

# **Neuropeptide Diversity in the Insect Nervous System: what are the functional implications?**

**DICK R. NÄSSEL**

Department of Zoology, Stockholm University, S-10691 Stockholm, Sweden

## **Introduction**

Neuropeptides are the most numerous and diverse of all the different types of chemical messengers known in the nervous system of metazoans (Mutt, 1989; Häkfelt, 1991; Cooper *et al.*, 1991; Kobayashi and Susuki, 1992; Masler *et al.*, 1993; Nassel, 1993a; Schoofs *et al.*, 1993). There is a large number of structurally and functionally distinct neuropeptides in a given species and it is not unusual that a given neuropeptide exists in multiple structural isoforms in a species. Furthermore specific neuropeptides often display some species variations in their amino acid sequences. The diversity is also seen in that many given neuropeptides are localized to a variety of neuron types as well as to neurosecretory or endocrine cells, indicating multiple functions of a given neuropeptide. In addition to the complexity in peptide structure and distribution, we can also expect that the receptors of the neuropeptides exist in several subtypes. Do we have any means of understanding this apparent complexity in peptide signalling systems? With respect to insects, we have some indications about functional roles of neuropeptides at peripheral targets, but a justified question is whether we at present understand the physiological role of any neuropeptide in the central nervous system. The present paper attempts to address some of the problems in revealing the functions of neuropeptides. For this purpose the nervous system of insects is chosen as an example.

The specific focus of the present review is on two aspects of insect neuropeptides that may be of fundamental importance in the evolution of complex signalling systems. The first aspect is the functional significance of the presence of multiple neuropeptide isoforms in a given species. Is the existence of multiple isoforms of neuropeptides reflecting the development of increasing degrees of fine-tuning of neural transmission or are we observing poorly regulated mechanisms subserving peptide biosynthesis, processing and expression, leading to appearance of novel peptide isoforms with no specific functions? The second aspect discussed here is the variation in expression of different neuropeptides in the insect nervous system. Some neuropeptides are very abundant, indicating multiple functions, others are expressed in few neurons or neurosecretory cells, which may mean that they have a limited range of actions. Of specific interest is that some given neuropeptides display different patterns of expression in different insect species. Thus certain neuron types may contain the specific peptide in most species (probably homologous populations of neurons), whereas certain populations of neurons only express the peptide in species-specific manners. Do these "additional" populations of neurons, seen only in some insect species, express a functional peptidergic phenotype or are we observing occasional superfluous expression caused by poor gene regulation (Bowers, 1994). In other words,

is it so that additional to the poor regulation leading to production of initially non-functional peptide isoforms, we may also encounter poor gene regulation leading to peptide expression in cells not yet utilizing the peptides? Portions of the present account have occurred in a recent review (Nässel, 1994).

### 1. The detection of neuropeptides and the concept of multiple peptide actions

Neuropeptides are commonly isolated and purified from tissue extracts by monitoring a biological activity in a sensitive bioassay (e. g. stimulation of muscle contraction). After the bioactive components have been brought to homogeneity by high performance liquid chromatography (HPLC), using different columns systems and solvent gradients, the amino acid sequence can be obtained by Edman degradation. Antisera can be raised to the purified or customs synthesized neuropeptides for use in immunocytochemical detection of peptides in tissue of

Table 1. Amino acid sequences of insect neuropeptides representing major peptide groups<sup>1</sup>

Neuropeptides	abbreviation	species	sequence
AKH-like	Lom-AKH-I	<i>Locusta</i>	pQLNFTPNGWT-NH2
	Drm-AKH	<i>Drosophila</i>	pQLTFSPDW-NH2
Corazonin		<i>Periplaneta</i>	pQTFQYSRGWTN-NH2
proctolin		<i>Periplaneta</i>	RYLPT
myokinin	LK-I	<i>Leucophaea</i>	DPAFN SWTG-NH2
	Lom-K-I	<i>Locusta</i>	AFSSWG-NH2
tachykinin-like	Lom-TK-I	<i>Locusta</i>	GPSGFYGVN-NH2
pyrokinins myotropins PBANs	Lem-PK	<i>Leucophaea</i>	pQTSFTPRL-NH2
	Lom-MT-I	<i>Locusta</i>	GAVPPAAQFSPRL-NH2
	Hez-PBAN	<i>Heliothis</i>	LSDDMPATPADQEMYROD- -PEQIDSR TKYFSPRL-NH2
Acc. gland myotropin	Lom-AG-MT-I	<i>Locusta</i>	GFKNVALSTARGF-NH2
sulphakinins	Lem-SK-I	<i>Leucophaea</i>	EQFEDY(SO <sub>3</sub> )GHMRF-NH2
	Drm-SK-I	<i>Drosophila</i>	FDDY(SO <sub>3</sub> )GHMRF-NH2
FMRFamide-like	CalliFMRFa I	<i>Colliphora</i>	TPQQDFMRF-NH2
	DrosoFMRFa I	<i>Drosophila</i>	DPKQDFMRF-NH2
	Lem-MS-I	<i>Leucophaea</i>	pQDV D HVFLRF-NH2
	MasFLRFa	<i>Manduca</i>	pQDVVHSFLRF-NH2
cardioactive peptides	CCAP	<i>Locusta</i>	PFCNAFTGC-NH2
myoinhibitory	Lom-MIP	<i>Locusta</i>	AWQDLNAGW-NH2
	Lom-MIH	<i>Locusta</i>	pQXYXKQSAFNAVS-NH2
vasopressin-like	F1 <sup>2</sup>	<i>Locusta</i>	CLITNCPRG-NH2

Table 1. (continued)

diuretic hormones	Mas-DH	<i>Manduca</i>	PRMSLSIDLPM SVLRQKLSLE- -KERK VHALRAAANRNFLNDI-NH2
	Lom-DH	<i>Locusta</i>	MGMG PSLSVNPM DVLRQRLLLEIAR- -RRLRDEEQIKANKDFLQOI-NH2
pigment-disp. factors	Rom-PDF	<i>Romalea</i>	NSEIINSLLGLPKLLNDA-NH2
eclosin hormones	Mass-EH	<i>Manduca</i>	NPAIATGYDPMEICIENCAQCKKMLG- -AWFEGPLCAESCIKFKGKLIPECEDF- -ASIAPFLNIKL
bombyxin	bombyxin II	<i>Bombyx</i>	A-chain GIVDECCLRPCSVDVLLSYC B-chain pQQPQAVHTYCGRHLAR- -TLADLCWEAGVD
allatotropins	Mas-AT	<i>Manduca</i>	GFKNVEMMTARGF-NH2
allatostains	Dip-ASB2	<i>Diptera</i>	AYSYVSEYKRLPVYNFGL-NH2
	Mas-AS	<i>Manduca</i>	pQVRFRCYFNPISCF

This table is altered from Nässel (1993) **Footnotes:** 1. Only one or a few representatives of each group are given to save space. References to literature are given in text. 2. Two other forms of vasopressin like peptide exist which are parallel and antiparallel dimers of F1.

for use in immunoassays to detect and quantify the peptides in tissue extracts. Such immunoassays can be used for the detection of related neuropeptides in other animal species and as monitors for the subsequent purification and isolation in these species. More recently molecular cloning techniques have been successfully applied in detection of genes coding for neuropeptide precursors and been instrumental in the detection of multiple neuropeptide isoforms. A large number of insect peptides have been isolated and sequenced by now (exemplified in Table 1). The largest number of peptides isolated from a single species of insect are from the locust *Locusta migratoria*, where the number is approaching 40 (Schoofs *et al.*, 1993). The total number of different peptides from all insect species studied is nearing 200 and with molecular cloning techniques, and deduction of peptides from their precursor codons, this number is steadily increasing.

The impact of immunohistochemistry on our understanding of the chemical heterogeneity of neurons and complexity of chemical signalling has been great. Certainly, our understanding has also benefited from the availability of synthetic peptides (as a result of improved isolation and sequencing techniques) for pharmacological experiments, progress in electrophysiological recording techniques, advances in molecular biology leading both to cloning of peptide precursors and receptors as well as other components in the signal transduction pathway (see Dores *et al.*, 1990; Restifo and White, 1990; Buchner, 1991; Battey *et al.*, 1993; Benjamin and Burke, 1994).

