2.2 subtype K^+ channels (EC_{50} = 21.4 nM). However, stromatoxin also weakly inhibits Kv2.1 and Kv2.1/Kv9.3 subtypes (Escoubas et al., 2002).

Heteroscodratoxins 1 and 2 (HmTx1 and HmTx2), from the tarantula spider *Heteroscodra maculata* venom, bind to the voltage sensor and inhibit the K^+ channel (EC_{50} = 100–300 nM for both). Heteroscodratoxin 2 seems to be specific for Kv2 channels, whereas heteroscodratoxin 1 also inhibits the Kv4 subtype with the same potency. Heteroscodratoxin 1 is the first peptide described to be effective on the Kv4.1 (Escoubas et al., 2002).

Finally, the *P. nigriventer* peptide toxin 3–1 (PnTx3–1), at micromolar concentration, selectively and reversibly

Table 2
Toxins acting on K^+ channels

Toxin	Source	Chemistry	Main action(s)	References
Argiotoxin-636 (Arg-636)	*Argiope* sp.	Acylpolyamine, 636 Da	Inhibits voltage-gated K^+ channels, blocking the inward-rectifying channel Kir2.1 subtype	Lee et al. (1999)
Hanatoxins 1 and 2 (HaTx1 and HaTx2)	*G. spatulata*	Peptides, 35 amino acids	Block Shab-related Kv2.1 channel from rat brain, shifting the channel opening to more depolarized voltages	Takahashi et al. (2000)
Heteropodatoxins 1–3 (HpTX1 to HpTX3)	*H. venatoria*	Peptides, 29–32 amino acids	Block voltage-dependent K^+ current in Kv4.2 channel, slowing the time course of current activation and inactivation and shifting the voltage dependence of current inactivation to more positive potentials	Sanguinetti et al. (1997)
Heteroscodratoxins 1 and 2 (HmTx1 and HmTx2)	*H. maculata*	Peptides, 34–38 amino acids	Bind to the voltage-sensing domains and inhibit the K^+ channel; HmTx2 seems to be specific for Kv2 channels, whereas HmTx1 also inhibits the Kv4 subtype with the same potency	Escoubas et al. (2002)
P. nigriventer peptide toxin 3–1 (PnTx3–1)	*P. nigriventer*	Peptide, 40 amino acids	Inhibits A-type K^+ currents in GH3 cells	Carneiro et al. (2003)
Phrixotoxins 1 and 2 (PaTx1 and PaTx2)	*P. auratus*	Peptides, 29–31 amino acids	Block Kv4.3 and Kv4.2 subtypes elicited currents, altering the gating properties of these channels	Diochot et al. (1999)
Stromatoxin-1 (ScTx1)	*S. calceata*	Peptides, 34–38 amino acids	Inhibits voltage-dependent potassium channels Shab (Kv2) and Shab (Kv4) subfamilies by modulating the voltage-dependence of the channel and by inducing a shift in the conductance–voltage relationship to more depolarized potentials	Escoubas et al. (2002)

inhibits A-type K^+ currents in GH3 cells without blocking other K^+ currents (Kushmerick et al., 1999; Carneiro et al., 2003).

4. Toxins acting on Ca^{2+} channels

It is well known that Ca^{2+} ions are involved in several physiological processes, including secretion of hormones and neurotransmitters, regulation of ion channel activity, enzymatic modulation, gene expression, cell proliferation, apoptosis, and cell death.

A variety of voltage-gated Ca^{2+} channels are required to mediate the functionality of Ca^{2+} ions in excitable cells. These proteins are classified into low-voltage-activated channels that inactivate rapidly, and high-voltage-activated channels that show no inactivation (Bean, 1989). High-voltage-activated Ca^{2+} channels are subdivided according to their pharmacological and biophysical characteristics into L-type (cardiac, smooth, and skeletal muscles; endocrine cells; neurons), N-type, P-type, Q-type (nerve terminals and dendrites), and R-type (neurons). Low-voltage-activated channels are only of T-type (cardiac and skeletal muscles, neurons) (Catterral, 2000). Recently, a more genetically derived nomenclature dividing Ca^{2+} channels into Ca_v1 (L-type Ca^{2+} channels), Ca_v2 (P-type, Q-type, N-type, and R-type Ca^{2+} channels), and Ca_v3 (T-type Ca^{2+} channels) families, respectively, has been proposed (Ertel et al., 2000).

All voltage-gated Ca^{2+} channels are heteromers formed by a pore-forming α_1-subunit and auxiliary regulatory subunits (β and disulfide-linked $\alpha_2\delta$ dimer). The α_1-subunit is responsible for ion conduction, voltage sensing, and binding site for drugs and toxins (Table 3). An additional auxiliary subunit-denominated γ-subunit, initially identified in skeletal muscle Ca^{2+} channels, has been also found in neurons (Takahashi et al., 1987; Letts et al., 1998).

