

Review

Antimicrobial and cytolytic peptides of venomous arthropods

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Abstract. As a response to invading microorganisms, the innate immune system of arthropods has evolved a complex arrangement of constitutive and inducible antimicrobial peptides that immediately destroy a large variety of pathogens. At the same time, venomous arthropods have developed an additional offensive system in their venom glands to subdue their prey items. In this complex venom system, several enzymes, low-molecular-mass compounds, neurotoxins, antimicrobial and cytolytic peptides

interact together, resulting in extremely rapid immobilization and/or killing of prey or aggressors. This review provides an overview of antimicrobial peptides identified in the hemolymph of venomous arthropods, and especially of cytolytic peptides in their venom. For these peptides a dual role is proposed: acting as antimicrobials as well as increasing the potency of the venom by influencing excitable cells.

Key words. Antimicrobial peptides; cytolytic peptides; venom; arthropods; synergism.

Introduction

As strategies in antagonistic relationships, prokaryotic and eukaryotic organisms have developed hundreds of different cytolytic peptides and proteins during their evolution in different phyla of the plant [1] and animal kingdom [2]. Many cytolytic peptides are specific antimicrobially acting peptides that are part of the innate immune system of invertebrates and vertebrates. They serve as primary defense weapons against invading prokaryotic and eukaryotic microorganisms, a fact which is well documented by a constantly increasing number of publications and reviews [3–6].

Gene-encoded antimicrobially acting peptides show great variety in amino acid sequence, structure and target specificity. Many of them are cationic, amphipathic peptides with molecular masses lower than 10 kDa and show a higher specificity to prokaryotic than to eukaryotic cells [7]. The main site of antimicrobial activity is the plasma

membrane of bacteria and parasitic protozoans. The unstructured antimicrobial peptides are electrically attracted to negatively charged groups of the cell surface, where they adopt an α -helical conformation and accumulate on the membrane. This can result in the formation of transient pores, membrane perturbation and cell lysis [8]. Antimicrobial peptides in vertebrates and invertebrates can either be induced by microbial infection and inflammation or produced constitutively. In vertebrates, biogenesis occurs in circulating phagocytes, destroying intracellularly phagocytosed pathogens by defensins and likewise in different tissues and epithelia from where they are actively secreted into the lumen, as has been shown for enteric α -defensins [9]. In some crustaceans [10, 11] and chelicerates, these peptides are stored constitutively in hemocytes and released into the hemolymph by exocytosis after microbial injury as an ancient form of host defense [12, 13]. Insects exhibit a further mode of action in response to microbial challenge. Depending on the immunogenic character of the microbial invaders, the transcription of different antimicrobial peptides, mainly in the fat body, is induced by two different pathways. The

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Imd pathway controls the immune response to Gram-negative bacteria, and the Toll pathway is activated after infection with fungi and Gram-positive bacteria [5]. After induction, a rapid release of diverse antimicrobial peptides into the hemolymph follows within hours [14]. Further constitutive expressions of these peptides are also reported in hemocytes, salivary glands [15] and in the midgut of *Anopheles gambiae* [16, 17].

Additionally, other cytolytic peptides are used facilitatively for the improvement of living conditions and access to nutrition. Bacteria that produce cytolytic peptides seem to have a growth advantage over their natural competitors for the same growth substrate and in the same ecological niche [18, 19]. This bactericidal activity can be specifically targeted at bacteria of the same group [20] or may occur as a more unspecific cytolytic activity against prokaryotic and eukaryotic cell membranes, as in the case of gramicidin S [21]. Cytolytic peptides from the parasitic protozoans *Entamoeba histolytica* and *Entamoeba dispar* (amoebapores and disparpores) primarily destroy the phagocytosed bacteria as food. The cytolytic activity toward eukaryotic cells that some of them exert seems to be a mere side effect [22, 23].

On the other hand, arthropods such as scorpions, spiders and stinging hymenopterans have developed venom glands that mainly produce three different groups of components, based on their molecular mass. The first group consists of proteins, including several enzymes such as hyaluronidases, phospholipases and sphingomyelinases. The second group is represented by a peptide fraction with molecular masses around and lower than 10 kDa, containing several neurotoxic and cytolytic compounds. A third group is composed of low-molecular-mass substances such as ions, free amino acids, biogenic amines, neurotransmitters, acylpolyamines, heterocyclic compounds and alkaloids [24, 25]. The cooperation of all these mentioned components in the venom enables these arthropods to paralyze and/or to kill other arthropods in terms of foraging and defense. As a reproduction strategy, parasitic wasps also paralyze other arthropods and lay an egg inside or outside of the prey, which serves as a nutrition source for their offspring. This review will focus on antimicrobial and cytolytic peptides from the hemolymph and venom glands of poisonous arthropods identified over the last 40 years. Common to all peptides is their amphipathic structure and their ability to disturb membrane structures. In context with the fact that all external world-facing epithelia, ducts and glands dispose of growth-inhibiting substances against potential invaders, a possible dual role, especially for the peptides identified in the venom, will be discussed.

Antimicrobial peptides in the hemolymph of venomous arthropods

Antimicrobial peptides isolated from the hemolymph of actively venomous arthropods have quite different structures from the cytolytic peptides identified in their venoms. According to their structure, antimicrobial peptides can be arranged into four families: α -helical, linear peptides devoid of cysteines, the proline-rich and glycine-rich peptides, and the cysteine-rich, open-ended cyclic peptides such as insect defensins [14].

In the hemolymph of the scorpion *Leiurus quinquestriatus* (Buthidae) [26] a 4-kDa scorpion defensin was identified with a high sequence homology to an insect defensin from the dragonfly *Aeschna cyanea* [27]. The scorpion defensin is a cationic 39-residue peptide with a CS $\alpha\beta$ fold and three intramolecular disulfide bridges, a common structural motif also present in scorpion toxins [28]. A further defensin from the hemolymph of the scorpion *Androctonus australis*, also belonging to the family Buthidae, showed 95% identity with the *Leiurus* defensin, both acting mainly on Gram-positive bacteria [29]. These high sequence similarities suggest a common ancient peptide structure for scorpion neurotoxins and insect defensins [30]. From the same hemolymph, two further antimicrobially acting peptides were characterized: buthinin, with three disulfide bridges, active against both Gram-positive and Gram-negative bacteria, and androctonin, with two disulfide bridges. Androctonin is bactericidal in the micromolar range to both Gram-positive and Gram-negative bacteria. The peptide additionally exhibits a fungicidal activity and is nonhemolytic up to a concentration of 150 μ M [31]. Its sequence shows a close similarity to antimicrobial tachypleusins and polyphemusins isolated from the hemolymph of the horseshoe crabs *Tachypleus tridentatus* and *Limulus polyphemus*, belonging to the old marine arthropod group Xiphosura [32, 33]. As assumed for cytolytic peptides from the venom of scorpions, antimicrobial peptides in the hemolymph also seem to be constitutively present, because their concentration does not increase after bacterial injury [26, 29]. Antimicrobial peptides from the hemolymph of spiders are currently known from one unchallenged mygalomorph species, *Acanthoscurria gomesiana* (Theraphosidae), from which gomesin had been characterized [34]. This peptide contains four cysteines and is active against different Gram-negative bacteria (0.4–6.25 μ M), Gram-positive bacteria (0.2–12.5 μ M) and fungi (0.4–25 μ M). Gomesin exhibits hemolytic activity of 16% at 1 μ M, which does not increase up to concentrations of 100 μ M. Interestingly, the viability of *Leishmania amazonensis* promastigotes was reduced to 50% in the presence of 2.5 μ M gomesin. Structural and sequence alignment of gomesin with protegrin from porcine leukocytes [35] and androctonin revealed several similarities in their amino

acid sequences as well in the structural distribution of hydrophilic and hydrophobic residues [36, 37].