## 2. What make neuropeptides special?

Neuropeptides are formed as cleavage products from large precursor proteins encoded in the genome. Neu-

ropeptide precursor synthesis takes place in the cell body of the neuron or neurosecretory cell by the same mechanisms as for regular protein synthesis. The primary structure is encoded in exons of a gene, together with initiating, processing and regulatory sequences. After transcription into pro-mRNA, splicing leads to removal of introns (non-coding sequences) and the coding mRNA is translated into a preprohormone. The preprohormone commonly contains a signal sequence and one or several copies of neuropeptide(s). By posttranslational processing the final bioactive peptide products are produced. Posttranslational processing includes enzymatic cleavage of the peptide(s) from the precursor, and other modifications such as C-terminal amidation, glycosylation, sulfation and N-terminal pyroglutamate formation. These posttranslational modifications occur within the secretory pathway, the endoplasmic reticulum, the Golgi apparatus and in the secretory vesicles. After release and action the peptides are degraded by enzymes (endo- and exopeptidases) associated with the cell membrane (see Masler *et al.*, 1993).

The primary structure of neuropeptides is thus directly dependent on the nucleotide codons and single point mutations suffice to change amino acids in a peptide. It is presumed that the neuropeptide diversity we see in organisms is the result of different forms of gene duplications followed by diversification by mutations (Ohno, 1970; Doolittle, 1981; Otsuka and Yoshioka, 1993). Repeated internal gene duplications may result in generation of multiple coding sequences for bioactive peptides with the same primary structure. Since one or a few of these will be sufficient for synthesis of physiological amounts of peptide, other coding sequences can be altered by mutations without serious drawbacks for the organism. With time these novel neuropeptides may get their own physiological functions after co-evolution of novel receptors. Duplications of whole genes also seems to have occurred, after which the peptides encoded on the duplicate gene were available for alteration by mutations. The new genes may thus eventually encode similar but functionally different neuropeptides. Examples of possible gene duplications and internal gene duplications are provided by the two known precursors of the FMRFamide-related peptides (FaRPs) in *Drosophila*. Two different precursors have been isolated which contain FaRPs. One precursor contains only two copies of FaRPs the drosulfakinins I and II (Nichols *et al.*, 1988). The other gene contains 13 FaRP sequences, 5 of which are identical, and an additional sequence resembling corticotropin releasing hormone (Nambu *et al.*, 1988; Schneider and Taghert, 1988). In this gene one FaRP sequence seems to have become duplicated several times and then some copies may have undergone mutational changes, resulting in five intact DPKQDFMRFamide sequences and eight changed forms. A third FaRP precursor seems to be present in *Drosophila*, since a new FaRP was recently isolated biochemically that is not encoded on any of the two known precursors (Nichols, 1992).

Several insect neuropeptide precursors have been deduced from molecular cloning (some from several species): AKH, bombyxin, eclosion hormone, prothoracicotropic hormone, diapause hormone (with pheromone biosynthesis activating neuropeptide), locust insulin-like peptide, diuretic hormone, neuroparsin A, allatostatins, corazonin and two different FaRP encoding precursors (for original references see Nagasawa, 1992, 1993; Nässel 1993a; Donley *et al.*, 1993; Sato *et al.*, 1993; Veenstra, 1994). Several of these are organized similar to the FaRP precursors with several peptides encoded (natural analog series), like the allatostatin and diapause hormone precursors (Donley *et al.*, 1993; Sato *et al.*, 1993).

### 3. The different patterns of peptide expression

Specific neuropeptides are each expressed in stereotyped patterns of neurons or in neurons and additional

cell types such as neurosecretory cells and endocrine cells. The pattern of expression may give us some indications of the function or at least sites of release of the peptides. Thus one of the first aims after isolation of novel neuropeptides is to map their distribution. The most common method to display peptide expression is by immunocytochemical detection using antisera raised against chosen portions of the peptide or the whole molecule (in each case commonly a peptide sequence conjugated to a carrier protein). More recently molecular biology has provided us with probes such as labeled nucleotide probes that in tissue can detect messenger RNAs encoding the neuropeptides in question.

Using these techniques, especially immunocytochemistry, we have quite good ideas how insect neuropeptides are distributed in the brain and other parts of the nervous system in some groups of insects (see Nässel, 1987, 1991, 1993a; Nässel *et al.*, 1994; Schoofs *et al.*, 1993; Agricola and Bräunig, 1994, Homberg, 1994). The best studied insect species are not representatives of major groups or types of insects, but they rather represent some of the major laboratory insects that have been used over the years in physiology and endocrinology as well as anatomical studies. Most published work on neuropeptide distribution thus relates to locusts, moths, cockroaches and dipteran flies (blowflies and *Drosophila*); the runner ups would be crickets, hemipteran bugs (*Rhodnius*) and the coleopterans (e. g. Colorado potato beetle and *Tenebrio*). Studies on neuropeptide action have also been performed on the same limited selection of insects, although certain aspects have in addition been employing mosquitos and stick insects. The following account will concentrate on work on locusts, cockroaches and flies, although mention of other insects is made when appropriate.

Four main types of peptide expression patterns can be distinguished in the nervous system. (1) Peptide distributed only in neurosecretory cells (or predominantly in these cells); (2) Peptide restricted to a small number of interneurons; (3) Peptide expressed in larger population of interneurons (interneurons of many types); and (4) Peptide found in a mixture of many neurosecretory and interneuron types (or even motoneurons and other efferents). We shall also see that some neuropeptides are distributed in similar patterns in different insect species (maybe even in homologous neurons or neuron clusters), whereas other neuropeptides appear to have species variations in expression pattern. This variation commonly manifests itself as the existence of a certain rudimentary population which is invariant (found in the different species) and a superimposed variable set of differing size and location in different species. In the next sections I will briefly deal with the four types of peptide expression and also discuss the possible relevance of several peptide isoforms in those cases they have been indicated. The four patterns will be exemplified with a few neuropeptides only to avoid too much confusion: (1) Predominant neurosecretory cell distribution: Corazonin, adipokinetic hormones, eclosion hormone and others; (2) Expression in small number of interneurons: pigment-dispersing factors; (3) Large population of interneurons: insect tachykinins; and finally (4) Mixed population: myokinins or leucokinin-related peptides.

### **1) Pattern 1: Predominant expression in neurosecretory cells**

Some neuropeptides have only been demonstrated in neurosecretory cells or endocrine cells, like adipokinetic hormones (Schooneveld *et al.*, 1986; Orchard, 1987), eclosion hormone (Copenhaver and Truman, 1986; Ishizaki and Susuki, 1992), prothoracicotropic hormone and bombyxin (Ishizaki and Susuki, 1992),

*Manduca* and *Locusta* diuretic hormones (Veenstra and Hagedorn, 1991; Patel *et al.*, 1994) and neuropeptides (Tamarelle and Girardie, 1989). One would assume that these neuropeptides act solely as neurohormones released into the circulation. This does not preclude that they each may have a spectrum of physiological actions as has been demonstrated for e. g. adipokinetic hormones (Orchard, 1987; Gäde, 1990; O'Shea and Rayne, 1992). Also, as we shall see at the end of this section, the possibility exists that a neuropeptide has different actions in different species (even if the peptide structure is well preserved). Certainly it is much easier to search for the actions of neurohormonal neuropeptides than interneuronal neuropeptides.

It is of interest that some neuropeptides are expressed predominantly in neurosecretory cells and a minor population of what may be interneurons. One such peptide is corazonin which was first isolated from *Periplaneta americana* (Veenstra, 1989), and then demonstrated in identical form in other insects including *Drosophila* (Veenstra, 1991, 1994). In *Periplaneta* both interneurons and neurosecretory cells of the lateral neurosecretory cell group (LNC) were demonstrated by immunocytochemistry (Veenstra and Davis, 1993; Predel *et al.*, 1994). However, in the cockroach *L. maderae* (Predel *et al.*, 1994) and the blowfly *Phormia terraenovae* (Cantera *et al.*, 1994) the number of interneurons is very small. In *P. terraenovae* there are actually only two interneurons in the brain (as compared to 14 cells in the LNC group). Could it be assumed that the neurohormonal role of corazonin in the blowfly is more prominent than its role in the two interneurons or is it even possible that the expression of corazonin-immunoreactivity in the two interneurons is due to cross reactivity or non-functional expression due to poor gene regulation?

