Chapter 8

Neuropeptide Signaling in Insects

Miriam Altstein* and Dick R. Nässel

Abstract

Neuropeptides represent the largest single class of signal compounds and are involved in regulation of development, growth, reproduction, metabolism and behavior of insects. Over the last few years there has been a tremendous increase in our knowledge of neuropeptide signaling due to genome sequencing, peptidomics, gene micro arrays, receptor characterization and targeted gene interference combined with physiological and behavior analysis. In this chapter we review the current knowledge of structure and distribution of insect neuropeptides and their receptors, as well as their diverse functions. We also discuss peptide biosynthesis, processing and expression, as well as classification of insect neuropeptides. Special attention is paid to the role insect neuropeptides play as potential targets for pest management and as a basis for development of insect control agents employing the rational/structural design approaches.

Introduction

Neuropeptides and peptide hormones play critical roles in regulation of almost every aspect of insect life.[1-4] Thus, secreted peptides orchestrate important events during development and are vital regulators of adult physiology and behavior. Of special interest here is that many aspects of growth, reproduction and homeostasis rely on peptide hormones. Therefore, insects do not only provide good models for analysis of basic endocrine mechanisms of general interest, but we can also utilize our knowledge for the generation of insect control agents based on antagonists that cause interference with peptide signaling pathways.

Over the years intense research has targeted peptide function and endocrine regulation in a large variety of insects, many of which are severe medical and agricultural pests. One rational for this has been that peptides and their receptors are more species specific than classical neurotransmitters and monoamines and thus targeted interference will be less wide and unspecific. By means of traditional biochemical and molecular techniques a large number of neuropeptides and hormonal peptides have been identified from a variety of insects and their putative functions tested in different bioassays. More recently, several complete insect genomes have been sequenced and provided information about genes encoding both peptides and G-protein coupled receptors (GPCRs) likely to have peptide ligands.[1,5-7] So far, most of the sequenced insect genomes are derived from insects that are not pests; exceptions are the mosquitos *Anopheles gambiae* and *Aedes aegypti*. Even so, the available genomic information provides us with a great resource for identifying components in peptide signaling in a range of insects and is of great use also for research on pest insects.

Based on information on annotated genomes of several species of *Drosophila* and from *Anopheles gambiae*, *Aedes aegypti, Bombyx mori* and *Apis mellifera* we know that in each species there are about 30-40 genes encoding neuropeptide precursors and a slightly larger number of genes encoding peptide GPCRs.[1,5,6,8] Since several of these peptide precursors encode more than one predicted neuropeptide it is possible that there are more than 40 functional neuropeptides

*Corresponding Author: Miriam Altstein—Department of Entomology, The Volcani Center, Bet Dagan, 50250 Israel. Email: vinnie2@agri.gov.il

Neuropeptide Systems as Targets for Parasite and Pest Control, edited by Timothy G. Geary and Aaron G. Maule.

or peptide hormones in a species. As discussed in more detail below, it is important to confirm the expression of processed peptides from nervous or other tissues by mass spectrometry, since it turns out that not all predicted peptide cleavage sites on the precursors are utilized.[4,9] The genomic information combined with available biochemical expression data provides us with a starting point for analysis of peptide functions in insects. As a first step of such analysis the novel peptides need to be tested in bioassays or in activation of orphan GPCRs in cellular expression systems.[1,8]

Neuropeptides: Their Biosynthesis, Processing and Expression

Insect neuropeptides consist of 5 to about 80 amino acid residues linked by peptide bonds. Some larger proteins are also known to act as hormones. Examples of these are prothoracicotropic hormone (PTTH) and the heterodimeric cystin knot protein bursicon, both known to have important roles during development.[2] The molecular structures of insect neuropeptides are immensely varied and they also display a great diversity in their distribution patterns, modes of action and their functional roles.[2,3]

It is not clear how many functional neuropeptides there are in a given species. We can make an estimate by looking into genomic information for different insects. In *Drosophila* there are about 35 neuropeptide encoding genes and 48 encoding peptide and protein GPCRs and these numbers are about 36 and 37, respectively, in the honey bee *Apis mellifera*.[1,5,6] However, as we shall see in the following, the number of functional peptides processed from the genes is not totally predictable and there is some variation between insect species. Furthermore, the pairing between all known peptides and specific peptide GPCRs has not been completed for any insect species yet; some orphan peptide GPCRs need to be matched up with their naturally occurring neuropeptide ligands and for some peptides receptors are still unidentified. In addition, some neuropeptides, such as insulin-like peptides, exert their activity through tyrosin kinase receptors,[4,10] and yet others may activate guanylate cyclase-type receptors. There are no genes in insects encoding orthologs of the ion channel-type of FMRFamide receptors[5] known from mollusks.[11]

