Chapter 16
The Cytotoxic Mode of Action of the Venom of *Cupiennius salei* (Ctenidae)

Lucia Kuhn-Nentwig and Wolfgang Nentwig

16.1 Introduction

The venom of the ctenid spider *Cupiennius salei* (Fig. 16.1) is rich in components which belong to different functional groups. Besides low molecular mass compounds, the venom contains several disulphide-rich peptides, also called mini-proteins, which act as neurotoxins on ion channels or as enhancers of neurotoxins. Likewise, a variety of small cytolytic peptides, which destroy membranes very efficiently, and enzymes are present in the venom. Neurotoxins with cytolytic activity, cytolytic α-helical small cationic peptides and enzymes most probably attacking connective tissue and phospholipid membranes cause the overall cytotoxic effect of this venom. Synergistic and enhancing interactions between components enable the spider to achieve a maximum of toxicity with a minimum of venom quantity.

16.2 Low Molecular Mass Compounds

The ion concentrations in the venom of *Cupiennius salei* are determined as Na^+ 8.9 mM, K^+ 215 mM and Ca^{2+} 0.94 mM (Kuhn-Nentwig et al. 1994). The high K^+ ion content synergistically increases the insecticidal activity of the main neurotoxins CsTx-1 and CsTx-9 (Wullschleger et al. 2005). These concentrations are the reverse of the concentrations found in the hemolymph of *Cupiennius salei* (Na^+ 223 mM, K^+ 6.79 mM and Ca^{2+} 4.0 mM) (Loewe et al. 1970).

The venom contains all 20 standard amino acids, most of which at concentrations below 25 pmol/µl; only glycine is more common (43.3 pmol/µl).

L. Kuhn-Nentwig (✉) • W. Nentwig
Institute of Ecology and Evolution, University of Bern, Bern, Switzerland
e-mail: lucia.kuhn@iee.unibe.ch

W. Nentwig (ed.), *Spider Ecophysiology*,
DOI 10.1007/978-3-642-33989-9_16,

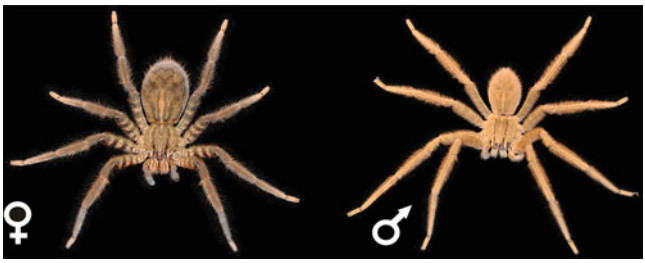

Fig. 16.1 Adult *Cupiennius salei* (Ctenidae). *Left* female spider, *right* male spider, dorsal view

Remarkable is the frequent occurrence of taurine (70.0 pmol/ml). Histamine was determined with a concentration of 5.7 nmol/µl, and the polyamines putrescine and cadaverine could be detected only in traces (3–18 pmol/µl) (Kuhn-Nentwig et al. 1994). Histamine is a known enhancer of the neurotoxic activity of the main neurotoxin CsTx-1 (Wullschleger et al. 2005).

16.3 Disulphide-Rich Peptides

Until now, 43 different cysteine-containing peptides have been identified from the cDNA library of *Cupiennius salei* venom glands (Kuhn-Nentwig, unpublished results), and for 17 of them, the amino acid sequence data have been published (Kuhn-Nentwig et al. 2004; Trachsel et al. 2012). Their molecular masses range between 3.5 and 9.9 kDa, and some of them exhibit a C-terminal amidation as posttranslational modification. Most of them contain an inhibitor cystine knot (ICK) motif where the disulphide bridge bonds are between C1–C4, C2–C5, C3–C8 and C6–C7. However, some of the peptides are characterised by the presence of only two cysteines (CsTx-16), and some contain up to 14 cysteines.

The expressed neurotoxins and neurotoxin-like structures can be divided into three groups: The first and most frequently expressed group (78.4 %) comprises the main neurotoxin CsTx-1, followed by CsTx-9, CsTx-10 and CsTx-11 and the enhancer peptides CsTx-8, CsTx-12 and CsTx-13 (Trachsel et al. 2012; Wullschleger et al. 2004) (Fig. 16.2). Interestingly, C-terminally truncated homologues of CsTx-1 (described as CsTx-2a, b), CsTx-9 (CsTx-7), CsTx-8 and CsTx-12 (CsTx-14) and CsTx-13 (CsTx-15) have been isolated in small quantities from the venom and seem to be rather posttranslational products than true translation products. All these peptides exhibit a high homology to peptides also identified in a cDNA library of *Lycosa singoriensis* (Zhang et al. 2010).