It is noteworthy that, although the peptide structure is well preserved, the action of corazonin seems to vary between species. Corazonin was originally isolated from corpora cardiaca by its cardioacceleratory action in *P. americana* (Veenstra, 1989). The peptide also induces contractions in hyperneural muscle and the antennal pulsatile organ of the same species (Predel *et al.*, 1994). The response to corazonin of these muscles was tested in 8 different cockroach species and it was found that only in three species did the heart respond and clear effects on the other muscles were seen only in *P. americana*. Obviously, there must be other targets for corazonin in these species. One may even ask whether the main function of corazonin is as a cardioacceleratory hormone. Corazonin, like a number of other myoactive peptides (see Holman *et al.*, 1991; Schoofs *et al.*, 1993) were isolated by convenient and sensitive assays which may not tell us much about the main physiological functions of the peptides.

## 2) Pattern 2: Peptide expression in small population of interneurons

It is rare to see a neuropeptide expressed only in a small population of interneurons in the insect brain. A few examples are a neurokinin A-like peptide in the cockroach optic lobe (Nässel, 1991; Nässel *et al.*, 1992b), sulfakinins in a small set of descending neurons of *Drosophila* and *Calliphora* (Tibbets and Nichols, 1993; Duve *et al.*, 1994) and pigment-dispersing hormone-related peptide in neurons associated with the optic lobes of a variety of insects as discussed in the following.

Several closely related octadecapeptides have been isolated from crustaceans and insects by their ability to disperse pigment in chromatophores of fiddler crabs (Rao *et al.*, 1985, 1991). These peptides were designated pigment-dispersing hormones (PDH) in crustaceans and pigment-dispersing factors (PDF) in insects. The sequence of cockroach (*Periplaneta americana*) PDF is NSELINSLGLPKVLNDAamide (Mohrherr *et*

*al.*, 1991) and other insect PDFs are quite similar. In insects only one isoform of PDFs has been detected in those species where analysis has been made, whereas more isoforms may be present in some crustaceans (Rao *et al.*, 1991; Klein *et al.*, 1994). In orthopteran, blattarian and dipteran insects the peptide of PDF type seems to be expressed in well defined populations of interneurons in the brain. These interneurons are associated with the optic lobes (Homberg *et al.*, 1991; Nässel *et al.*, 1991, 1993; Helfrich-Förster and Homberg, 1993; Stengl and Homberg, 1994). In orthopterans (except crickets), cockroaches and flies some of these PDF containing neurons form strong bilateral connections between optic lobes and have processes in the so called accessory medulla, a small neuropil which in some insects receive inputs from extraocular photoreceptors (Hagberg, 1986, Nässel *et al.*, 1988; Fleissner *et al.*, 1993). The morphology and projections of these optic lobe PDF neurons suggested that they are incorporated into a bilaterally synchronized circadian pacemaker system (Homberg *et al.*, 1991). A more extensive study in the cockroach *L. maderae*, has provided further evidence for the role of PDF containing neurons in circadian pacemaker functions (Stengl and Homberg, 1994). In this study the circadian locomotor activity of the cockroaches was examined after lesions of the PDF neurons (subsequently probed by immunolabeling). The circadian locomotor rhythm was abolished after lesion of both optic tracts (severing the bilateral PDF pathway). In *Drosophila* some of the PDF containing neurons also contain the *period* protein, a gene product involved in circadian regulation (Helfrich-Förster, 1994; see also Zerr *et al.*, 1990).

The PDF-containing neurons in the brain of many insects thus seem to be a rather homogeneous population of neurons (two types of optic lobe neurons, one of which has not been so well characterized). In the brain of these species the PDF-like peptide may thus have a single function as a regulator in circadian activity patterns. It is, however, not yet clear for any insect what the actions of PDFs are and by what mechanisms they act. And as usual, things are never as simple as one wants them to be. In some insect species there appear to be additional populations of PDF containing neurons. For instance in the blowfly brain there is a small population of lateral neurosecretory cells displaying PDF-immunoreactivity. In the same species, as well as in *Drosophila*, there are also six large efferent neurons in the last neuromeres of the abdominal ganglion with processes innervating the hindgut which are PDF immunoreactive. The finding of additional PDF-immunoreactive neurons could have several explanations: (1) that in some species PDF-like peptide has additional functions, i. e. neurohormonal actions and myotropic action on hindgut muscle; (2) that additional PDF isoforms exist in some species and these are expressed in additional neurons, (3) that the PDH antiserum cross reacts with a non-PDF-like peptide in the additional neurons (this peptide must share some epitope with PDH) and (4) that in some species there are additional neurons expressing PDF-like peptide due to poor regulation of gene transcription and further translation and processing.

### **3) Pattern 3: Peptide expression in diverse population of interneurons, exemplified by insect tachykinins**

Tachykinins constitute a large family of peptides found both in vertebrates and invertebrates. The best known tachykinin is substance P (Otsuka and Yoshioka, 1993). The originally isolated tachykinins all have a C-terminal pentapeptide with the sequence FXGLMamide. More recently, however, some forms of ta-

chykinin-like peptides were isolated from invertebrates which have sequence identities in other portions of the molecule and a C-terminus pentapeptide FXGVRamide. Substance P is a multifunctional messenger in mammals and can act both as an excitatory neurotransmitter and as a modulator involved in regulation of sensory processing, movement control, gastric motility, vasodilation, salivation, and micturation (Hershey *et al.*, 1991; Otsuka and Yoshioka, 1993). As we shall see later, the tachykinin-related peptides of insects are also diverse in structure and may share some of the functions with their mammalian relatives. There are, however, so far no reports on the structure of insect tachykinin precursors or the genes encoding them.

The first insect peptides resembling tachykinins, locustatachykinin I-IV (LomTK I-IV) were isolated from the locust, *Locusta migratoria* (Schoofs *et al.*, 1990a,b), by means of the *Leucophaea maderae* hindgut contraction bioassay. The locustatachykinins (Table 3) display about 30-40% amino acid homologies with the vertebrate tachykinins, but in the C-terminal pentapeptides the differences are so large that the insect peptides have been placed in a separate subfamily (Schoofs *et al.*, 1993). Since the four peptides were isolated from extracts of dissected brains with corpora cardiaca-corpora allata complexes, the exact tissue distribution of LomTK I-IV was initially obscure. By *in vitro* assays several actions of locustatachykinins were established: stimulatory action on locust foregut and oviduct muscle, stimulatory action on locust hindleg extensor tibia muscle (Schoofs *et al.*, 1993) and pheromonotropic activity on moth (*Bombyx mori*) pheromone glands (Fonagy *et al.*, 1992). Later, two tachykinin-related peptides, callitachykinin I and II, were isolated from the blowfly *Calliphora vomitoria* (Lundquist *et al.*, 1994a).

Antisera were raised against LomTK I (Nässel, 1993b) and it was found that large numbers of neurons (about 800) were immunolabeled in the locust brain (Nässel, 1993b). The cell bodies of the locustatachykinin-immunoreactive (LomTK-IR) neurons in the brain are distributed in all neuromeres and have processes in most neuropil regions. Most, if not all, neurons in central ganglia appear to be interneurons. A few LomTK-IR neurons in the protocerebrum project to the glandular lobe of corpora cardiaca. They form terminals innervating the adipokinetic hormone (AKH) producing glandular cells and since LomTK I induces release of AKH I from locust corpora cardiaca *in vitro*, these LomTK-IR neurons may be involved in control of release (Nässel *et al.*, 1995). LomTK-IR cell bodies and processes were also found in the frontal ganglion and processes in the hypocerebral ganglion. The distribution of LomTK-IR neurons and fibers indicate involvement in sensory processing (antennal lobes, optic lobes, ocellar neuropil), higher motor control (central body complex), control of foregut (frontal ganglion) and processing in higher multimodal centers such as the mushroom bodies. It is important to note that the LomTK antisera we raised recognize LomTK I-III and to a lesser extent LomTK IV (Lundquist *et al.*, 1994a) and we thus display all these peptides in immunocytochemistry. Therefore we have no data on the distribution of the individual isoforms: colocalization versus differential distribution remains an open question.