Among stinging hymenopteran insects, antimicrobial peptides are best investigated from the hemolymph of the honeybee *Apis mellifera*, as a recent review reports [38]. After bacterial injury, the proline-rich 18-residue apidaecins, the 34-residue abaecins and the glycine-rich hymenoptaecin with 93 residues are present in micromolar concentration in the hemolymph [39–41]. All three peptide groups are devoid of cysteine. Apidaecins are active against Gram-negative bacteria and lack a pore-forming activity. This peptide group shows a stereospecific interaction with bacterial targets and a direct effect on protein biosyntheses [42]. Apidaecins are also present in the hemolymph of the bumblebees *Bombus terrestris* and *Bombus pascuorum*, as well as in the wasps *Vespa maculifrons* and *Vespa maculata*. They exhibit a conserved C-terminal part that seems to be specific for hymenopterans [43, 44]. Aباecin is, like hymenoptaecin, active against both Gram-positive and Gram-negative bacteria, but compared with apidaecins it is less bactericidal. This peptide has also been identified in the hemolymph of *Bombus pascuorum*. *Apis* defensin is less common in the hemolymph even after bacterial challenge [45] and differs from royalisin, isolated from honeybee royal jelly, only in one C-terminal amino acid residue and a C-terminal amidation [38, 46]. *Bombus* defensin from the hemolymph of *Bombus pascuorum* also possesses an amidated C-terminus and features similarities to *Apis* defensin and royalisin. In contrast to other insect defensins and royalisin, it is active against Gram-positive and Gram-negative bacteria [44]. The hymenopteran defensins identified consist of 51 residues, contain the characteristic motif of three intramolecular disulfide bridges and are larger than other insect defensins.

Sequence similarities to royalisin and defensin from *Apis mellifera* and *Bombus pascuorum* have been found in a 40-residue defensin from the European ant *Formica rufa*, also belonging to the Hymenoptera. This defensin also contains three intramolecular disulfide bridges without any further additional modifications [44, 46, 47]. The formaeccins 1 and 2, from the hemolymph of the Australian ant *Myrmecia gulosa* have also been characterized as belonging to the proline-rich peptide family [48]. These small cationic, *O*-glycosylated antimicrobial peptides (2 kDa) are active against growing *Escherichia coli* and inactive against Gram-positive and several other stationary cells. The *O*-glycosylation of Thr11 seems to be important for its activity. An overview of these antimicrobial peptides in the hemolymph of venomous arthropods is given in Table 1.

Antimicrobial and cytolytic peptides in the venom of arthropods

Besides the previously mentioned antimicrobial peptides from their hemolymph, scorpions, spiders and stinging hymenopterans have, during their evolution, also developed antimicrobial, cytolytic peptides in their venom glands together with a complex mixture of enzymes, neurotoxins and low-molecular-mass compounds. Despite their different compositions, these venoms have two main purposes: first, they are used to subdue mainly arthropods as food. Second, they serve as a defense weapon against invertebrate and vertebrate predators. The venom, which is injected, has the job of immobilizing and/or killing prey or enemies as soon as possible. The better and more effective the different venom components work together, the less venom has to be injected. Venom is principally precious because it takes *Cupiennius salei* several days to regenerate, and a venomless period is a defenseless time [49]. As shown for the spider *Cupiennius salei* and the scorpion *Parabuthus transvaalicus*, venom investment is allotted as economically as possible [50–54]. Generally, neurotoxins identified in various arthropod venoms modify ion conductance (ion channel toxins), affect neurotransmitter release (presynaptic toxins) and interfere with the binding of neurotransmitters (postsynaptic toxins) [55]. For example, diverse voltage-gated sodium channels in muscle cells and the central and peripheral nervous system of insects and mammals are targets of α and β toxins (scorpions) and μ -agatoxins (spiders), leading to fast excitatory paralysis [56–58]. In addition, many neurotoxins act on voltage-gated calcium channels, which play an important role in cardiac, muscular and neuronal function, resulting in a flaccid paralysis [58]. So the diverse predators have developed different strategies to paralyze a prey and to deter aggressors.

Before discussing the role of antimicrobial peptides in the context of the other venom compounds, a detailed overview of the peptides so far characterized is given. The majority of the cytolytic peptides identified so far in the venom of arthropods are linear, highly cationic and amphipathic peptides without cysteine residues and have isoelectric points above pH 8.57. They adopt an α -helical structure in the presence of membranes and membrane mimicking substances such as trifluoroethanol. Also, a defensin-like peptide, scorpine [59], which contains three disulfide bridges, was isolated.

Scorpions

First reports on the hemolytic activity of scorpion venom have been available since 1918 [60]. Starting in 1996, possible pore-forming peptides from the venom of *Scorpio maurus palmatus* and *Opisthophthalmus carinatus*

Table 1. Antimicrobial peptides in the hemolymph of venomous arthropods.

| Source ^a | Peptide [n] | Residue | pI ^b | Molecular mass [Da] ^c | Amino acid sequence | Reference |
|---------------------|---------------------------------|----------------------------|-----------------|----------------------------------|---|---|
| I | <i>Acanthoscurria gomesiana</i> | Gomesin | 18 | 2270.7 | ZCRRLLCYKQRCVTVYCRGR* | Z pyroglutamate 34 |
| II | <i>Androctonus australis</i> | Androctonin | 25 | 3076.6 | RSVCRQIKICRRRRGGCYKCTNRPY | 29 |
| | | Buthinin | 34 | 3968.6 | SIVPIRCRSNRDCRRFCGFRGRCTYARQCLCGY | |
| | <i>Androctonus defensionis</i> | | 37 | 4205.8 | GFGCFNQGACHRHCRSIRRRGGYAGLFGKQTCTCYR | |
| | <i>Leiurus quinquestriatus</i> | | 38 | 4319.9 | GFGCFNQGACHRHCRSIRRRGGYAGLFGKQTCTCYRN | 26 |
| III | <i>Myrmecia gulosa</i> | Formaecin 1 | 16 | 1997.2 | GRPNPVNKKPTPHPRLL | O-linked N-acetylgalactosamine to Thr-11 48 |
| | | Formaecin 2 | 16 | 2010.3 | GRPNPVNKKPTPYPRLL | |
| | <i>Formica rufa</i> | <i>Formica</i> defensionis | 40 | 4118.7 | FTCDLLSGAGVDHSACAACHLLRGLGKTGGRCNSDRVCVCRA | 47 |
| IV | <i>Apis mellifera</i> | Abaecin | 34 | 3878.5 | YVPLPNVPPQGRRRPFTFPQGGPFNPKIKWPQGY | 40 |
| | | Apidaecin Ia | 18 | 2108.4 | GNNRPVYIP-QPRPPHPRI | 39 |
| | | Apidaecin Ib | 18 | 2108.4 | GNNRPVYIP-QPRPPHPRL | |
| | | Apidaecin II | 18 | 2122.5 | GNNRFYIIP-QPRPPHPRL | 43 |
| | | Apidaecin III | 18 | 2098.4 | GNNRPVYIS-QPRPPHPRL | |
| | Hymenoptaecin | 93 | 9.69 | 10269.4 | ZERGSIVIQGTKEGKSRSLDIDYKQRYVDKNGMTGDAYGGLNIRPGQPSR QHAGFEFGKEYKNGFIKQSEVQRGPGGRLSPYFGINGGFRF | Z pyroglutamate 41 |
| | <i>Apis</i> defensionis | 51 | 8.64 | 5518.3 | VTCDLLSFKQVNDSSACAANCLSLGKAGHCEKGVCI | CRKTSFKDLWDRF* 45 |
| | Royalisin | 51 | 8.30 | 5526.3 | VTCDLLSFKQVNDSSACAANCLSLGKAGHCEKGVCI | CRKTSFKDLWDRF* 46 |
| | <i>Bombus</i> defensionis | 51 | 8.65 | 5529.5 | VTCDLLSFKGVAEHSACAANCLSMGKAGGRCENGIICRKTTFKELWDRF* | 44 |
| | Abaecin | 39 | 10.00 | 4395.0 | FVPYNPRPGQSKPFPFPFGHGPFNPKIQWPYLPNPGH | |
| | Apidaecin | 17 | 11.71 | 1963.3 | GNNRPVYIP-PPRPPHPRL | |
| | Apidaecin Bb +A | 17 | 11.71 | 1977.3 | ANRPVYIP-PPRPPHPRL | 43 |
| | Apidaecin Bb -A | 16 | 11.71 | 1906.3 | NRPVYIP-PPRPPHPRL | |
| | Apidaecin Yj +S | 18 | 12.30 | 2101.4 | SNKPRPQQVPPRPPHPRL | 43 |
| | Apidaecin Yj -S | 17 | 12.30 | 2014.4 | NKPRPQQVPPRPPHPRL | |
| | Apidaecin Ho + | 17 | 12.30 | 1957.3 | GKPRPQQVPPRPPHPRL | 43 |
| | Apidaecin Ck A | 16 | 11.71 | 1868.2 | NRPYVYIP-A-PPRPPHPRL | |
| | Apidaecin Ck P | 16 | 11.71 | 1894.2 | NRPYVYIP-PPRPPHPRL | |
| | Apidaecin Cd 1+ | 20 | 12.48 | 2281.7 | GKPNRFPAPAIQPRPPHPRL | 43 |
| | Apidaecin Cd 1- | 17 | 12.48 | 1999.3 | NRPFPAPAIQPRPPHPRL | |
| | Apidaecin Cd 2+ | 20 | 12.31 | 2253.7 | GKPNRFPAPAIKPRPPHPRL | |
| | Apidaecin Cd 3+ | 20 | 12.31 | 2226.7 | GKPSKPRPAPAIKPRPPHPRL | |
| | Apidaecin Cd 3- | 17 | 12.31 | 1944.3 | SKPRPAPAIKPRPPHPRL | |