The second group is composed of neurotoxin-like structures (20 %) and we named some of them (5.4 %) “ancient” neurotoxins or neurotoxin-like structures because their structure is related to neurotoxins which have been published for the agelenids *Agelenopsis aperta* and *Agelena orientalis*, the ctenid *Phoneutria nigriventer* and the lycosid *Geolycosa* sp. In the *Cupiennius salei* venom, this group comprises

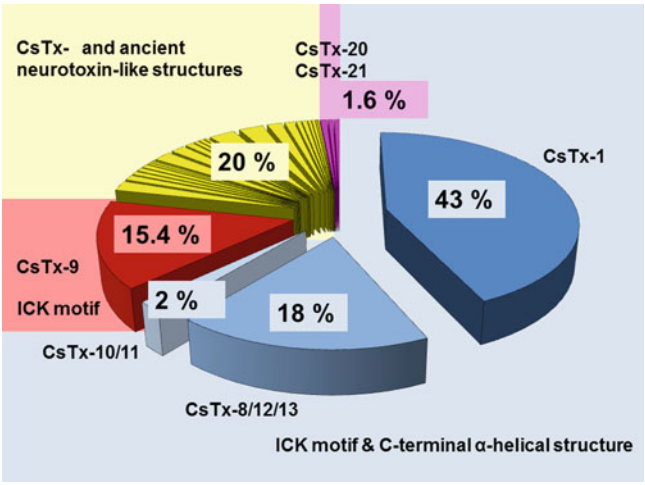

Fig. 16.2 Distribution of expressed neurotoxins and neurotoxin-like structures ($N = 625$ contigs; 90 % assemblage) in the venom of *Cupiennius salei*. Group 1 peptides (compare text) exhibit the ICK motif and possess C-terminally an α-helical structure (coloured in *blue*) but include also CsTx-9, exhibiting only the ICK motif (coloured in *red*). Group 2 peptides (CsTx peptides and ancient neurotoxin-like structures) are expressed only in low amounts (marked in *yellow*). Group 3 peptides (CsTx-20/21) exhibit no propeptide after the signal peptide (coloured in *pink*)

24 peptides; they account only for 20 % of the total expressed neurotoxin-like structures, and their concentrations in the venom are very low (Fig. 16.2). Therefore, we assume that a functional context can be excluded for some of them and evolutionary reasons could explain their existence.

The third peptide group differs from group 1 and 2 by the absence of an acidic propeptide between the signal peptide and the mature peptide (1.6 %). CsTx-20 and CsTx-21 homologues are more acidic peptides with isoelectric points (pI's) between 4.85 and 6.06, exhibiting 10 cysteines and molecular masses between 7.2 and 9.9 kDa (Kuhn-Nentwig, unpublished results; Trachsel et al. 2012) (Fig. 16.3).

16.3.1 Neurotoxins

The most abundant neurotoxin in the venom of *Cupiennius salei* is CsTx-1 with concentrations between 1.4 and 3.3 mM. CsTx-1 exhibits an ICK motif and is composed of 74 amino acid residues with a highly cationic amidated C-terminus. This peptide is the most insecticidal neurotoxin in the venom, and its insecticidal activity (LD_{50} 0.35 pmol/mg *Drosophila*) is three to four times increased through synergistic interactions with other neurotoxins, enhancers or cytolytic peptides