In the cockroach *L. maderae* a similar distribution of LomTK-IR neurons was seen in the brain (Muren *et al.*, 1995). In addition endocrine cells of the midgut were labeled and immunoreactive fibers were found on muscle of the foregut, midgut and on the pharyngeal dilator muscles in the head (these structures were not studied in the locust). Thus tachykinin-like peptides may have additional roles in intestinal function and control of skeletal muscle (Muren *et al.*, 1995). In the blowfly, the callitachykinins are distributed in interneurons and endocrine cells of the intestine only (Lundquist *et al.*, 1994b; Nässel *et al.*, 1995).

What are the possible functions of tachykinin-like peptide in the nervous systems of locust, cockroach



and flies? In the locust and cockroach brain the distribution of immunoreactive fibers in nearly every neuropil region indicates a wide distribution of release sites and thus presumably actions are widespread. The rich supply of LomTK-LI fibers to the antennal lobes, central complex, optic lobes, ocellar neuropil, mushroom bodies, tritocerebrum, pars intercerebralis and glandular lobe of corpora cardiaca, suggest that locust tachykinin-like peptides are involved in sensory processing, higher motor control, learning, feeding and release of hormones. These are types of actions known also for the mammalian tachykinins (Otsuka and Yoshioka, 1993). Whether the action of the LomTKs in the CNS are as primary neurotransmitters or as neuromodulators is not known, but it seems likely that these peptides, like their mammal counterparts (with some exceptions) act as neuromodulators via second messengers. An important issue that is now being addressed in our laboratory is the possibility that the different isoforms have different actions and have different physiological roles.

#### **4) Pattern 4: Peptide expression in large mixed populations of cells, exemplified by leucokinin-like peptides**

A number of insect neuropeptides are expressed both in neurosecretory cells and neurons. Examples are the FMRFamide-related peptides, proctolin, allatostatins and leucokinins (see Nässel, 1993a; Homberg, 1994). Here we shall deal with the leucokinins since they illustrate quite well problems connected with species variations, the question of differential distribution and function of neuropeptide isoforms and the dilemma caused by lack of systematic screening of peptide actions.

In the late eighties, eight myotropic peptides, designated leucokinin I-VIII (LK I-VIII), were isolated from whole heads of the cockroach *Leucophaea maderae*, with the aid of a sensitive hindgut contraction bioassay (Holman *et al.*, 1986, 1990, 1991). With the use of the same bioassay and similar purification schemes closely related peptides were isolated from a cricket and a locust species: five achetakinins (AK I-V) and one locustakinin (LomK) (Holman *et al.*, 1990, 1991; Schoofs *et al.*, 1992). More recently three leucokinin-like peptides, culekinin depolarizing peptides (CDP I-III), were isolated from the mosquito *Culex salinarius* (Clottens *et al.*, 1993) and another three from *Aedes aegypti* (Veenstra, 1994). The leucokinins were the first clear example in insects of a natural analog series of isopeptides in one species. The leucokinins have nearly identical C-terminal pentapeptides; the carboxy terminal pentapeptide has the structure FXSWGamide, where X is either N, S, H or Y (in locustakinin X is S). The achetakinins have the C-terminus pentapeptide FX<sub>1</sub>X<sub>2</sub>WGamide, where X<sub>1</sub> Y, S, N or H and X<sub>2</sub> is P or S.

The action of leucokinins on the cockroach hindgut is an increase in the frequency and amplitude of spontaneous phasic contractions at lower concentrations and with a tonic component in higher concentrations (Holman *et al.*, 1986; Cook and Wagner, 1991). These actions are calcium dependant. Leucokinins also act on the foregut and oviduct, but only at 100-1000 times higher concentrations and the heart did not give consistent responses (Cook and Wagner, 1991). The dose concentrations that give half maximal response (ED<sub>50</sub>) on the hindgut for the eight leucokinins vary somewhat and LK VIII, LK VI and LK V are the most potent. It was also shown that the leucokinins, achetakinins and locustakinin induce fluid secretion in Malpighian tubules of mosquitos, crickets and locusts (Hayes *et al.*, 1989; Coast *et al.*, 1990; Patel *et al.*, 1994) and again the potencies varied. In the cricket assay AK V is the most potent with an ED<sub>50</sub> of 0.018 nM and AK

III is least potent with an  $ED_{50}$  of 0.324 nM (Coast *et al.*, 1990).

The achetakinins I, II and V are also potent in causing lipid mobilization and inhibiting protein synthesis in cricket and locust fat body, similar to *Acheta* adipokinetic hormone (Goldsworthy *et al.*, 1991). The similar actions of achetakinins and AKH could be explained by the molecular conformations (secondary structure) of the two peptides being similar, but Goldsworthy *et al.* (1991), however, argue that AKHs and achetakinins probably have different receptors on fat body (at least in the locust) and act via different second messenger systems. We will return to the action of leucokinins/achetakinins on the visceral muscles and Malpighian tubules later, but we should note that there are indications that the different myokinins may have different receptors and thus possibly have different physiological functions.

The distribution of leucokinin immunoreactive (LK-IR) peptide in the nervous system of the cockroach *L. maderae* was surprisingly diverse (Nässel *et al.*, 1992a). About 160 LK-IR neuronal cell bodies were found in the brain. All of these were located in the protocerebrum, although their processes were found also in deuto and tritocerebrum. The brain neurons appear to be of 8 main types: lateral and median neurosecretory cells, local optic lobe neurons, descending neurons connecting protocerebrum with antennal lobes and the ventral cord, and about four types of local or bilateral interneurons in the midbrain (Nässel *et al.*, 1992a). The LK-IR lateral and median neurosecretory cells send axons to the neurohemal organ termed corpora cardiaca where they terminate in the storage lobe. In the subesophageal ganglion 8 weakly leucokinin immunoreactive neurons were seen and in each of the thoracic ganglia 4-6 strongly labeled and two clusters of 4-6 weakly labeled cell bodies were revealed. Each of the abdominal neuromeres contain two pairs of efferent neurons which could be labeled with their axons projecting through the posterior abdominal nerve roots, via the link nerve to varicose terminations in the lateral cardiac nerve.

A substantial component of the LK-IR neurons are neurosecretory cells with release sites either in the cephalic neurohemal organ, corpora cardiaca, or in the abdominal release sites in the lateral cardiac nerves adjacent to the contractile aorta (heart). It can thus be proposed that the peripheral actions of leucokinins at e. g. Malpighian tubules and hindgut are mediated by hormonally released leucokinin(s).

A first indication of the differential distribution of leucokinins I-VIII has been obtained with the use of a specific antiserum raised to achetakinin I, which also specifically recognizes LK VIII (Meola *et al.*, 1994). When the AK I antiserum was applied to the central nervous system of *L. maderae* Meola *et al.* (1994) found labeling of only a small subset of the neurons labeled with our LK I antiserum. In the median neurosecretory cell group only 24 of the about 100 LK-IR cell bodies could be labeled and in the lateral group only two pairs as compared to seven pairs (see Fig 1). No other neurons were found in the brain with the AK I antiserum. Similarly, only a smaller number of neurons were labeled in the thoracic ganglia. The findings of Meola *et al.* (1994) indicate that LK VIII has a restricted neuronal distribution in the cockroach nervous system and since the AK-IR neurons were barely detectable with our more general leucokinin antiserum it could well be that the other leucokinins are not colocalized with LK VIII.

Leucokinin-like immunoreactive material has been demonstrated in neurons in many species of insects: cockroaches, locusts, a cricket, blowflies, *Drosophila*, mosquitos, a crane fly, the honeybee, and moths (Nässel and Lundquist, 1991; Nässel *et al.*, 1992a; Cantera and Nässel, 1992; Cantera *et al.*, 1992; Clottens *et al.*, 1993; Nässel, 1993b; Chen *et al.*, 1994a,b; Meola *et al.*, 1994). When comparing different insects it is obvious that there are species differences in immunolabeling with LK I antiserum (also seen when using

LK IV antiserum). Interestingly, however, some main types of LK-IR neurons can be found in insects representing Diptera, Lepidoptera, Orthoptera, Blattodea and Hymenoptera. These are the LK-IR lateral neurosecretory cells of the abdominal ganglia which in all studied species have axons leaving a posterior abdominal nerve root and supply processes to neurohemal organs and/or areas in the abdomen (Nässel, 1994). Also, in all species, except the bee (Chen *et al.*, 1994a), LK-IR neurosecretory cells are found in the cerebral ganglia (Nässel, 1994).

