Invertebrate Protein and Peptide Hormones 5

Contents

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When analyzing invertebrates in the 1930s and 1940s, Berta and Hans Schaller developed the idea of neurosecretion using the large oceanic snail *A. californica*[1] (Califonia sea hare) with its few and relatively large neurons. Neuropeptides have been found in all metazoans. Where whole genome sequences are available such as in bees *A. mellifera*[2]) sequence motifs (signal peptides, cleaving sites of prohormone convertase, terminal glycine) could be investigated. In bees, 200 different potential neuropeptides were identified and the majority of those could be confirmed by chemical analysis (Hummon et al. 2006). In further insects, that is, *D. melanogaster* or *C. elegans*,[3] the two model organisms of developmental biology, the potential inventory of neuropeptides is identified.

Within deuterostomes some tunicates (e.g., *C. intestinalis*[4]) and echinoderms (*S. purpurata*) genomes have been fully sequenced. In these species, the focus has been on vertebrate hormone homologues. Since 2008, when we first looked this up, several peptides have been found related to RF-amides, or to GnRH in echinoderms and tachykinins and GnRH in tunicates (Kawada et al. 2011; Roch et al. 2014; Rowe et al. 2014). These hormones have been discussed in chapter 4. Therefore, when we talk about invertebrates in the following, mostly this is about protostomes.[5]

Table 5.1 summarizes structural motifs and other characteristics of invertebrate hormones. Individual hormones are described in the following sections.

[1] Aplysia californica.

[2] Apis mellifera.

[3] Caenorhabditis elegans.

[4] Ciona intestinalis.

[5] As far as PubMed is concerned; Aug. 2008

Table 5.1 Sequence motifs of invertebrate neuropeptides

Name	Motif
Adipokinetic hormones	p**ELNFx**$_{4/5}$-NH_2
Allatotropins	**TARGF**-NH_2
Allatostatins type-A	**Y/F-x-FG-L/I**-NH_2
type-B	**Wx**$_6$**W**-NH_2
type-C	**PISCF**-OH
Bombyxin—insulin-like peptide	B chain → C-peptide → A chain
CAP	nonapeptides with intramolecular disulfide-bridge like oxytocin/vasopressin
Enterins	**PxxxHxxFV**-NH_2
FMRF/RFamide	**FMRF**-NH_2 or **RF**-NH_2
Cardioexcitatory peptide	**N**D**WF**-NH_2
Leucokinins	**FxxWG**-NH_2
Short neuropeptide-F	**xxxxRLRF**-NH_2
MIP	**PxFY**-NH_2 (y is F, I, V)
Orcokinins	H_2N-**NxDEI**
PTTH	Cysteine knot like gonadotropins/TSH/NGF
Pyrokinins	**FxPRL**-NH_2
Sulfakinins	Tyrosine-sulfated **YGHMRF**-NH_2
Tachykinin	**FxGLM**-NH_2

5.1 Metabolically Active Peptide Hormones

5.1.1 Crustacean Hyperglycemic Hormone

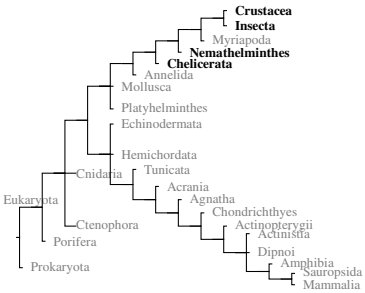
Fact sheet 5.1: Crustacean hyperglycemic hormone (CHH)

Sequence: Fig. 5.1.

Synthesis and target: CHH and related peptides are formed in neurosecretory cells of the X-organ and-after axonal transport into the neurohemal organ sinus gland-are released into the hemolymph.

Function: CHH controls carbohydrate metabolism.

Receptor: Transmembrane guanylate cyclase (mGC).



5.1.1.1 Introduction

CHH of crustacea is a hormone of the X organ in the eye-stalk released in the sinus gland. CHH is the prototype of a peptide family: CHH/MIH/MOIH/GIH/

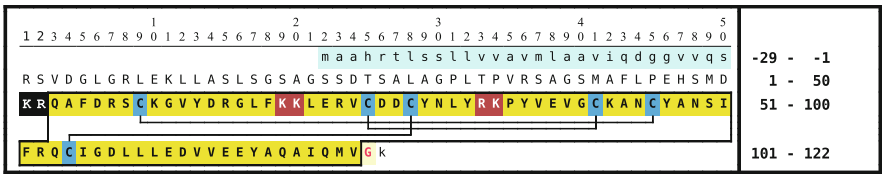


Fig. 5.1 The crustacean hypoglycemic hormone (CHH) of the common European hermit crab (*P. bernardus* [Pagurus bernhardus]): signal peptide (first line, *light blue*); the precursor CHH amino acids 1–126 (*uppercase*) is cut by prohormone convertase (PC) 1 after the motif KR (inverse) to release CHH amino acids 53–126 which is then processed to the amidated CHH (amino acids 53–124 *boxed*; *bold* on *yellow*); unused PC2 motifs are shown *white* on *dark red*; *black lines* between cysteine residues (*black* on *blue*) are analogous to Yasuda et al. (1994). The phenylalanine (amino acid 55) may be epimerized; the N-terminal glutamine in the secreted peptide is modified to pyroglutamate (Source: GenBank DQ450960)