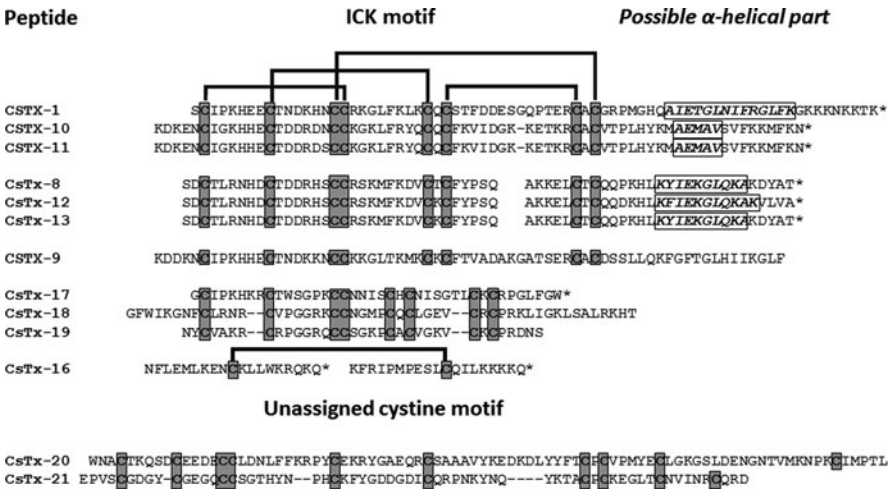

Fig. 16.3 Overview of cysteine-containing neurotoxins and neurotoxin-like structures identified in the venom of *Cupiennius salei*. Cysteine residues are in *bold type* and shaded in *grey*. The disulphide bridge pattern [ICK motif; single interchain disulphide bridge (CsTx-16) or unassigned disulphide bridge pattern (CsTx-20/21)] are indicated above the sequences. Possible α-helical parts in the C-terminal part of the peptides are boxed in *bold* and *italic*. C-terminal amidation is indicated by an *asterisk*

(Kuhn-Nentwig et al. 2004; Wullschleger et al. 2004, 2005). Furthermore, CsTx-1 blocks insect (cockroach) mid/low voltage-activated and high voltage-activated Ca_v channels and vertebrate L-type (GH3 cells) Ca_v channels (Kubista et al. 2007).

Reducing the C-terminal part of CsTx-1 results in considerable differences in the LD_{50} values obtained in bioassays on *Drosophila* flies. In the case of CsTx-2a (lacking the last 13 amino acid residues), the insecticidal activity is reduced to only 14 % and for CsTx-2b (lacking the last 14 amino acid residues) to less than 1 % (Fig. 16.3). It is obvious that the C-terminal part of CsTx-1 plays an important role in its toxicity even though a synthetically produced peptide containing only the C-terminal last 13 amino acids, which we named CT1-short, shows no insecticidal activity. Also, CT1-short has no effect on the insecticidal activity of CsTx-1, CsTx2a or CsTx2b when administered together (Kuhn-Nentwig et al. 2000).

Secondary structure prediction of the C-terminal part of CsTx-1 resulted in a putative α-helix. However, such a putative C-terminal α-helical structure is only possible in CsTx-1 and in CT1-long (this synthetically produced peptide corresponds to amino acid residues 45–74 of CsTx-1) and not in CsTx-2a, CsTx-2b and CT1-short, as verified by CD measurements of these peptides in the presence of membrane-mimicking trifluoroethanol. Investigations of CsTx-1 and CT1-long on prokaryotic and eukaryotic cell membrane systems exhibit an unspecific membranolytic activity of both peptides in the micromolar range on prokaryotic

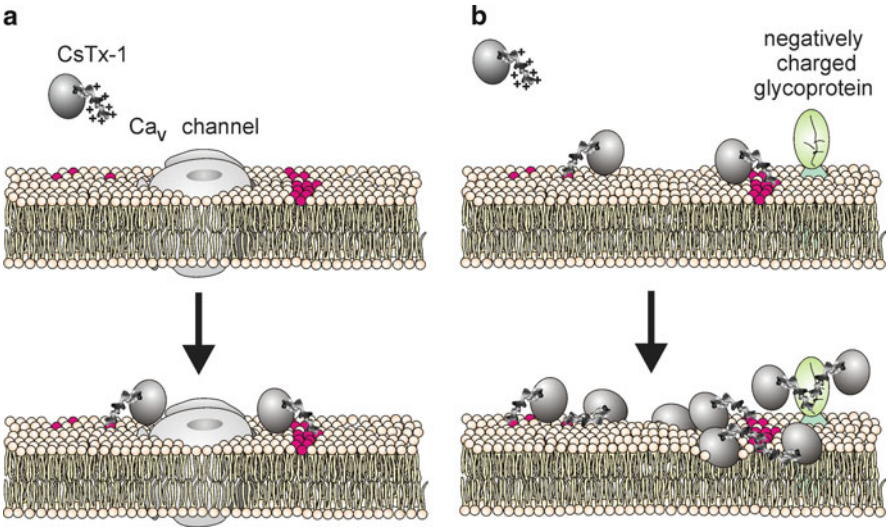

Fig. 16.4 (**a**) CsTx-1 inhibits a Ca^{2+} channel after attraction and anchoring with its highly cationic C-terminus to the cell membrane at negatively charged cell structures such as phospholipids with negatively charged head groups (*red*) or rafts of such negatively charged phospholipids. Ion-channel inhibition may take place by direct binding to the ion channel or binding to negatively charged lipid rafts, thus influencing the membrane architecture surrounding the ion channel. (**b**) In higher concentrations and in absence of the target ion channel, CsTx-1 acts membranolytic by disturbing the membrane architecture after binding to negatively charged phospholipids, negatively charged lipid rafts or negatively charged glycoproteins

and eukaryotic cells. This membranolytic activity exhibits different preferences of CsTx-1 and CT1-long depending on the tested membrane system (Kuhn-Nentwig et al. 2012).