The pattern of interneurons in the brain displaying leucokinin immunoreactivity, however, varies between the insect orders. In fact we have even detected a significant difference between two species of blowfly *Calliphora vomitoria* and *Phormia terraenovae* (Nässel, 1994). Only a few neurons in the brain of *C. vomitoria* label with antisera to leucokinin I and IV (Nässel, 1994), whereas many more were labeled in the brain of *P. terraenovae* (Lundquist *et al.*, 1993; Nässel, 1994). In the *Drosophila* brain more or less the same small number of LK-IR neurons were seen as in *Calliphora* (Nässel, 1994). The LK-IR neurons of *Drosophila* and *Calliphora* are likely to be homologs of a subpopulation of the LK-IR neurons in *Phormia*.

What can the comparative immunocytochemistry tell us about possible functions of leucokinin-like peptides in the insect nervous system? The cells that appear to contain leucokinin related peptide in all studied insect species are neurosecretory cells of the brain (except the honey bee) and abdominal ganglia. Thus hormonal action(s) of leucokinin-related peptides seem to be important in all studied species. Suggested hormonal actions are stimulation of secretion in Malpighian tubules (Hayes *et al.*, 1989, 1994; Coast *et al.*, 1990; Patel *et al.*, 1994), possibly stimulation of contraction in visceral muscle (Holman *et al.*, 1990, 1991) and activation of lipid mobilization and inhibition of protein synthesis (Goldsworthy *et al.*, 1992). In the locust it was found that locustakinin is involved in water balance at two sites: LemK increases fluid secretion in Malpighian tubules and stimulate water reabsorption by the rectum (Schoofs *et al.*, 1993). Hormonal release of leucokinins has been indicated by demonstration of calcium dependent, potassium stimulated release of LK-IR material from the *L. maderae* corpora cardiaca *in vitro* and presence of physiological levels of immunoreactive material in the hemolymph (Muren *et al.*, 1993). Interneuronal functions of leucokinin-related peptides are not known, but it appears that in the cockroach CNS leucokinins may act in many types of circuits both within restricted areas and in projection pathways.

### Concluding remarks

In the previous sections we have seen that different neuropeptides have distinct distributions in small subsets of neurons of the insect central nervous system. Commonly a given insect neuropeptide is distributed in less than 0.1% of the neurons of the brain or ventral cord (Nässel 1991, 1993a; Nässel *et al.*, 1994; Agricola and Bräunig, 1994; Homberg, 1994). Some neuropeptides are present in very few neurons: for example in the entire adult central nervous system of the blowfly there are 10 neurons (4 of these in the brain) reacting with antiserum to crustacean cardioactive peptide (CCAP) (unpublished) and 16 neurons and neurosecretory cells containing corazonin (Cantera *et al.*, 1994). In *Drosophila* an antiserum to pigment dispersing hormone labels 22 neurons in the entire central nervous system (Helfrich-Förster and Homberg, 1993; Nässel *et al.*, 1993). On the other hand some peptides, like LomTK-like peptides and FaRPs, are located in several hundred neurons and neurosecretory cells in different insect species, but still less than 0.1% of the

total number (Nässel, 1993a,b, 1994).

Commonly insect neuropeptides, like the leucokinins described above, seem to be present both in a variety of interneurons and in neurosecretory cells (Raabe, 1989; Nässel, 1993a; Schoofs *et al.*, 1993; Homberg, 1994) or even like proctolin additionally in motoneurons (O'Shea and Schaffer, 1985; Orchard *et al.*, 1989). Some neuropeptides have, however, been detected only in interneurons or only in neurosecretory cells of the brain. For example in the brains of locust, cockroach, blowfly and *Drosophila* tachykinins appear to be expressed by interneurons only (Nässel, 1993, 1994; Lundquist *et al.*, 1994a; Muren *et al.*, 1995). In the *Drosophila*, cockroach and locust brain PDH is exclusively expressed in small populations of interneurons associated with the visual system (Homberg *et al.*, 1991; Helfrich-Förster and Homberg, 1993; Nässel *et al.*, 1993). Peptides such as corazonin, eclosion hormone and PTTHs have been demonstrated only in neurosecretory cells and authentic AKHs only in glandular cells of the corpora cardiaca (Schooneveld *et al.*, 1986). Thus insects have explored different neuropeptides to varying extents, ranging from peptides with discrete functions as neurohormones or single-action central messengers to multifunctional molecules with extremely diverse sites of release and action.

It seems justified to ask whether peptide-containing interneurons constitute peptidergic systems and thus whether peptides are primary signalling molecules in the CNS, or if they commonly act in modulation of synaptic transmission. Primary actions seems to be at hand in some cases: the action of eclosion hormone and cardioactive peptides on behaviour have been monitored during development of the moth *Manduca sexta* (Truman, 1973; Tublitz *et al.*, 1986, 1991). These peptides trigger behaviors by initiation of specific motor patterns. A complex behavior or a physiological response may be programmed into the synaptic connections of a circuit of neurons and the only message encoded into the neuropeptide is to switch on the circuit. A second role that has become evident from studies of the lobster stomatogastric ganglion is that peptides can modulate the activity in neural circuits or induce shifts between functional networks within a given set of interconnected neurons (Marder *et al.*, 1993; Selverston, 1993; Marder, 1994).

A distinct action of many neuropeptides in the mammalian CNS is to modulate synaptic transmission (Hökfelt, 1991). By regulating one or several of the mechanisms involved in synaptic transmission, peptides can impart fine tuning of signal transfer mediated by primary neurotransmitters. In this capacity the neuropeptide may be utilized in various neuronal circuits with entirely different functions and the peptide cannot be ascribed a primary function in behavior or physiology. In insects peptides have been found to act as cotransmitters with modulatory action at peripheral targets (Adams and O'Shea, 1983; O'Shea and Schaffer, 1985). Colocalization of for instance GABA and several neuropeptides in the insect brain suggests similar action centrally (see Nässel, 1993a, 1994; Homberg, 1994). It is also likely that insect neuropeptides act as releasing factors or in the activation or inhibition of protein synthesis. Peptides such as prothoracicotropic hormone, PBAN, AKH, allatostatins and allatotropins have these actions in glands or fat body (Raabe, 1989; Ishizaki and Susuki, 1992; Masler *et al.*, 1993). The LomTK-LI fiber terminals innervating the AKH producing glandular cells of the locust corpora cardiaca and the inducement of AKH I release by LomTK I *in vitro* suggest a physiological role of LomTKs in control of AKH release (Nässel *et al.*, 1995). In the brain involvement in regulation of neurohormone release is suggested from the abundant peptidergic innervation of the pars intercerebralis where the neurosecretory cells have collateral processes (Raabe, 1989; Nässel, 1993a; Homberg, 1994).

At present the most promising approaches for analysis of peptide function and mechanisms in peptide action still rely on peripheral tissues, such as visceral, skeletal and heart muscles, Malpighian tubules, salivary glands, pheromone glands, fat body and endocrine tissues (e. g. Raabe, 1989; O'Shea and Schaffer, 1985; O'Shea *et al.*, 1985; Osborne *et al.*, 1990; Holman *et al.*, 1991, Goldsworthy *et al.*, 1992; Masler *et al.*, 1993; Schoofs *et al.*, 1993; Stay and Woodhead, 1993; Evans, 1994). This would restrict the investigator to peptides expressed in neurosecretory systems, endocrine cells and neurons with efferent axons to peripheral targets. It is, however, hoped that experimental approaches will be developed to also enable assay of *central functions* of peptides in small brains such as those in insects. Focal injections and miniature assays of regionalized release of peptides are not yet feasible at a relevant resolution in insects. The hopes are instead probably to be put on advances in molecular genetic approaches to analysis of peptidergic signalling (see Segal, 1993). Some progress has already been made with respect to cloning of neuropeptide receptors and components of the second messenger systems. Quite a bit more has been accomplished with respect to the molecular biology and genetics of the components of the signalling pathways of acetylcholine, the amino acid transmitters and the monoamines (see Restifo and White, 1990; Buchner, 1991) and it is likely that we will see further advances also for peptidergic pathways.