Voltage-gated Ca^{2+} channels present wide molecular diversity. Molecular cloning has revealed the existence of at least 10 different genes showing considerable homology (77–90%), encoding α_1-subunit (Moreno, 1999). The $\alpha_2\delta$ dimer has been identified by a single gene; nevertheless, extensive alternative splicing produces five distinct isoforms (Williams et al., 1992; Powers et al., 1994). At least, four β-subunit genes were established, and alternative splicing has been shown for two β genes (Birnbaumer et al., 1998). Only one γ-subunit gene was found and no alternative splicing was detected (Powers et al., 1993). Such diversity is highly significant since the precise nature of the α_1, β, $\alpha_2\delta$, and γ

Table 3
Toxin acting on Ca^{2+} channels

Toxin	Source	Chemistry	Main action(s)	References
ω-Agatoxin IA (ω-Aga-IA)	*A. aperta*	Peptide, 69 amino acids	Inhibits Ca^{2+} channel currents in cultured rat dorsal root ganglion neurons and cerebellar granule cells, and blocks presynaptic Ca^{2+} in insects' motoneurons	Adams et al. (1990), Scott et al. (1990)
ω-Agatoxin IIA (ω-Aga-IIA)	*A. aperta*	Peptide, approximately 9.2 kDa	Blocks presynaptic Ca^{2+} channels in insects' motoneurons and neurosecretory cells and Ca^{2+} channels in rat and chick synaptosomal membranes	Venema et al. (1992), Olivera et al. (1994)
ω-Agatoxin IIIA (ω-Aga-IIIA)	*A. aperta*	Peptide, 76 amino acids	Blocks N-type and P-type currents in a voltage-dependent manner in rat sensory and central neurons; unlike ω-aga-IA and ω-aga-IIA, it does not block insects motoneurons at nanomolar concentrations; it also blocks Ca^{2+} entry into chick brain synaptosomes	Mintz et al. (1991), Uchitel (1997)
ω-Agatoxin IVA (ω-Aga-IVA)	*A. aperta*	Peptide, approximately 5.2 kDa	Inhibits P-type and Q-type Ca^{2+} channels by altering the voltage-dependent gating so that very large depolarizations are needed for channel opening	Adams et al. (1990), Baltazar et al. (1997)
ω-Grammotoxin SIA (GrTx)	*G. spatulata*	Peptide, 36 amino acids	Blocks P-type and N-type Ca^{2+} channels potently and specifically; some authors have suggested that this toxin also acts in Q-type Ca^{2+} channels in rat hippocampal neurons	Keith et al. (1995), Piser et al. (1995)
ω-*P. nigriventer* peptide toxin 3–3 (ω-PnTx3–3)	*P. nigriventer*	Peptide, 34 amino acids	Decreases intracellular release of Ca^{2+} in synaptosomes and blocks the synaptic vesicles exocytosis by inactivation of intrasynaptosomal P/Q-type Ca^{2+} channels	Guatimosim et al. (1997), Vieira et al. (2003)
ω-Phonetoxin IIA	*P. nigriventer*	Peptide, 76 amino acids	Blocks N-type Ca^{2+} channels in rat dorsal root ganglion neurons and L-type channels in rat beta pancreatic cells	Cassola et al. (1998)
CNS 2103	*D. okefinokensis*	Acylpolyamine	Blocks L-type and R-type Ca^{2+} channels in N1E-115 cells	Kobayashi et al. (1992)
Funnel web spider (FTX)	*A. aperta*	Acylpolyamine, <500 Da	Blocks P-type Ca^{2+} channels in Purkinje cells and squid giant synapse	Uchitel (1997)
SNX-325	*S. florentina*	Peptide, 49 amino acids	Inhibits the majority of Ca^{2+} currents, with some specificity to N-type channels	Newcomb et al. (1995)

gene products defines the pharmacological and biophysical characteristics of the expressed Ca^{2+} channel heteromers (Catterral, 2000).

ω-Agatoxins and the acylpolyamine funnel web spider (FTX) were the first toxins isolated from *A. aperta* spider venom found to be active on voltage-gated Ca^{2+} channels. ω-Agatoxins are heterologous polypeptides rich in cysteine and disulfide bridges, which vary from 5 to 10 kDa in length and are classified according to their homology, selectivity, and mode of action (Uchitel, 1997).

ω-Agatoxins group I (ω-Aga-IA, ω-Aga-IB, and ω-Aga-IC) and group II (ω-Aga-IIA and ω-Aga-IIB) are composed of potent blockers of insect neuromuscular transmission having low homology between them. ω-Agatoxins from groups I and II seem to be selective for insect L-type and N-type Ca^{2+} channels, respectively. ω-Agatoxins group III (exemplified by ω-Aga-IIIA) are inactive in insects, but block the binding of [^{125}I]ω-conotoxin GVIA to chick or rat brain membranes, as do toxins from group I. In this system, K^+-stimulated Ca^{2+} entry is inhibited by ω-agatoxins-IIA and ω-agatoxins-IIIA, but not by the toxins from group I. Group IV is comprised of ω-agatoxins, like ω-agatoxins-IVA, which is inactive in insects and avian species but is a potent P/Q-type Ca^{2+} channel blocker in mammalian species (Adams et al., 1990; Olivera et al., 1994; Uchitel, 1997).