Insect neuropeptides, like those of other animals, are processed from larger precursor proteins encoded by genes. Some neuropeptides are present in single copies on the precursors, but often several copies of identical or slightly diversified peptides (isopeptides) can be seen. So far the largest number of insect peptides that was shown to be processed from a single precursor was 23 peptides from a cockroach FMRFamide precursor.[12] Since it has been shown that predicted peptide cleavage sites on precursor proteins are not always utilized, it is critical that genomic data is followed up by biochemical determination of peptide complement in the same species. This information about the "peptidome" can now be obtained by very sensitive mass spectrometry techniques. Thus, for *Drosophila* and *Apis* the genome predictions have been tested against peptidome analysis.[6,9,13,14] There is some ambiguous information for some of the larger peptides and some peptides may have escaped detection, but estimates of processed peptides expressed in tissues can now be made more accurately. So, for instance in the honey bee about 100 peptides derived from 36 genes have been identified by mass spectrometry.[6] In the cockroach *Periplaneta americana* about 80 neuropeptides have been identified biochemically, but genomic information is more scarce for this species.[12,15] A further problem that has been only partly addressed is to what extent all expressed neuropeptides, including closely related isoforms, play functional roles in the organism. Most of the neuropeptides originally identified by biochemical means display activities in bioassay systems, but not all peptides predicted from genomic data and then confirmed by mass spectrometry, have been tested. One estimate of the complexity of peptide signaling in a species might be derived from the number of neuropeptide GPCRs. If so, the number of distinct peptide signaling systems would be in the order of about 40-50 in a species. In this number we have taken into account that a few neuropeptides are known to activate more than one GPCR.[1,2] The complexity may be further increased given the fact that GPCRs can diversify the functions by coupling to different G-proteins thus stimulating different downstream secondary messenger pathways. In the next section the peptide genes and GPCRs will be presented.

Many of the neuropeptides are C-terminally alpha-amidated and display other posttranslational modifications of structures that generate varying degrees of stability to peptidases and which are also important for their biological activity.[4] Some peptides contain cysteins that form disulfide bridges that provide structural constraints. For several of the insect neuropeptides the structure-activity relationship (SAR) has been extensively analyzed, either in bioassays or by testing of recombinant GPCRs expressed in cells.[16-25] Examples of peptides analyzed in this respect are: peptides related to pyrokinins (PKs) and pheromone biosynthesis activating neuropeptide (PBAN), dark color-inducing neurohormone (DCIN), tachykinin-related peptides, corticotropin releasing factor (CRF)-like diuretic hormones (DH) and adipokinetic hormones (AKH). For these peptides we thus have information about active cores, as well as residues critical for activity and metabolic stability. These structural data have provided important information on functional similarities within neuropeptide families in different taxa and also about possible sites of peptide modifications to provide stable agonists and antagonists.

In the insect central nervous system (CNS) neuropeptides are produced by neurosecretory cells and interneurons and more rarely by motoneurons or sensory neurons.[3,26] Peptides can also be detected in endocrine cells of the intestinal tract or at other peripheral sites.[3,27] The major release site for peptide hormones produced in neurosecretory cells are: the corpora cardiaca and allata, segmental neurohemal organs associated with the ventral nerve cord (perivisceral organs; PVOs), as well as axon terminations on the anterior aorta, peripheral nerves (including abdominal heart nerves), the intestine and body wall muscles.[14] Thus, peptides can act as circulating hormones, as local neurohormones that are released nonsynaptically within the nervous system, or at muscles or glands. Neuropeptides are also known to be released nearby synapses and act as synaptic modulators or even as co-transmitters, thereby modifying the action of fast-acting "classical" neurotransmitters. These modes of actions in the CNS have been studied more extensively in mammals, mollusks and crustaceans[28-31] and less data is available for insects.

Each neuropeptide or set of peptides derived from a single precursor is distributed in a stereotypic pattern in specific neurons, neurosecretory cells or endocrine cells.[3,26,32] Commonly there are very few neurons or neurosecretory cells expressing each neuropeptide precursor. For example, only two neurons express eclosion hormone in *Drosophila*[33] and four neurons produce SIFamide in the same insect.[34] Commonly there are in the order of 20-50 peptidergic neurons, but in some cases a few hundred can be seen (e.g., tachykinin-related peptides or allatotropin in locusts).[26] Based on the pattern of distribution in the CNS and periphery (if applicable) one can propose that some peptides may play multiple functional roles and others may be only circulating hormones or signal in restricted circuits within the CNS.

Neuropeptides Families, GPCRs and Peptide Functions

Traditionally insect neuropeptides were grouped into "neuropeptide families" which were based either upon homologies in sequences or functional similarities shared by peptides of different taxa. This followed the same principles that were used for vertebrate peptide families. Now that genomic data are available for several organisms it seems more relevant to compare neuropeptide precursor genes and peptides derived from these genes in different species. In Table 1 the genes encoding neuropeptides in *Drosophila* are listed as an example. Orthologs of most of the same genes can be identified in the other insect species analyzed. Some genes could not be found in *Drosophila* although they are known in other insects: genes encoding allatotropin, orcokinin and PBAN. Conversely some of the *Drosophila* genes were not detected in honey bees: genes encoding proctolin, leucokinin, myoinhibitory peptides (MIP) and allatostatin C.[6] Thus, the total number of insect peptide genes appears to be larger than that seen in a single species. It is also likely that all the neuropeptides have not yet been identified in any insect species (including *Drosophila*).