Synergistic interactions between neurotoxins and low molecular mass compounds or between neurotoxins and enhancer peptides or cytolytic peptides are well documented (Adams 2004; Wullschleger et al. 2004, 2005), but a synergistic interaction within one peptide points to a further possibility to increase the toxicity of venom compounds. CsTx-1 exhibits two structurally different domains: In the N-terminal position, the ICK motif is responsible for the Ca^{2+} channel inhibition, and in the C-terminal position, an α-helix motif exhibits cytolytic activity (Kuhn-Nentwig et al. 2012) (Fig. 16.4).

Besides CsTx-1, so far two further neurotoxins in the venom of *Cupiennius salei*, CsTx-10 and CsTx-11, and the enhancer peptides, CsTx-8, CsTx-12 and CsTx-13, possess a comparable C-terminal extension in which secondary structure predictions identified a putative C-terminal α-helical structure. Also from another spider, the miturgid *Cheiracanthium punctorium*, a two-domain modular toxin CpTx-1a has been reported (Vassilevski et al. 2010), which could point to a common strategy of higher entelegyne spiders for the enhancement of the toxicity of one peptide. This strategy is also known for some scorpion venom-derived

peptides exhibiting a putative α-helical N-terminus and a CSαβ motif fold originated from three disulphide bridges located C-terminally (Kuhn-Nentwig 2009).

16.3.2 Enhancer Peptides

CsTx-8, CsTx-12, CsTx-13 and the C-terminally truncated peptides CsTx-14 and CsTx-15 stand for the enhancer peptides that enhance in non-toxic concentrations the insecticidal activities of CsTx-1 and CsTx-9 (Trachsel et al. 2012; Wullschleger et al. 2004, 2005). These peptides differ from the above-mentioned neurotoxins by a further posttranslational modification in which, after amino acid residue 34, six amino acid residues are posttranslationally removed (Kuhn-Nentwig, unpublished results). This results in two peptide chains A and B, connected by the two disulphide bridges C3–C8 and C6–C7. Identification of the disulphide bridge pattern of CsTx-13 by nanoelectrospray tandem MS revealed the ICK motif as described above for CsTx-1 (Wullschleger et al. 2004). The C-terminus of chain B is also posttranslationally amidated in CsTx-8, CsTx-12 and CsTx-13. Interestingly, peptide chains B of CsTx-8, CsTx-12 and CsTx-13 exhibit, after secondary structure predictions, an α-helix motif which may act membranolytically as described for CsTx-1. The insecticidal activity of CsTx-13 (LD_{50} value 16.3 pmol/mg *Drosophila* fly) seems to be the weakest when compared with CsTx-9 (LD_{50} value 3.12 pmol/mg fly) and CsTx-2a (LD_{50} value 2.58 pmol/mg fly).

16.4 Cytolytic Peptides

16.4.1 Overview

The venom of *Cupiennius salei* contains many membranolytically acting peptides (cupiennins) with molecular masses between 1.5 and 4.2 kDa. They exert a strong cytolytic activity towards prokaryotic and eukaryotic cells (Kuhn-Nentwig et al. 2002b). The cupiennin 1 (a–d) and cupiennin 2 (a–e) families are characterized by 35 amino acid residues, pI's between 10.2 and 10.5 and net charges from +6 to +7. These highly cationic peptides exhibit hydrophobic N-termini composed of six amino acid residues which are followed by six repeats (cupiennin 1 family) or five repeats (cupiennin 2) of four amino acids, which form the central part of the peptide chain, with lysine always in first position. The C-termini are more polar, and more than 40 % of all amino acid residues are hydrophobic. Due to well-defined

hydrophobic and hydrophilic areas within the α-helix, the peptides reach an amphiphilic conformation which is essential for their cytolytic activity (Powers and Hancock 2003).

Furthermore, small cationic peptides (SCP), as the cupiennin 3 (SCP 3a–d; 27 amino acid residues) and cupiennin 4 (SCP 4a, b; 27 amino acid residues) families, are characterised by the absence of two to three repeats in the central part of the peptides when compared with the cupiennin 1 family. Nevertheless, these peptides exhibit pI's between 10.4 and 11.2, have net charges from +5 to +7 and are also C-terminally amidated such as the cupiennin 1 and 2 families. Besides these, several N-terminally or C-terminally truncated forms of the cupiennin 1 (SCP 1a–h) and 4 families (SCP 4c–g) have been identified (Trachsel et al. 2012). Currently, it is not clear if some of them are posttranslational products of known cupiennins or products of simple, binary or complex precursors as described for the latarcins, membranolytic peptides from the venom of the zodariid spider *Lachesana tarabaevi* (Kozlov et al. 2006). Interestingly, the SCP families 6 and 7 have introduced besides lysine also arginine into the peptide chain to obtain a cationic character as it is known from other spider venom-derived cytolytic peptides as oxyopinins and latarcins (Kozlov et al. 2006).