Table 5.2 CHH family members

Abbreviation	Name	Alternative name
CHH	*Crustacean hyperglycemic hormone*	Ion transport peptide (ITP)
MIH	*Molt-inhibiting hormone*	
MOIH	*Mandibular organ-inhibiting hormone*	
GIH/VIH	*Gonad/vitellogenesis-inhibiting hormone*	

VIH. Crustacean hyperglycemic peptide stimulates the carbohydrate metabolism; molting-inhibiting hormone (MIH) suppresses the molt required for growth in ecdysozoans; mandibular-organ (MO)–inhibiting hormone blocks methylfarnesoate synthesis in the mandibular organ; gonad-inhibiting/vitellogenesis-inhibiting hormones (GIH/VIH) modify gonadal functions.

The CHH homologue of insects, ion transport protein (ITP), is formed and released in the corpora cardiaca.

5.1.1.2 Biochemistry and Structure

The members of the CHH/MIH/MOIH/GIH/VIH-family (abbreviations in Table 5.2) are peptides with about 80 amino acids with six characteristic cysteines and thus three disulfide bridges (see also Fig. 5.21). The CHH precursor bears another, CHH precursor-related peptide (CPrP; amino acids 1–50) whose function remains yet unknown. In crustaceans CHH is formed in the X organ (XO) and released in the sinus gland (SG). The CHH homologue of insects, ion transport protein (ITP), is formed and released in the corpora cardiaca (CC). An alternative ITP was identified by Dircksen et al. (2001) in the pericardial organ. By alternative splicing of the same RNA a C-terminal modified peptide was generated.

5.1.1.3 Physiology

The CHH receptors in crustaceans are transmembrane guanylate cyclases (mCG), which after CHH-binding stimulate intracellular cGMP production. CHH stimulates amylase release in the midgut. This in turn increases sugar content in the

hemolymph. CHH (as MIH) may inhibit ecdysteroid biosynthesis which delays molting. ITP similarly binds to an mGC and regulates diuresis in flies.

5.1.1.4 Phylogeny

CHH/ITP and related peptides have been found in chelicerates, nematodes, crustaceans, and insects.

5.1.2 Bombyxin and Insulin-Like Peptides (ILP)

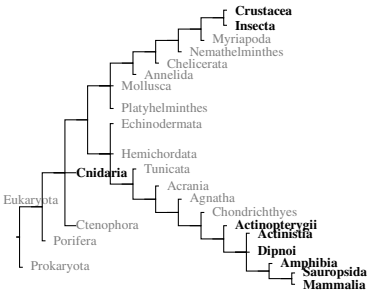
Fact sheet 5.2: Bombyxin and insulin-related peptides (ILP)

Sequence: Fig. 5.2.

Synthesis and target: Bombyxins/ILPs are synthesized by neurosecretory cells of the CNS, as well as in the gut (in *D. melanogaster*).

Function: Bombyxin/ILPs have important functions for growth.

Receptor: ILP/bombyxin receptor is (like the insulin receptor) a transmembrane tyrosine kinase. An insulin receptor substrate (IRS) is needed for signal induction.



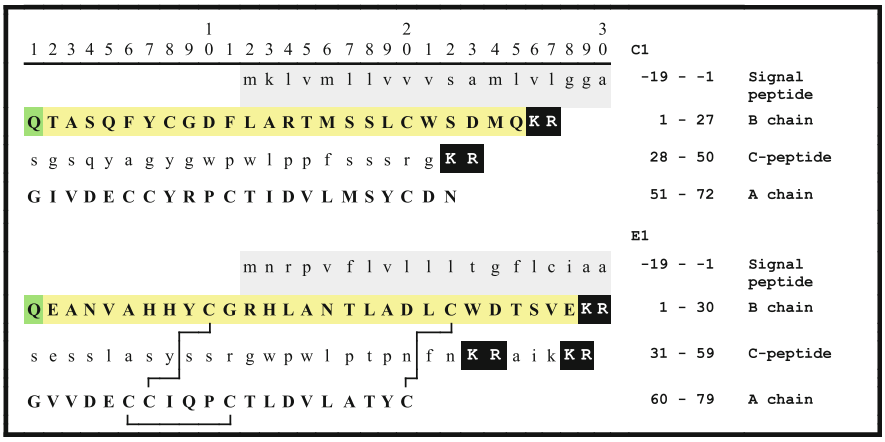


Fig. 5.2 Primary bombyxin sequences from *B. mori* (Bombyx mori): signal peptide amino acids −19 to −1 in the first row with *grey background*. Prohormone convertase-1 (PC1) cuts off the C-peptide (*lowercase*) (**KR** are shown *white on black*); *black lines* between cysteine residues of the A- and B-chains indicate disulfide bonds according to Maruyama et al. (1992); N-terminal glutamine (**Q**) cycled pyroglutamates (p**E**, on *green*) (Source: GenBank P21808, P15410)

Table 5.3 Differential expression of ILP in *D. melanogaster* (From Brogiolo et al. 2001)

