CMLS, Cell. Mol. Life Sci. 60 (2003) 2651–2668
1420-682X/03/122651-18
DOI 10.1007/s00018-003-3106-8
© Birkhäuser Verlag, Basel, 2003

CMLS Cellular and Molecular Life Sciences

Review

Antimicrobial and cytolytic peptides of venomous arthropods

L. Kuhn-Nentwig

Zoological Institute, University of Bern, Baltzerstrasse 6, 3012 Bern (Switzerland), Fax: +41 31 631 48 88,
e-mail: lucia.kuhn@zos.unibe.ch,URL: http://www.zoology.unibe.ch/ecol/

Received 17 March 2003; received after revision 11 June 2003; accepted 17 June 2003

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

* Corresponding author.

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 µM [31]. Its sequence shows a close similarity to antimicrobial tachyplesins 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 µM), Gram-positive bacteria (0.2–12.5 µM) and fungi (0.4–25 µM). Gomesin exhibits hemolytic activity of 16% at 1 µM, which does not increase up to concentrations of 100 µM. Interestingly, the viability of *Leishmania amazonensis* promastigotes was reduced to 50% in the presence of 2.5 µM 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 *Vespula maculifrons* and *Vespula maculata.* They exhibit a conserved C-terminal part that seems to be specific for hymenopterans [43, 44]. Abaecin 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 formaecins 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 mammalians 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 *Opistophthalmus 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 *Acanthoscurria gomesiana*	Gomesin	18	9.93	2270.7	ZCRRLCYKQRCVTYCRGR*	Z pyroglutamate	34
II *Androctonus australis*	Androctonin	25	10.22	3076.6	RSVCRQIKICRRRGGCYYKCTNRPY		29
	Buthinin	34	9.80	3968.6	SIVPIRCRSNRDCRRFCGFRGGRCTYARQCLCGY		
	Androctonus defensin	37	9.69	4205.8	GFGCPFNQGACHRHCRSIRRRGGYCAGLFKQTCTCYR		
Leiurus quinquestriatus	*Leiurus* defensin	38	9.69	4319.9	GFGCPLNQGACHRHCRSIRRRGGYCAGFFKQTCTCYRN		26
III *Myrmecia gulosa*	Formaecin 1	16	12.01	1997.2	GRPNPVNNKPTPHPRL	O-linked N-acetylgalactosamine to Thr-11	48
	Formaecin 2	16	11.00	2010.3	GRPNPVNTKPTPYPRL		
Formica rufa	*Formica* defensin	40	8.36	4118.7	FTCDLLSGAGVDHSACAAHCLLRGKTGGRCNSDRVCVCRA		47
IV *Apis mellifera*	Abaecin	34	10.45	3878.5	YVPLPNVPQPGRRPFPTFPGQGPFNPKIKWPQGY		40
	Apidaecin Ia	18	11.71	2108.4	GNNRPVYIP-QPRPPHPRI		39
	Apidaecin Ib	18	11.71	2108.4	GNNRPVYIP-QPRPPHPRL		
	Apidaecin II	18	11.71	2122.5	GNNRPIYIP-QPRPPHPRL		
	Apidaecin III	18	11.71	2098.4	GNNRPVYIS-QPRPPHPRL		43
	Hymenoptaecin	93	9.69	10269.4	ZERGSIVIQGTKEGKSRPSLDIDYKQRVYDKNGMTGDAYGGLNIRPGQPSR QHAGFEFGKEYKNGFIKGQSEVQRGPGGRLSPYFGINGGFRF	Z pyroglutamate	41
	Apis defensin	51	8.64	5518.3	VTCDLLSFKGQVNDSACAANCLSLGKAGGHCEKGVCICRKTSFKDLWDKRF*		45
	Royalisin	51	8.30	5526.3	VTCDLLSFKGQVNDSACAANCLSLGKAGGHCEKGVCICRKTSFKDLWDKYF		46
Bombus pascuorum	*Bombus* defensin	51	8.65	5529.5	VTCDLLSIKGVAEHSACAANCLSMGKAGGRCENGICLCRKTTFKELWDKRF*		44
	Abaecin	39	10.00	4395.0	FVPYNPPRPGQSKPFPSFPGHGPFNPKIQWPYPLPNPGH		
	Apidaecin	17	11.71	1963.3	GNRPVYIP-PPRPPHPRL		
Bombus terrestris	Apidaecin Bb +A	17	11.71	1977.3	ANRPVYIP-PPRPPHPRL		43
	Apidaecin Bb –A	16	11.71	1906.3	NRPVYIP-PPRPPHPRL		
V *Vespula maculifrons*	Apidaecin Yj +S	18	12.30	2101.4	SNKPRPQQVPPRPPHPRL		43
	Apidaecin Yj –S	17	12.30	2014.4	NKPRPQQVPPRPPHPRL		
Vespula maculata	Apidaecin Ho +	17	12.30	1957.3	GKPRPQQVPPRPPHPRL		
VI *Sphecius speciosus*	Apidaecin Ck A	16	11.71	1868.2	NRPTYVP-APRPPHPRL		43
	Apidaecin Ck P	16	11.71	1894.2	NRPTYVP-PPRPPHPRL		
VII *Coccygomimus disparis*	Apidaecin Cd 1+	20	12.48	2281.7	GKPNRPRPAPIQPRPPHPRL		43
	Apidaecin Cd 1–	17	12.48	1999.3	NRPRPAPIQPRPPHPRL		
	Apidaecin Cd 2+	20	12.31	2253.7	GKPNKPRPAPIKPRPPHPRL		
	Apidaecin Cd 3+	20	12.31	2226.7	GKPSKPRPAPIKPRPPHPRL		
	Apidaecin Cd 3–	17	12.31	1944.3	SKPRPAPIKPRPPHPRL		