16.4.2 Membranolytic Activity of the Cupiennin 1 Family

The cupiennin 1 family is the best investigated membranolytically acting peptide family from *Cupiennius salei*. CD measurements of cupiennin 1a in water exhibit a random coiled structure. In the presence of membrane-mimicking trifluoroethanol (50 %) or negatively charged phospholipid vesicles, the formation of an α-helix occurs. It is supposed that the peptides may be attracted to the cell surfaces by electrostatic interactions between their positively charged side chains of lysine and negatively charged membrane phospholipid head groups and other negatively charged components of bacteria, protozoa and eukaryotic cells (Kuhn-Nentwig et al. 2002b; Pukala et al. 2007a).

Determination of the solution structure of cupiennin 1a by nuclear magnetic resonance spectroscopy exhibits a helix-hinge-helix structure, a structural motif, which has frequently been identified in cationic cytolytic peptides. Well-defined helices are located between residues Gly3-Ala21 and Tyr28-Lys32, and the hinge region is supposed to be initiated by Gly25 (Pukala et al. 2007a). Analysing the role of the N- and C-terminal segments of cupiennin 1d shows that the cytolytic activity depends on the hydrophobic N-terminus and is modulated by the polar C-terminus (Kuhn-Nentwig et al. 2002a). With a length of ~30 Å of the N-terminal helix of cupiennin 1a, the peptide is able to span the bilayer of bacterial cell membranes and phosphatidylcholine bilayers, resulting in pore formation and membrane destruction (Cornell and Separovic 1983; Pukala et al. 2007a).

16.4.3 Biological Activity of the Cupiennin 1 Family

The cupiennin 1a family acts cytolytically on a variety of different bacteria cell membrane types in the submicromolar range (minimal inhibitory concentrations from 0.08 to 5 μM). Additionally, the eukaryotic pathogens trypanosomes and plasmodia, causing sleep sickness and malaria, are destroyed in submicromolar concentrations (IC_{50} values 0.029 to 0.658 μM). Eukaryotic cells, which dispose of negatively charged cell membrane structures, such as erythrocytes, rat skeletal myoblasts or different human leukemic and tumour cells are destroyed likewise in the sub- and micromolar range. In the case of human erythrocytes, it could be demonstrated that binding of cationic peptides is mediated by attraction to negatively charged sialic acids on the outer leaflet of these cells. A stereospecific mode of action of cupiennin 1a could be excluded (Kuhn-Nentwig et al. 2011).

Besides the direct effects of cupiennin 1a on membrane systems, this peptide also inhibits the formation of nitric oxides by neuronal nitric oxide synthase. The mechanism involves a complexation with calmodulin. Calmodulin is the regulatory protein for a variety of kinase phosphorylating enzymes and the eukaryotic cytoskeleton and it is essential for operations of neuronal nitric oxide synthase (Pukala et al. 2007b). Likewise, the production of superoxide by the NADPH oxidase in phorbol myristate acetate-stimulated granulocytes is additionally inhibited by cupiennin 1a (Kuhn-Nentwig et al. 2011). It is supposed that cupiennin 1a will interfere with many cellular functions and that it simultaneously destroys membrane parts of the neuronal tissues and muscle cells leading to a collapse of the cellular and neuronal functions.

16.5 Enzymes

Besides low molecular mass compounds and peptides with molecular masses up to 10 kDa, several proteins with molecular masses between 25 and 97 kDa have been identified in the *Cupiennius salei* venom. One of these proteins exhibits hyaluronidase activity and cleaves hyaluronan into fragments of varied molecular size (Kuhn-Nentwig et al. 1994).

Up to now the presence of hyaluronan (hyaluronic acid), a large linear polymer of repeating disaccharides of glucuronic acid and GlcNAc in arthropods, is still controversially discussed. On the one side, it is stated that hyaluronan, common in vertebrates, has not been found in arthropods and in *Drosophila* only chondroitin sulphate and heparan sulphate have been identified (Takeo et al. 2004; Toyoda et al. 2000). Additionally, no hyaluronan synthase genes were found searching the genomic sequencing project for *Drosophila* (DeAngelis 2002).