^a Taxa: I Theraphosidae, II Buthidae, III Formicidae, IV Apidae, V Vespidae, VI Sphecidae, VII Ichneumonidae ^{b,c} using for calculations of pI ExPASy-PeptideMass and average mass of the occurring amino acid residues and given peptide mass as [M] (www.expasy.org/cgi-bin/peptide-mass.pl). Nonidentical residues are boxed in yellow, and dashes indicate gaps introduced to optimize the alignment. * amidated C-Terminus.

(both Scorpionidae) were assumed to be responsible for the induction of leak currents in *Xenopus laevis* oocytes [61] and cardiac cells of the rat [62].

Meanwhile, cytolytic peptides have been identified in the venoms of three out of four scorpion superfamilies. Purified and characterized cytolytic peptides have been described for *Parabuthus schlechteri* (Buthidae) [63, 64], *Pandinus imperator* [59, 65] and *Opisthophthalmus carinatus* (Scorpionidae) [64], *Opisthacanthus madagascariensis* (Ischnuridae) [66] and *Hadrurus aztecus* (Luridae) [67]. From the venom gland of the Chinese scorpion *Buthus martensii* (Buthidae) a full-length cDNA was isolated that encodes an insect defensin-like peptide named BmTXKS2 [68]. BmTXKS2 shows a similar CS $\alpha\beta$ structural motif and high sequence homology with *Leiurus* and *Androctonus* defensins from the hemolymph of these scorpions also belonging to the Buthidae family [26, 29]. The pore-forming peptide parabutoporin from the South African scorpion *Parabuthus schlechteri* shows a high content (26.6%) of basic amino acid residues, mainly lysine, which is remarkable. Additionally, five negatively charged amino acid residues are located in the C-terminal part of the peptide (table 2). At submicromolar concentrations the peptide causes degranulation of human granulocytes, which is comparable to the effect of mastoparan, a pore-forming peptide from wasp venom. The peptide-containing fraction was also able to depolarize nociceptive nerve cells of dorsal rat ganglions [63].

Opistoporin 1 and 2, from the South African scorpion *Opisthophthalmus carinatus*, have also been purified. The peptides differ from each other only in one position (table 2, 3). Synthesized parabutoporin and opistoporin 1 were most bactericidal to different Gram-negative bacteria (1.6–50 μM), less so to Gram-positive bacteria (6.3 to $\geq 50 \mu\text{M}$), and fungicidal to different fungi and one yeast species in micromolar concentrations. Opistoporin 1 is less hemolytic than melittin and parabutoporin in the micromolar range. CD spectra of parabutoporin and opistoporin 1, in the presence of negatively charged (Myr₂Gro-PGro) and zwitterionic (Myr₂Gro-PCho) small unilamellar vesicles, showed no great differences in secondary structure. The peptides show synergistic effects with some conventional antibiotics. When an N- and C-terminal truncated form of parabutoporin (amino residues 7–22) and a C-terminal truncated form of parabutoporin (amino acid residues 1–28) were tested, almost no biological effect could be found at a concentration of 50 μM . Comparison of primary structures of further cytolytic peptides described for different scorpion venom peptides by CLUSTAL W sequence alignment revealed a typical scorpion conserved sequence: S(x)3KxWxS(x)5L [64]. Currently, one of the longest described antimicrobial peptides from the venom glands of arthropods is scorpine from the West and Central African scorpion *Pandinus imperator*, with 75 amino acid residues (table 3) [59]. The

secondary structure prediction of scorpine [69] shows an α -helical conformation for the N-terminal residues 5–16 and 18–46, which exhibits similarities to cecropin B (from the Chinese oak silk moth, *Antheraea pernyi*) [70], sarcotoxin Ic (from the flesh fly, *Sarcophaga peregrina*) [71] and cecropin P1 (from the pig intestine, *Sus scrofa*) [72]. Scorpine possesses three disulfide bridges in the C-terminal region that bear resemblance to the defensins from the dragon fly *Aeschna cyanea* [27] and from the scorpion *Leiurus quinquestriatus hebraeus* [26]. Depending on the purification procedure, scorpine corresponds to approximately 1.4% of the total venom. Scorpine inhibits bacterial growth of *Bacillus subtilis* and *Klebsiella pneumoniae* in the micromolar range and also the fecundation and ookinete formation of the eukaryotic parasite *Plasmodium berghei*, the organism causing malaria.

Pandinin 1 and 2, further cationic antimicrobial peptides from *P. imperator*, have demonstrated antimicrobial activity in the micromolar range against various Gram-positive bacteria (2.4–5.2 μM) and Gram-negative bacteria (2.4–38.2 μM). Pandinin 2 also exhibits fungicidal (*Candida albicans*) and hemolytic activity (11.1–44.5 μM) stronger than magainin 1 against sheep erythrocytes. C-terminal amidation of synthetic pandinin 2 has no effect on the antimicrobial and hemolytic activity of the peptide. Both peptides seem to be present only in small amounts in the venom of *P. imperator*. The N-terminal part of pandinin 2 is predominated by hydrophobic amino acid residues comparable to melittin, magainin and cupiennin 1 and is composed of a single α -helix. Pandinin 1 shows two distinct α -helices that are separated by a coil region of higher flexibility. One-dimensional ¹H NMR spectra of pandinin 2 in dodecylphosphocholine (DPC) micelles suggest that the N-terminal part is more deeply buried in the lipid phase than the C-terminal region [65]. A further cationic antimicrobial peptide has been purified from the venom of the Mexican scorpion *Hadrurus aztecus* [67] (table 2, 3). When the antimicrobial activity of native hadrurin against *E. coli* was compared with that of synthesized all-L-hadrurin in the free or amidated C-terminal form and all-D-hadrurin (with the exchange of Leu in position 2, 6, 9 and 27 with Ile), no clear differences could be observed. A difference in the hemolytic effect against human erythrocytes was only measured for the all-D-enantiomer, which is in the same range as the effect of melittin (100% hemolysis at a concentration of 5 μM). The hemolytic effect of the other forms of hadrurin mentioned are reduced to 80% lysis at a concentration of 20 μM . Hadrurin preferentially lyses zwitterionic phosphatidylcholine liposomes at a low concentration. For the lysis of acidic liposomes (composed of phosphatidylcholine and phosphatidylserine), a higher critical molar ratio peptide/lipid is required.

Opisthacanthus madagascariensis, a scorpion endemic to Madagascar, is the source of the shortest cytolytic pep-