	Embryo	Larva
dIlp1	Not expressed (NE)	Not analyzed
dIlp2	**in midgut**, in mesodermal stages 12–16	In imaginal disks[a], **in 7 neurosecretory cells in *partes intercerebrales* (PI) and in salivary glands**
dIlp3	NE	**In 7 neurosecretory PI cells** (see dIlp2)
dIlp4	**in mesodermal stages 2–6**, in the anterior midgut rudiment	**In midgut**
dIlp5	NE	**In 7 neurosecretory PI cells** (s. dIlp2), in gut
dIlp6	NE	in gut
dIlp7	Ubiquitous but yolk sack, in midgut	**In 10 neurons of the ventral nerve cord**

Strong, moderate, low expression
[a]Give rise to wings, gonads, limps, eyes, and antennae

5.1.2.1 Introduction

Bombyxins and other ILPs represent neuropeptides involved in regulation of growth, development, fecundity, metabolic homeostasis, and longevity. Nagasawa et al. (1986) identified the small neuropeptide of the prothoracic gland as an insulin of insects.

5.1.2.2 Biochemistry and Structure

The insulin-like peptides show a similar structure to vertebrate insulin. The precursor bears a signal peptide, B-chain, C-peptide, and the A-chain. In contrast to vertebrate insulin, in bombyxin only the PC1 is required for cleaving of the precursor. In addition, the terminal glutamine can be internally closed to the pyroglutamate ring.

B. mori and *D. melanogaster* each have several ILP genes (Kawakami et al. 1989; Brogiolo et al. 2001), which are expressed in different cells and due to developmental stage differentially regulated. In *D. melanogaster* seven dILP were identified; these are chronologically and topologically differentially expressed (see Table 5.3).

5.1.2.3 Physiology

ILPs/Bombyxins control indispensable growth functions of insects. Synthesized by a few neurosecretory cells in the pars intercerebralis, ILPs are released in the corpora cardiaca. Release depends on available food.

The insulin receptor of flies (coded for by a singular gene and alternatively spliced to different proteins) is related to the vertebrate IR. In contrast to this IR, an analogous insulin–receptor substrate (IRS) phosphorylated upon ligand binding and thus initiating signal transduction is integrated into the receptor protein chain.

However, a separate IRS gene exists in *D. melanogaster* which codes for chico, which when expressed and phosphorylated initiates further signal cascades. The insulin receptor (InsR) is indispensible in insects. Without the InsR irreparable developmental defects of the fly nervous system occur (Fernandez et al. 1995). To which degree insulin signaling influences growth and differentiation is discussed later. Receptor knockout is lethal for the embryo.

A special function has been reported for the Ilp1 of bees. Expression of Ilp1 is due to feeding of the larva by royal jelly thus fed as a queen to be. Only when royal jelly has been fed, Ilp1 is expressed with the consequence that special checkpoints in development are taken that ultimately lead to the raising of a new queen (Wheeler et al. 2006).

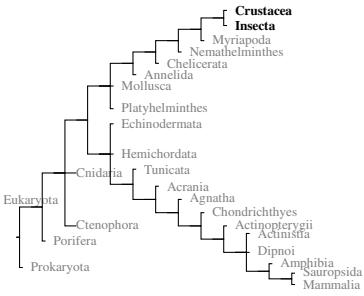
5.1.2.4 Phylogeny

ILPs appear ubiquitously in eumetazoans.

5.1.3 AKH, RPCH, and HrTH

Fact sheet 5.3: Adipokinetic hormone (AKH), red-pigment–concentrating hormone (RPCH), hypertrehalosemic peptide (HrTH)

Sequence:	Fig. 5.3.
Synthesis and target:	AKH is formed in the corpora cardiaca by neurosecretory cells. It acts on GPCR on adipocytes of the fat body and stimulates protein kinase A.
Function:	AKH controls metabolism pathways providing energy for flying.
Receptor:	Heptahelical G-protein-coupled transmembrane receptor.



5.1.3.1 Introduction

Adipokinetic hormones (AKH) are released in the corpora cardiaca of insects and target the fat body. They induce release of sugars and lipids that can be used by equally AKH-stimulated wing muscle cells providing the required forces for flying.

5.1.3.2 Biochemistry and Genes

AKH originates from a preproprotein precursor. At first the signal peptide is cleaved off by the signal peptidase, afterward, by PC1 or PC2, the AKH associated peptide(AAP) (Fig. 5.3); finally, the C-terminal glycine is oxidized to amide by peptidylglycine alpha-hydroxylating monooxygenase (PHM) and the N-terminal glutamine transformed into Pyroglutamate. Characteristic for AKH are

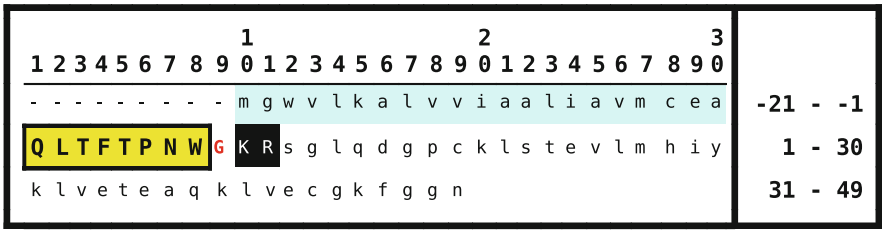


Fig. 5.3 Primary sequence of the adipokinetic hormone (AKH) of *Periplaneta americana*. The signal peptide is shown on *gray background*, the AKH peptide *boxed* and on *yellow* background, and the PC1 motif *black on white* (Source: GenBank AAV41425)