ω-Agatoxin IVA has been particularly useful in the elucidation of the involvement of several types of Ca^{2+} channels on neurotransmitter release. ω-Agatoxin IVA markedly inhibits the Ca^{2+}-dependent fraction of the evoked [^{3}H]γ-aminobutyric acid (GABA) release and the elevation of Ca^{2+} induced by high [K^+] in mouse brain synaptosomes. Nevertheless, the Na^+-dependent, Ca^{2+}-independent, carrier-mediated GABA release by veratridine is not modified by this toxin (Sitges and Chiu, 1995). Furthermore, it has been shown that ω-agatoxin IVA, at micromolar concentration, blocks GABA, glutamate, dopamine, serotonin, and acetylcholine release from rat brain slices (Harvey et al., 1996). This toxin also acts on the endocrine system and has been useful for the understanding of the role of P-type Ca^{2+} channels in the release of catecholamines from adrenal chromaffin cells, causing a biphasic, dose-dependent inhibition of secretion (Baltazar et al., 1997).

Although present in the same venom, ω-agatoxins usually decrease neurotransmitter release while μ-agatoxins increase it. This apparent puzzle could be explained by the different phases of venom action. In the first, μ-agatoxins would have a quicker and reversible effect, while the ω-agatoxins would have a slower and irreversible effect (Olivera et al., 1994).

Besides ω-agatoxins, the venom of the spider *A. aperta* is the source of other important Ca^{2+} channel blockers (Llinás et al., 1989, 1992). FTX (molecular weight 200–400 Da) blocks Ca^{2+} channels in Purkinje cells and the squid giant synapse. FTX also inhibits acetylcholine release from the neuromuscular junction of mice and humans, and catecholamine release from bovine adrenal chromaffin cells (Protti et al., 1996; Uchitel et al., 1992; Duarte et al., 1993). The synthetic analog FTX 3–3, which has the same structure as the natural toxin, and sFTX 3–3, an amide-like analogue, have similar activities and are selective for P-type Ca^{2+} channels. Norris et al. (1996) have shown that FTX3–3 (EC_{50} = 130 μM) and sFTX3-3 (EC_{50} = 240 μM) antagonize P-type, N-type, and L-type Ca^{2+} channels in mammalian Purkinje cells and superior cervical ganglia neurons.

Another example of an acylpolyamine toxin is CNS2103, from *Dolomedes okefinokensis* spider venom, which reversibly blocks L-type and R-type Ca^{2+} channels in N1E-115 cells, without affecting the function of Na^+, K^+, or T-type Ca^{2+} channels (Kobayashi et al., 1992; Stromgaard et al., 2001).

ω-*P. nigriventer* peptide toxin 3–3 (PnTx3–3) decreases intracellular release of Ca^{2+} induced by KCl depolarization in rat cerebrocortical synaptosomes. In addition, it blocks the synaptic exocytosis of vesicles by inhibition of P/Q-type Ca^{2+} channels (EC_{50} = 1 nM) (Guatimosim et al., 1997). In whole cell patch clamp experiments in cerebellar granule neurons, this toxin inhibits high-voltage-activated Ca^{2+} channels, being more efficient against P/Q-type and R-type (Leão et al., 2000). Cassola et al. (1998) have described ω-phonetoxin IIA, also isolated from *P. nigriventer* spider venom. This toxin is a peptide of 8 kDa, 76 amino acid residues, and seven disulfide bridges, which blocks N-type high-voltage-activated Ca^{2+} channels in rat dorsal root ganglion neurons (K_d = 0.7 nM) and L-type channels in rat β pancreatic cells (K_d = 28 nM). It shows significant homology with the ω-agatoxin III family, and it has been suggested that this toxin interacts with ω-conotoxin-binding sites (Dos Santos et al., 2002). Additionally, peptides that inhibit L-type Ca^{2+} channels from *P. nigriventer* venom (as Tx3-2 and Tx3-5) have been characterized (Kalapothakis et al., 1998).

SNX-325 is a peptide from the venom of the Asian spider *Segestria florentina* that inhibits the majority of Ca^{2+} currents (at 2 μM), without affecting Na^+ or K^+ currents. At 10 nM, SNX-325 has been shown to be a selective blocker of the cloned expressed class B N-type Ca^{2+} channels. SNX-325 is approximately equipotent with the N-type Ca^{2+} channel-selective ω-conopeptides (GVIA and MVIIA) in blocking the K^+-induced release of [^{3}H]norepinephrine from hippocampal slices and in blocking the barium current through cloned expressed N-type Ca^{2+} channels in oocytes (Newcomb et al., 1995). Other three polypeptides with insecticidal activity have been characterized in the spider venom *S. florentina*. They are F5.5, F5.6, and F5.7, with molecular weight of 4973, 4993, and 5159 Da, respectively. These peptides cause complete flaccid paralysis of *H. virescens* (Lepidoptera: Noctuidae) larvae, without affecting vertebrates. Some structural comparisons have suggested that this family of polypeptides might share structural and functional relationships with other small

spider neurotoxins, several of which are known to be highly selective agonists/antagonists of different voltage-dependent Ca^{2+} channels (Lipkin et al., 2002).