Table 2. Antimicrobial and cytolytic peptides in the venom of arthropods

| Source ^a | Peptide | Residue [n] | pI ^b | Molecular mass [Da] | Amino acid sequence | Reference |
|--------------------------------------|---------------|-------------|-----------------|---|--|-----------|
| I <i>Cupiennius salei</i> | Cupiennin 1a | 35 | 10.30 | 3798.6 | GFGALF K FLA K VA K TVAK Q AA K QGA K YV V N K OME* | 82 |
| | Cupiennin 1b | 35 | 10.30 | 3800.6 | GFGSLF K FLA K VA K TVAK Q AA K QGA K Y I AN K OME* | |
| | Cupiennin 1c | 35 | 10.30 | 3770.5 | GFGSLF K FLA K VA K TVAK Q AA K QGA K Y I AN K QTE* | |
| | Cupiennin 1d | 35 | 10.30 | 3795.0 | GFGSLF K FLA K VA K TVAK Q AA K QGA K YV V AN K HME* | |
| | Lycotoxin I | 25 | 10.60 | 2843.5 | IWL T AL K FL G HA K HLA K Q-- Q LS L * | 79 |
| | Lycotoxin II | 27 | 10.22 | 3206.9 | K I K W F K T M K S I A F I A K E Q M K -- K HL G GE | |
| | Oxyopinin 2a | 37 | 10.79 | 4126.9 | G K FSV F G K IL S IA K V F G V G K -V R Q F K T AS D L D K N Q | 80 |
| | Oxyopinin 2d | 37 | 10.79 | 4156.9 | G K FSV F S K IL S IA K V F G V G K -V R Q F K T AS D L D K N Q | |
| | Oxyopinin 2b | 37 | 10.30 | 4146.9 | G K FS F A K IL S IA K F F G V G K -V R Q F K E AS D L D K N Q | |
| | Oxyopinin 2c | 37 | 10.46 | 4064.8 | G K LS G IS K VL R AI A K F F G V G K - A R Q F K E AS D L D K N Q | |
| Oxyopinin 1 | 48 | 11.26 | 5221.3 | F R G L A K L L K I G L S F A R V L K K V L P K A A K A K A L A K S M A D E N A I - R Q N Q | | |
| II <i>Opisthophthalmus carinatus</i> | Opistoporin 1 | 44 | 9.78 | 4836.6 | G K V D W I K ST A K L W N S E P V K E L K N T A L N A A K N L V A E K I G A T P S | 64 |
| | Opistoporin 2 | 44 | 9.78 | 4870.6 | G K V D W I K ST A K L W N S E P V K E L K N T A L N A A K N F V A E K I G A T P S | |
| | Pandimin 1 | 44 | 9.78 | 4799.5 | G K V D W I K SA K K I W S E P V S Q L K G V L N A A K N V V A E K I G A T P T | 65 |
| | Hadrurin | 41 | 10.30 | 4436.2 | G I L D T I S I A S K W N S K I V Q D L K R K G I N W V A N K L G V S P Q A A | 67 |
| | Parabutoporin | 45 | 10.00 | 4995.0 | F L G S F L K A N S K L A K I R A K E M L K D Y A K L L E G S E E V P G Q | 63 |
| | BmKbpp | 47 | 10.08 | 5321.3 | F E R G S F L K K V W S K L A K L R S K Q L L K D Y A N K V L N G P E E E A A A P A E | |
| | Pandimin 2 | 24 | 10.00 | 2612.1 | F W G A L A K G A L K L I P S L F S F S F S K D | 65 |
| | IsCT | 13 | 8.59 | 1502.9 | I L G I W E G I K S L F* | 66 |
| | IsCT2 | 13 | 8.75 | 1464.8 | I F G A I W N G I K S L F* | |
| | Scorpine | 75 | 8.80 | 8449.8 | G W I N E E K I Q K I D E R M G N T V L G R M A K A I V H K M A K N E F Q C M A N M D M L G N C E K H C Q T S G E K G Y C H G T K K C G T P L S Y | 59 |
| III <i>Pachycondyla goeldii</i> | Ponericin W1 | 25 | 10.70 | 2710.4 | W L G S A L K I G A L L P S V V G L F K K K K K | 125 |
| | Ponericin W2 | 25 | 10.70 | 2710.4 | W L G S A L K I G A L L P S V V G L F Q K K K K K | |
| | Ponericin W3 | 26 | 11.43 | 2864.6 | G I W G T L A K I G I K A V P R V I S M L K K K K K | |
| | Ponericin W4 | 26 | 10.60 | 2852.5 | G I W G T A L K W G V K L L P L V G M A Q T K K Q | |
| | Ponericin W5 | 24 | 10.60 | 2600.2 | F W G A L L K G A A K L I P S V V G L F - K K K Q | |
| | Ponericin W6 | 20 | 10.00 | 2029.5 | F I G T A L G I A S - A I P A I V K L F K* | |
| | Ponericin G2 | 30 | 10.13 | 3307.9 | G W Q D W L K K K E W L K A K G P G I V K A A L Q A A T Q | |
| | Ponericin G3 | 30 | 10.22 | 3383.1 | G W Q D W L N K G E W L K K K G P G I M K A A L K A A T Q | |
| | Ponericin G4 | 29 | 9.70 | 3164.8 | D F K D W M K T A G E W L K K K G P G I L K A A M A A A T - | |
| | Ponericin G5 | 30 | 10.30 | 3108.8 | G L K D W K I A G G W L K K K G P G I L K A A M A A A T Q | |
| Ponericin G1 | 30 | 10.48 | 3213.9 | G W D W A K K A G G W L K K K G P G M A A L K A A M Q | | |
| Ponericin G6 | 18 | 9.70 | 1818.3 | G L V D V L G K V G G L I K K L L P * | | |
| Ponericin G7 | 19 | 9.70 | 1876.4 | G L V D V L G K V G G L I K K L L P G | | |

Table 2 (continued)

| | | | | | | | |
|---|--|---------------------|-------|--------|--|-----------------|-----|
| | Ponericin L1 | 24 | 10.30 | 2595.3 | LLKELWTKMKGAGAVLGIKGLL* | 130 | |
| | Ponericin L2 | 24 | 10.30 | 2577.3 | LLKELWTKIKGAGAVLGIKGLL* | | |
| <i>Myrmecia pilosula</i> | Pilosulin 1 | 56 | 10.45 | 6052.4 | GLGSVFGRLARILGKVIPIKVAKGLGPKVAKVLPIKVMKEAIPMAVEMAQSQEE QQPQ | | |
| IV <i>Megabombus pennsylvanicus</i> | Bombolitin I | 17 | 10.30 | 1835.4 | IKITTMLAKLGLVLAHV* | 108 | |
| | Bombolitin II | 17 | 9.70 | 1805.2 | SKITDILAKLGLVLAHV* | | |
| | Bombolitin III | 17 | 9.70 | 1861.4 | IKIMDILAKLGLVLAHV* | | |
| | Bombolitin IV | 17 | 9.70 | 1872.4 | INIKDILAKLGLVLAHV* | | |
| | Bombolitin V | 17 | 8.76 | 1730.2 | INVLGILGLLGLKALSHL* | | |
| <i>Apis mellifera</i> | Melittin <i>A. m.</i> | 26 | 12.02 | 2846.5 | GIGAVIKVLTITGLPALISWIKRKRQQ* | 87 | |
| | Melittin <i>A. d.</i> | 26 | 11.10 | 2847.5 | GIGAILKVLSTGLPALISWIKRKRQE* | 89 | |
| | Melittin <i>A. f.</i> | 26 | 11.33 | 2818.5 | GIGAILKVLATGLPLIISWIKNKRKQ* | 88 | |
| V <i>Vespa mandarinia</i> <i>Vespa xanthoptera</i> <i>Vespa orientalis</i> <i>Vespa levisii</i> <i>Vespa crabro</i> <i>Vespa basalis</i> | Mastoparan M | 14 | 10.30 | 1478.9 | INLKATAALAKKLL* | 111 | |
| | Mastoparan X | 14 | 10.30 | 1556.0 | INWKGIAAAMAKKLL* | 112 | |
| | HR-I | 14 | 10.30 | 1493.0 | INLKATAALVKKVL* | 113 | |
| | Mastoparan | 14 | 10.30 | 1478.9 | INLKATAALAKKLL* | 109 | |
| | Mastoparan C | 14 | 10.30 | 1507.0 | INLKALLAVAKKLL* | 113 | |
| | Mastoparan B | 14 | 10.48 | 1612.1 | LKLSIVSWAKKVL* | 110 | |
| | HR-II | 14 | 10.00 | 1523.0 | FLPLILGLVKGLL* | 113 | |
| | Crabrolin | 13 | 11.00 | 1496.0 | FLPLILRKIVTAL* | 113 | |
| | <i>Polistes jadwigae</i> | Polistes Mastoparan | 14 | 8.57 | 1635.0 | VDWKKIGQHILSVL* | 114 |
| | VI <i>Anterhynchium flavomargarinatum micado</i> | Eumenine Mastoparan | 14 | 10.30 | 1523.0 | INLKIAGIISKSL* | 115 |
| VII <i>Anoplius samariensis</i> | Anoplin | 10 | 11.17 | 1153.5 | GLLKRRIKILL* | 116 | |

^a Taxa: I Araneae, II Scorpiones, III Formicidae, IV Apidae, V Vespidae, VI Eumenidae, VII Pompilidae. ^{b,c} using for calculations of pI ExpASY-PeptideMass and average mass of the occurring amino acid residues and given peptide mass as [M] (www.expasy.org/cgi-bin/peptide-mass.pl). Nonidentical residues are boxed in yellow, and dashes indicate gaps introduced to optimize the alignment. Lys and Arg are boxed in red to indicate structural similarities. * amidated C-Terminus.