Over the last few years quite a number of the *Drosophila* GPCRs classified as peptide and protein receptors, based on sequence homologies,[5] have been deorphaned, i.e.,their ligands identified. Thus, out of 48 *Drosophila* peptide GPCRs, more than 25 have their ligands identified.[1,2] The GPCRs in *Anopheles* and *Apis* and others can be classified and tentatively assigned ligands by sequence

***Table 1. Neuropeptide precursor genes identified from* D. melanogaster[1]**

Neuropeptide Precursor (Peptides)	Gene	CG number[2]	Sequence[3]
Adipokinetic hormone	*akh*	CG1171	pQLTFSPDWa
Amnesiac product(s)	*amn*	CG11937	*
Allatostatin-A (Drostatin-A-1-4)	*ast*	CG13633	VERYAFGLa
Allatostatin-B[4] (Drostatin-B-1-5)	*mip*	CG6456	AWQSLQSSWa
Allatostatin-C (Drostatin-C)	*ast2*	CG14919	pEVRYRQCYFNPISCF
Bursicon	*burs*	CG13419	*
Partner of Bursicon (bursicon beta)	*pburs*	CG15284	*
Capability (CAP-1-2, PK-1)	*capa*	CG15520	GANMGLYAFPRVa TGPSASSGLWFGPRLa
Crustacean cardioactive peptide	*ccap*	CG4910	PFCNAFTGCa
Corazonin	*crz*	CG3302	pQTFQYSRGWTNa
Diuretic hormone (CRF-like; DH_{44})	*Dh*	CG8348	*
Diuretic hormone (Calcitonin-like)	*Dh31*	CG13094	TVDFGLARGYSGTQEAKH- RMGLAAANFAGGPa
dFMRFamides (dFMRFa-1-8)	*fmrfa*	CG2346	DPKQDFMRFa
Drosulfakinins (DSK-1-2)	*dsk*	CG18090	FDDYGHMRFa
Dromyosuppressin (DMS)	*dms*	CG6440	TDVDHVFLRFa
Ecdysis triggering horm. (ETH-1-2)	*eth*	CG18105	DDSSPGFFLKITKNVPRLa
Eclosion hormone	*eh*	CG6400	*
Hugin/pyrokinin-2 (hug-γ, PK-2)	*hug,*	CG6371	LRQLQSNGEPAYRVRTPRLa SVPFKPRLa
Insulin-like peptide 1	*Dilp-1*	CG14173	*
Insulin-like peptide 2	*Dilp-2*	CG8167	*
Insulin-like peptide 3	*Dilp-3*	CG14167	*
Insulin-like peptide 4	*Dilp-4*	CG6737	*
Insulin-like peptide 5	*Dilp-5*	AE003550[5]	*
Insulin-like peptide 6	*Dilp-6*	CG14049	*
Insulin-like peptide 7	*Dilp-7*	CG13317	*
Ion transport peptide (CHH-like)	*itp*	CG13586	*
IPNamide (of NPLP-1 precursor)	*nplp1*	CG3441	NVGTLARDFQLPIPNa
Leucokinin-like	*lk*	CG13480	NSVVLGKKQRFHSWGa
Neuropeptide F (long)	*npf*	CG10342	SNSRPPRKNDVNTMADA- YKFLQDLDTYYGDRARVRFa
Neuropeptide F (short) (sNPF-1-4)	*snpf*	CG13968	AQRSPSLRLRFa PQRLRWa
Pigment-dispersing factor	*pdf*	CG6496	NSELINSLLSLPKNMNDAa
Proctolin	*Proct*	CG7105	RYLPT
Prothoracicotropic hormone (PTTH)	*ptth*	CG13687	*
SIFamide	*Ifamide*	CG4681	AYRKPPFNGSIFa
Tachykinin-related (DTK-1-6)	*dtk*	CG14734	APTSSFIGMRa

[1]Compiled from refs 3-5. [2]Celera Genomics accession numbers. [3]Sequences given for representative peptides of each precursor. [4]These peptides are also designated myoinhibitory peptides (MIPs). [5]GenBank accession number for gene cluster. *Sequences too long to be given here.

homologies.[1,35] GPCRs from other insects have been identified by more traditional homology cloning and tests of ligands.[8] The identification of peptide GPCRs is of immense value since they can now be expressed in cell systems for assays of activation or blocking by receptor-selective and/or non-selective-agonists and antagonists.

Table 2. Functions of neuropeptides and hormonal peptides in insects[1]

Functions	Peptides
Development	PTTH, allatotropin, allatostatins
Molting	PTTH, allatotropin, allatostatins, eclosion hormone, ecdysis triggering hormone, pre-ecdysis triggering hormone, CCAP, corazonin, FMRFa, myoinhibitory peptide, bursicon
Feeding	NPF, sNPF, Hugin (pyrokinin)
Growth	Insulin-like peptides
Reproduction[2]	Neuroparsins, insulin-like peptides, PBAN, sNPF, SIFamide
Metabolism[3]	AKH, insulin-like peptides
Water and ion regul.	Diuretic hormones (DH_{44}, DH_{31}), CAPA[4], leucokinin, ion transport peptide
Specific behaviors	IPNamide, SIFamide, PDF, NPF
Myotropic	Proctolin, FMRFamides, myosuppressins, PKs, and many others
Multifunctional	Allatotropin, CCAP, tachykinin-related, sNPF, proctolin
Pigmentation	Melanization and reddish coloration hormone (PK), DCIN/corazonin

Note that these peptides may have several additional functions. References and acronyms are given in the text. Both reproduction physiology and reproductive behavior. Carbohydrate and lipid metabolism. Capability (CAPA) gene encodes perviscerokinins (CAP_{2B}) and pyrokinin.