The peptide ω-grammotoxin SIA (GrTx) (36 amino acid residues with three disulfide bridges) was isolated from the venom of the spider *G. spatulata*. It has a potent action and apparent specificity for P-type and N-type Ca^{2+} channels (EC_{50} = 180 nM). It causes a complete, dose-dependent inhibition of K^+-evoked influx of $^{45}Ca^{2+}$ into either rat or chick brain synaptosomes. Similar inhibitory profiles are generated for the inhibition of the release of either D-[^{3}H]aspartate or [^{3}H]norepinephrine from rat hippocampal, or of [^{3}H]norepinephrine from chick cortical brain slice preparations, evoked by K^+ depolarization (Lampe et al., 1993). Other authors have suggested that this toxin also acts on Q-type channels in rat hippocampal neurons (Piser et al., 1995; Keith et al., 1995).

Finally, insecticidal toxins active on Ca^{2+} channels have been isolated from the spider *H. curta* venom (*Hololena* toxin, 16 kDa) (Bowers et al., 1987), *H. versuta* venom (ω-atracotoxin, peptides with 36–37 amino acid residues) (Wang et al., 1999), and *Plectreurys tristes* venom (PLTXs, 6–7 kDa) (Branton et al., 1987). Crude venom from *Anemesia* spider species contains components that increase neuronal excitability, which in part appears to be mediated by enhancing Ca^{2+} influx through N-type voltage-activated Ca^{2+} channels (Kalikulov et al., 2001).

There has not been a great deal of literature describing wasp toxins that directly alters the functions of Ca^{2+} or K^+ channels.

5. Toxins acting on glutamate receptors

L-glutamate is considered to be the most important mediator of excitatory signals in both the mammalian central nervous system and the insect peripheral nervous system. Glutamate is also involved in most aspects of mammalian brain function, including cognition, memory, and learning (Fonnum, 1984; Collingridge and Lester, 1989).

Most neurons and glial cells have glutamate receptors in their plasma membranes (Bergles et al., 2000). These proteins are categorized into two major classes, according to their differential intracellular signal transduction mechanisms and molecular homologies, namely, metabotropic receptors (mGluRs) and ionotropic receptors (iGluRs) (for reviews, see Ozawa et al., 1998; Coutinho and Knopfel, 2002).

Metabotropic receptors mediate their function via intracellular G-proteins and are divided into eight functionally distinct receptors, referred to as mGluR1–mGluR8 (Pin and Duvoisin, 1995; Conn and Pin, 1997).

On the other hand, ionotropic receptors are ion channels permeable to particular cations, and are subclassified into *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA; historically termed the "quisqualate receptor"), and kainate receptors (Hollmann and Heinemann, 1994). Each of these proteins is a glutamate-gated ion channel. The NMDA and AMPA receptors mediate the bulk of fast excitatory synaptic transmission and coexist at many synapses in the brain. NMDA receptors differ from AMPA receptors in two important ways: (a) NMDA-gated channels are permeable to Ca^{2+} and Na^+, while AMPA receptors are permeable to both Na^+ and K^+; and (b) inward ionic current through NMDA-gated channels is voltage-dependent. Kainate receptors also exist throughout the brain, but their functions are poorly understood. A major difficulty in the study of the functions of kainate receptors has been the inability to distinguish between individual subunits, and the lack of drugs to distinguish between kainate and AMPA receptors (Bear et al., 1996).

Excessive activation of glutamate receptors is involved and partly responsible for the brain damage found following a stroke and also in several neurodegenerative chronic diseases such as Huntington, Alzheimer, Parkinson, amyotropic lateral sclerosis, ischemia, and epilepsy (Coutinho-Netto et al., 1981; Olney, 1990; Vajda, 2002). The development of glutamate antagonists capable of blocking specific subtypes of glutamate receptors should therefore be of major importance for the generation of therapies for these diseases.

The characterization of glutamate receptor antagonists isolated from spider venoms began in the early 1980s (Table 4). Kawai et al. (1982, 1983, 1991) characterized the toxin Joro spider toxin (JSTX), isolated from the venom of the *Nephila clavata*, which suppresses excitatory postsynaptic potentials (EPSPs) in lobster neuromuscular junction and specifically blocks AMPA receptors in cortical synaptosomes. This pioneering work led to several publications on antagonists of ionotropic glutamate receptor, derived from spider and wasp venoms. Because up to this date compounds derived from venoms that act on metabotropic receptors have not been reported, we will focus interest on described pharmacological actions of spider and wasp toxins on ionotropic receptors.

Usherwood colleagues have extensively characterized the venoms of the spiders *Argiope trifasciata* and *Araneus gemma*, which contain components that act as antagonists of the glutamate receptor in the neuromuscular junction of the locust. Ionophoretic and neurally evoked twitch contraction of locust metathoracic retractor unguis muscle studies indicate that the venoms act only when the glutamate receptor channel complex is activated by an agonist. The authors concluded that the active principles in these spider venoms were molecules of low molecular weight (less than 1000 Da) (Usherwood et al., 1984). Subsequently, these toxins were characterized as the previously cited acylpolyamines, which nowadays are a well-established, wide source of glutamate receptor antagonists in these venoms (Blagbrough et al., 1992; Green et al., 1996).