[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 *Opistophthalmus carinatus* (Scorpionidae) [64], *Opisthacanthus madagascariensis* (Ischnuridae) [66] and *Hadrurus aztecus* (Iuridae) [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 *Androctonu*s 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 charge 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 *Opistophthalmus 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 ≥ 50 µM), 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 (Myr_2Gro-*P*Gro) and zwitterionic (Myr_2Gro-*P*Cho) 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 berghi*, 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 ^{1}H 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 phophatidyl-choline and phosphatidyl-serine), 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 *Cupiennius salei*	Cupiennin 1a	35	10.30	3798.6	GFGALFKFLAKKVAKTVAKQAAKQGAKYVVNKQME*	82
	Cupiennin 1b	35	10.30	3800.6	GFGSLFKFLAKKVAKTVAKQAAKQGAKYIANKQME*	
	Cupiennin 1c	35	10.30	3770.5	GFGSLFKFLAKKVAKTVAKQAAKQGAKYIANKQTE*	
	Cupiennin 1d	35	10.30	3795.5	GFGSLFKFLAKKVAKTVAKQAAKQGAKYVANKHME*	
Hogna carolinensis	Lycotoxin I	25	10.60	2843.5	IWLTALKFLGKHAAKHLAKQ---QLSKL*	79
	Lycotoxin II	27	10.22	3206.9	KIKWFKTMKSIAKFIAKEQMK----KHLGGE	
Oxyopes takobius	Oxyopinin 2a	37	10.79	4126.9	GKFSVFGKILRSIAKVFKGVGK-VRKQFKTASDLDKNQ	80
	Oxyopinin 2d	37	10.79	4156.9	GKFSVFSKILRSIAKVFKGVGK-VRKQFKTASDLDKNQ	
	Oxyopinin 2b	37	10.30	4146.9	GKFSGFAKILKSIAKFFKGVGK-VRKQFKEASDLDKNQ	
	Oxyopinin 2c	37	10.46	4064.8	GKLSGISKVLRAIAKFFKGVGK-ARKQFKEASDLDKNQ	
	Oxyopinin 1	48	11.26	5221.3	FRGLAKLLKIGLKSFARVLKKVLPKAAKAGKALAKSMADENAI-RQQNQ	
II *Opistophthalmus carinatus*	Opistoporin 1	44	9.78	4836.6	GKVWDWIKSTAKKLWNSEPVKELKNTALNAAKNLVAEKIGATPS	64
	Opistoporin 2	44	9.78	4870.6	GKVWDWIKSTAKKLWNSEPVKELKNTALNAAKNFVAEKIGATPS	
Pandinus imperator	Pandinin 1	44	9.78	4799.5	GKVWDWIKSAAKKIWSSEPVSQLKGQVLNAAKNYVAEKIGATPT	65
Hadrurus aztecus	Hadrurin	41	10.30	4436.2	GILDTIKSIASKVWNSKTVQDLKRKGINWVANKLGVSPQAA	67
Parabuthus schlechteri	Parabutoporin	45	10.00	4995.0	FKLGSFLKKAWKSKLAKKLRAKGKEMLKDYAKGLLEGGSEEVPGQ	63
Buthus martensii	BmKbpp	47	10.08	5321.3	FRFGSFLKKVWKSKLAKKLRSKGKQLLKDYANKVLNGPEEEAAAPAE	
Pandinus imperator	Pandinin 2	24	10.00	2612.1	FWGALAKGALKLIPSLFSSFSKKD	65
Opisthacanthus mada-gascariensis	IsCT	13	8.59	1502.9	ILGKIWEGIKSLF*	66
	IsCT2	13	8.75	1464.8	IFGAIWNGIKSLF*	
Pandinus imperator	Scorpine	75	8.80	8449.8	GWINEEKIQKKIDERMGNTVLGRMAKAIVHKMAKNEFQCMANMDMLGNCEKH CQTSGEKGYCHGTKCKCGTPLSY	59
III *Pachycondyla goeldii*	Ponericin W1	25	10.70	2710.4	WLGSALKIGAKLLPSVVGLFKKKKQ	125
	Ponericin W2	25	10.70	2710.4	WLGSALKIGAKLLPSVVGLFQKKKK	
	Ponericin W3	26	11.43	2864.6	GIWGTLAKIGIKAVPRVISMLKKKKQ	
	Ponericin W4	26	10.60	2852.5	GIWGTALKWGVKLLPKLVGMAQTKKQ	
	Ponericin W5	24	10.60	2600.2	FWGALIKGAAKLIPSVVGLF-KKKQ	
	Ponericin W6	20	10.00	2029.5	FIGTALGIAS-AIPAIVKLFK*	
	Ponericin G2	30	10.13	3307.9	GWKDWLKKGKEWLKAKGPGIVKAALQAATQ	
	Ponericin G3	30	10.22	3383.1	GWKDWLNKGKEWLKKKGPGIMKAALKAATQ	
	Ponericin G4	29	9.70	3164.8	DFKDWMKTAGEWLKKKGPGILKAAMAAAT-	
	Ponericin G5	30	10.30	3108.8	GLKDWVKIAGGWLKKKGPGILKAAMAAATQ	
	Ponericin G1	30	10.48	3213.9	GWKDWAKKAGGWLKKKGPGMAKAALKAAMQ	
	Ponericin G6	18	9.70	1818.3	GLVDVLGKVGGLIKKLLP*	
	Ponericin G7	19	9.70	1876.4	GLVDVLGKVGGLIKKLLPG	

Table 2 (continued)

	Species	Peptide	Length	pI	Mass	Sequence	Ref.
		Ponericin L1	24	10.30	2595.3	LLKELWTKMKGAGKAVLGKIKGLL*	
		Ponericin L2	24	10.30	2577.3	LLKELWTKIKGAGKAVLGKIKGLL*	
	Myrmecia pilosula	Pilosulin 1	56	10.45	6052.4	GLGSVFGRLARILGRVIPKVAKKLGPKVAKVLPKVMKEAIPMAVEMAKSQEE QQPQ	130
IV	*Megabombus pennsylvanicus*	Bombolitin I	17	10.30	1835.4	IKITTMLAKLGKVLAHV*	108
		Bombolitin II	17	9.70	1805.2	SKITDILAKLGKVLAHV*	
		Bombolitin III	17	9.70	1861.4	IKIMDILAKLGKVLAHV*	
		Bombolitin IV	17	9.70	1872.4	INIKDILAKLVKVLGHV*	
		BombolitinV	17	8.76	1730.2	INVLGILGLLGKALSHL*	
	Apis mellifera	Melittin *A. m.*	26	12.02	2846.5	GIGAVLKVLTTGLPALISWIKRKRQQ*	87
	Apis dorsata	Melittin *A. d.*	26	11.10	2847.5	GIGAILKVLSTGLPALISWIKRKRQE*	89
	Apis florea	Melittin *A. f.*	26	11.33	2818.5	GIGAILKVLATGLPTLISWIKNKRKQ*	88
V	*Vespa mandarinia*	Mastoparan M	14	10.30	1478.9	INLKAIAALAKKLL*	111
	Vespa xanthoptera	Mastoparan X	14	10.30	1556.0	INWKGIAAMAKKLL*	112
	Vespa orientalis	HR-I	14	10.30	1493.0	INLKAIAALVKKVL*	113
	Vespula lewisii	Mastoparan	14	10.30	1478.9	INLKALAALAKKIL*	109
	Vespa crabro	Mastoparan C	14	10.30	1507.0	LNLKALLAVAKKIL*	113
	Vespa basalis	Mastoparan B	14	10.48	1612.1	LKLKSIVSWAKKVL*	110
	Vespa orientalis	HR-II	14	10.00	1523.0	FLPLILGKLVKGLL*	113
	Vespa crabro	Crabrolin	13	11.00	1496.0	FLPLILRKIVTAL*	113
	Polistes jadwigae	*Polistes* Mastoparan	14	8.57	1635.0	VDWKKIGQHILSVL*	114
VI	*Anterhynchium flavomarginatum micado*	Eumenine Mastoparan	14	10.30	1523.0	INLLKIAKGIIKSL*	115
VII	*Anoplius samariensis*	Anoplin	10	11.17	1153.5	GLLKRIKTLL*	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 CTerminus.