Can neuropeptides and their genes be loosely organized into functional groups? Are there peptides that are primarily involved in regulation of development and others that regulate reproduction and so on? In an attempt to organize peptides somewhat into functional categories we will list peptides after certain functions that have been assigned to them (Table 2). This may also provide an idea of the complex regulation of various aspects of insect physiology and behavior. It is likely that there are some peptide hormones that may sub-serve a single hormonal function (or be part of a single hormonal cascade). This is underscored by their very restricted distribution in a small number of neurosecretory cells (and no presence in interneurons). Such peptides may have distinct hormonal roles orchestrating single aspects of insect life. For example, eclosion hormone and ecdysis triggering hormone in moths and flies are present in small populations of neurosecretory cells and display distinct functions in ecdysis behavior.[27] It is, however, likely that many of the neuropeptides (and peptide hormones) sub-serve multiple functions since they are produced by multiple and diverse interneuron types. For these peptides the distribution of release sites and their receptors in circuits within the CNS determine their functions.[26] Additionally we know that several insect neuropeptides can act both within the CNS and at peripheral targets further expanding possible regulatory roles.[3] The functions assigned to peptides in Table 2 are based on studies of various insect species, including *Drosophila*. Many of these peptides are involved in several functions and are thus listed under more than one category.[3,4,28,36,37]

So what are neuropeptides doing in insects? Many of the peptides have only been investigated in vitro and about half of the known peptides display myostimulatory or myoinhibitory activities. Here, we will only discuss peptides where in vivo functions can be suggested. Insect peptides have been shown to play major roles in regulation of molting,[27] feeding and growth,[38-41] reproduction,[4,36] pheromone production,[22,42] pigmentation,[17,43,44] metabolism of lipids and carbohydrates,[45,46] water and ion transport.[18]

Furthermore it has been shown by targeted gene interference that specific behaviors in *Drosophila* are regulated by neuropeptides. Pigment-dispersing factor (PDF) is an output peptide from the lateral clock neurons of the brain regulating circadian locomotor activity under constant light conditions.[47] Two further peptides have been implicated in the *Drosophila* clock: IPNamide and Neuropeptide F (NPF). SIFamide is present in four brain neurons with extensive arborizations that are especially important for male reproductive behavior.[34] NPF is critical for

regulation of feeding, foraging and social feeding behavior and there is a convergence of NPF and *Drosophila* insulin-like peptide (DILP) signaling in regulation of motivated food ingestion.[40,41] Another peptidergic system has been implicated in feeding in *Drosophila*: about 20 neurons in the subesophageal ganglion that express the neuropeptide precursor gene *hugin* (*hug*) expressing a pyrokinin.[39] The *hug*-derived peptide is important for initiation of feeding, dependent on food quality and it acts in circuitry that modulate feeding behavior based on chemosensory and nutrient signals. Furthermore, peptides of the sNPF (short neuropeptide F) gene appear to be important regulators of larval and adult feeding.[48] A peptide similar to the sNPFs has been identified in the mosquito *Aedes* and plays a role in female host seeking behavior.[49] The pyrokinins, NPFs and sNPFs are likely to play similar roles in other insect species and are thus relevant lead peptides for disruption of insect viability.

Insect Neuropeptides as Potential Targets for Pest Management

As evident from the recent studies described above, insect neuropeptides regulate many physiological and behavioral processes during development, reproduction and senescence and maintain growth, homeostasis, osmoregulation, water balance, metabolism and visceral activities. Peptides involved in regulation of vital functions are prime targets for advancement of the understanding of the physiology of insects and also targets for the development of novel insect-control strategies based on interference with their activity.

Although insect neuropeptides have been studied intensely in the past few decades, the mechanisms by which they exert their action are far from being fully characterized or understood. The possibilities of gaining a better insight into the mode of action and of exploiting insect neuropeptides for pest management rely primarily on our understanding the cellular and molecular basis of their actions. One way of obtaining a better insight into the mode of activity and functional diversity of peptides is by use of receptor-selective agonists and antagonists. Despite the vast scientific and insecticidal/insect control potential of antagonists (and to some extent agonists), their application has not been widely implemented so far in insects. This is mainly because of lack of defined methods for obtaining antagonists on the basis of a known neuropeptide agonist and because of the inability to predict which conformation will lead to a highly potent inhibitory or stimulatory receptor-selective activity. In addition, peptides are highly susceptible to proteolytic degradation and have a poor bioavailability. Therefore their conversion into an insecticide prototype requires rendering them resistant to peptidase degradation and to design them with a high bioavailability. Similar problems are also common in the pharmaceutical industry where immense efforts are being made in attempts to convert mammalian neuropeptides into therapeutic drugs. For many years, the most common approach used by the pharmaceutical industry for drug discovery was based on random screening of large chemical libraries of non-peptide compounds and further optimization of a lead molecule with respect to selectivity and pharmacokinetic properties. This approach has produced receptor-subtype-specific bioavailable ligands with nanomolar affinity (similar to that of the endogenous ligand) for peptide receptors some of which have been approved for clinical application and some which are in clinical trials.[50] An example of this is aprepitant (MK 869), a neurokinin-1 antagonist used for the treatment of chemotherapy-induced emesis and treatment of major depressions.[51] In the past decade, a parallel approach, based on rational drug design (or structure-based design) has evolved which integrates and implements the vast amount of information on the genes encoding GPCRs as well as the SAR of neuropeptides and their receptors. The approach has been applied to somatostatin, bradykinin, neurokinin and luteinizing hormone releasing hormone (LHRH) and resulted in the discovery of a few highly potent agonists and antagonists.[52]