A mixture of low-molecular-weight compounds, known as argiotoxins, was obtained from the venom of the orb

Table 4
Toxins acting on glutamate receptors

Toxin	Source	Chemistry	Main action(s)	References
Argiopines, argiopinines, and pseudoargiopinines	*Argiope* sp.	Acylpolyamine	Block glutamate-activated and kainate-activated ion currents in a voltage-dependent manner; at higher concentrations, they also block acetylcholine-gated receptor channels	Kiskin et al. (1989)
Argiotoxin-636 (Arg-636), α-agatoxin-489 (Agel-489), and α-agatoxin-505 (Agel-505)	*A. lobata* and *A. aperta*	Acylpolyamine, 636, 489, and 505 Da, respectively	Potent AMPA and/or NMDA receptors blockers in cultured cerebellar granule cells	Adams et al. (1989), Fagni and Bockaert (1995), Williams (1993)
Joro spider toxin (JSTX)	*N. clavata*	Acylpolyamine	Blocks specifically AMPA receptors in cortical synaptosomes in a noncompetitive manner	Kawai et al. (1982, 1983)
Joro spider toxin 3 (JSTX-3)	*N. clavata*	Acylpolyamine	Specific antagonist of AMPA receptors in the mammalian central nervous system	Himi et al. (1990)
Nephilatoxin-3 (NSTX-3)	*N. maculata*	Acylpolyamine	Noncompetitive blocker of glutamate receptor	Blagbrough et al. (1996)
δ-Philanthotoxin (δ-PhTX)	*P. triangulum*	Acylpolyamine	Blocks open channels gated both by junctional and extrajunctional glutamate receptors on locust muscle; in *Xenopus* oocytes expressing receptor clones GluR1, GluR2, and NMDA, kainate and NMDA elicited antagonist-reversible responses in a noncompetitive manner	Clark et al. (1982), Eldefrawi et al. (1988), Brackley et al. (1993)
P. nigriventer peptide toxin 4(5−5)	*P. nigriventer*	Peptide, 5175 Da	Reversibly inhibits NMDA receptor-generated currents in rat hippocampal neurons	De Figueiredo et al. (2001)

weaver spider *Argiope aurantia*. They block neuromuscular transmission and cause reversible paralysis in insects. Their structures reveal common features and consist of a basic residue of arginine, an acylpolyamine chain, and an asparagine that is connected to an aromatic moiety (Adams et al., 1987). Jackson and Parks (1989) have shown that acylpolyamine toxins with amino acid(s) in their structure have similar modes of action at invertebrate neuromuscular junctions, by exerting noncompetitive blocking of AMPA-sensitive receptors.

Several compounds have also been purified from the venom of the spider *Argiope lobata*, which selectively block ionic currents elicited by glutamate and its agonist kainate in rat hippocampal neuron membranes. Three groups of these compounds, argiopine, argiopinines, and pseudoargiopinines, block glutamate-activated and kainate-activated ion currents in a voltage-dependent manner (Kiskin et al., 1989).

JSTX-3 (synthetic Joro spider toxin), nephilatoxin (NSTX) isolated from *Nephila maculata* spider venom, and argiotoxin-636 are structurally related to Joro spider toxin. Intracerebroventricular injections of high doses of JSTX-3 (more than 12 nmol per brain) were shown to increase motor activity of mice. However, at lower doses (4.7 nmol per brain), the toxin did not generate any atypical behavior, although it did specifically antagonize AMPA-induced convulsions, suggesting JSTX-3 as a selective antagonist of AMPA receptors in the mammalian central nervous system (Himi et al., 1990).

The acylpolyamine toxin argiotoxin-636 has been shown to block isolated NMDA receptors in cultured cerebellar granule cells by decreasing the probability of channel opening in a voltage-dependent manner and NMDA-activated ion channels expressed in *Xenopus* oocytes (Draguhn et al., 1991; Fagni and Bockaert, 1995). Still, argiotoxin-636 acts as an AMPA open channel blocker in a voltage-dependent manner and discriminates between the functionally diverse AMPA receptors (Herlitze et al., 1993).

α-Agatoxin-489 (Agel-489) and α-agatoxin-505 (Agel-505) are acylpolyamine toxins isolated from *A. aperta* spider venom. A α-agatoxin-489 (0.1 nM−1 μM) antagonizes activated NMDA receptor and/or open NMDA-activated ionic channels in a voltage-dependent manner in rat hippocampus neurons (Kiskin et al., 1992). α-Agatoxin-489 and α-agatoxin-505 (3−30 μM) enhance the binding of [^{3}H]dizocilpine (MK-801) to NMDA receptors on membranes prepared from rat brain. However, higher concentrations of both toxins inhibit the binding of this drug. At concentrations considerably lower than those inhibiting [^{3}H]dizocilpine binding in membranes, α-agatoxin-505 inhibits responses to NMDA with an EC_{50} of 13 nM in *Xenopus* oocytes voltage-clamped at −70 mV, in a strongly voltage-dependent manner. These actions may be attributed to an interaction with an Mg^{2+} binding site (Williams, 1993).

The *P. nigriventer* peptide toxin 4(5−5) [PnTx4(5−5)] is a single-chain insecticidal molecule composed of 47 amino

acid residues, including 10 cysteines, that reversibly inhibits NMDA receptor-generated currents in rat hippocampal neurons. This toxin is highly toxic to the housefly (*Musca domestica*), cockroach (*Periplaneta americana*), and cricket (*Acheta domesticus*) (De Figueiredo et al., 2001).