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]	Hydrohobic amino acids [%][e]
Spiders					
Cupiennin 1a	+ 8	– 0.138	0.023	62.9	48.6
Cupiennin 1b	+ 8	– 0.155	0.023	80.0	45.8
Cupiennin 1c	+ 8	– 0.168	0.012	74.3	42.9
Cupiennin 1d	+ 8	– 0.152	0.028	77.1	45.8
Lycotoxin I	+ 6	– 0.083	0.068	92.0	48.0
Lycotoxin II	+ 6	– 0.219	0.127	70.4	37.0
Oxyopinin 1	+10	– 0.211	0.196	93.8	50.2
Oxyopinin 2a	+ 8	– 0.231	0.210	62.2	37.8
Oxyopinin 2b	+ 7	– 0.230	0.188	64.9	37.8
Oxyopinin 2c	+ 7	– 0.261	0.229	70.3	37.8
Oxyopinin 2d	+ 8	– 0.243	0.219	64.9	37.8
Ants					
Ponericin W1	+ 6	– 0.058	0.230	68.0	48.0
Ponericin W2	+ 6	– 0.058	0.242	68.0	48.0
Ponericin W3	+ 7	– 0.113	0.089	65.4	46.1
Ponericin W4	+ 5	– 0.048	0.025	57.7	42.2
Ponericin W5	+ 5	0.018	0.228	75.0	54.1
Ponericin W6	+ 4	0.217	0.107	85.0	70.0
Ponericin G1	+ 7	– 0.168	0.219	50.0	40.0
Ponericin G2	+ 5	– 0.160	0.220	60.0	36.6
Ponericin G3	+ 6	– 0.213	0.245	63.3	33.2
Ponericin G4	+ 3	– 0.119	0.256	69.0	44.7
Ponericin G5	+ 5	– 0.046	0.205	36.7	46.6
Ponericin G6	+ 3	0.086	0.292	55.6	55.7
Ponericin G7	+ 2	0.090	0.276	52.6	52.7
Ponericin L1	+ 6	– 0.049	0.232	62.5	45.9
Ponericin L2	+ 6	– 0.030	0.250	62.5	45.8
Pilosulin 1	+ 7	– 0.142	0.044	75.0	53.6
Scorpions					
BmKbpp	+ 7	– 0.260	0.067	70.2	40.6

Cytolytic peptide	Net charge[a]	H[b]	μ[c]	Predicted α-helix [%][d]	Hydrophobic amino acids [%][e]
Scorpions					
Parabutoporin	+7	– 0.235	0.059	71.1	35.5
Scorpine[f]	+4	– 0.257	0.013	54.7	36.8
Pandinin 1	+4	– 0.121	0.064	65.9	38.5
Pandinin 2	+3	– 0.005	0.220	66.7	50.1
Opistoporin 1	+4	– 0.165	0.046	65.9	38.5
Opistoporin 2	+4	– 0.163	0.053	65.9	38.5
IsCT[g]	+2	0.113	0.331	23.1	46.2
IsCT2[g]	+2	0.221	0.266	30.8	53.9
Hadrurin	+5	0.006	0.094	36.6	39.1
Wasps[g]					
Mastoparan	+4	0.046	0.145	71.4	71.5
Mastoparan B	+5	– 0.064	0.065	57.1	49.9
Mastoparan C	+4	0.052	0.136	78.6	71.3
Mastoparan M	+4	0.046	0.144	71.4	71.5
Mastoparan X	+4	0.009	0.134	64.3	57.1
P. Mastoparan	+2	0.010	0.068	7.1	42.9
E. Mastoparan	+4	0.051	0.207	21.4	57.1
HR-I	+4	0.067	0.144	71.4	71.4
HR-II	+3	0.222	0.214	35.7	71.3
Crabrolin	+3	0.141	0.360	61.5	77.0
Anoplin	+4	– 0.117	0.228	10.0	50.0
Bumblebees/Bees					
Bombolitin I	+4	0.058	0.257	58.8	58.9
Bombolitin II	+3	– 0.004	0.282	76.5	53.0
Bombolitin III	+3	0.080	0.298	76.5	64.7
Bombolitin IV	+3	0.044	0.168	70.6	58.7
BombolitinV	+2	0.206	0.095	64.7	58.9
Melittin *A. m.*	+6	– 0.086	0.215	46.2	46.1
Melittin *A. d.*	+5	– 0.058	0.157	53.9	46.1
Melittin *A. f.*	+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 [g] 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 ≥ 150 μM), 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 IsTC2 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 tachyplesins 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 scorpine 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 Lycosidoidea (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 $^{45}Ca^{2+}$. 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 Gly^{19}-Val^{20}-Gly^{21}. 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 phospatidylcholine, 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 LD_{50} values than the LD_{50} 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 nonamidated 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 LD_{50} values (5–8 pmol/mg fly) are significantly higher than the LD_{50} 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 µl of different cupiennins in LD_{50} 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 A_2 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 A_2 [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 A_2 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 jadwigae* [114]. The cytolytic eumenine mastoparan from the solitary eumenine wasp *Anterhynchium flavomarginatum micado* [115] and anoplin 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 leucin-residue (table 2, 3).

Mastoparans show many biological activities: low hemolysis, mast-cell degranulation and release of histamine, further facilitation of phospholipase A_2 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 A_1 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 A_2 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, Ecitoninae, 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 ponericin 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 ponericin 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 ponericins W1–6 with gaegurin 5, an antimicrobial peptide from the frog *Rana rugosa* [126], and melittin show about 70% sequence similarity. The ponericins L1 and 2 are similar to the dermaseptin family from the skin of the frog *Phyllomedusa sauvagii* [127, 128]. All members of the ponericin families are highly active against Gram-positive and Gram-negative bacteria, with lower MIC values compared with melittin, cecropin and dermaseptin. Members of the ponericin G and W families also show fungicidal activity against the yeast *Saccharomyces cerevisiae*. The ponericins W1–6 are most hemolytic against horse and sheep erythrocytes, whereas the ponericin G and L families showed no cytolytic activity in the tested concentration of 0.4–0.5 mM. Interestingly, all ponericin families exhibited an insecticidal activity with LD_{50} between 73.8 and 607 µg/g cricket (*Acheta domesticus*), 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 arthropod 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 dipeptidylaminopeptidases 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, parabutoporin depolarizes rat dorsal ganglion cells [63], and oxiopinins 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 eukaroytic 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 *Hadrurus* 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.