Table 5.4 Several adipokinetic hormones in insects

Species	Hormone	Sequence	Dibasic motif	GenBank entry
L. migratoria	AKH I	p**ELNFTPNWGT**-NH_2	GKR	P55319
	AKH II	p**ELNFSAGW**-NH_2	*GRR*	P08379
	AKH III	p**ELNFTPWW**-NH_2	GKR	P19872
Schistocerca gregaria	AKH I	p**ELNFTPNWGT**-NH_2	GKR	P18829
Schistocerca nitens	AKH II	p**ELNFSTGW**-NH_2	*GRR*	P53807
M. sexta[a]	AKH	p**ELTFTSSWG**-NH_2	GKR	P67788.1
D. melanogaster	AKH	p**ELTFSPNW**-NH_2	GKR	P61855
C. maenas[b]	RPCH	p**ELNFSPGW**-NH_2	GKR	Q26324

[a]Manduca sexta
[b]Carcinus maenas

the N-terminal **ELFN** sequences. Some locusts have been shown to express several AKH peptides (Table 5.4).

5.1.3.3 Physiology

After AKH binding to the heptahelical G-protein coupled transmembrane receptor in adipocytes of the fat body the protein kinase A is stimulated by cAMP. This enzyme phosphorylates the lipid storage droplet protein-1 (LSDP-1) and the triglycerol lipase (TG lipase). Both events induce rapid lipid release into the hemolymph (Patel et al. 2005).

Red-pigment–concentrating hormone (RPCH) of crustaceans is built like AKH. Some X organ neurons secrete RPCH in the sinus gland of the eye stalks. In addition, RPCH/AKH is expressed in the stomatogastric ganglion. Chung and Webster (2004) have shown that RPCH was initially found in X-organs/sinus glands, but was formed in other neurons, presumably in the postcommissural organ. RPCH seems relevant for the rhythmic movements in the gastrointestinal tract (the pyloric rhythm, gastric mill) controlled by 28 neurons of the stomatogastric nerve system (Thirumalai and Marder 2002).

Recently RPCH was also found to be a modulator of the heart ganglion in *Cancer borealis* (Cruz-Bermudez and Marder 2007). Because different peptides and further substances influence the pulses of heart beating the author suggests that due to expression in the heart ganglion an accelerated distribution of hormones in a given animal appears feasible and thus a shortening of the response time to hormonal release.

5.1.3.4 Phylogeny

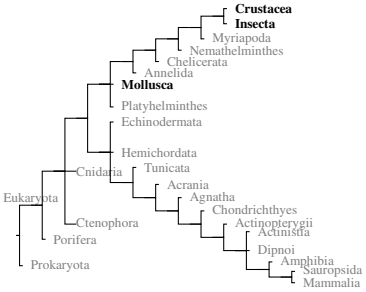
Thus far AKH/RPCH have been found in crustaceans and insects.

5.2 Regulation of Heart Frequency and Pressure by Neuropeptides

5.2.1 Cardioacceleratory Peptides: CAP

Fact sheet 5.4: Cardioacceleratory peptide (CAP)

Sequence:	Fig. 5.4.
Synthesis and target:	CAP are neuronally synthesized in insects and crustacea and released in neurohemal organs.
Function:	CAP stimulates heart frequency.
Receptor:	CAP receptor is a heptahelical GPCR.



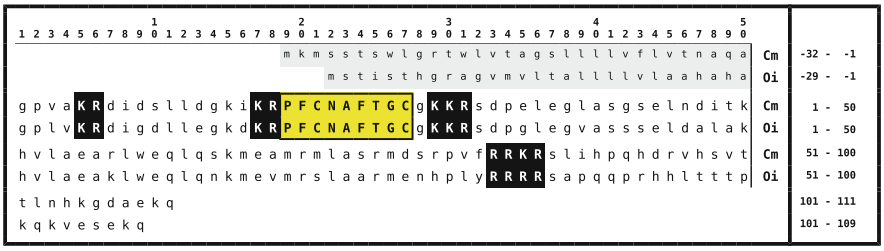


Fig. 5.4 Primary sequences of crustacean cardioacceleratory peptide (CCAP) of Carcinus maenas and Orconectes immunis. The signal peptides are shown on *gray background*; CAP are *boxed* and in *yellow*. Dibasic peptide motifs are shown *white on black* (Source: GenBank ABB46291 and ABB46293)

5.2.1.1 Introduction

The first cardioacceleratory peptide of crustaceans (CCAP) has been identified by Stangier et al. (1987) in the pericardial organ. Injections of this peptide increased the heart frequency. CAP has also been found in insects and molluscs. These molecules resemble the posterior pituitary hormones such as oxytocin or vasopressin.