On the other hand, histochemical investigations of the mesenteric connective tissue of cockroaches and locusts indicate the presence of hyaluronan in various instars (Ashhurst and Costin 1971; Francois 1978; Treherne et al. 1982), but also chondroitin sulphate and heparan sulphate have been identified in internal organs of cockroaches (dos Santos et al. 2006). From *Hippasa partita* (Lycosidae), a highly substrate-specific hyaluronidase is known, which only cleaves hyaluronan, but not chondroitin and heparan sulphate (Nagaraju et al. 2007).

It has intensively been discussed that hyaluronidase acts as spreading factor, facilitating the access of neurotoxic and cytolytic venom components to their targets (Kuhn-Nentwig et al. 2011). This assumption is convincing for large mygalomorph spiders which may have small vertebrates as prey and which may need to defend themselves against vertebrate predators. In contrast to this, most araneomorph spiders do not target vertebrates. Nevertheless, hyaluronidase activity has been identified in their venoms, but its function as spreading factor still needs further clarification in terms of substrate specificity of the hyaluronidase and possible substrate availability within various prey items, e.g. such as basement membranes surrounding nerve and muscle tissues or connective tissues.

Preliminary results from the cDNA library of *Cupiennius salei* venom glands also show that it contains, besides several other enzymes, a hyaluronidase sequence with a high similarity to the hyaluronidase BmHYA1 [UniProtKB/TrEMBL: D1MBU1], identified from the venom of the Chinese red scorpion *Buthus martensii* Karsch (Feng et al. 2010). Additionally, a phospholipase C sequence with a high similarity to phospholipase C-like protein [UniProtKB/TrEMBL: C5J8D0] from the scorpion *Opisthacanthus cayaporum* venom glands (Silva et al. 2009) and putative phospholipase C [UniProtKB/TrEMBL: B7Q2N6 and B7P6Q6] similar to the tick *Ixodes scapularis* have been identified with the Blast algorithm (Kuhn-Nentwig and Piquemal, unpublished results).

16.6 Conclusions

The venom of *Cupiennius salei* is characterized by (1) a high diversity of cytolytic compounds (linear cytolytic peptides and a cysteine-rich peptide exhibiting two domains: the ICK motif and an α-helical cytolytically acting domain), (2) the neurotoxic activity of ion-channel inhibitors, (3) a highly active hyaluronidase and (4) synergistic interactions between many of these components (Fig. 16.5). The combined effects of synergistic and enhancing interactions between various components enable *Cupiennius salei* to inject a maximum of toxicity with a minimum of venom quantity, thus optimizing its venom investment.

Acknowledgements We thank Johann Schaller and Stefan Schürch for long-term cooperation in spider venom peptide amino acid sequence analyses and MS measurements and Reto Stöcklin (Atheris Laboratories).

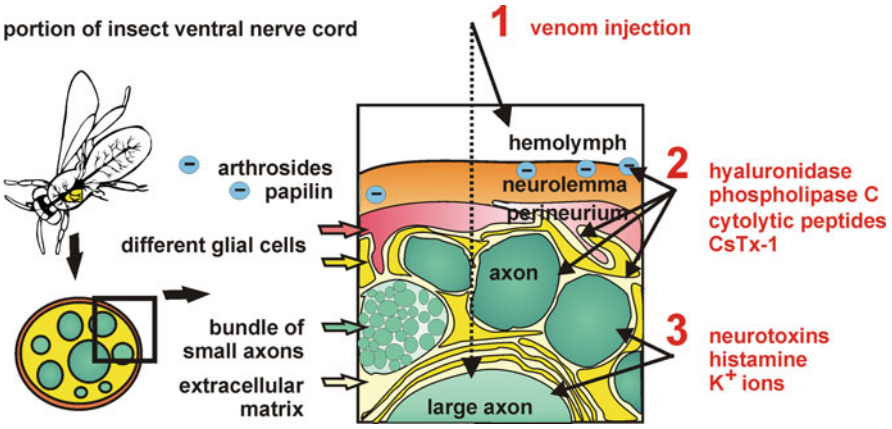

Fig. 16.5 The brain and the nerve system of insects are protected from direct contact with the hemolymph by the hemolymph-brain barrier (Treherne 1985). The rather permeable acellular neurolemma [containing glycoaminoglycans, negatively charged papilins and arthrosides (Kramerova et al. 2000; Sickmann et al. 1992)] is followed by the main barrier: the perineurium. Further, glial cells and axons are embedded in different glycosaminoglycans containing extracellular matrix (Francois 1978; Treherne et al. 1982). The envenomation process is shown here in three steps: *1*. *Cupiennius salei* venom can get directly or via hemolymph in contact with the neuronal tissue. *2*. Hyaluronidase and phospholipase C may act as spreading factor. The cytolytic peptides can be electrostatically attracted to negatively charged compounds and adopt an α-helical conformation which leads to cell membrane destruction. *3*. As a result, neurotoxins and other direct-acting substances have a better access to their neuronal targets cells