Regarding wasps, at least four acylpolyamine toxins named α-philanthotoxin, β-philanthotoxin, γ-philanthotoxin, and δ-philanthotoxin (PhTX) were purified from the venom of the digger wasp *Philanthus triangulum*. δ-philanthotoxin was shown to block EPSP on locust muscle by blocking of open channels gated by both junctional and extrajunctional glutamate receptors (Clark et al., 1982; Stromgaard et al., 1999). At that time, it was not known what type of glutamate receptor was involved in the process, until it was shown that δ-philanthotoxin did not antagonize CA1 field EPSPs mediated by AMPA/kainate receptors at Schaffer collateral/commissural CA1 synapses. If this toxin was present during tetanization, it was able to suppress the induction of the long-term potentiation, probably by antagonizing the postsynaptic NMDA receptor (Matthies et al., 1992).

6. Toxins acting on other neurotransmitter receptors

Although most publications regarding toxins acting on neurotransmitter receptors are related to glutamate receptors, some have been described to act on acetylcholine receptors.

The venom of *P. triangulum* has been shown to reduce twitch tension and tetanic contractions in a rat phrenic nerve–diaphragm preparation. It also reduces the temperature sensitivity and voltage sensitivity of acetylcholine receptor-activated ion channels, at the motor end plate, and shortens the decay time of the miniature end-plate currents, in analogy to a block described for the δ-philanthotoxin on insect glutamate-activated channels (Van Wilgenburg et al., 1984). In addition, in *Xenopus* oocytes expressing rat brain RNA or RNA transcribed from the excitatory amino acid receptor clones 1 and 2 and NMDA receptor 1, kainate antagonist and NMDA antagonist responses elicited by δ-philanthotoxin are reversible and noncompetitive (Brackley et al., 1993).

The parasitoid wasp *Ampulex compressa* stings the first thoracic ganglion of cockroaches and induces a transient flaccid paralysis of the front legs and an excessive grooming followed by a 2- to 5-week-long lethargic state. Assays have demonstrated that the flaccid paralysis is mainly accounted for by the presence of an active component in the venom, which induces a postsynaptic block of nicotinic receptors (Haspel and Libersat, 2003; Libersat, 2003). Antagonists of insect nicotinic receptors were also found in solitary wasp venoms (*Campsomeriella annulata annulata*, *Carinoscolia melanosoma fascinata*, and *Megacampsomeris prismatica*); these toxins were characterized as bradykinins, which play a major role in the paralyzing action for prey capture (Konno et al., 2002).

7. Toxins acting on other synaptic functions: neurotransmitter release and transport

The nerve cell body produces enzymes that are involved in the synthesis of most neurotransmitters, subsequently stored in synaptic vesicles by a proton gradient-dependent uptake system. At the synapse, neurotransmitters are released via a Ca^{2+}-triggered exocytotic fusion of synaptic vesicles with the presynaptic plasma membrane. Following its exocytotic release, neurotransmitters achieve specific receptors and mediate excitatory or inhibitory neurotransmission (Danbolt, 2001). Compounds acting on Ca^{2+} channels, like some toxins described in Section 3, can affect neurotransmitter release. In addition, other toxins can affect neurotransmitter release by interaction with specific proteins involved in the cycle of synaptic vesicles. Others can form pores in plasma membrane that are permeable to cations (Henkel and Sankaranarayanan, 1999).

The action of neurotransmitters in the synaptic cleft is usually terminated by high-affinity transporters, localized in the membrane of neuronal and glial cells and are typically dependent on the Na^{+} gradient (Danbolt, 2001). Neurotransmitter transporters are also involved in the modulation of electrical signals, transport of water and ions, and, recently, it has been proposed to play a dynamic role in control of brain excitability (Amara and Fontana, 2002; Richerson and Wu, 2003). It has also been proposed that changes in the ion concentration in the plasmatic membrane alter transport function up to the point of reversing its activity, resulting in net neurotransmitter release (Attwell et al., 1993). This release is distinguished from exocytotic release by being independent of Ca^{2+} influx via selective voltage-dependent channels (Reis et al., 1999; Phillis and O'Regan, 2003).

Excessive stimulation or blockade of neurotransmitter release and transport may result in a disturbance of the neural synapse. It is therefore perhaps not surprising that receptors located on membranes, ion channels, and proteins involved in neurotransmitter release are targeted by most toxins. However, toxins acting directly on neurotransmitter transporters have not been as widely described; on the other hand, new studies have pointed to the fact that this possibly constitutes a mechanism of action displayed by these toxins (Fontana et al., 2003).

A group of high-molecular-weight polypeptides, purified from the venom of the black widow spider *Latrodectus* species, induces massive neurotransmitter secretion from affected nerve endings. These compounds can be highly selective for vertebrates (α-latrotoxin, α-LTX), insects (α-latroinsectotoxin, LIT), or crustaceans (α-latrocrustatoxin, α-LCT), resulting in a blockade of nerve transmission and leading to muscle paralysis (Pinto and Rothlin, 1974; Henkel and Sankaranarayanan, 1999).