5.2.1.2 Biochemistry and Structure

CCAP are nonapeptides with an intramolecular disulfide bridge. At the C-terminus these peptides are amidated. With the genome of *D. melanogaster* fully sequenced Park et al. (2002) expressed vasopressin receptor-like GPCR in cell lines and observed calcium mobilization or cAMP enhancement when using CCAP as ligand. However, the CCAP concentrations required for these stimulations were very high which made a specific interaction of CCAP with these receptors unlikely and suggested an unspecific stimulation.

5.2.1.3 Physiology

CCAP in crustaceans and insects has not only been found in the pericardial organ, but in several brain neurons (e.g., in the tobacco hornworm (*M. sexta*) in the subesophageal, thoracic, abdominal, and terminal ganglia (Loi et al. 2001). In crustaceans Trube et al. (1994) found CCAP in neurons and neurosecretory cells of the ventral nerve cord. Veelaert et al. (1997) observed neurosecretory cells of the pars intercerebralis of the locust brain secreting CAP into the corpora cardiaca, therein stimulating AKH release, an analogy to the hypothalamic–pituitary axis.

CCAP is preferentially expressed during late stages of molting when the CCAP content in the hemolymph increases (Phlippen et al. 2000; Gammie and Truman 1999; Loi et al. 2001). In order to meet the enhanced water uptake during molting CCAP is thought to provide the required stimulation of heart frequency and thus increased pumping. Gammie and Truman (1997) have additionally demonstrated (again in *M. sexta*), that CAP induces those essential movements during the molt, resulting in breaking of the old cuticle and in its stripping off. In *D. melanogaster* Davis et al. (2007) provided evidence of CAP activating those enzymes coloring the newly built skeleton: tyrosine hydroxylase (TH) and DOPA carboxylase, which are dealt with in more detail in the chapter on vertebrate catecholamine biosynthesis.

Table 5.5 Cardioacceleratory peptides (M-CAP) of molluscs, compared to a CCAP (Source Vehovszky et al. 2005)

Species	Hormone	Sequence
Helix pomatia	M-CAP I	**PF C NSYG C YNS**-NH_2
	M-CAP II	**LF C NGYGG C QNL**-NH_2
Orconectes immunis	CCAP	**PF C NAFTG C** -NH_2

CAP of molluscs (M-CAP) are related to the CCAP: cyclic peptides with some amino acid exchanges (Table 5.5). Vehovszky et al. (2005) have shown that M-CAP influences the central motor of ingestion.

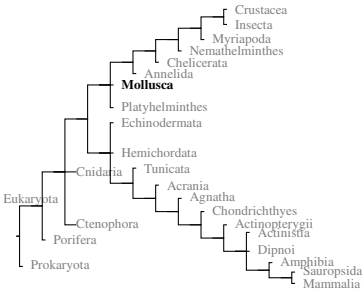
5.2.1.4 Phylogeny

So far CAP have been observed in molluscs, crustaceans, and insects. The presence of a GPCR of the vasopressin family does not allow extrapolating an expression of CAP/vasotocin/vasopressin-like molecules in all bilateria.

5.2.2 Cardioexcitatory Peptide, NDWF-Amide

Fact sheet 5.5: Cardioexcitatory peptide NDWF-NH_2)

Sequence	**NDWF**-NH_2; unknown precursor protein.
Synthesis and target	**NDWF**-NH_2 positive brain neurons target cardiovascular muscles in molluscs.
Function	**NDWF**-NH_2 stimulates blood flow and increases blood pressure in molluscs.
Receptor	(unknown).



The molluscan cardioexcitatory peptide is a tripeptide with a D-amino acid: D-tryptophan (DW): asparaginyl-D-trytophanyl-phenylalanyl-amide (**N**D **WF**-NH_2). Morishita et al. (1997) used it to stimulate in *A. kurodai*[6] heart contraction, but not heart frequency. Later it was found in *A. californica* that not only in the heart, but in all tissues with **N**D**WF**-positive neurons muscle contractions can be induced by this peptide. The peptide was further identified in snails (Morishita et al. 2003a,b).

[6] Aplysia kurodai.

D-Amino acids

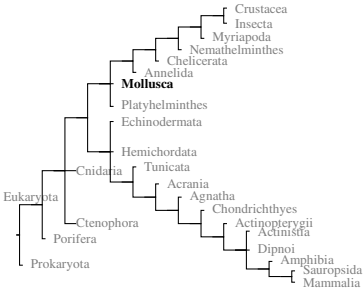
In ribosomes amino acid incorporation is restricted to L-amino acids. The observation of D-isomers presupposes a posttranslational isomerization by a racemase or isomerase. D-Aspartate has been shown to be an neurotransmitter, as well as D-serine. Further D-amino acids incorporated into proteins were found in the animal kingdom. Isomerase acts mainly close to the N-terminus. The spider enzyme (from *Agelenopsis aperta*) uses an **LxF** motif as a substrate and isomerizes the x amino acid; the **NxF** of molluscs is closely related to the spider motif.

Neither the gene coding for **N**D**WF**amide, nor the enzyme isomerizing the tryptophan have thus far been identified (see also the box "D-amino acids"). **N**D**WF**amide has thus far been found only in molluscs.

5.2.3 Enterins

Fact sheet 5.6: Enterins

Sequence:	Fig. 5.5.
Synthesis and target:	Enterins are formed in cerebral and buccal ganglia of molluscs.
Function:	Enterins are effective in aorta and gut and inhibit muscle contraction.
Receptor:	(thus far unknown).