Table 3. Structural data of antimicrobial and cytolytic peptides from arthropod venoms

| Cytolytic peptide | Net charge ^a | H ^b | μ^c | Predicted α -helix [%] ^d | Hydrophobic amino acids [%] ^e | Cytolytic peptide | Net charge ^a | H ^b | μ^c | Predicted α -helix [%] ^d | Hydrophobic amino acids [%] ^e |
|-------------------|-------------------------|----------------|---------|--|--|---------------------------|-------------------------|----------------|---------|--|--|
| <i>Spiders</i> | | | | | | | | | | | |
| Cupiennin 1a | + 8 | - 0.138 | 0.023 | 62.9 | 48.6 | Parabutopeptin | +7 | - 0.235 | 0.059 | 71.1 | 35.5 |
| Cupiennin 1b | + 8 | - 0.155 | 0.023 | 80.0 | 45.8 | Scorpine ^f | +4 | - 0.257 | 0.013 | 54.7 | 36.8 |
| Cupiennin 1c | + 8 | - 0.168 | 0.012 | 74.3 | 42.9 | Pandinin 1 | +4 | - 0.121 | 0.064 | 65.9 | 38.5 |
| Cupiennin 1d | + 8 | - 0.152 | 0.028 | 77.1 | 45.8 | Pandinin 2 | +3 | - 0.005 | 0.220 | 66.7 | 50.1 |
| Lycotoxin I | + 6 | - 0.083 | 0.068 | 92.0 | 48.0 | Opistoposin 1 | +4 | - 0.165 | 0.046 | 65.9 | 38.5 |
| Lycotoxin II | + 6 | - 0.219 | 0.127 | 70.4 | 37.0 | Opistoposin 2 | +4 | - 0.163 | 0.053 | 65.9 | 38.5 |
| Oxyopinin 1 | +10 | - 0.211 | 0.196 | 93.8 | 50.2 | IsCT ^g | +2 | 0.113 | 0.331 | 23.1 | 46.2 |
| Oxyopinin 2a | + 8 | - 0.231 | 0.210 | 62.2 | 37.8 | IsCT2 ^g | +2 | 0.221 | 0.266 | 30.8 | 53.9 |
| Oxyopinin 2b | + 7 | - 0.230 | 0.188 | 64.9 | 37.8 | Hadrurin | +5 | 0.006 | 0.094 | 36.6 | 39.1 |
| Oxyopinin 2c | + 7 | - 0.261 | 0.229 | 70.3 | 37.8 | <i>Wasps</i> ^h | | | | | |
| Oxyopinin 2d | + 8 | - 0.243 | 0.219 | 64.9 | 37.8 | Mastoparan | +4 | 0.046 | 0.145 | 71.4 | 71.5 |
| <i>Ants</i> | | | | | | | | | | | |
| Ponericin W1 | + 6 | - 0.058 | 0.230 | 68.0 | 48.0 | Mastoparan B | +5 | - 0.064 | 0.065 | 57.1 | 49.9 |
| Ponericin W2 | + 6 | - 0.058 | 0.242 | 68.0 | 48.0 | Mastoparan C | +4 | 0.052 | 0.136 | 78.6 | 71.3 |
| Ponericin W3 | + 7 | - 0.113 | 0.089 | 65.4 | 46.1 | Mastoparan M | +4 | 0.046 | 0.144 | 71.4 | 71.5 |
| Ponericin W4 | + 5 | - 0.048 | 0.025 | 57.7 | 42.2 | Mastoparan X | +4 | 0.009 | 0.134 | 64.3 | 57.1 |
| Ponericin W5 | + 5 | 0.018 | 0.228 | 75.0 | 54.1 | P Mastoparan | +2 | 0.010 | 0.068 | 7.1 | 42.9 |
| Ponericin W6 | + 4 | 0.217 | 0.107 | 85.0 | 70.0 | E. Mastoparan | +4 | 0.051 | 0.207 | 21.4 | 57.1 |
| Ponericin G1 | + 7 | - 0.168 | 0.219 | 50.0 | 40.0 | HR-I | +4 | 0.067 | 0.144 | 71.4 | 71.4 |
| Ponericin G2 | + 5 | - 0.160 | 0.220 | 60.0 | 36.6 | HR-II | +3 | 0.222 | 0.214 | 35.7 | 71.3 |
| Ponericin G3 | + 6 | - 0.213 | 0.245 | 63.3 | 33.2 | Crabrolin | +3 | 0.141 | 0.360 | 61.5 | 77.0 |
| Ponericin G4 | + 3 | - 0.119 | 0.256 | 69.0 | 44.7 | Anoplin | +4 | - 0.117 | 0.228 | 10.0 | 50.0 |
| Ponericin G5 | + 5 | - 0.046 | 0.205 | 36.7 | 46.6 | <i>Bumblebees/Bees</i> | | | | | |
| Ponericin G6 | + 3 | 0.086 | 0.292 | 55.6 | 55.7 | Bombolitin I | +4 | 0.058 | 0.257 | 58.8 | 58.9 |
| Ponericin G7 | + 2 | 0.090 | 0.276 | 52.6 | 52.7 | Bombolitin II | +3 | - 0.004 | 0.282 | 76.5 | 53.0 |
| Ponericin L1 | + 6 | - 0.049 | 0.232 | 62.5 | 45.9 | Bombolitin III | +3 | 0.080 | 0.298 | 76.5 | 64.7 |
| Ponericin L2 | + 6 | - 0.030 | 0.250 | 62.5 | 45.8 | Bombolitin IV | +3 | 0.044 | 0.168 | 70.6 | 58.7 |
| Pilosulin 1 | + 7 | - 0.142 | 0.044 | 75.0 | 53.6 | Bombolitin V | +2 | 0.206 | 0.095 | 64.7 | 58.9 |
| <i>Scorpions</i> | | | | | | | | | | | |
| BmKbpp | + 7 | - 0.260 | 0.067 | 70.2 | 40.6 | Melittin <i>A. m.</i> | +6 | - 0.086 | 0.215 | 46.2 | 46.1 |
| | | | | | | Melittin <i>A. d.</i> | +5 | - 0.058 | 0.157 | 53.9 | 46.1 |
| | | | | | | Melittin <i>A. f.</i> | +6 | - 0.050 | 0.220 | 50.0 | 46.1 |

^a The net charge of the peptides was calculated assuming that H is not charged under physiological conditions; ^b The mean hydrophobicity, H, and ^c the hydrophobic moment, μ , were calculated using the Eisenberg consensus scale [146]; ^d the helicity α was calculated using secondary structure consensus prediction [69] DPM, GOR4, HNNC, PHD, Predator, SIMPA96, SOPM and ^e additionally, DSC and without Predator; ^e Ala, Val, Phe, Pro, Met, Ile, Leu; ^f amino acid residues 1–38.

tides, IsCT [73] and IsCT2 [66], from scorpion venom. They are composed of 13 amino acid residues with an amidated C-terminus and differ from each other in only 3 amino acid residues (table 2, 3). Both peptides are bactericidal against Gram-negative bacteria (3.3 to $\geq 150 \mu\text{M}$), more effective against Gram-positive bacteria (0.7–17.1 μM), and show hemolysis of sheep erythrocytes of about 20% (IsCT2) and 30% (IsCT) in a concentration of 200 μM . IsCT releases histamine from rat peritoneal mast cells to a higher degree than mastoparan in a comparable concentration. The lytic ability of IsCT and IsCT2 is in a relatively low peptide-to-lipid ratio higher for phosphatidic acid than for phosphatidylcholine containing unilamellar vesicles. From the venom, two C-terminal truncated forms of both peptides, which lack the last two amino acid residues, were isolated. These nonamidated forms showed neither bactericidal nor hemolytic activity and were shown to be the result of proteolytic activity of the scorpion venom [66]. Analyses of the cDNA encoding the precursors of IsCT and IsCT2 revealed that, besides the signal peptide of 23 amino acid residues, both contained an unusual acidic propeptide (3 Glu/7 Asp) at the C-terminal with unknown function. This precursor structure is similar to the precursors of tachypleins isolated from the hemocytes of horseshoe crabs [74], which are cysteine-rich antimicrobial peptides composed of 17 amino acid residues. However, the precursor of neurotoxins [75, 76] and the antimicrobially acting scorpiene from scorpion venom [59] show a different structure.