### References

- Adams, M.E. and O'Shea, M. 1983. Peptide cotransmitter at a neuromuscular junction. *Science* **221**: 286-288.
- Agricola, H.J. and Bräunig, P. 1994. Comparative aspects of peptidergic signalling pathways in the nervous system of arthropods. In: O Breidbach, W Kutsch (Eds) *The nervous systems of invertebrates: an evolutionary and comparative approach*. Birkhäuser Verlag, Basel pp. 303-328.
- Batthey, J.F., Fathi, Z., Wada, E., Hellmich, M.R. and Way, J.M. 1993. Molecular genetic approaches to the analysis of neuropeptide receptors. *Cell Physiol Biochem* **3**: 213-230.
- Benjamin, P.R. and Burke, J. F. 1994. Alternative mRNA splicing of the FMRFamide gene and its role in neuropeptidergic signalling in a defined neural network. *BioEssays* **16**: 335-342.
- Bowers, C.W. 1994. Superfluous neurotransmitters? *Trends Neurosci* **17**: 315-320.
- Buchner, E. 1991. Genes expressed in the adult brain of *Drosophila* and effects of their mutations on behavior: A survey of transmitter- and second messenger-related genes. *J. Neurogenet* **7**: 153-192.
- Cantera, R. and Nässel, D.R. 1992. Segmental peptidergic innervation of abdominal targets in larval and adult dipteran insects revealed with an antiserum against leucokinin I. *Cell Tissue Res* **269**: 459-471.
- Cantera, R., Hansson, B.S., Hallberg, E. and Nässel, D.R. 1992. Postembryonic development of leucokinin I immunoreactive neurons innervating a neurohemal organ in the turnip moth *Agrotis segetum*. *Cell Tissue Res* **269**: 65-77.
- Cantera, R., Veenstra, J.A., and Nässel, D.R. 1994. Postembryonic development of corazonin-containing neurons in the blowfly *Phormia terranova*. *J Comp Neurol* **350**: 559-572.
- Champagne, D.E. and Ribeiro, J. 1994. Sialokinin I and II: Vasodilatory tachykinins from the yellow fever mosquito *Aedes aegypti*. *Proc Natl Acad Sci USA* **91**: 138-142.
- Chen, Y., Veenstra, J.A., Davis, N.T. and Hagedorn, H.H. 1994a. A comparative study of leucokinin-immunoreactive neurons in insects. *Cell Tissue Res* **276**: 69-83.
- Chen, Y., Veenstra, J.A., Davis, N.T. and Hagedorn, H.H. 1994b. Leucokinin and diuretic hormone immunoreactivity in the tobacco hornworm, *Manduca sexta*, and co-localization of this immunoreactivity in lateral neurosecretory cells

- of the abdominal ganglia. *Cell Tissue Res* **278**: 493-507.
- Clottens, F.L., Meola, S.M., Coast, G.M., Hayes, T.K., Wright, M.S., Nachman, R.J. and Holman, G.M. 1993. Characterization of an antiserum against an acetakinin I-analog and its use for the localization of culekinin depolarizing peptide II in the mosquito, *Culex salinarius*. *Regul Pept* **49**: 145-157.
- Coast, G.M., Holman, G.M. and Nachman, R.J. 1990. The diuretic activity of a series of cephalo-yotropic neuropeptides, the acetakinins, on isolated Malpighian tubules of the house cricket, *Acheta domesticus*. *J Insect Physiol* **36**: 481-488.
- Cook, B.J. and Wagner, R.M. 1991. Myotropic neuropeptides. Physiological and pharmacological actions. In: Menn J.J., Kelly, T.J. and Masler, E.P., (Eds) *Insect neuropeptides: Chemistry, Biology and Action*. American Chemical Soc, Washington, D.C., (ASC Symposium Series 453), pp 51-64.
- Cooper, J.R., Bloom, F.E. and Roth, R.H. 1991. *The biochemical basis of neuropharmacology* (6th edition) Oxford University press, New York.
- Copenhaver, P.F. and Truman, J.W. 1986. Identification of the cerebral neurosecretory cells that contain eclosion hormone in the moth *Manduca sexta*. *J Neurosci* **6**: 1738-1747.
- Donly, B.C., Ding, Q., Tobe, S.S. and Bendena, W.G. 1993. Molecular cloning of the gene for the allatostatin family of neuropeptides from the cockroach *Diploptera punctata*. *Proc Natl Acad Sci USA* **90**: 8807-8811.
- Doolittle, R.F. 1981. Similar amino acid sequences: chance or common ancestry? *Science* **214**: 149-159
- Dores, R.M., McDonald, L.K., Stevenson, T.C. and Sei, C.A. 1990. The molecular evolution of neuropeptides: prospects of the '90s. *Brain Behav Evol* **36**: 80-99.
- Duve, H., Rehfeld, J.F., East, P. and Thorpe, A. 1994. Localisation of sulfakinin neuronal pathways in the blowfly *Calliphora vomitoria*. *Cell Tissue Res* **275**: 177-186.
- Evans, P.D. 1994. The effects of myomodulin and structurally related neuropeptides on skeletal neuromuscular transmission in the locust. *J Exp Biol* **190**: 253-264.
- Fleissner, G., Fleissner, G. and Frisch, B. 1993. A new type of putative non-visual photoreceptors in the optic lobe of beetles. *Cell Tissue Res* **273**: 435-445.
- Fonagy, A., Matsumoto, S., Schoofs, L., De Loof, A. and Mitsui, T. 1992. In vivo and in vitro pheromonotropic activity of two locustatachykinin peptides in *Bombyx mori*. *Biosci Biotech Biochem* **56**: 1692-1693.
- G. 1990. The adipokinetic hormone/red pigment-concentrating hormone peptide family: structures, interrelationships and functions. *J Insect Physiol* **36**: 1-12.
- Goldsworthy, G., Coast, G., Wheeler, C., Cusinato, O., Kay, I. and Khambay, B. 1992. The structure and functional activity of neuropeptides. In: Crampton JM, Eggleston P (Eds) *Insect molecular science*. Academic Press, London, pp. 205-225.
- Hagberg, M. 1986. Ultrastructure and central projections of extraocular photoreceptors in caddisflies (Insecta: trichoptera). *Cell Tissue Res* **245**: 643-648.
- Hayes, T.K., Pannabecker, T.L., Hinckley, D.J., Holman, G.M., Nachman, R.J., Petzel, D.H. and Beyenbach, K.W. 1989. Leucokinins, a new family of ion transport stimulators and inhibitors in insect Malpighian tubules. *Life Sci* **44**: 1259-1266.
- Hayes, T.K., Holman, G.M., Pannabecker, T.L., Wright, M.S., Strey, A.A., Nachman, R.J., Hoel, D.F., Olson, J.K. and Beyenbach, K.W. 1994. Culekinin depolarizing peptide: A mosquito leucokinin-like peptide that influences insect Malpighian tubule ion transport. *Regul Pept* **52**: 235-248.
- Helfrich-Förster C. 1995. The period clock gene is expressed in central nervous system neurons which also produce a neuropeptide that reveals the projections of circadian pacemaker cells within the brain of *Drosophila melanogaster*. *Proc Natl Acad Sci USA* **920**: 612-616.