5.2.3.1 Introduction

Enterins were identified as characteristic neuropeptides of aplysia (*A. californica* and *A. kurodai*) (Furukawa et al. 2001). No homologue has ever been found in other phyla. Enterins in concert with AMrP are antagonists of **N**D**WF** of molluscs and inhibit contractions of the aorta as well as of the gut.

5.2.3.2 Biochemistry and Structure

The enterin-precursor protein is relatively large. Dibasic and monobasic cleavage sites for prohormone convertases are present. The peptides shown in Fig. 5.5 *boxed* have actually been isolated. The enterin peptide motif is **PxxxHxxFV**-NH_2. The nonactive additional peptides have been isolated as well, which support the model of precursor cleavage as shown in Fig. 5.5 (Furukawa et al. 2001).

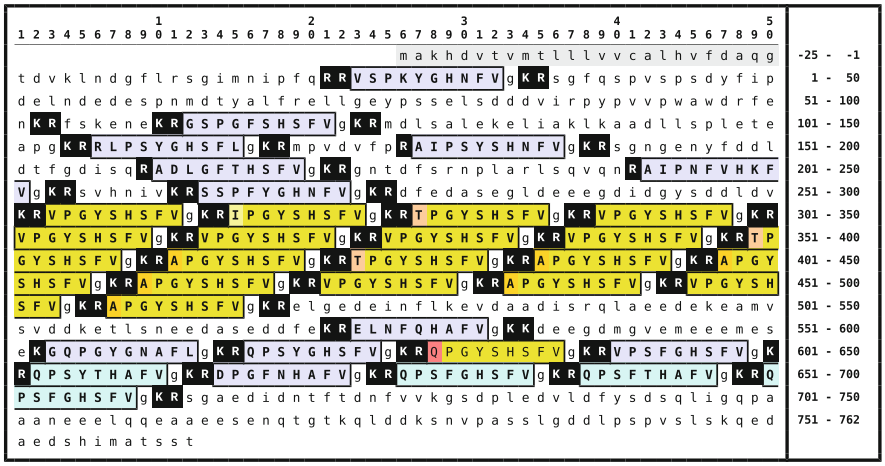


Fig. 5.5 Primary sequence of the enterins-preproprotein from *A. californica*. The signal peptide is shown on *gray background*; the enterins are *boxed* and with *gray background*. Monobasic and dibasic peptide motifs are shown *white on black*. C-terminal glycines are usually oxidized to amides. N-terminal glutaminyl amino acids are internally cycled to pyroglutamate (Origin: Furukawa et al. 2001 GenBank Q95P23)

5.2.3.3 Physiology

Enterins inhibit gut contractions either as neurotransmitters or in an endocrine manner. Their presence in cerebral and buccal ganglia suggests a role in food ingestion. Whether enterins exhibit additional regulatory functions is an open question (Furukawa et al. 2001).

5.2.3.4 Phylogeny

Enterins have only been identified in molluscs.

5.2.4 *Mytilus* Inhibitory Peptides (MIP; AMrP)

5.2.4.1 Introduction

MIP were first found in the pedal ganglia of the clam *Mytilus edulis* (Hirata et al. 1988). In the meanwhile related peptides were observed in other mussels and in snails. The characteristic feature of MIP is inhibition of muscle contractions..

5.2.4.2 Biochemistry and Structure

The characteristic motif of MIP and related, "aplysia-MIP–like peptides" (AMrP) is **PxFF/I/V**-NH_2. The precursor protein of *A. californica* (Fig. 5.6) encloses 24 peptides, six or seven amino acids long; 21 of these contain this motif, and two others bear a methionine or leucine at the last position. **GSPRFF**-NH_2 is found elevenfold.

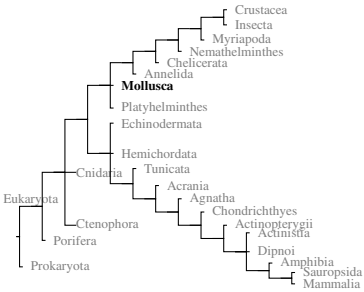
Fact sheet 5.7: *Mytilus* Inhibitory Peptides

Sequence: Fig. 5.6.

Synthesis and target: MIP are neuropeptides in several ganglia, especially the pleural and abdominal ganglia.

Function: MIP inhibit muscle contractions.

Receptor: (thus far unknown).