The best known of these toxins is α-latrotoxin, and models of its action have been proposed to explain the massive release of neurotransmitters induced in a variety of neurosecretory cells. One of these models proposes that

the toxin inserts itself into the phospholipid bilayer, where its molecules aggregate, forming a constantly open channel-like pore, permeable to several cations including Ca^{2+}, Na^{+}, K^{+}, and Mg^{2+}, by triggering exocytosis (Finkelstein et al., 1976; Hurlbut et al., 1994; Barnett et al., 1996). Cryoelectron microscopy showed that α-latrotoxin inserts itself into a bilayer of liposomes in a tetrameric form. These studies have also extended our knowledge about the size of the α-latrotoxin pore now estimated to be 10–25 Å in diameter (Krasilnikov and Sabirov, 1992; Orlova et al., 2000). These data help to explain why small molecules, apart from ions, pass in or out of cells. Studies have been performed in order to obtain more information about the precise orientation of the tetramer in membranes (Ichtchenko et al., 1998; Davletov et al., 1998; Khvotchev and Sudhof, 2000).

By using molecular techniques, it has also been discovered that α-latrotoxin interacts with two types of receptors on membranes, of different structures and belonging to distinct families of proteins. Neurexin-1α binds α-latrotoxin in a Ca^{2+}-dependent manner and is specifically expressed in nerve tissue; CIRL/latrophilin is a Ca^{2+}-independent receptor for α-latrotoxin and belongs to the family of G-protein-coupled receptors (Ushkaryov et al., 1992; Davletov et al., 1996; Krasnoperov et al., 1996; for a review of the actions of α-latrotoxin, see Ushkaryov, 2002).

Neurexin-1α, a 160- to 220-kDa protein, has a large extracellular domain, which seems to be related to an adhesion function. Its intracellular domain binds to the cytoplasm domain of synaptotagmin, a Ca^{2+} sensor involved in the final stages of exocytosis, and to Ca^{2+}/calmodulin-dependent serine protein kinase, suggesting a role in intracellular signaling of exocytosis (Petrenko et al., 1991; Ushkaryov et al., 1992; Geppert et al., 1994). The association of α-latrotoxin to neurexin-1α may be a way for the toxin to induce massive neurotransmitter release via second messengers.

CIRL/latrophilin is a glycosylated polypeptide of 120 kDa that binds to α-latrotoxin, in a Ca^{2+}-dependent or Ca^{2+}-independent manner, depending on the cell type. CIRL/latrophilin is tightly associated with syntaxin 1A, suggesting that it could be recruited to vesicle release sites. This protein triggers exocytosis, possibly via a second messenger mechanism whose pathway is still not well established. This is the main model for the explanation of the massive Ca^{2+}-independent release of neurotransmitter induced by α-latrotoxin in some types of cells (Krasnoperov et al., 1997).

Although there still exists uncertainty about the mode of action of α-latrotoxin, it should be considered that the toxin acts in different ways depending on the targeted cell type and on concentration. For instance, it is more probable that at higher concentrations, the toxin acts by forming an ion channel, and at lower concentrations, the toxin acts by a mechanism dependent on receptors like neurexin-1α and/or CIRL/latrophilin (Henkel and Sankaranarayanan, 1999).

α-Latrocrustotoxin (α-LCT), another toxin from *Latrodectus* species, acts as a lethal presynaptic neurotoxin in the central and peripheral nervous systems of the crayfish, causing distortions of its electromyography and motor activity pattern (LD_{50} = 0.1 μg/g of body weight). In the isolated nerve–muscle preparation, this toxin causes intense transmitter release, resulting in the complete blockade of neuromuscular transmission and destructive alterations in the nervous cells (Burmistrov et al., 1997). α-Latrocrustotoxin binds to a G-protein-coupled receptor found in invertebrates independently of Ca^{2+}. However, it requires extracellular Ca^{2+} to stimulate exocytosis via G-proteins and second messengers. The toxin produces an increase in the frequency of spontaneously occurring miniature excitatory postsynaptic potentials at crayfish neuromuscular junctions, without affecting their amplitude distribution. It has been suggested that the induction of an extracellular Ca^{2+} influx into nerve terminals may explain the action of α-latrocrustotoxin on transmitter release at crayfish neuromuscular junctions (Elrick and Charlton, 1999).

α-Latroinsectotoxin, at nanomolar concentrations, causes an increase in the frequency of miniature excitatory postsynaptic potentials in neuromuscular preparations of blowfly larvae. It is believed that the α-latroinsectotoxin molecules incorporate into the lipid bilayer membrane in an oriented manner, and that dimerization might be a prerequisite for channel formation. The ion channels formed are cation-selective; the most permeable ions are Ba^{2+}, Ca^{2+}, and Mg^{2+} (Magazanik et al., 1992; Shatursky et al., 1995).

It has been suggested that the selectivity of α-latrotoxin and α-latroinsectotoxin could be due to differences in the structure of neurotoxin receptors in nerve endings of vertebrates and insects, respectively, but both toxins increase the conductance of bilayer lipid membranes by inducing channel-like activity (Magazanik et al., 1992).

The crude venom of *Steatoda paykulliana* spider increases the release of neurotransmitters from PC12 cells, probably by forming pores permeable to monovalent and bivalent cations (Cavalieri et al., 1987).

The *P. nigriventer* peptide toxin 3–6 (PnTx3–6) abolishes Ca^{2+}-dependent glutamate release (EC_{50} = 74.4 nM) by inactivating intrasynaptosomal P/Q-type Ca^{2+} channels (Vieira et al., 2003).