In the past few years a novel integrated approach termed backbone cyclic neuropeptide-based antagonist (BBC-NBA) has been developed in which rationally designed BBC conformational libraries were synthesized, based on a detailed SAR study[53] of the insect neuropeptide PK/PBAN family and screened for occurrence of antagonists.[54,55] The backbone cyclization approach resulted in the discovery of conformationally constrained, highly potent, selective and nonselective,

metabolically stable and highly bioavailable BBC PK/PBAN antagonists active in the nmole range.[42,42b,54,56,57] Recently, a few PK/PBAN GPCRs have been cloned from various moth species[58-61] revealing structural differences within receptors that mediate different functions (e.g., sex pheromone production, melanization and pupal diapause).[61] The information gained on the selective and nonselective conformationally constrained BBC antagonists as well as the structural information of the PK/PBAN receptors can further serve for rational design of nonpeptidergic small molecule libraries (NPSML). In these, the bioactive biophores (deduced from structural analysis of the BBC antagonists and from their interaction with the receptors) will be applied on simple inexpensive scaffolds for the development of highly potent, metabolically stable, bioavailable and inexpensive insect specific and environment friendly insect control agents. An alternative approach to address the difficulties associated with the development of improved insect neuropeptide active compounds (agonists) was introduced with the same neuropeptide family by Nachman, Altstein and coworkers. The approach was based on design of pseudopeptides in which various amino acids have been substituted in a manner that rendered the molecule more stable to peptidase attack and more bioavailable.[62-65]

Conclusion

Due to the vast amount of information currently available on insect neuropeptides and the restricted space in this review we have highlighted only some of the major issues related to this important group of signaling molecules. Many topics have been omitted and they can be found in the reviews that are cited in this chapter. Much of the recent progress in revealing specific functions of neuropeptide signaling in vivo has been made in *Drosophila* by means of targeted interference with genes of peptide precursors or GPCRs or by cell-specific expression of apoptosis genes.[2,33,40,41,47] In parallel with these studies the in vitro characterization of peptide GPCRs and analysis of peptide and GPCR distributions in various insects has advanced our understanding of neuropeptide signaling tremendously. Comparative experimental studies, combined with information from annotated genomes from multiple insect species, will also improve our insight into the evolution of neuropeptide signaling.

The use of neuropeptides as a basis for drug design made a leap forward in the past decade due to the vast amount of novel information on GPCR and neuropeptide genes and their sequences. This information, together with the rapid developments in bioinformatics, molecular engineering, proteomics and chemical analysis (mainly liquid chromatography coupled to mass spectrometry) generated large amounts of data. This provides a basis for a better understanding of signaling mechanisms of mammalian and insect neuropeptides as well as for development of drugs and insect control agents based on rational/structural design. Both the pharmaceutical and agrochemical fields are still in their infancy and although this strategic approach has been used to develop a few vertebrate neuropeptide antagonist and antagonists the technology has not yet been optimized. Thus, new approaches to the generation of neuropeptide agonists/antagonists and to their further conversion into NPSM compounds with desired features need to be developed. It is anticipated that once these strategies have been worked out and the approaches expanded, it will be possible to implement them to a large variety of neuropeptides for tailoring highly potent drugs or insect control agents.

Addendum

In the last few years, a number of important advances have been made in insect neuropeptide research. A few relevant examples are given here. Sequencing of a few more insect genomes have been completed and thus new information about insect neuropeptide and GPCR genes is available (see refs. 66-68). From the new data we can conclude that some neuropeptide genes may have been lost over evolution in certain species, others seem to have diversified. A promising novel approach that employs quantitative mass spectrometry has been utilized to analyze peptide expression in honey bees under different foraging conditions.[69] This study indicates that peptide expression is dynamic in adult insects. An elegant technique has been developed that makes it

possible to determine the peptidome of selected neuron types.[70] The technique is based on marking genetically defined neuron populations with GFP (by Gal4-UAS technique), followed by dissociation of neurons and fluorescent cell sorting, and then analysis by mass spectrometry. In principle it is now possible to determine the pantheon of neuropeptides in major types of neurons that can be defined by promoter Gal4 lines or other means of fluorescent marking in vivo. A novel type of peptide receptor has been identified in endocrine cells of the Oriental fruit-fly, a membrane bound receptor guanylate cyclase activated by eclosion hormone.[71] This finding may be of significance for specific chemical interference with development in pest insects. Finally, major advances have been made in understanding insulin signaling in insects, especially in regulation of growth, metabolism and life span (see refs. 72-74). Another novel avenue of research relates to the bioavailability of neuropeptides. Recent studies have shown that linear and cyclic peptides of different length and polarities are highly bioavailable and can penetrate through the cuticle when applied in aqueous or organic solutions, and reach and activate the target organ.[75-77] These results contradict the common notion that peptides have low bioavailability, and may lead to a dramatic simplification of the strategies needed to be employed for design of neuropeptide based agonist and antagonists insect control agents.