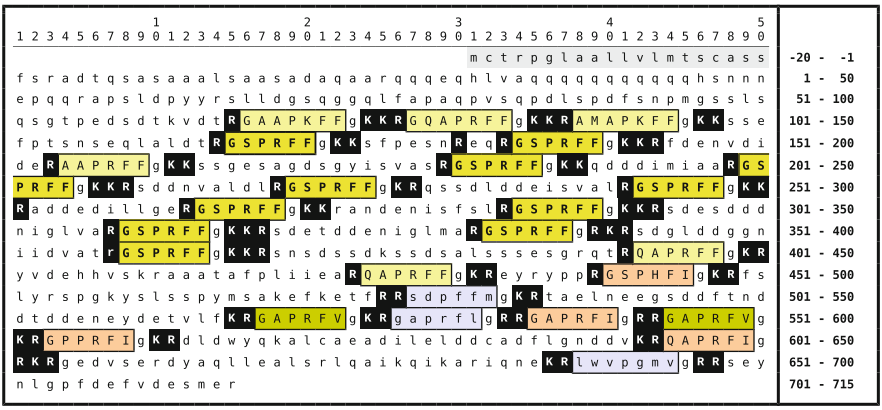


Fig. 5.6 Primary sequence of mytilus inhibitory peptide (MIP, AMrP) from *A. californica*. The signal peptide is shown on *gray background*; the MIP are *boxed* and with *colored background*. The prevalent peptide **GSPRFF**-NH_2 is shown in *uppercase*. Monobasic and dibasic peptide motifs are shown *white on black*. C-terminal glycines are oxidized to amides. N-terminal glutaminyl amino acids may be internally cycled to pyroglutamate (Origin: Fujisawa et al. 1999; GenBank AAF80382)

5.2.4.3 Physiology

MIP/AMrP neurons were observed in several ganglia, most prominent in pleural and abdominal ganglia, additionally in cerebral, buccal, and pedal ganglia. MIP/AMrP inhibit muscle contractions: contractions of the esophagus, of the Penis retraction muscle, or body wall muscle were differentially inhibited by several *A. californica*-AMrP. Most active were **GAPFRV**-NH_2, **GAPFRI**-NH_2, and **GPPFRI**-NH_2 (Fujisawa et al. 1999). In *A. kurodai* Sasaki et al. (2004) analyzed stimulation of the aorta vasoconstrictor muscle by **N**D**WF**amide and its inhibition by enterins and AMrP (**GSPRFF**-NH_2). In cultivated pleural ganglion neurons McDearmid

et al. (2002) identified a 4-aminopyridine-sensitive potassium channel that could be stimulated by **GAPRFV**-NH_2 and **GSPRFF**-NH_2.

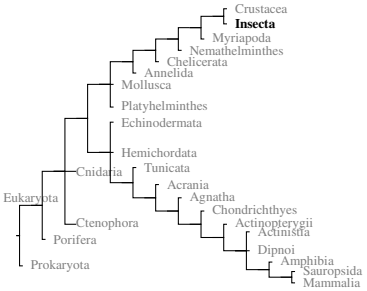
5.2.4.4 Phylogeny

To date MIP/AMrP seem to be restricted to molluscs. So-called myoinhibitory peptides (MIP = leucomyosuppressin) from the prothoracic gland of insects are structurally unrelated to the molluscan MIP.

5.2.5 Diuretic Hormones; (DiuH)[7]

Fact sheet 5.8: Diuretic Hormones (DiuH)

Sequence:	Fig. 5.7.
Synthesis and target	DiuH are formed by neurosecretory cells of the brain and of the corpora cardiaca and act on GPCR in malpighian tubules.
Function:	DiuH stimulate sodium chloride secretion and water transport.
Receptor:	A GPCR of the secretin receptor family.



5.2.5.1 Introduction

Diuresis in insects takes place in the malpighian tubules (MT), appendices to the mid gut. When a mosquito or a bug has fed on a blood meal, the animal or human blood is hypotonic compared to the insect hemolymph: approximately 280 mOsm in blood

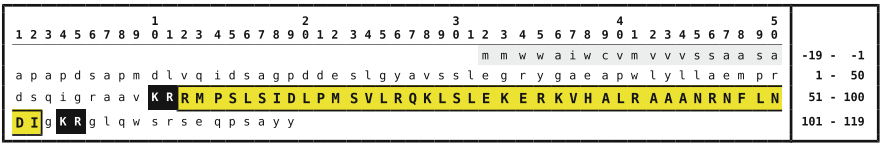


Fig. 5.7 Primary sequence of DiuH from Manduca sexta.The signal peptide is shown on *gray* background; the diuretic hormone is *boxed* and on *yellow background*. Dibasic peptide motifs are shown *white on black*. The C-terminal glycine will be oxidized to amide (Origin: Kataoka et al. 1989 GenBank P21819)

[7]Because there are two hormones that are abbreviated DH (diuretic hormone and diapause hormone) we abbreviate diuretic hormone as DiuH.

and about 370 mOsm in the hemolymph. By secreting sodium and chloride ions into the lumen of the MT and simultaneous uptake of water the food becomes isotonic for the insect and can be ingested and digested. These processes are stimulated by diuretic hormone.

Vasopressin/oxytocin-like peptides as well as CRH-related peptides have been found active as DiuH,[8] furthermore there exist some calcitonin-related diuretic peptides. Because they are analyzed in great detail, CRH-like insect peptides and calcitonin-related peptides are regarded as *the* DiuH being in vitro much more active as inotocin or similar peptides (see Gaede 2004).

5.2.5.2 Biochemistry and Structure

DiuH are formed by the action of the PC1 (and PC2) from precursor peptides. They are C-terminally amidated. Whether the associated peptides (see Fig. 5.7) are physiologically active is not known. In some species some genes were identified as coding for active peptides with amino acid chain lengths of 30 to 50. A differential expression has not been described in the literature. Apart from DiuH, mainly kinins and CAP are stimulators of diuresis.