Acknowledgement. I thank Dr. W. Nentwig, Dr. H. Murray and two unknown referees for helpful discussion and critical comments on the manuscript.

1 Broekaert W. F., Cammue B. P. A., De Bolle M. F. C., Thevissen K., De Samblanx G. W. and Osborn R. W. (1997) Antimicrobial peptides from plants. Crit. Rev. Plant Sci. **16:** 297–323
2 Andreu D. and Rivas L. (1998) Animal antimicrobial peptides: an overview. Biopolymers **47:** 415–433
3 Otvos L. Jr. (2000) Antibacterial peptides isolated from insects. J. Peptide Sci. **6:** 497–511
4 Hancock R. E. W. and Diamond G. (2000) The role of cationic antimicrobial peptides in innate host defenses. Trends Microbiol. **8:** 402–410
5 Hoffmann J. A. and Reichhart, J.-M. (2002) *Drosophila* innate immunity: an evolutionary perspective. Nat. Immunol. **3:** 121–126
6 Zasloff M. (2002) Antimicrobial peptides of multicellular organisms. Nature **415:** 389–395
7 Matsuzaki K. (1999) Why and how are peptide-lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Biochim. Biophys. Acta Bio-Membr. **1462:** 1–10
8 Giangaspero A., Sandri L. and Tossi A. (2001) Amphipathic α-helical antimicrobial peptides – A systematic study of the effects of structural and physical properties on biological activity. Eur. J. Biochem. **268:** 5589–5600
9 Schröder J.-M. (1999) Epithelial antimicrobial peptides: innate local host response elements. Cell. Mol. Life Sci. **56:** 32–46
10 Relf J. M., Chisholm J. R. S., Kemp G. D. and Smith V. J. (1999) Purification and characterization of a cysteine-rich 11.5-kDa antibacterial protein from the granular haemocytes of the shore crab, *Carcinus maenas*. Eur. J. Biochem. **264:** 350–357
11 Muñoz M., Vandenbulcke F., Saulnier D. and Bachère E. (2002) Expression and distribution of penaeidin antimicrobial peptides are regulated by haemocyte reactions in microbial challenged shrimp. Eur. J. Biochem. **269:** 2678–2689
12 Iwanaga S., Kawabata S.-I. and Muta T. (1998) New types of clotting factors and defense molecules found in horseshoe crab hemolymph: their structures and functions. J. Biochem. (Tokyo) **123:** 1–15
13 Destoumieux D., Bulet P., Loew D., Van Dorsselaer A., Rodriguez J. and Bachère E. (1997) Penaeidins, a new family of antimicrobial peptides isolated from the shrimp *Penaeus vannamei* (Decapoda). J. Biol. Chem. **272:** 28398–28406
14 Hetru C., Hoffmann D. and Bulet P. (1998) Antimicrobial peptides from insects. In: Molecular Mechanisms of Immune Responses in Insects, pp. 40–66, Brey P. T. and Hultmark D. (eds), Chapman and Hall, London
15 Lamberty M., Zachary D., Lanot R., Bordereau C., Robert A., Hoffmann J. A. et al. (2001) Insect immunity. Constitutive expression of a cysteine-rich antifungal and a linear antibacterial peptide in a termite insect. J. Biol. Chem. **276:** 4085–4092
16 Vizioli J., Richman A. M., Uttenweiler-Joseph S., Blass C. and Bulet P. (2001) The defensin peptide of the malaria vector mosquito *Anopheles gambiae*: antimicrobial activities and expression in adult mosquitoes. Insect Biochem. Mol. Biol. **31:** 241–248
17 Vizioli J., Bulet P., Hoffmann J. A., Kafatos F. C., Müller H.-M. and Dimopoulos G. (2001) Gambicin: a novel immune responsive antimicrobial peptide from the malaria vector *Anopheles gambiae*. Proc. Natl. Acad. Sci. USA **98:** 12630–12635
18 Vining L. C. (1990) Functions of secondary metabolites. Annu. Rev. Microbiol. **44:** 395–427
19 Dykes G. A. (1995) Bacteriocins: ecological and evolutionary significance. Trends Ecol. Evol. **10:** 186–189
20 Hentschel U., Schmid M., Wagner M., Fieseler L., Gernert C. and Hacker J. (2001) Isolation and phylogenetic analysis of bacteria with antimicrobial activities from the Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola.* FEMS Microbiol. Ecol. **35:** 305–312
21 Kondejewski L. H., Farmer S. W., Wishart D. S., Kay C. M., Hancock R. E. W. and Hodges R. S. (1996) Modulation of structure and antibacterial and hemolytic activity by ring size in cyclic gramicidin S analogs. J. Biol. Chem. **271:** 25261–25268
22 Nickel R., Ott C., Dandekar T. and Leippe M. (1999) Pore-forming peptides of *Entamoeba dispar* – Similarity and divergence to amoebapores in structure, expression and activity. Eur. J. Biochem. **265:** 1002–1007
23 Leippe M. (1999) Antimicrobial and cytolytic polypeptides of amoeboid protozoa-effector molecules of primitive phagocytes. Dev. Comp. Immunol. **23:** 267–279
24 Bettini S. (ed) (1978) Arthropod venoms, Springer, Heidelberg
25 Piek T. (1986) Venoms of the Hymenoptera, Piek, T. (ed), Academic Press, London
26 Cociancich S., Goyffon M., Bontems F., Bulet P., Bouet F., Ménez A. et al. (1993) Purification and characterization of a scorpion defensin, a 4-kDa antibacterial peptide presenting structural similarities with insect defensins and scorpion toxins. Biochem. Biophys. Res. Commun. **194:** 17–22
27 Bulet P., Cociancich S., Reuland M., Sauber F., Bischoff R., Hegy G. et al. (1992) A novel insect defensin mediates the inducible antibacterial activity in larvae of the dragonfly *Aeschna cyanea* (Paleoptera, Odonata). Eur. J. Biochem. **209:** 977–984
28 Bontems F., Roumestand C., Gilquin B., Ménez A. and Toma F. (1991) Refined structure of charybdotoxin: common motifs in scorpion toxins and insect defensins. Science **254:** 1521– 1523
29 Ehret-Sabatier L., Loew D., Goyffon M., Fehlbaum P., Hoffmann J. A., Van Dorsselaer et al. (1996) Characterization of