Spiders

So far, cytolytic peptides from the venom of spiders have only been described for labidognath spiders, especially from members of the superfamily Lycosoidea (wolf-spider-like spiders), which are mainly non-web-building, omnivorous hunting spiders. These investigations concern two species from the genera *Hogna* and *Lycosa* (wolf spiders, Lycosidae), one *Oxyopes* species (lynx spiders, Oxyopidae) and *Cupiennius salei* (ctenids, Ctenidae). Cytotoxic effects on cultured cell lines of venoms from Araneidae (*Neoscona arabesca*, *Eriophora edax*), Lycosidae (*Lycosa helluo*, *Lycosa sp.*, *Trochosa sp.*), Oxyopidae (*Peucetia viridans*, *Oxyopes sp.*), Salticidae (*Phidippus ardens*, *P. johnsoni*, *P. octopunctatus*), Theridiidae (*Latrodectus hesperus*) and the orthognath spider *Aphonopelma sp.* give evidence for further possible sources of cytolytic peptides [77].

The first description of antimicrobial activity was published in 1989 for *Lycosa singoriensis* from China, a species widespread in the palearctic. The purified peptide was active against *E. coli* and composed of 43 to 45 amino acid residues, one-fourth of them basic and one-third hydrophobic amino acid residues [78]. From *Hogna*

carolinensis (mentioned as *Lycosa carolinensis*), a species widely distributed in Mexico and in the United States, the antimicrobial peptides lycotoxin I and II were purified. They occur in the crude venom at a concentration of 1–5 mM. These cationic peptides are characterized by lysine repeats occurring at every fourth or fifth position. Lycotoxins inhibit the growth of Gram-negative and, to a higher degree, Gram-positive bacteria at micromolar concentrations. Beside fungicidal (against *Candida glabrata*) and hemolytic activity in higher concentrations, they are able to dissipate voltage gradients across insect muscle membranes. These cytolytic peptides seem to interfere with the ability of rat synaptosomes to sequester Ca^{2+} , and lycotoxin I dissipates ion gradients of synaptosomes preloaded with $^{45}\text{Ca}^{2+}$. When applied together, the lycotoxins show mainly an additive antimicrobial effect, assuming only little or no synergistic action [79].

Oxyopinins are large amphipathic peptides with a non-amidated C-terminus isolated from the lynx spider *Oxyopes takobius* (erroneously identified as *Oxyopes kitabensis*) [80]. They can be divided into two peptide groups: oxyopinin 1 includes a Pro at position 24, indicating a possible flexible hinge region that could separate two segments of α -helices in the peptide. Oxyopinins 2a, b, c and d exhibit highly similar sequences and 27 conserved amino acid residues. Both peptide groups are characterized by a 6- to 9-fold repeat of 3 to 4 amino acid residues, always starting with lysine or arginine (table 2, 3). Similar to oxyopinin 1, a possible flexible hinge region is given by Gly¹⁹-Val²⁰-Gly²¹. No data are available about their concentrations in the crude venom, but the relative concentrations of the pure peptides oxyopinin 1 and oxyopinin 2 are about 4.6 times higher than the concentration of the isolated neurotoxin OxyTx1. Synthetic oxyopinin 1 shows strong antimicrobial activity toward both Gram-negative and Gram-positive bacteria at micromolar concentrations. Synthetic oxyopinin 2b is more lytic to *Staphylococcus aureus* than to *E. coli*. The hemolytic effect of the oxyopinins on various mammal erythrocytes seems to differ in dependence on the content of phosphatidylcholine in the different erythrocytes. Oxyopinin 1, and to a lesser degree oxyopinin 2b, is able to bind to different phospholipid bilayers. The leakage of their content, probably mediated by the oxyopinins, is strong for bilayers containing phosphatidylcholine, followed by phosphatidylethanolamine and phosphatidic acid. Up to a peptide concentration of 200 μM , no cytolytic effect on negatively charged hemocytes of the cutworm *Spodoptera litura* could be observed. Both oxyopinin groups produce a drastic reduction of *Spodoptera frugiperda* pupal ovary cell (Sf9) membrane resistance by opening nonselective ion channels, as shown by electrophysiological recordings. Injection of oxyopinin 1 and 2b into *S. litura* larvae resulted in necrotic spots at the site of

the needle insertion after 60–90 min postinjection. Coinjection of the neurotoxin OxyTx1 and of oxyopinin 1 in a molar ratio of 1:5 into *S. litura* larvae showed significant lower LD₅₀ values than the LD₅₀ values of the neurotoxin alone. This cooperative lethality effect of the peptide mixture leads to an enhancement of the paralytic and lethal activity in a dose- and time-dependent manner.

The neotropical spider *Cupiennius salei*, which lives in Central America, also has cytolytic peptides in its venom, and a first peptide family was named cupiennin 1 [81, 82]. Cupiennin 1a, b, c and d are characterized by a high total charge of +8 because of lysine residues (table 2, 3). These cupiennins have an amidated C-terminus. The amino acid sequences are highly conserved; only in the hydrophobic N-terminal part and in the more hydrophilic C-terminal part are 5 out of 35 amino acid residues exchanged. The central part of the cupiennins consists of a 6-fold repeat of 4 amino acids, with lysine at every first position. The amphipathic motif is characterized by a right-handed ribbon of positively charged side groups of lysine and polar amino acids winding around the α -helix of the cupiennins. Cupiennin 1a occurs in a concentration of 1.2 mM in crude venom, whereas the concentrations of cupiennin 1b, 1c and 1d are ten times lower. Cupiennin 1a, 1d, the synthetic cupiennin 1a* (with Glu as a non-amidated C-terminal amino acid) and the synthetic cupiennin 1d* (with Gln as a nonamidated C-terminal amino acid) are active against Gram-negative and Gram-positive bacteria in the submicromolar range. The cupiennins also show a hemolytic effect on human erythrocytes, which is lower than the effect of melittin by a factor of 8 to 14. The insecticidal effect of cupiennins on *Drosophila melanogaster* flies, quantified as paralysis and death, is dose-dependent, and the LD₅₀ values (5–8 pmol/mg fly) are significantly higher than the LD₅₀ value of the main neurotoxin CSTX-1 (0.35 pmol/mg fly) but in the same concentration range as CSTX-2a [83, 84] and CSTX-9 [85], two other neurotoxins from the venom of *C. salei*. No difference in the cytolytic activity is found between the C-terminal amidated and nonamidated forms. Injections of 0.05 μ l of different cupiennins in LD₅₀ concentrations into *D. melanogaster* were impeded by the fact that very often the injected volumes flowed out between the injection opening and the glass capillary. We interpret this effect as a direct cytolytic action of cupiennins on the tissue next to the injection capillary. This effect was only observed for the cytolytic peptides cupiennin and melittin but never during the injection of different neurotoxins or other proteins, even in high millimolar concentrations. Further experiments with synthetic N- and C-terminal truncated forms of cupiennin 1d* showed that the major determinants of structure and cytolytic activity are located in the hydrophobic N-terminal chain segment. Removal of the first six N-terminal amino acids implicated no further biological activities of the truncated peptide. It

seems that the more-polar C-terminus modulates the peptide accumulation at negatively charged cell surfaces via electrostatic interaction [86].

Insects

Over three decades ago, direct hemolytic activities had been observed in venoms of different bees, wasps and ants [24]. The venoms of stinging hymenopterans contain a variety of biologically active substances. Besides high levels of hyaluronidase and phospholipases, neurotoxins, kinins, hemolysins and low-molecular-mass compounds like histamine and serotonin [25], more and more cytolytically active peptides have been identified and characterized. Apocritan hymenopterans are roughly divided into parasitic wasps (such as Ichneumonidae and Braconidae), spider wasps (Pompilidae), ants (Formicidae), wasps (Vespidae and Eumenidae), and bees and bumblebees (Apidae).