References

Adams ME (2004) Agatoxins: ion channel specific toxins from the american funnel web spider, *Agelenopsis aperta*. Toxicon 43:509–525

Ashhurst DE, Costin NM (1971) Insect mucosubstances. II. The mucosubstances of the central nervous system. Histochem J 3:297–310

Cornell BA, Separovic F (1983) Membrane thickness and acyl chain length. Biochim Biophys Acta 733:189–193

DeAngelis PL (2002) Evolution of glycosaminoglycans and their glycosyltransferases: implications for the extracellular matrices of animals and the capsules of pathogenic bacteria. Anat Rec 268:317–326

dos Santos AV, Onofre GR, Oliveira DM, Machado EA, Allodi S, Silva LC (2006) Heparan sulfate is the main sulfated glycosaminoglycan species in internal organs of the male cockroach, *Periplaneta americana*. Micron 37:41–46

Feng L, Gao R, Meng J, Gopalakrishnakone P (2010) Cloning and molecular characterization of BmHYA1, a novel hyaluronidase from the venom of Chinese red scorpion *Buthus martensii* Karsch. Toxicon 56:474–479

Francois J (1978) The ultrastructure and histochemistry of the mesenteric connective tissue of the cockroach *Periplaneta americana* L. (Insecta, Dictyoptera). Cell Tissue Res 189:91–107

Kozlov SA, Vassilevski AA, Feofanov AV, Surovoy AY, Karpunin DV, Grishin EV (2006) Latarcins, antimicrobial and cytolytic peptides from the venom of the spider *Lachesana tarabaevi* (Zodariidae) that exemplify biomolecular diversity. J Biol Chem 281:20983–20992

Kramerova IA, Kawaguchi N, Fessler LI, Nelson RE, Chen YL, Kramerov AA, Kusche-Gullberg M, Kramer JM, Ackley BD, Sieron AL, Prockop DJ, Fessler JH (2000) Papilin in development; a pericellular protein with a homology to the adamts metalloproteinases. Development 127: 5475–5485

Kubista H, Mafra RA, Chong Y, Nicholson GM, Beirao PS, Cruz JS, Boehm S, Nentwig W, Kuhn-Nentwig L (2007) CSTX-1, a toxin from the venom of the hunting spider *Cupiennius salei*, is a selective blocker of L-type calcium channels in mammalian neurons. Neuropharmacology 52:1650–1662

Kuhn-Nentwig L (2009) In: Lima ME (ed) Animal toxins: state of the art. Editora UFMG, Belo Horizonte, pp 249–268

Kuhn-Nentwig L, Schaller J, Nentwig W (1994) Purification of toxic peptides and the amino acid sequence of CSTX-1 from the multicomponent venom of *Cupiennius salei* (Araneae: Ctenidae). Toxicon 32:287–302

Kuhn-Nentwig L, Schaller J, Kämpfer U, Imboden H, Malli H, Nentwig W (2000) A lysine rich C-terminal tail is directly involved in the toxicity of CSTX-1, a neurotoxic peptide from the venom of the spider *Cupiennius salei*. Arch Insect Biochem Physiol 44:101–111

Kuhn-Nentwig L, Dathe M, Walz A, Schaller J, Nentwig W (2002a) Cupiennin 1d*: the cytolytic activity depends on the hydrophobic N-terminus and is modulated by the polar C-terminus. FEBS Lett 527:193–198

Kuhn-Nentwig L, Müller J, Schaller J, Walz A, Dathe M, Nentwig W (2002b) Cupiennin 1, a new family of highly basic antimicrobial peptides in the venom of the spider *Cupiennius salei* (Ctenidae). J Biol Chem 277:11208–11216

Kuhn-Nentwig L, Schaller J, Nentwig W (2004) Biochemistry, toxicology and ecology of the venom of the spider *Cupiennius salei* (Ctenidae). Toxicon 43:543–553

Kuhn-Nentwig L, Willems J, Seebeck T, Shalaby T, Kaiser M, Nentwig W (2011) Cupiennin 1a exhibits a remarkably broad, non-stereospecific cytolytic activity on bacteria, protozoan parasites, insects, and human cancer cells. Amino Acids 40:69–76