Regarding wasps, mastoparan toxins, which are usually found as tetradecapeptides, have been reported to be the major peptide class in the venom of many species of hornet wasps (Nakajima et al., 1985). Several such compounds have been characterized as causing release of histamine, GABA, serotonin, and other chemical substances, such as hormones, from several cell types (Ohara-Imaizumi et al., 2001; Wilson and Guild, 2002; Kruger et al., 2003). The main action of mastoparan appears to involve multiple signal transduction pathways through G-proteins, leading to phospholipase C activation and phosphatidyl inositol breakdown (Nakajima et al., 1985).

Finally, orientotoxin is a compound isolated from the giant hornet *Vespa orientalis* venom, which has lysophospholipase activity and blocks the release of neurotransmitters from presynaptic nerve membranes (Tuichibaev et al., 1984).

Regarding transporters, it has been demonstrated that Joro spider toxin, cited above as a specific blocker of glutamate receptors, inhibits glutamate uptake by synaptosomes in a dose-dependent manner, without altering glycine, leucine, and tyrosine uptake (Pan-Hou et al., 1989). In contrast to the reported inhibitory effects of acylpolyamine toxins on high-affinity neurotransmitter uptake, two acylpolyamine-like spider toxins JSTX-3 and NSTX-3 significantly increase the high-affinity uptake of glutamate as demonstrated by high-resolution autoradiography (Van Marle et al., 1989).

The *P. nigriventer* peptide fraction 4 (PnTx4) inhibits glutamate uptake in a dose–response manner in rat cortical synaptosomes (EC_{50} = 2.35 μg/ml). This fraction is composed of seven peaks, referred to as *P. nigriventer* peptide toxins 4(1–7) [PnTx4(1–7)]. Among them, *P. nigriventer* peptide toxins 4(3–7) also inhibit glutamate uptake (Mafra et al., 1999). *P. nigriventer* peptide toxin 3–4 from the same venom has been reported to decrease glutamate release from synaptosomes. This toxin also inhibits glutamate uptake in synaptosomes in a time-dependent manner, leading to a decrease in Ca^{2+}-independent glutamate release, dependent on the redox state of cysteine residues of the toxin (Reis et al., 1999, 2000).

Two fractions isolated from *Parawixia bistriata* (*P. bistriata* fraction A2) and *Scaptocosa raptoria* (*S. raptoria* toxin 1; SrTx1) spiders venoms were demonstrated to have important anticonvulsive effects when injected intracerebroventricularly in rats. *S. raptoria* toxin 1 and *P. bistriata* fraction A2 abolished rat convulsive tonic–clonic seizures induced by convulsant drugs. They also reduced the mortality of experimental animals, most probably due to an inhibitory effect on GABA uptake, as shown by experiments on rat synaptosomes (Rodrigues et al., 2001; Cairrão et al., 2002). Still, the crude venom *P. bistriata* spider was shown to paralyze termites in a dose-dependent manner, and to stimulate glutamate and inhibit GABA uptakes in rat cortical synaptosomes (Fontana et al., 2000, 2003). Recently, a compound purified from this venom, referred to as *P. bistriata* toxin 1.2.3 (PbTx1.2.3), was shown to stimulate glutamate uptake into rat cortical synaptosomes by a mechanism that appears to be independent of glutamate receptor activation. In addition, *P. bistriata* toxin 1.2.3 prevents neuronal death during retinal acute ischemia, implying that the enhanced transport activity probably limits the excitotoxicity (Fontana et al., 2003).

Regarding wasp toxins acting on neurotransmitter transporters, a few reports on the venom of *P. triangulum* have been made. In insects, the venom has central and peripheral effects; the latter consist of a presynaptic as well as a postsynaptic blockade of skeletal neuromuscular transmission. The presynaptic block is probably caused by an inhibition of glutamate uptake, producing paralysis of the prey (Piek, 1982). Two components of the venom of *P. triangulum*, β-philanthotoxin and δ-philanthotoxin, reduce the high-affinity uptake of glutamate in rat hippocampus (10 μM for both). The second one reduces the uptake more efficiently than β-philantotoxin (Van Marle et al., 1986).

In addition, the venom of the social wasp *Agelaia vicina* nearly abolishes glutamate and GABA uptake in a noncompetitive manner in rat cerebral cortex synaptosomes, suggesting that some components of this venom might be a useful tool for further characterization of GABA and glutamate uptake systems (Pizzo et al., 2000).

Finally, peptides of 9–18 amino acid residues containing a bradykinin-like sequence, known as kinins, are found in wasp venom. Kinins are neurotoxic components that act on the insect central nervous system, causing a presynaptic block of the cholinergic transmission by irreversible depletion of neurotransmitters, probably caused by a noncompetitive inhibition of choline uptake (Piek, 1991).

8. Conclusions

Toxins are evolutionary products acting to immobilize prey and also used in self-defense. Several toxins show high specificity and affinity for ion channels, receptors, and transporters in invertebrates and vertebrates. The study of neurotoxins has come into evidence over the last 30 years. These molecules represent an incredibly rich source of probes useful for understanding synaptic transmission events, for identifying insecticide targets, and for aiding in the design of novel drugs for the treatment of neurological disorders.

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