Bees and bumblebees

Apidae use their venoms mainly for defending themselves and their colonies against invertebrate and vertebrate aggressors. One of the best-investigated cytolytic peptides from the bee family is melittin, the major component of the honeybee *Apis mellifera* venom [87–89]. Hundreds of publications are available on its biological activity, structure and membrane interactions. The reader is referred for more information to detailed reviews [90–93]. Briefly, melittin is characterized by a hydrophobic N-terminus and a helix kink induced by the GXP motif followed by a more polar amidated C-terminal part (table 2, 3). It is a bent α -helical rod as determined from crystals grown in aqueous solutions [94, 95]. At 40% of the dry weight, melittin is the main component in bee venom followed by phospholipase A₂ with 10–12% [96]. The peptide causes lysis of erythrocytes from different species and induces release of histamine from mast cells [97], bilayer micellization, membrane fusion and voltage-dependent ion channels across planar lipid bilayers [91, 93, 98]. Melittin interacts with many proteins such as calmodulin [99], calsequestrin [100], myosin light chains [101], negatively charged lipids and gangliosides [91, 102]. Its affinity for negatively charged lipids is about 100-fold greater than for zwitterionic lipids [103]. Melittin facilitates the activity of phospholipase A₂ [97], the mast cell degranulating peptide P401 from honeybee venom [96], protein kinase C and Ca/calmodulin-dependent protein kinase II [104]. Inhibitory activities of melittin have been described for many ion-motive ATPases [105]. In addition to the effects just described, melittin shows an insecticidal activity toward *D. melanogaster*,

acts antimicrobially to Gram-positive and Gram-negative bacteria and to fungi in the micromolar range, and is nearly as active as cytolytic peptides from spider venoms [64, 82, 106, 107].

Bombolitins, cytolytic peptides isolated from the venom of the bumblebee *Megabombus pennsylvanicus* [108], are five structurally related heptadecapeptides possessing one to three cationic residues and an amidated C-terminus (table 2, 3). They constitute about 25% of the dry weight venom. Bombolitin II is at 33% the most abundant of all bombolitins. As with other cytolytic peptides from wasp and bee venoms, the bombolitins lyse erythrocytes, facilitate the activity of phospholipase A₂ and cause the release of histamine from mast cells. Bombolitin V shows the same lytic activity towards erythrocytes as melittin and is five times more potent in releasing histamine than mastoparan.

Wasps

Social and solitary wasps of the families Vespidae and Eumenidae and the solitary spider wasps of the family Pompilidae use their venom for prey capture and defense. In contrast to social wasps, which feed their brood with pulpified prey items, solitary wasps store paralyzed prey to feed their larvae. So far, many cytolytic peptides in the venom of social wasps have been characterized: mastoparan from *Vespula lewisii* [109], mastoparan B from *Vespa basalis* [110], mastoparan M from *Vespa mandarinia* [111], mastoparan X from *Vespa xanthoptera* [112], mastoparan C and crabrolin from *Vespa crabro*, HR-I and II from *Vespula orientalis* [113] and *Polistes* mastoparan from *Polistes jadvigae* [114]. The cytolytic eumenine mastoparan from the solitary eumenine wasp *Anterhynchium flavomarginatum micado* [115] and anoplins from the solitary spider wasp *Anoplius samariensis* [116] have been described. All these mastoparans and HR-I are very similar, being able to adopt an amphipathic α -helical structure and being composed of 14 residues with two to four cationic amino acids. All hitherto characterized cytolytic peptides from the venom of these wasps possess an amidated C-terminal leucine-residue (table 2, 3).

Mastoparans show many biological activities: low hemolysis, mast-cell degranulation and release of histamine, further facilitation of phospholipase A₂ from different sources [117] and activation of GTP-binding protein [118]. The enzymatic activities of pig brain protein kinase C, rat cerebral cortex Na,K-ATPase and also the proliferation of TPA-induced HL60 cells are inhibited by mastoparan and analogues [119]. Mastoparan D and its all-D analogue are bactericidal, but the all-D analogue exhibits bactericidal activity that is twice as high. Investigation with scanning-beam electron microscopy of all-

D mastoparan-treated bacteria and tumor cell lines documented blastlike bleb extrusions on the surface for *S. aureus*, swelling on the end of *E. coli* and hollow, shrunk and collapsed structures of tumor cells. These observations indicate a direct nonstereospecific cell-disrupting mechanism of mastoparans [120, 121]. Minimal inhibitory concentrations for the observed bactericidal and fungicidal effects of mastoparan are in the micromolar range and slightly higher when compared with melittin and cytolytic peptides from the scorpion *O. carinatus* [64]. A synergistic hemolytic effect of mastoparan B with the 'lethal protein' that possesses phospholipase A₁ activity, both from the venom of the hornet *Vespa basalis*, is reported [110].

Crabrolin is less hemolytic and less active in releasing histamine and facilitating phospholipase A₂ than mastoparan [113, 122]. However, experiments with crabrolin and analogues suggested that the α -helical conformation is necessary for its hemolytic activity, but is not a prerequisite for its bactericidal activity [122]. Anoplin, with 10 residues the shortest cytolytic peptide isolated from wasp venom, shows a high homology to crabrolin and HR-II. Both peptides show an α -helical structure in the presence of trifluoroethanol as revealed by CD-spectroscopy [116]. Interestingly, anoplin also shows antimicrobial activity against Gram-positive and Gram-negative bacteria, histamine release and very slight hemolytic activity [116].

Ants

Stinging ants of different subfamilies such as Ponerinae, Myrmicinae, Ectoninae, Pseudomyrmecinae and Myrmeciinae possess complex venom mixtures rich in hyaluronidase, phospholipases, neurotoxins, histamine and further low-molecular-mass compounds. Many of them also contain hemolysins, active substances causing damage of erythrocytes [123, 124], which have become more and more interesting during the search for new antimicrobial peptides. Ants use their venoms mainly for prey capture, as a defense strategy against invertebrate and vertebrate predators and for communication. Overpowered and paralyzed or killed prey items are mainly carried into the nest whole or cut into small pieces and directly used as food for adults and/or for their larval brood. *Pachycondyla goeldi*, a ponerine ant from French Guiana, expresses three different antibacterial and insecticidal peptide families in its venom gland, called ponerin G, W and L families [125]. They exhibit molecular masses of between 1.8 and 3.4 kDa, consist of 18 to 30 amino acid residues and are present in concentrations between 0.3 and 23 mM in the venom. The ponerin G family is composed of seven different peptides sharing high sequence similarities with each other and also with ce-

cropin-like peptides. Comparisons of the ponerics W1–6 with gaegurin 5, an antimicrobial peptide from the frog *Rana rugosa* [126], and melittin show about 70% sequence similarity. The ponerics L1 and 2 are similar to the dermaseptin family from the skin of the frog *Phyllomedusa sauvagii* [127, 128]. All members of the ponerin families are highly active against Gram-positive and Gram-negative bacteria, with lower MIC values compared with melittin, cecropin and dermaseptin. Members of the ponerin G and W families also show fungicidal activity against the yeast *Saccharomyces cerevisiae*. The ponerics W1–6 are most hemolytic against horse and sheep erythrocytes, whereas the ponerin G and L families showed no cytolytic activity in the tested concentration of 0.4–0.5 mM. Interestingly, all ponerin families exhibited an insecticidal activity with LD₅₀ between 73.8 and 607 µg/g cricket (*Acheta domestica*), whereas only two peptides (W3-desK and W4) showed a toxic effect on the ants themselves.

Hemolytic activity in the venom of the Australian ant *Myrmecia pilosula* was first reported in 1964 [129]. Meanwhile, pilosulin 1, a cationic cysteine-free and probably α -helical peptide of 56 residues has been identified. It causes lysis of erythrocytes at micromolar concentrations and is only four times less active than melittin. The cytotoxic effect was also demonstrated on further eukaryotic cells such as peripheral mononuclear blood cells, various white blood cells, and Eppstein-Barr-transformed B-cells [130].

A further hemolytic peptide, barbatolysin, was isolated from the venom of the red harvester ant *Pogonomyrmex barbatus*, showing cytolytic activity on erythrocytes. Using gel filtration experiments, its molecular mass was determined to be ca. 3.5 kDa. Interestingly, the amino acid composition shows a high content of His, Gly and Glx and a total absence of Lys, Arg, Ile and Leu residues [131]. Unfortunately, no further structural data are available.