Kuhn-Nentwig L, Fedorova IM, Lüscher BP, Kopp LS, Trachsel C, Schaller J, Vu XL, Seebeck T, Streitberger K, Nentwig W, Sigel E, Magazanik LG (2012) A venom-derived neurotoxin, CsTx-1, from the spider *Cupiennius salei* exhibits cytolytic activities. J Biol Chem 287: 25640–25649

Loewe R, Linzen B, von Stackelberg W (1970) Die gelösten Stoffe in der Hämolymphe einer Spinne, *Cupiennius salei* Keyserling. Z Vergl Physiol 66:27–34

Nagaraju S, Devaraja S, Kemparaju K (2007) Purification and properties of hyaluronidase from *Hippasa partita* (funnel web spider) venom gland extract. Toxicon 50:383–393

Powers JP, Hancock RE (2003) The relationship between peptide structure and antibacterial activity. Peptides 24:1681–1691

Pukala TL, Boland MP, Gehman JD, Kuhn-Nentwig L, Separovic F, Bowie JH (2007a) Solution structure and interaction of cupiennin 1a, a spider venom peptide, with phospholipid bilayers. Biochemistry 46:3576–3585

Pukala TL, Doyle JR, Llewellyn LE, Kuhn-Nentwig L, Apponyi MA, Separovic F, Bowie JH (2007b) Cupiennin1a, an antimicrobial peptide from the venom of the neotropical wandering spider *Cupiennius salei*, also inhibits the formation of nitric oxide by neuronal nitric oxide synthase. FEBS Lett 274:1778–1784

Sickmann T, Weske B, Dennis RD, Mohr C, Wiegandt H (1992) Chemical distribution of glycosphingolipids in third-instar larval organs of the blowfly, *Calliphora vicina* (Insecta: Diptera). J Biochem 111:662–669

Silva EC, Camargos TS, Maranhao AQ, Silva-Pereira I, Silva LP, Possani LD, Schwartz EF (2009) Cloning and characterization of cDNA sequences encoding for new venom peptides of the Brazilian scorpion *Opisthacanthus cayaporum*. Toxicon 54:252–261

Takeo S, Fujise M, Akiyama T, Habuchi H, Itano N, Matsuo T, Aigaki T, Kimata K, Nakato H (2004) In vivo hyaluronan synthesis upon expression of the mammalian hyaluronan synthase gene in *Drosophila*. J Biol Chem 279:18920–18925

Toyoda H, Kinoshita-Toyoda A, Fox B, Selleck SB (2000) Structural analysis of glycosaminoglycans in animals bearing mutations in sugarless, sulfateless, and tout-velu. *Drosophila* homologues of vertebrate genes encoding glycosaminoglycan biosynthetic enzymes. J Biol Chem 275:21856–21861

Trachsel C, Siegemund D, Kämpfer U, Kopp LS, Bühr C, Grossmann J, Lüthi C, Cunningham M, Nentwig W, Kuhn-Nentwig L, Schürch S, Schaller J (2012) Multicomponent venom of the spider *Cupiennius salei*: a bioanalytical investigation applying different strategies. FEBS J 279:2683–2694

Treherne JE (1985) Blood-brain barrier. In: Kerkut GA, Gilbert LC (eds) Comprehensive insect physiology biochemistry and pharmacology. Pergamon, Oxford, pp 115–137

Treherne JE, Schofield PK, Lane NJ (1982) Physiological and ultrastructural evidence for an extracellular anion matrix in the central nervous system of an insect (*Periplaneta americana*). Brain Res 247:255–267

Vassilevski AA, Fedorova IM, Maleeva EE, Korolkova YV, Efimova SS, Samsonova OV, Schagina LV, Feofanov AV, Magazanik LG, Grishin EV (2010) Novel class of spider toxin: active principle from the yellow sac spider *Cheiracanthium punctorium* venom is a unique two-domain polypeptide. J Biol Chem 285:32293–32302

Wullschleger B, Kuhn-Nentwig L, Tromp J, Kämpfer U, Schaller J, Schürch S, Nentwig W (2004) CSTX-13, a highly synergistically acting two-chain neurotoxic enhancer in the venom of the spider *Cupiennius salei* (Ctenidae). Proc Natl Acad Sci USA 101:11251–11256

Wullschleger B, Nentwig W, Kuhn-Nentwig L (2005) Spider venom: enhancement of venom efficacy mediated by different synergistic strategies in *Cupiennius salei*. J Exp Biol 208: 2115–2121

Zhang Y, Chen J, Tang X, Wang F, Jiang L, Xiong X, Wang M, Rong M, Liu Z, Liang S (2010) Transcriptome analysis of the venom glands of the Chinese wolf spider *Lycosa singoriensis*. Zoology (Jena) 113:10–18