novel cysteine-rich antimicrobial peptides from scorpion blood. J. Biol. Chem. **271:** 29537–29544
30 Dimarcq J.-L., Bulet P., Hetru C. and Hoffmann J. (1998) Cysteine-rich antimicrobial peptides in invertebrates. Biopolymers **47:** 465–477
31 Hetru C., Letellier L., Oren Z., Hoffmann J. A. and Shai Y. (2000) Androctonin, a hydrophilic disulphide-bridged non-haemolytic antimicrobial peptide: a plausible mode of action. Biochem. J. **345:** 653–664
32 Nakamura T., Furunaka H., Miyata T., Tokunaga F., Muta T. and Iwanaga S. (1988) Tachyplesin, a class of antimicrobial peptide from the hemocytes of the horseshoe crab (*Tachypleus tridentatus*). Isolation and chemical structure. J. Biol. Chem. **263:** 16709–16713
33 Miyata T., Tokunaga F., Yoneya T., Yoshikawa K., Iwanaga S., Niwa M. et al. (1989) Antimicrobial peptides isolated from horseshoe crab hemocytes, tachyplesin II and polyphemusins I and II: chemical structures and biological activity. J. Biochem. (Tokyo) **106:** 663–668
34 Silva P. I. Jr., Daffre S. and Bulet P. (2000) Isolation and characterization of gomesin, an 18-residue cysteine-rich defense peptide from the spider *Acanthoscurria gomesiana* hemocytes with sequence similarities to horseshoe crab antimicrobial peptides of the tachyplesin family. J. Biol. Chem. **275:** 33464–33470
35 Fahrner R. L., Dieckmann T., Harwig S. S., Lehrer R. I., Eisenberg D. and Feigon J. (1996) Solution structure of protegrin-1, a broad-spectrum antimicrobial peptide from porcine leukocytes. Chem. Biol. **3:** 543–550
36 Mandard N., Sy D., Maufrais C., Bonmatin J.-M., Bulet P., Hetru C. et al. (1999) Androctonin, a novel antimicrobial peptide from scorpion *Androctonus australis*: solution structure and molecular dynamics simulations in the presence of a lipid monolayer. J. Biomol. Struct. Dyn. **17:** 367–380
37 Mandard N., Bulet P., Caille A., Daffre S. and Vovelle F. (2002) The solution structure of gomesin, an antimicrobial cysteine-rich peptide from the spider. Eur. J. Biochem. **269:** 1190–1198
38 Casteels P. (1998) Immune response in hymenoptera. In: Molecular Mechanisms of Immune Responses in Insects, pp. 93–110, Brey P. T. and Hultmark D. (eds), Chapman and Hall, London
39 Casteels P., Ampe C., Jacobs F., Vaeck M. and Tempst P. (1989) Apidaecins: antibacterial peptides from honeybees. EMBO J. **8:** 2387–2391
40 Casteels P., Ampe C., Riviere L., Van Damme J., Elicone C., Fleming M. et al. (1990) Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (*Apis mellifera*). Eur. J. Biochem. **187:** 381–386
41 Casteels P., Ampe C., Jacobs F. and Tempst P. (1993) Functional and chemical characterization of hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the honeybee (*Apis mellifera*). J. Biol. Chem. **268:** 7044–7054
42 Casteels P. and Tempst P. (1994) Apidaecin-type peptide antibiotics function through a non-pore-forming mechanism involving stereospecificity. Biochem. Biophys. Res. Commun. **199:** 339–345
43 Casteels P., Romagnolo J., Castle M., Casteels-Josson K., Erdjument-Bromage H. and Tempst P. (1994) Biodiversity of apidaecin-type peptide antibiotics. Prospects of manipulating the antibacterial spectrum and combating acquired resistance. J. Biol. Chem. **269:** 26107–26115
44 Rees J. A., Moniatte M. and Bulet P. (1997) Novel antibacterial peptides isolated from a European bumblebee, *Bombus pascuorum* (Hymenoptera, Apoidea). Insect. Biochem. Mol. Biol. **27:** 413–422
45 Casteels-Josson K., Zhang W., Capaci T., Casteels P. and Tempst P. (1994) Acute transcriptional response of the honeybee peptide-antibiotics gene repertoire and required post-translational conversion of the precursor structures. J. Biol. Chem. **269:** 28569–28575
46 Fujiwara S., Imai J., Fujiwara M., Yaeshima T., Kawashima T. and Kobayashi K. (1990) A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. J. Biol. Chem. **265:** 11333–11337
47 Taguchi S., Bulet P. and Hoffmann J. A. (1998) A novel insect defensin from the ant *Formica rufa*. Biochimie **80:** 343–346
48 Mackintosh J. A., Veal D. A., Beattie A. J. and Gooley A. A. (1998) Isolation from an ant, *Myrmecia gulosa*, of two inducible O-glycosylated proline-rich antibacterial peptides. J. Biol. Chem. **273:** 6139–6143
49 Boevé J.-L., Kuhn-Nentwig L., Keller S. and Nentwig W. (1995) Quantity and quality of venom released by a spider (*Cupiennius salei*, Ctenidae). Toxicon **33:** 1347–1357
50 Rein J. O. (1993) Sting use in two species of *Parabuthus* scorpions (Buthidae). J. Arachnol. **21:** 60–63
51 Malli H., Kuhn-Nentwig L., Imboden H., and Nentwig W. (1999) Effects of size, motility and paralyzation time of prey on the quantity of venom injected by the hunting spider *Cupiennius salei*. J. Exp. Biol. **202:** 2083–2089
52 Wigger E., Kuhn-Nentwig L. and Nentwig W. (2002) The venom optimisation hypothesis: a spider injects large venom quantities only into difficult prey types. Toxicon **40:** 749–752
53 Wullschleger B. and Nentwig W. (2002) Influence of venom availability on a spider's prey-choice behavior. Funct. Ecol. **16:** 802–807
54 Inceoglu B., Lango J., Jing J., Chen L., Doymaz F., Pessah I. N. et al. (2003) One scorpion, two venoms: prevenom of *Parabuthus transvaalicus* acts as an alternative type of venom with distinct mechanism of action. Proc. Natl. Acad Sci. USA **100:** 922–927
55 Zlotkin E. (1996) The role of hymenopterous venoms in nature. In: Bee Products, pp. 185–201, Mizrahi A. and Lensky Y. (eds), Plenum Press, New York
56 Gordon D. and Gurevitz M. (2003) The selectivity of scorpion a-toxins for sodium channel subtypes is determined by subtle variations at the interacting surface. Toxicon **41:** 125–128
57 Escoubas P., Diochot S. and Corzo G. (2000) Structure and pharmacology of spider venom neurotoxins. Biochimie **82:** 893–907
58 Zlotkin E., Fishman Y. and Elazar M. (2000) AaIT: from neurotoxin to insecticide. Biochimie **82:** 869–881
59 Conde R., Zamudio F. Z., Rodríguez M. H. and Possani L. D. (2000) Scorpine, an anti-malaria and anti-bacterial agent purified from scorpion venom. FEBS Lett. **471:** 165–168
60 Balozet L. (1971) Scorpionism in the old world. In: Venomous animals and their venoms, vol. III, Venomous invertebrates, pp. 349–371, Bücherl W. and Buckley E. E. (eds), Academic Press, New York, London
61 Debont T., Daenens P. and Tytgat J. (1996) An improved fractionation and fast screening method for the identification of new and selective neurotoxins. Neurosci. Res. **24:** 201–206
62 Du Plessis J., Badenhorst C., Van der Walt J., Tytgat J., Debont T. and Verdonck F. (1999) Toxic effects of venom from *Opistophthalmus carinatus*. A comparison with the Buthidae family. Toxicon **37:** 308–309
63 Verdonck F., Bosteels S., Desmet J., Moerman L., Noppe W., Willems J. et al. (2000) A novel class of pore-forming peptides in the venom of *Parabuthus schlechteri* Purcell (Scorpions: Buthidae). Cimbebasia **16:** 247–260
64 Moerman L., Bosteels S., Noppe W., Willems J., Clynen E., Schoofs L. et al. (2002) Antibacterial and antifungal properties of α-helical, cationic peptides in the venom of scorpions from southern Africa. Eur. J. Biochem. **269:** 4799–4810
65 Corzo G., Escoubas P., Villegas E., Barnham K. J., He W., Norton R. S. et al. (2001) Characterization of unique amphi-