Antimicrobial peptides from the hemolymph and venom: similarities and differences

So far, antimicrobial peptides identified in the hemolymph of venomous arthropods can be subdivided into the cysteine-containing peptides, the proline-rich and the glycine-rich peptides. Most of the cationic peptides isolated from their venoms, however, belong to the α -helical linear peptides. The isolation of scorpine [59] and the identification of BmTXKS2 [68], both belonging to the cysteine-rich peptides, indicate the possibility of further structurally different peptides in venom. The peptides in both groups are characterized by a small size, a positive charge at neutral pH with pI's above pH 8.3 and a broad antimicrobial activity. Some of the peptides from arthro-

pod venoms show additionally hemolytic and cytotoxic activities in low micromolar concentrations [65, 82, 90, 108, 125], in contrast to gomesin from the hemolymph, which is weakly hemolytic in high concentrations [34]. Synthesis of antimicrobial peptides in the hemolymph of aculeate hymenopterans is induced upon infection with different microbes, in contrast to scorpions, which exhibit a constitutive expression [26, 29]. In the hemolymph of arthropods, the antimicrobial peptides occur in the micromolar range, whereas the concentration of some peptides in the venom reach the millimolar range [79, 82, 96, 125].

For the honeybee, it could be shown that the sequential processing of the melittin precursor by dipeptidyl-aminopeptidases in a late step of the biosynthesis occurs after the precursor has entered the venom sac. The chitin wall of the venom sac prevents contact of melittin with phospholipid bilayers and a damage of the venom gland by its own components [132]. Antimicrobial- and cytolytic-acting peptides seem to be constitutively present in the venom glands, but great variations are also reported for melittin [133] and the degranulating activity from the venom of *Parabuthus schlechterii* [63], which is explained as a function of seasonal factors, food and the development stage of the arthropods.

Why are these cytolytic peptides in venom?

So far, investigations of the cytolytic peptides presented above have focused more on effects on different vertebrate cells as well as on prokaryotic and eukaryotic microorganisms but have neglected arthropods, which represent the most probable target of these predators and their cytolytic peptides. The venoms of scorpions, spiders and aculeate hymenopterans contain a complex mixture of different compounds with the aim of paralyzing and/or killing a given prey item, but also to deter invertebrate and vertebrate aggressors as quickly as possible.

Prey catching is a dangerous act for a predator because many arthropods that may become the prey of a scorpion, spider or predacious wasp possess defense weapons (chewing mouth parts or strong legs with thorns) that could hurt the predator considerably. In the case of social bees or ants, invertebrate or vertebrate predators could do tremendous damage to their colony or nest. Therefore, the evolution of arthropod venoms must have tended toward fast-acting venom. Because the reasons for using venom may be different, venom composed of different substances may be useful. These compounds focus on rapid paralysis, fast death and/or short, sharp pain. Assuming that venom composition is also liable to optimization pressure, one would expect minimized concentrations of single compounds, synergisms between compounds, and highly effective substances. This may reduce energy costs

for biosynthesis of the constitutively venomous substances as well as the danger of injury by prey.

Injection of venom into an invertebrate prey results in directly affecting the central nervous system, as concluded for some solitary aculeate wasps [134] and the scorpion *Leiurus quinquestriatus* [135]. In contrast, it is reported that *Philanthus* stings the honeybee in the thorax ganglion region from where the venom is then distributed by the hemolymph, causing peripheral muscle paralysis [136]. Venom injection of spiders also occurs in the back of a fly's head and in the thorax, abdomen and femur [137]. Frequently, venom injection by aculeate hymenopterans, spiders and scorpions takes place in the soft parts of the thorax and is perhaps injected directly into the skeletal muscles and/or hemolymph of arthropods, where the diverse paralyzing and neurotoxic substances still must pass several cellular barriers to reach their targets.

Although the venoms of scorpions, spiders and hymenopterans are composed of different neurotoxic substances, they show parallels in the presence of different enzymes and low-molecular-mass substances such as histamine and potassium ions [24, 25, 54, 83, 138]. A first well-known step in quickly reaching a cellular target is a high hyaluronidase content, which is present in many hymenopteran [25], scorpion and spider venoms [24, 83, 139]. This enzyme acts as a spreading agent and hydrolyzes the macropolysaccharide polymers, hyaluronic acid and chondroitin sulphate that constitute the bulk of animal connective tissue [124]. A second widespread enzyme, phospholipase A2 and/or B, is also present in all arthropod venoms except those of spiders. It catalyzes the hydrolysis of lipids and achieves, in combination with melittin, the breakdown of membrane structures [96].

The brain and the nervous system of insects are protected from direct contact with the hemolymph by the hemolymph-brain barrier [140]. The rather permeable acellular neurolemma (containing glycoaminoglycans, negatively charged papilin and arthrosides) [140–141] is followed by the main barrier: the perineurium. Further, glial cells and axons are embedded in the hyaluronic-acid-containing extracellular matrix [141, 142].

Peripheral axons are encapsulated by glial cells [143] and separated from the hemolymph, as well as the CNS and muscles, by the basement membrane containing negatively charged papilin, MDP-1, and arthrosides [144–146]. At the neuromuscular endplate of insects, the axon may lie in a groove formed in the surface of the muscle fiber or completely engulfed by the muscle cells. Axons that synapse on the muscle surface are normally capped by a layer of glial cells comparable to the neuromuscular endplate of vertebrates and are not freely accessible for neurotoxins. On the other hand, it has also been shown for insects that some axon terminals and those that penetrate the muscle fibers are not ensheathed by Schwann cells [147] and are therefore accessible for neurotoxins.

It is conceivable that the linear cytolytic peptides become attracted by the negatively charged basement membranes, resulting in an α -helical conformation that disturbs membranes and influences cell excitability. Peptides such as lycotoxin I cause the dissipation of ion and voltage gradients across nerve membranes and block neuromuscular transmission in insect body wall muscles [79]. Mastoparan acts on G proteins [118]. Further, parabuto-porin depolarizes rat dorsal ganglion cells [63], and ox-iopinins disturb the membrane potential of insect cells up to their lysis [80]. As mentioned above, melittin exhibits both facilitation and inhibition of enzyme activities. Further, the peptide associates spontaneously with the negatively charged cardiolipin and ganglioside GM1, a sialic-acid-containing glycosphingolipid present mainly in the outer eukaryotic plasma membrane of nerve cells [102, 104]. No further information is available on the synergistic effects of these peptides with other venom compounds [80, 82, 125].

In favor of a dual role of the cytolytic peptides, they could (i) facilitate venom activity as explained above and (ii) be a part of the antimicrobial defense system inside the venom glands, aimed at possible infectious microorganisms from the ingestion of infected prey animals or infection of the venom glands during venom injection [59, 64, 66, 67, 79]. Further, it has been proposed that cytolytic peptides could play a conserving role in paralyzed caterpillars, which many solitary wasps provide as nutrition for their offspring [116]. There are also a number of other suggestions as to the reason for cytolytic peptides in the venom of arthropods: one author group [148] reported that threatened *Hadrorurus* scorpions spray venom against aggressors; another group [67] observed that scorpions clean their bodies with their own venom from 'dirty and possible saprophytic organisms', and it was assumed that ants use their venom as an aerosol to protect their brood from microorganisms [125, 149]. Today it is difficult to decide whether these are biologically relevant aspects. They certainly require more detailed confirmation.

Conclusions

Despite their excellent antimicrobial activities, we have to realize that antimicrobial and cytolytic peptides from arthropod venom are at present not well suited for new antibiotic drugs since many of them do not sufficiently discriminate between microorganisms (pathogens) and erythrocytes (eukaryotic cells). Up to now, numerous efforts have attempted to elucidate the structural basis of their broad cytolytic activity, but the underlying mechanisms are still not well understood [150]. Meanwhile, the antifungal peptide ETD-151 derived from an antifungal peptide from insect hemolymph is a promising drug candidate that shows no signs of toxicity in animal models

even in high concentrations [151]. The cytolytic peptides presented here derive from only a few venomous arthropod species. Most possess several cytolytic peptides, and we can easily anticipate that among the more than 1 million arthropod species (of which at least 20% have venom glands), a much larger molecule variety must be expected. The hitherto isolated and characterized cytolytic peptides from arthropod venom are therefore only the tip of the iceberg. The more new structures we identify, the better we can predict structural features that help us to reduce undesirable side effects on eukaryotic cells. So in the long run, the possibility that cytolytic peptides may yet provide new antimicrobial drugs should not be excluded.

Finally, the effective and broad activity of cytolytic peptides can be a valuable instrument in enhancing, together with arthropod neurotoxins, the insecticidal activity of genetically modified insect-specific baculoviruses as biopesticides [80, 152]. Incorporated into the genome of genetically engineered anophelines, cytolytic peptides could amplify the innate immune defense system in achieving a malaria-resistant insect [59]. Since there are many ecological problems that have to be solved when following such an approach, I again believe that this option will work only in the long run.

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