References

1. Hauser F, Cazzamali G, Williamson M et al. A review of neurohormone GPCRs present in the fruitfly Drosophila melanogaster and the honey bee Apis mellifera. Prog Neurobiol 2006; 80:1-19.
2. Johnson EC. Postgenomic approaches to resolve neuropeptide signaling in Drosophila. In: Satake H, ed. Invertebrate Neuropeptides and Hormones: Basic Knowledge and Recent Advances. Trivandrum: Transworld Research Network 2006:179-224.
3. Nässel DR. Neuropeptides in the nervous system of Drosophila and other insects: multiple roles as neuromodulators and neurohormones. Prog Neurobiol 2002; 68:1-84.
4. Taghert PH, Veenstra JA. Drosophila neuropeptide signaling. Adv Genet 2003; 49:1-65.
5. Hewes RS, Taghert PH. Neuropeptides and neuropeptide receptors in the Drosophila melanogaster genome. Genome Res 2001; 11:1126-1142.
6. Hummon AB, Richmond TA, Verleyen P et al. From the genome to the proteome: Uncovering peptides in the Apis brain. Science 2006; 314:647-649.
7. Riehle MA, Garczynski SF, Crim JW et al. Neuropeptides and peptide hormones in Anopheles gambiae. Science 2002; 298:172-175.
8. Meeusen T, Mertens I, De Loof A et al. G protein-coupled receptors in invertebrates: A state of the art. Int Rev Cytol 2003; 230:189-+.
9. Baggerman G, Boonen K, Verleyen P et al. Peptidomic analysis of the larval Drosophila melanogaster central nervous system by two-dimensional capillary liquid chromatography quadrupole time-of-flight mass spectrometry. J Mass Spectrom 2005; 40:250-260.
10. Garofalo RS. Genetic analysis of insulin signalling in Drosophila. Trends Endocrinol Metablo 2002; 13:156-162.
11. Cottrell GA. The first peptide-gated ion channel. J Exp Biol 1997; 200:2377-2386.
12. Predel R, Neupert S, Wicher D et al. Unique accumulation of neuropeptides in an insect: FMRFamide-related peptides in the cockroach, Periplaneta americana. Eur J Neurosci 2004; 20:1499-1513.
13. Baggerman G, Cerstiaens A, De Loof A et al. Peptidomics of the larval Drosophila melanogaster central nervous system. J Biol Chem 2002; 277:40368-40374.
14. Predel R, Wegener C, Russell WK et al. Peptidomics of CNS-associated neurohemal systems of adult Drosophila melanogaster: A mass spectrometric survey of peptides from individual flies. J Comp Neurol 2004; 474:379-392.
15. Predel R, Neupert S, Roth S et al. Tachykinin-related peptide precursors in two cockroach species. FEBS J 2005; 272:3365-3375.
16. Altstein M, Dunkelblum E, Gabay T et al. PBAN-Induced sex-pheromone biosynthesis in Heliothis-Peltigera: Structure, dose and time-dependent analysis. Arch Insect Biochem Physiol 1995; 30:307-319.
17. Altstein M, Gazit Y, Ben Aziz O et al. Induction of cuticular melanization in Spodoptera littoralis larvae by PBAN/MRCH: Development of a quantitative bioassay and structure function analysis. Arch Insect Biochem Physiol 1996; 31:355-370.
18. Coast GM, Orchard I, Phillips JE et al. Insect diuretic and antidiuretic hormones. Advan In Insect Phys 2002; 29:279-409.
19. Gäde G. The explosion of structural information on insect neuropeptides. Fortschr Chem Org Naturst 1997; 71:1-128.