pathic antimicrobial peptides from venom of the scorpion *Pandinus imperator*. Biochem. J. **359:** 35–45
66 Dai L., Corzo G., Naoki H., Andriantsiferana M. and Nakajima T. (2002) Purification, structure-function analysis and molecular characterization of novel linear peptides from scorpion *Opisthacanthus madagascariensis*. Biochem. Biophys. Res. Commun. **293:** 1514–1522
67 Torres-Larios A., Gurrola G. B., Zamudio F. Z. and Possani L. D. (2000) Hadrurin, a new antimicrobial peptide from the venom of the scorpion *Hadrurus aztecus*. Eur. J. Biochem. **267:** 5023–5031
68 Zhu S., Li W., Jiang D. H. and Zeng X.-C. (2000) Evidence for the existence of insect defensin-like peptide in scorpion venom. IUBMB Life **50:** 57–61
69 Combet C., Blanchet C., Geourjon C. and Deléage G. (2000) NPS@: network protein sequence analysis. Trends Biochem. **25:** 147–150
70 Qu X.-M., Steiner H., Engström Å., Bennich H. and Boman H. G. (1982) Insect immunity: isolation and structure of cecropins B and D from pupae of the Chinese oak silk moth, *Antheraea pernyi*. Eur. J. Biochem. **127:** 219–224
71 Okada M. and Natori S. (1985) Primary structure of sarcotoxin I, an antibacterial protein induced in the hemolymph of *Sarcophaga peregrina* (flesh fly) larvae. J. Biol. Chem. **260:** 7174–7177
72 Lee J.-Y., Boman A., Chuanxin S., Andersson M., Jörnvall H., Mutt V. et al. (1989) Antibacterial peptides from pig intestine: isolation of a mammalian cecropin. Proc. Natl. Acad. Sci. USA **86:** 9159–9162
73 Dai L., Yasuda A., Naoki H., Corzo G., Andriantsiferana M. and Nakajima, T. (2001) IsCT, a novel cytotoxic linear peptide from scorpion *Opisthacanthus madagascariensis*. Biochem. Biophys. Res. Commun. **286:** 820–825
74 Shigenaga T., Muta T., Toh Y., Tokunaga F. and Iwanaga S. (1990) Antimicrobial tachyplesin peptide precursor. cDNA cloning and cellular localization in the horseshoe crab (*Tachypleus tridentatus*). J. Biol. Chem. **265:** 21350–21354
75 Wu J.-J., Dai L., Lan Z.-D. and Chi C.-W. (1999) Genomic organization of three neurotoxins active on small conductance Ca^{2+}-activated potassium channels from the scorpion *Buthus martensii* Karsch. FEBS Lett. **452:** 360–364
76 Wang C.-G., He X.-L., Shao F., Liu W., Ling M.-H., Wang D.-C. et al. (2001) Molecular characterization of an anti-epilepsy peptide from the scorpion *Buthus martensii* Karsch. Eur. J. Biochem. **268:** 2480–2485
77 Cohen E. and Quistad G. B. (1998) Cytotoxic effects of arthropod venoms on various cultered cells. Toxicon **36:** 353–358
78 Xu K., Ji Y. and Qu X. (1989) Purification and characterization of an antibacterial peptide from venom of *Lycosa singoriensis*. Acta Zool. Sinica **35:** 300–305
79 Yan L. and Adams M. E. (1998) Lycotoxins, antimicrobial peptides from venom of the wolf spider *Lycosa carolinensis*. J. Biol. Chem. **273:** 2059–2066
80 Corzo G., Villegas E., Gómez-Lagunas F., Possani L. D., Belokoneva O. S. and Nakajima, T. (2002) Oxyopinins, large amphipathic peptides isolated from the venom of the wolf spider *Oxyopes kitabensis* with cytolytic properties and positive insecticidal cooperativity with spider neurotoxins. J. Biol. Chem. **277:** 23627–23637
81 Haeberli S., Kuhn-Nentwig L., Schaller J. and Nentwig, W. (2000) Characterisation of antibacterial activity of peptides isolated from the venom of the spider *Cupiennius salei* (Araneae: Ctenidae). Toxicon **38:** 373–380
82 Kuhn-Nentwig L., Müller J., Schaller J., Walz A., Dathe M. and Nentwig, W. (2002) 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
83 Kuhn-Nentwig L., Schaller J. and 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
84 Kuhn-Nentwig L., Schaller J., Kämpfer U., Imboden H., Malli H. and 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
85 Schaller J., Kämpfer U., Schürch S., Kuhn-Nentwig L., Haeberli S. and Nentwig W. (2001) CSTX-9, a toxic peptide from the spider *Cupiennius salei*: amino acid sequence, disulphide bridge pattern and comparison with other spider toxins containing the cystine knot structure. Cell. Mol. Life Sci. **58:** 1538–1545
86 Kuhn-Nentwig L., Dathe M., Walz A., Schaller J. and Nentwig W. (2002) Cupiennin 1d*: the cytolytic activity depends on the hydrophobic N-terminus and is modulated by the polar C-terminus. FEBS Lett. **527:** 193–198
87 Habermann E. and Jentsch J. (1967) Sequenzanalyse des Melittins aus den tryptischen und peptischen Spaltstücken. Hoppe-Seyler Z. Physiol. Chem. **348:** 37–50
88 Kreil G. (1973) Structure of melittin isolated from two species of honeybees. FEBS Lett. **33:** 241–244
89 Kreil G. (1975) The structure of *Apis dorsata* melittin: phylogenetic relationships between honeybees as deduced from sequence data. FEBS Lett. **54:** 100–102
90 Tosteson M. T., Holmes S. J., Razin M. and Tosteson D. C. (1985) Melittin lysis of red cells. J. Membr. Biol. **87:** 35–44
91 Dempsey C. E. (1990) The actions of melittin on membranes. Biochim. Biophys. Acta **1031:** 143–161
92 Saberwal G. and Nagaraj R. (1994) Cell-lytic and antibacterial peptides that act by perturbing the barrier function of membranes: facets of their conformational features, structure-function correlations and membrane-perturbing abilities. Biochim. Biophys. Acta **1197:** 109–131
93 Bechinger B. (1997) Structure and functions of channel-forming peptides: magainins, cecropins, melittin and alamethicin. J. Membrane Biol. **156:** 197–211
94 Eisenberg D., Terwilliger T. C. and Tsui F. (1980) Structural studies of bee melittin. Biophys. J. **32:** 252–254
95 Terwilliger T. C., Weissman L. and Eisenberg D. (1982) The structure of melittin in the form I crystals and its implication for melittin's lytic and surface activities. Biophys. J. **37:** 353– 361
96 Banks B. E. C. and Shipolini R. A. (1986) Chemistry and pharmacology of honeybee venom. In: Venoms of the Hymenoptera, pp. 329–416, Piek T. (ed), Academic Press, London
97 Habermann E. (1972) Bee and wasp venoms. Science **177:** 314–322
98 Bernheimer A. W. and Rudy B. (1986) Interactions between membranes and cytolytic peptides. Biochim. Biophys. Acta **864:** 123–141
99 Kataoka M., Head J. F., Seaton B. A. and Engelman D. M. (1989) Melittin binding causes a large calcium-dependent conformational change in calmodulin. Proc. Natl. Acad. Sci. USA **86:** 6944–6948
100 He Z., Dunker A. K., Wesson C. R. and Trumble W. R. (1993) Ca^{2+}-induced folding and aggregation of skeletal muscle sarcoplasmic reticulum calsequestrin. J. Biol. Chem. **268:** 24635–24641
101 Malencik D. A. and Anderson S. R. (1988) Association of melittin with the isolated myosin light chains. Biochemistry **27:** 1941–1949
102 Chatterjee C. and Mukhopadhyay C. (2002) Melittin-GM1 interaction: a model for a side-by-side complex. Biochem. Biophys. Res. Commun. **292:** 579–585
103 Batenburg A. M., Van Esch J. H. and De Kruijff B. (1988) Melittin-induced changes of the macroscopic structure of phosphatidylethanolamines. Biochemistry **27:** 2324–2331