- Helfrich-Förster, C. and Homberg, U. 1993. Pigment-dispersing hormone-immunoreactive neurons in the nervous system of wild-type *Drosophila melanogaster* and of several mutants with altered circadian rhythmicity. *J Comp Neurol* **337**: 177-190.
- Hershey A.D., Polenzani, L., Woodward, R.M., Miledi, R. and Krause, J.E. 1991. Molecular and genetic characterization, functional expression, and mRNA expression patterns of a rat substance P receptor. *Ann Ny Acad Sci* **632**: 63-78.
- Hökfelt, T. 1991. Neuropeptides in perspective: the last ten years. *Neuron* **7**: 867-879.
- Holman, G.M., Cook, B.J. and Nachman R.J. 1986. Isolation, primary structure and synthesis of two neuropeptides from *Leucophaea maderae*: members of a new family of cephalomyotropins. *Comp Biochem Physiol* **88C**: 205-211.
- Holman, G.M., Cook, B.J. and Nachman, R.J. 1987. Isolation, primary structure and synthesis of leucokinins VII and VIII: the final members of this new family of cephalomyotropic peptides isolated from head extracts of *Leucophaea maderae*. *Comp Biochem Physiol* **88C**: 31-34.
- Holman, G.M., Nachman, R.J. and Wright, M.S. 1990. Insect neuropeptides. *Annu Rev Entomol* **35**: 201-217.
- Holman, G.M., Nachman, R.J., Schoofs, L., Hayes, T.K., Wright, M.S. and De Loof, A. 1991. The *Leucophaea maderae* hindgut preparation: A rapid and sensitive bioassay tool for the isolation of insect myotropins of other insect species. *Insect Biochem* **21**: 107-112.
- Homberg, U. 1994. Distribution of neurotransmitters in the insect brain. *Progr Zool* **40**: 1-85.
- Homberg, U., Wörden, S., Dirksen, H. and Rao, K.R. 1991b. Comparative anatomy of pigment-dispersing hormone-immunoreactive neurons in the brain of orthopteroid insects. *Cell Tissue Res* **266**: 343-357.
- Ishizaka, H. and Susuki, A. 1992. Brain secretory peptides of the silkworm *Bombyx mori*: prothoracicotropic hormone and bombyxin. In: J Joosse, RM Buijs, FJH Tilders (Eds) *Progr Brain Res* **92**: 1-14.
- Klein, J.M., Mohrherr, C.J., Sleutels, F., Riehm, J.P. and Rao, K.R. 1994. Molecular cloning of two pigment-dispersing hormone (PDH) precursors in the blue crab *Callinectes sapidus* reveals a novel member of the PDH neuropeptide family. *Biochem Biophys Res Commun* **205**: 410-416.
- Kobayashi, M. and Suzuki, A. 1992. Invertebrate neuropeptides: their localization, structure and function. Multi-author review. *Experientia* **48**: 423-473.
- Lundquist, C.T., Brodin, E., Muren, J.E. and Nässel, D.R. 1993. Tachykinin- and leucokinin-related peptides in the nervous system of the blowfly: immunocytochemical and chromatographic diversity. *Peptides* **14**: 651-663.
- Lundquist, C.T., Clottens, F.L., Holman, G.M., Riehm, J.P., Bonkale, W. and Nässel, D.R. 1994a. Locustatachykinin immunoreactivity in the blowfly central nervous system and intestine. *J Comp Neurol* **341**: 225-240.
- Lundquist, C.T., Clottens, F.L., Holman, G.M., Nichols, R., Nachman, R.J. and Nässel, D.R. 1994b. Callitachykinin I and II, two novel myotropic peptides isolated from the blowfly, *Calliphora vomitoria*, that have resemblances to tachykinins. *Peptides* **15**: 761-768.
- Marder, E., 1994. Invertebrate neurobiology: Polymorphic neural networks. *Curr Biol* **4**: 752-754.
- Marder, E., Abbott, L.F., Buchholtz, F., Epstein, I.R., Golowasch, J., Hooper, S.L. and Kepler, T.B. 1993. Physiological insights from cellular and network models of the stomatogastric nervous system of lobsters and crabs. *Am Zool* **33**: 29-39.
- Masler, E.P., Kelly, T.J. and Menn, J.J. 1993. Insect neuropeptides: Discovery and application in insect management. *Arch Insect Biochem Physiol* **22**: 87-111.
- Meola, S.M., Clottens, F.L., Coast, G.M. and Holman, G.M. 1994. Localization of leucokinin VIII in the cockroach *Leucophaea maderae*, using an antiserum directed against an achetakinin-I analog. *Neurochem Res* **19**: 805-814.
- Mohrherr, C.J., Rao, K.R. and Riehm, J.P. 1991. Characterization of a pigment-dispersing factor from the american cockroach. *Soc Neurosci Abstr* **17**: 276.

- Muren, J.E., Lundquist, C.T. and Nässel, D.R. 1993 Quantitative determination of myotropic neuropeptide in the nervous system of the cockroach *Leucophaea maderae*: distribution and release of leucokinins. *J. Exp. Biol.*, **179**: 289-300.
- Muren, J.E., Lundquist, C.T. and Nässel, D.R. 1995. Abundant distribution of locustatachykinin-like peptide in the nervous system and intestine of the cockroach *Leucophaea maderae*. *Phil Trans R Soc (Lond) B* **348**: 423-444.
- Mutt, V. 1986. Questions answered and raised by work on the chemistry of gastrointestinal and cerebrogastrointestinal hormonal polypeptides. *Chemica Scripta* **26B**: 191-207.
- Nachman, R.J., Holman, G.M. and Haddon, W.F. 1993 Leads for insect neuropeptide mimetic development. *Arch Insect Biochem Physiol* **22**: 181-197.
- Nagasawa, H. 1992. Neuropeptides of the silkworm, *Bombyx mori*. *Experientia* **48**: 425-430.
- Nagasawa, H. 1993. Recent advances in insect neuropeptides. *Comp Biochem Physiol* **106C**: 295-300.
- Nambu, J.R., Murphy-Erdosh, C., Andrews, P.C., Feistner, G.J. and Scheller, R.H. 1988. Isolation and characterization of a *Drosophila* neuropeptide family. *Neuron* **1**: 55-61
- Nässel, D.R. 1987. Neuroactive substances in the insect CNS. In: M.A. Ali (ed) *Nervous systems in invertebrates*. NATO ASI Series Vol 141, Plenum Press, New York, pp. 171-212.
- Nässel, D.R. 1991. Neurotransmitters and neuromodulators in the insect visual system. *Progr Neurobiol* **37**: 179-254.
- Nässel, D.R. 1993a. Neuropeptides in the insect brain: a review. *Cell Tissue Res* **273**: 1-29.
- Nässel, D.R. 1993b. Insect myotropic peptides: differential distribution of locustatachykinin- and leucokinin-like immunoreactive neurons in the locust brain. *Cell Tissue Res* **274**: 27-40.
- Nässel, D.R. 1994. Neuropeptides, multifunctional messengers in the nervous system of insects. *Verh Dtsch Zool Ges* **87**: 59-81.
- Nässel, D.R. and Lundquist, C.T. 1991. Insect tachykinin-like peptide: distribution of leucokinin immunoreactive neurons in the cockroach and blowfly brains. *Neurosci Lett* **130**: 225-228.
- Nässel, D.R., Bayraktaroglu, E. and Dirksen, H. 1994. Neuropeptides in neurosecretory and efferent neural systems of insect thoracic and abdominal ganglia. *Zool Sci* **11**: 15-31.
- Nässel, D.R., Cantera, R. and Karlsson, A. 1992a. Neurons in the cockroach nervous system reacting with antisera to the neuropeptide Leucokinin I. *J Comp Neurol* **322**: 45-67.
- Nässel, D.R., Kim, M.-Y. and Lundquist, C.T. 1995. Several forms of callitachykinins are distributed in the central nervous system and intestine of the blowfly *Calliphora vomitoria*. *J. Exp. Biol.* (in press).
- Nässel, D.R., Lundquist, C.T. and Brodin, E. 1992b. Diversity in tachykinin-like peptides in the insect brain. *Acta Biol Hung* **43**: 175-188.
- Nässel, D.R., Shiga, S., Wikstrand, E.M. and Rao, K.R. 1991. Pigment-dispersing hormone-immunoreactive neurons and their relation to serotonergic neurons in the blowfly and cockroach visual system. *Cell Tissue Res* **266**: 511-523.
- Nässel, D.R., Shiga, S., Mohrherr, C.J. and Rao, K.R. 1993. Pigment-dispersing hormone-like peptide in the nervous system of the flies *Phormia* and *Drosophila*: immunocytochemistry and partial characterization. *J Comp Neurol* **331**: 183-198.
- Nässel, D.R., Holmqvist, M.H., Hardie, R.C., Håkanson, R. and Sundler, F. 1988. Histamine-like immunoreactivity in photoreceptors of the compound eyes and ocelli of flies. *Cell Tissue Res* **253**: 639-646.
- Nässel, D.R., Passier, PCCM., Elekes, K., Dirksen, H., Vullings, HGB. and Cantera, R. 1995. Evidence that locustatachykinin I is involved in release of adipokinetic hormone from locust corpora cardiaca. *Regul Peptides* **57**: 297-310.
- Nichols, R. 1992. Isolation and structural characterization of *Drosophila* TDVDHVFLRFamide and FMRFamide-containing neural peptides. *J Mol Neurosci* **3**: 213-218.