20. Gäde G, Goldsworthy GJ. Insect peptide hormones: a selective review of their physiology and potential application for pest control. Pest Manag Sci 2003; 59:1063-1075.
21. Nachman RJ, Roberts VA, Lange AB et al. Active conformation and mimetic analog development for the pyrokinin-PBAN-diapause-pupariation and myosuppressin insect neuropeptide families. Phytochemicals for Pest Control 1997; 658:277-291.
22. Rafaeli A, Jurenka R. PBAN regulation of pheromone biosynthesis in female moths. Insect Pheromone Biochemistry and Molecular Biology. NY: Academic Press, 2003; 107-36.
23. Schoofs L, Vandenbroeck J, Deloof A. The myotropic peptides of Locusta migratoria: Structures, distribution, functions and receptors. Insect Biochem Mol Biol 1993; 23:859-881.
24. Vanden Broeck J. Neuropeptides and their precursors in the fruitfly, Drosophila melanogaster. Peptides 2001; 22:241-254.
25. Yerushalmi Y, Bhargava K, Gilon C et al. Structure-activity relations of the dark-colour-inducing neurohormone of locusts. Insect Biochem Mol Biol 2002; 32:909-917.
26. Nässel DR, Homberg U. Neuropeptides in interneurons of the insect brain. Cell Tissue Res 2006; 326:1-24.
27. Ewer J, Reynolds S. Neuropeptide control of molting in insects. In: Pfaff DW, Arnold AP, Fahrbach SE et al, eds. Hormones, Brain and Behavior. San Diego: Academic Press, 2002:1-92.
28. Kastin A. Handbook of biologically active peptides. San Diego: Elsevier, 2006.
29. Kiss T, Pirger Z. Neuropeptides as modulators and hormones in terrestrial snails: their occurrence, distribution and physiological significance. In: Satake H, ed. Invertebrate Neuropeptides And Hormones: Basic Knowledge and Recent Advances. Transworld Research Network, Trivandrum, 2006:75-110.
30. Nusbaum MP, Blitz DM, Swensen AM et al. The roles of cotransmission in neural network modulation. Trends Neurosci 2001; 24:146-154.
31. Strand FL. Neuropeptides: Regulators of Physiological Processes. Cambridge, MA: The MIT Press, 1999.
32. Davis NT, Homberg U, Teal PEA et al. Neuroanatomy and immunocytochemistry of the neurosecretory system of the subesophageal ganglion of the tobacco hawkmoth, Manduca sexta: Immunoreactivity to PBAN and other neuropeptides. Microscopy Res Tech 1996; 35:201-229.
33. McNabb SL, Baker JD, Agapite J et al. Disruption of a behavioral sequence by targeted death of peptidergic neurons in Drosophila. Neuron 1997; 19:813-823.
34. Terhzaz S, Rosay P, Goodwin SF et al. The neuropeptide SIFamide modulates sexual behavior in Drosophila. Biochem Biophys Res Commun 2007; 352:305-310.
35. Hill CA, Fox AN, Pitts RJ et al. G protein-coupled receptors in Anopheles gambiae. Science 2002; 298:176-178.
36. De Loof A, Baggerman G, Breuer M et al. Gonadotropins in insects: An overview. Arch Insect Biochem Physiol 2001; 47:129-138.
37. Schoofs L, Clynen E, Cerstiaens A et al. Newly discovered functions for some myotropic neuropeptides in locusts. Peptides 2001; 22:219-227.
38. Broughton SL, Piper MD, Ikeya T et al. Longer lifespan, altered metabolism and stress resistance in Drosophila from ablation of cells making insulin-like ligands. Proc Natl Acad Sci USA 2005; 102:3105-3110.
39. Melcher C, Pankratz MJ. Candidate gustatory Interneurons modulating feeding behavior in the Drosophila brain. Plos Biology 2005; 3:1618-1629.
40. Wu O, Wen T, Lee G et al. Developmental control of foraging and social behavior by the Drosophila neuropeptide Y-like system. Neuron 2003; 39:147-161.
41. Wu O, Zhao Z, Shen P. Regulation of aversion to noxious food by Drosophila neuropeptide Y- and insulin-like systems. Nature Neurosci 2005; 8:1350-1355.
42. Altstein M. Role of neuropeptides in sex pheromone production in moths. Peptides 2004; 25:1491-1501.
42b. Altstein M, Hariton A. Rational design of insect control agents: The PK/PBAN family as a study case. In: Ishaaya I, Horowitz R, eds. Biorational Control of Arthropod Pests: Application and Resistance Management. New York: Springer, 2009:49-81.
43. Matsumoto S, Isogai A, Suzuki A et al. Purification and properties of the melanization and reddish colouration hormone (MRCH) in the armyworm, Leucania Separata (lepidoptera). Insect Biochem 1981; 11:725-733.
44. Tanaka S. Endocrine Mechanisms Controlling Body-Color Polymorphism in Locust. In: Loof AD, Hoffman KH, eds. Arch Insect Biochem Physiol 2001; 139-49.
45. Lee G, Park JH. Hemolymph sugar homeostasis and starvation-induced hyperactivity affected by genetic manipulations of the adipokinetic hormone-encoding gene in Drosophila melanogaster. Genetics 2004; 167:311-323.