104 Raynor R. L., Zheng B. and Kuo J. F. (1991) Membrane interactions of amphiphilic polypeptides mastoparan, melittin, polymyxin B and cardiotoxin. J. Biol. Chem. **266:** 2753–2758

105 Shorina E. A., Mast N. V., Lopina O. D. and Rubtsov A. M. (1997) Melittin-induced inhibition and aggregation of Ca-ATPase in skeletal muscle sarcoplasmic reticulum: a comparative study. Biochemistry **36:** 13455–13460

106 Fennell J. F., Shipman W. H. and Cole L. J. (1968) Antibacterial action of melittin, a polypeptide from bee venom. Proc. Soc. Exp. Biol. Med. **127:** 707–710

107 Mitchell H. K., Lowy P. H., Sarmiento L. and Dickson L. (1971) Melittin: toxicity to *Drosophila* and inhibition of acetylcholinesterase. Arch. Biochem. Biophys. **145:** 344–348

108 Argiolas A. and Pisano J. J. (1985) Bombolitins, a new class of mast cell degranulating peptides from the venom of the bumblebee *Megabombus pennsylvanicus*. J. Biol. Chem. **260:** 1437–1444

109 Hirai Y., Yasuhara T., Yoshida H., Nakajima T., Fujino M. and Kitada C. (1979) A new mast cell degranulating peptide 'mastoparan' in the venom of *Vespula lewisii*. Chem. Pharm. Bull. (Tokyo) **27:** 1942–1944

110 Ho C.-L. and Hwang L.-L. (1991) Structure and biological activities of a new mastoparan isolated from the venom of the hornet *Vespa basalis*. Biochem. J. **274:** 453–456

111 Hirai Y., Yasuhara T., Yoshida H. and Nakajima T. (1981) A new mast cell degranulating peptide, mastoparan-M, in the venom of the hornet *Vespa mandarinia*. Biomed. Res. **2:** 447–449

112 Hirai Y., Kuwada M., Yasuhara T., Yoshida H. and Nakajima T. (1979) A new mast cell degranulating peptide homologous to mastoparan in the venom of Japanese hornet (*Vespa xanthoptera*). Chem. Pharm. Bull. (Tokyo) **27:** 1945–1946

113 Argiolas A. and Pisano J. J. (1984) Isolation and characterization of two new peptides, mastoparan C and crabrolin, from the venom of the European hornet, *Vespa crabro*. J. Biol. Chem. **259:** 10106–10111

114 Hirai Y., Ueno Y., Yasuhara T., Yoshida H. and Nakajima T. (1980) A new mast cell degranulating peptide, polistes mastoparan, in the venom of *Polistes jadwigae*. Biomed. Res. **1:** 185–187

115 Delatorre P., Olivieri J. R., Ruggiero Neto J., Lorenzi C. C. B., Canduri F., Fadel V. et al. (2001) Preliminary cryocrystallography analysis of an eumenine mastoparan toxin isolated from the venom of the wasp *Anterhynchium flavomarginatum micado*. Biochim. Biophys. Acta **1545:** 372–376

116 Konno K., Hisada M., Fontana R., Lorenzi C. C. B., Naoki H., Itagaki Y. et al. (2001) Anoplin, a novel antimicrobial peptide from the venom of the solitary wasp *Anoplius samariensis*. Biochim. Biophys. Acta **1550:** 70–80

117 Argiolas A. and Pisano J. J. (1983) Facilitation of phospholipase A_2 activity by mastoparans, a new class of mast cell degranulating peptides from wasp venom. J. Biol. Chem. **258:** 13697–13702

118 Higashijima T., Uzu S., Nakajima T. and Ross E. M. (1988) Mastoparan, a peptide toxin from wasp venom, mimics receptors by activating GTP-binding regulatory proteins (G proteins). J. Biol. Chem. **263:** 6491–6494

119 Raynor R. L., Kim Y.-S., Zheng B., Vogler W. R. and Kuo J. F. (1992) Membrane interactions of mastoparan analogues related to their differential effects on protein kinase C, Na,K-ATPase and HL60 cells. FEBS Lett. **307:** 275–279