- Nichols, R., Schneuwly, S.A. and Dixon, J.E. 1988. Identification and characterization of a *Drosophila* homologue to the vertebrate neuropeptide cholecystokinin. *J Biol Chem* **263**: 12167-12170.
- Orchard, I. 1987. Adipokinetic hormone: an update. *J Insect Physiol* **33**: 451-463.
- Ohno, S. 1970. Evolution by gene duplication. New York, Heidelberg, Berlin, Springer Osborne RH, Banner SE Wood SJ (1990) The pharmacology of the gut of the desert locust *Schistocerca gregaria* and other insects. *Comp Biochem Physiol* **96C**: 1-9.
- O'Shea, M. and Rayne, R.C. 1992. Adipokinetic hormones: cell and molecular biology. *Experientia* **48**: 430-438.
- O'Shea, M. and Schaffer, M. 1985. Neuropeptide function: the invertebrate contribution. *Ann Rev Neurosci* **8**: 171- 198.
- Otsuka, M. and Yoshioka, K. 1993. Neurotransmitter functions of mammalian tachykinins. *Physiol Rev* **73**: 229- 308.
- Patel, M., Chung, J.S., Kay, I., Mallet, A.I., Gibbon, C.R., Thompson, K.S.J., Bacon, J.P. and Coast, G.M., 1994. Localisation of *Locusta*-DP in locust CNS and hemolymph satisfies initial hormonal criteria. *Peptides* **15**: 591- 602.
- Predel, R., Agricola, H., Linde, D., Wollweber, L., Veenstra, J.A. and Penzlin, H. 1994. The insect neuropeptide corazonin: physiological and immunocytochemical studies in Blattariae. *Zoology* **98**: 35-49.
- Raabe, M. 1989. *Recent developments in insect neurohormones*. Plenum, New York.
- Rao, K.R. and Riehm, J.P., 1989. The pigment-dispersing hormone family: Chemistry, structure-activity relations, and distribution. *Biol Bull* **177**: 225-229.
- Rao, K.R., Mohrherr, C.J., Bonomelli, S.L., Riehm, J.P. and Kingan, T.G. 1991 Insect neuropeptides: influence on color change in insects and chromatophoral pigment movements in crustaceans. In: Menn JJ, Kelly TJ, Masler EP (eds). *Insect Neuropeptides: Chemistry, Biology and Actions*, ACS Symposium Series 453. American Chemical Society, Washington, pp. 110-122.
- Restifo, L.L. and White, K. 1990. Molecular and genetic approaches to neurotransmitter and neuromodulator systems in *Drosophila*. *Adv Insect Physiol* **22**: 115-219.
- Sato, Y., Oguchi, M., Menjo, N., Imai, K., Saito, H., Ikeda, M., Isobe, M. and Yamashita, O. 1993. Precursor for multiple neuropeptides secreted from the subesophageal ganglion of the silkworm *Bombyx mori*: characterization of the cDNA encoding the diapause hormone precursor and identification of additional peptides. *Proc Natl Acad Sci USA* **90**: 3251-3255.
- Schneider, L.E. and Taghert, P.H. 1988. Isolation and characterization of a *Drosophila* gene that encodes multiple neuropeptides related to Phe-Met-Arg-Phe-NH<sub>2</sub> (FMRFamide). *Proc Natl Acad Sci USA* **85**: 1993- 1997.
- Schneider, L.E., Roberts, M.S. and Taghert, P.H. 1993. Cell type-specific transcriptional regulation of the *Drosophila* FMRFamide neuropeptide gene. *Neuron* **10**: 279-291.
- Schoofs, L., Vanden, Broeck, J., and De Loof, A. 1993. The myotropic peptides of *Locusta migratoria*: structures, distribution, functions and receptors. *Insect Biochem Molec Biol* **23**: 859-881.
- Schoofs, L., Holman, G.M., Hayes, T.K., Nachman, R.J. and De Loof, A. 1990a. Locustatachykinins I and II, two novel insect neuropeptides with homology to peptides of the vertebrate tachykinin family. *FEBS Lett* **261**: 397-401.
- Schoofs, L., Holman, G.M., Hayes, T.K., Kochansky, J.P. Nachman, R.J. and De Loof, A. 1990b Locustatachykinin III and IV: two additional insect neuropeptides with homology to peptides of the vertebrate tachykinin family. *Regul Peptides* **31**: 199-212.
- Schoofs, L., Holman, G.M., Proost, P., Van Damme, J., Hayes, T.K. and De Loof, A. 1992. Locustakinin, a novel peptide from *Locusta migratoria*, isolation, primary structure and synthesis. *Regul Peptides* **37**: 49-57.
- Schooneveld, H., Romberg-Privee, H.M. and Veenstra, J.A. 1986. Immunocytochemical differentiation between adipokinetic hormone (AKH)-like peptides in neurons and glandular cells in the corpus cardiacum of *Locusta migratoria* and *Periplaneta americana* with C-terminal and N-terminal specific antisera to AKH. *Cell Tissue Res* **243**: 9-14.

- Segal, D. 1993. Prospects of using *Drosophila* for insect neuroendocrine research. *Arch Insect Biochem Physiol* **22**: 199-231.
- Selverston, A.I. 1993. Neuromodulatory control of rhythmic behaviors in invertebrates. *Int Rev Cytol* **147**: 1-24.
- Stay, B. and Woodhead, A.P. 1993. Neuropeptide regulators of insect corpora allata. *Am Zool* **33**: 357-364.
- Stengl, M. and Homberg, U. 1994. Pigment-dispersing hormone-immunoreactive neurons in the cockroach *Leucophaea maderae* share properties with circadian pacemaker neurons. *J Comp Physiol A* **175**: 203-213.
- Tibbetts, M.F. and Nichols, R. 1993. Immunocytochemistry of sequence-related neuropeptides in *Drosophila*. *Neuropeptides* **24**: 321-325.
- Truman, J.W. 1973. How moths "turn on": a study of the action of hormones on the nervous system. *Am Sci* **61**: 700-706.
- Tublitz, N.J., Copenhauer, P.F., Taghert, P.H. and Truman, J.W. 1986. Peptidergic regulation of behavior: an identified neuron approach. *Trends, Neurosci.* **9**: 359-363.
- Tublitz, N., Brink, D., Broadie, K.S., Loi, P. and Sylwester, A.W. 1991. From behavior to molecules: an integrated approach to the study of neuropeptides. *Trends Neurosci* **14**: 254-259.
- Veenstra, J.A. 1989. Isolation and structure of corazonin, a cardioactive peptide from the American cockroach. *FEBS Lett* **250**: 231-234.
- Veenstra, J.A. 1991. Presence of corazonin in three insect species, and isolation and identification of [His<sup>7</sup>] corazonin from *Schistocerca americana*. *Peptides* **12**: 1285-1289.
- Veenstra, J.A. 1994. Isolation and structure of the *Drosophila* corazonin gene. *Biochem Biophys Res Commun* **204**: 292-296.
- Veenstra, J.A. and Davis, N.T. 1993. Localization of corazonin in the nervous system of the cockroach *Periplaneta americana*. *Cell Tissue Res* **274**: 57-64.
- Veenstra, J.A. and Hagedorn, H.H. 1991. Identification of neuroendocrine cells producing a diuretic hormone in the tobacco hornworm moth, *Manduca sexta*. *Cell Tissue Res* **266**: 359-364.
- Zerr, D.M., Hall, J.C., Rosbash, M. and Siwicki, K.K. 1990. Circadian fluctuations of period protein immunoreactivity in the CNS and the visual system of *Drosophila*. *J Neurosci* **10**: 2749-2762