46. Rulifson EJ, Kin SK, Nusse R. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science 2002; 296:1118-1120.
47. Renn SC, Park JH, Rosbash M et al. A pdf neuropeptide gene mutation and ablation of PDF neurons each cause severe abnormalities of behavioral circadian rhythms in Drosophila. Cell 1999; 99:791-802.
48. Lee KS, You KH, Choo JK et al. Drosophila short neuropeptide F regulates food intake and body size. J Biol Chem 2004; 279:50781-50789.
49. Brown MR, Klowden MJ, Crim JW et al. Endogenous regulation of mosquito host-seeking behavior by a neuropeptide. J Insect Physiol 1994; 40:399-406.
50. Hökfelt T, Bartfai T, Bloom F. Neuropeptides: opportunities for drug discovery. Lancet Neurol 2003; 2:463-472.
51. Quartara L, Altamura M. Tachykinin receptors antagonists: from research to clinic. Curr Drug Targets 2006; 7:975-992.
52. Gurrath M. Peptide-binding G protein-coupled receptors: new opportunities for drug design. Curr Med Chem 2001; 8:1605-1648.
53. Zeltser I, Gilon C, Ben-Aziz O et al. Discovery of a linear lead antagonist to the insect pheromone biosynthesis activating neuropeptide (PBAN). Peptides 2000; 21:1457-1465.
54. Altstein M, Ben-Aziz O, Daniel S et al. Backbone cyclic peptide antagonists, derived from the insect pheromone biosynthesis activating neuropeptide, inhibit sex pheromone biosynthesis in moths. J Biol Chem 1999; 274:17573-17579.
55. Altstein M. Novel insect control agents based on neuropeptide antagonists—The PK/PBAN family as a case study. Journal of Molecular Neuroscience 2003; 22:147-157.
56. Altstein M, Ben-Aziz O, Zeltser I et al. Inhibition of PK/PBAN-mediated functions in insects: Discovery of selective and nonselective inhibitors. Peptides 2007; 28:574-584.
57. Ben-Aziz O, Zeltser I, Bhargava K et al. Backbone cyclic pheromone biosynthesis activating neuropeptide (PBAN) antagonists: Inhibition of melanization in the moth Spodoptera Littoralis (Insecta, Lepidoptera). Peptides 2006; 27:2147-2156.
58. Cazzamali G, Torp M, Hauser F et al. The Drosophila gene CG9918 codes for a pyrokinin-1 receptor. Biochem Biophys Res Commun 2005; 335:14-19.
59. Choi MY, Fuerst EJ, Rafaeli A et al. Identification of a G protein-coupled receptor for pheromone biosynthesis activating neuropeptide from pheromone glands of the moth Helicoverpa zea. Proc Natl Acad Sci U S A 2003; 100:9721-9726.
60. Hull JJ, Ohnishi A, Moto K et al. Cloning and characterization of the pheromone biosynthesis activating neuropeptide receptor from the silkmoth, Bombyx mori—Significance of the carboxyl terminus in receptor internalization. J Biol Chem 2004; 279:51500-51507.
61. Zheng L, Lytle C, Njauw C-N et al. Cloning and characterization of the pheromone biosynthesis activating neuropeptide receptor gene in Spodoptera littoralis larvae. Gene 2007; 393:20-30.
62. Nachman RJ, Teal PEA, Ujvary I. Comparative topical pheromonotropic activity of insect pyrokinin/PBAN amphiphilic analogs incorporating different fatty and/or cholic acid components. Peptides 2001; 22:279-285.
63. Nachman RJ, Teal PEA, Strey A. Enhanced oral availability/pheromonotropic activity of peptidase-resistant topical amphiphilic analogs of pyrokinin/PBAN insect neuropeptides. Peptides 2002; 23:2035-2043.
64. Nachman RJ, Ben-Aziz O, Davidovitch M et al. Biostable β-amino acid PK/PBAN analogs: Agonist and antagonist properties. Peptides 2008; 30:608-615.
65. Nachman RJ, Teal PEA, Ben Aziz O et al. An amphiphilic, PK/PBAN analog is a selective pheromonotropic antagonist that penetrates the cuticle of a heliothine insect. Peptides 2009; 30:616-621.
66. Hauser F, Cazzamali G, Williamson M et al. A genome-wide inventory of neurohormone GPCRs in the red flour beetle Tribolium castaneum. Front Neuroendocrinol 2008; 29:142-165.
67. Roller L, Yamanaka N, Watanabe K et al. The unique evolution of neuropeptide genes in the silkworm Bombyx mori. Insect Biochem Mol Biol 2008; 38:1147-1157.
68. Yamanaka N, Yamamoto S, Zitnan D et al. Neuropeptide receptor transcriptome reveals unidentified neuroendocrine pathways. PLoS ONE 2008; 3:e3048.
69. Brockmann A, Annangudi SP, Richmond TA et al. Quantitative peptidomics reveal brain peptide signatures of behavior. Proc Natl Acad Sci USA 2009; 17;106:2383-2388.
70. Yew JY, Wang Y, Barteneva N et al. Analysis of neuropeptide expression and localization in adult Drosophila melanogaster central nervous system by affinity cell-capture mass spectrometry. J Proteome Res 2009; 8:1271-1284.
71. Chang JC, Yang RB, Adams ME, Lu KH. Receptor guanylyl cyclases in Inka cells targeted by eclosion hormone. Proc Natl Acad Sci USA 2009; 106:13371-13376.

72. Barnes AI, Wigby S, Boone JM et al. Feeding, fecundity and lifespan in female Drosophila melanogaster. Proc Biol Sci 2008; 275:1675-1683.
73. Broughton S, Partridge L. Insulin/IGF-like signalling, the central nervous system and aging. Biochem J 2009; 418:1-12.
74. Giannakou ME, Partridge L. Role of insulin-like signalling in Drosophila lifespan. Trends Biochem Sci 2007; 32:180-188.
75. Hariton A, Ben-Aziz O, Davidovitch M et al. Bioavailability of insect neuropeptides: The PK/PBAN family as a case study. Peptides 2009a; 30:1034-1041.
76. Hariton A, Ben-Aziz O, Davidovitch M et al. Bioavailability of β-amino acid and C-terminally derived PK/PBAN analogs. Peptides 2009b; 30:2174-2181
77. Hariton A, Ben Aziz O, Davidovitch M, Altstein M. Bioavailability of backbone cyclic PK/PBAN antagonists: inhibition of sex pheromone biosynthesis elicited by the natural mechanism in Heliothis peltigera females. FEBS J 2010; 277:1035-1044.