120 Li M.-L., Liao R.-W., Qiu J.-W., Wang Z.-J. and Wu T.-M. (2000) Antimicrobial activity of synthetic all-D mastoparan M. Int. J. Antimicrob. Agents **13:** 203–208

121 Wu T.-M. and Li M.-L. (1999) The cytolytic action of all-D mastoparan M on tumor cell lines. Int. J. Tissue React. **XXI:** 35–42

122 Krishnakumari V. and Nagaraj R. (1997). Antimicrobial and hemolytic activities of crabrolin, a 13-residue peptide from the venom of the European hornet, *Vespa crabro*, and its analogues. J. Pept. Res. **50:** 88–93

123 Blum M. S. and Hermann H. R. (1978) Venoms and venom apparatuses of the Formicidae: Myrmeciinae, Ponerinae, Dorylinae, Pseudomyrmecinae, Myrmicinae and Formicinae. In: Arthropod venoms, pp. 801–869, Bettini S. (ed), Springer, Heidelberg

124 Schmidt J. O. (1986) Chemistry, pharmacology and chemical ecology of ant venoms. In: Venoms of the Hymenoptera, pp. 425–508, Piek T. (ed), Academic Press, London

125 Orivel J., Redeker V., Le Caer J.-P., Krier F., Revol-Junelles A.-M., Longeon A. et al. (2001) Ponericins, new antibacterial and insecticidal peptides from the venom of the ant *Pachycondyla goeldii*. J. Biol. Chem. **276:** 17823–17829

126 Park J. M., Jung J.-E. and Lee B. J. (1994) Antimicrobial peptides from the skin of a Korean frog, *Rana rugosa*. Biochem. Biophys. Res. Commun. **205:** 948–954

127 Simmaco M., Mignogna G. and Barra D. (1998) Antimicrobial peptides from amphibian skin: what do they tell us? Biopolymers **47:** 435–450

128 Mor A. and Nicolas P. (1994) Isolation and structure of novel defensive peptides from frog skin. Eur. J. Biochem. **219:** 145–154

129 Cavill G. W. K., Robertson P. L. and Whitfield F. B. (1964) Venom and venom apparatus of the bull ant, *Myrmecia gulosa* (Fabr.) Science **146:** 79–80

130 Wu Q.-X., King M. A., Donovan G. R., Alewood D., Alewood P., Sawyer W. H. et al. (1998) Cytotoxicity of pilosulin 1, a peptide from the venom of the jumper ant *Myrmecia pilosula.* Biochim. Biophys. Acta **1425:** 74–80

131 Bernheimer A. W., Avigad L. S. and Schmidt J. O. (1980) A hemolytic polypeptide from the venom of the red harvester ant, *Pogonomyrmex barbatus.* Toxicon **18:** 271–278

132 Kreil G. (1990) Processing of precursors by dipeptidylaminopeptidases: a case of molecular ticketing. Trends Biochem. Sci. **15:** 23–26

133 Owen M. D. and Pfaff L. A. (1995) Melittin synthesis in the venom system of the honeybee (*Apis mellifera* L.). Toxicon **33:** 1181–1188

134 Piek T. (1990) Neurotoxins from venoms of the hymenoptera. Twenty-five years of research in Amsterdam. Comp. Biochem. Physiol. **96C:** 223–233

135 Shulov A. and Levy G. (1978) Venoms of buthinae. In: Arthropod venoms, pp. 309–317, Bettini S. (ed), Springer, Heidelberg

136 Rathmayer W. (1962) Das Paralysierungsproblem beim Bienenwolf, *Philanthus triangulum* F (Hym. Sphec). Z. Vergleich. Physiol. **45:** 413–462

137 Foelix R. F. (1996) How do crab spiders (Thomisidae) bite their prey? Rev. Suisse de Zool. **vol. hors série:** 203–210

138 Rash L. D., King R. G. and Hodgson W. C. (1998) Evidence that histamine is the principal pharmacological component of venom from an Australian wolf spider (*Lycosa godeffroyi*).Toxicon **36:** 367–375

139 Basu A., Gomes A., Gomes A., Dasgupta S. C. and Lahiri S. C. (1990) Histamine, 5-HT and hyaluronidase in the venom of the scorpion *Lychas laevifrons* (Pock). Indian J. Med. Res. **92:** 371–373

140 Treherne J. E. (1985) Blood-brain barrier. In: Comprehensive Insect Physiology Biochemistry and Pharmacology Vol. 5, pp. 115–137, Kerkut G. A. and Gilbert L. I. (eds), Pergamon Press, Oxford

141 Treherne J. E., Schofield P. K. and Lane N. J. (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

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

143 Jones B. W. (2001) Glial cell development in the *Drosophila* embryo. BioEssays **23:** 877–887

144 Hortsch M., Olson A., Fishman S., Soneral S. N., Marikar Y., Dong R. et al. (1998) The expression of MDP-1, a component of *Drosophila* embryonic basement membranes, is modulated by apoptotic cell death. Int. J. Dev. Biol. **42:** 33–42
145 Sickmann T., Weske B., Dennis R. D., Mohr C. and Wiegandt H. (1992) Chemical distribution of glycoshingolipids in third-instar larval organs of the blowfly, *Calliphora vicina* (Insecta: Diptera). J. Biochem. **111:** 662–669
146 Kramerova I. A., Kawaguchi N., Fessler L. I., Nelson R. E., Chen Y., Kramerov A. A., et al. (2000) Papilin in development; a pericellular protein with a homology to the ADAMTS metalloproteinases. Development **127:** 5475–5485
147 Osborne M. P. (1975) The ultrastructure of nerve-muscle synapses. In: Insect Muscle, pp. 151–205, Usherwood P. N. R. (ed), Academic Press, London
148 McCormick S. J. and Polis G. A. (1990) Prey, predators and parasites. In: The Biology of Scorpions, pp. 294–320, Polis G. A. (ed), Stanford University Press, Stanford
149 Obin M. S. and Vander Meer R. K. (1985) Gaster flagging by fire ants (*Solenopsis spp.*): functional significance of venom dispersal behavior. J. Chem. Ecol. **11:** 1757–1768
150 Shai Y. (1999) Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by α-helical antimicrobial and cell nonselective membrane-lytic peptides. Biochim. Biophys. Acta Bio-Membr. **1462:** 55–70
151 Dimarcq J-L. and Hunneyball I. (2003) Pharma-entomology: when bugs become drugs. Drug Discovery Today **8:** 107–110
152 Inceoglu A. B., Kamita S. G., Hinton A. C., Huang Q., Severson T. F., Kang K.-D. et al. (2001) Recombinant baculoviruses for insect control. Pest Manag. Sci. **57:** 981–987

To access this journal online:
http://www.birkhauser.ch