The DiuH-receptor in *A. aegyptii*[9] has been identified as a GPCR of the secretin receptor family, expressed on the surface of the MT (Jagge and Pietrantonio 2008).

5.2.5.3 Physiology

DiuH are formed in neurosecretory cells of the pars intercerebralis and of the corpora cardiaca and released thereof. In thoracic and abdominal ganglia an however much reduced expression could be observed (Audsley et al. 1997). DiuH acts via the hemolymph on receptors at the malpighian tubules which in turn increase intracellular cAMP. A similar enhanced intracellular cAMP formation was observed in DiuH receptor expressing cell lines (Gaede 2004).

Because the DiuH content in adult locusts was much reduced on the day after the final molt, as after a feeding, a role of DiuH in the postmolting processes was suggested.

5.2.5.4 Phylogeny

Diuretic hormones have been found in 20 different insect species, but not in other phyla.

5.3 Kinins

Named as kinins we describe neuropeptides and neurohormones isolated due to their myotropic or gut-stimulating properties. Different kinin families can be differentiated by characteristic sequence motifs: pyrokinins/pheromone-

[8] Diuretic hormone.

[9] Aedes aegyptii.

biosynthesis–activating neuropeptide (**FxPRL**-NH_2[10]), leucokinins/lymnokinins (**FxxWG**-NH_2), sulfakinins (sulfated **RF**-NH_2), or orcokinins (N-terminal **NxDEI** peptides.

5.3.1 Pyrokinin/Myotropin and Pheromone Biosynthesis Activating Neuropeptide (PBAN)

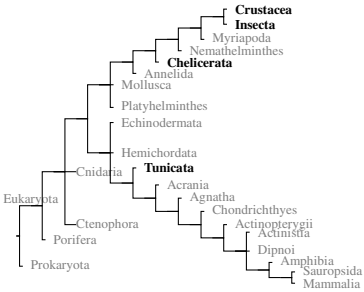
Fact sheet 5.9: Pyrokinins/PBAN

Sequence: Fig. 5.8.

Synthesis and target: PBAN is formed in the subesophageal gland and released in the corpora cardiaca.

Function: PBAN stimulates pheromone biosynthesis.

Receptor: (yet unknown).



5.3.1.1 Introduction

PBAN is formed by singular neurosecretory cells of the subesophageal gland and released in this gland or, after axonal transport, in the corpora cardiaca. PBAN acts on the enzymes of the pheromone glands. The presence of PBAN has been proven in

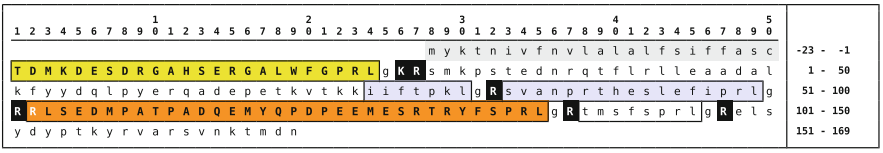


Fig. 5.8 The pheromone-biosynthesis–activating neuropeptide (PBAN) and diapause hormone (DiaH) primary sequence from Bombyx mori. The signal peptide is shown on *gray background*, the diapause hormone [1–24] and PBAN [102–132] are *boxed* on *yellow/orange background*. Monobasic and dibasic peptide motifs are shown *black on white*. C-terminal glycines are oxidized to amides. Three additional neuropeptide (α-SG [74–80], β-SG [83–99], and γ-SG [108–115]) are equally released by prohormone convertases. N-terminal glutamine amino acids are internally cycled to pyroglutamate (Origin: Sato et al. 1993; GenBank P09971)

[10]x represent any amino acid.

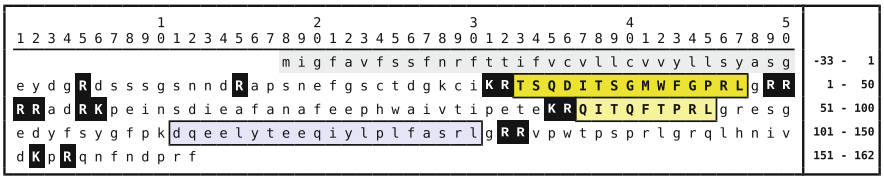


Fig. 5.9 The pyrokinin precursor of the honeybee (Apis mellifera). The signal peptide is shown on *gray background*; pyrokinins are *boxed*, in *uppercase* and on *yellow background*. Whether the peptides [111–130] and [134–142](in *lowercase*) are active as pyrokinins is not reported in the literature; Audsley and Weaver (2006) do not mention them. Monobasic and dibasic peptide motifs are shown *white on black*. Glycines at the C-terminus are always oxidized to amide. N-terminal glutamines may be internally cycled to Pyroglutamate (Origin: GenBank NP_001104182)

lepidopterans, however, in other insects there are PBAN-like peptides; the analogous peptide of *D. melanogaster* is called "hugin."

5.3.1.2 Biochemistry and Structure

Pyrokinins constitute a group of peptides characterized by a C-terminal **FxPRL**-NH_2 motif and exerting muscle contracting (myotropic) activity. Particular pyrokinins are the PBAN where precursor sequences have only been identified in lepidopterans (Figs. 5.8, 5.9).

The precursor of the silk moth (*B. mori*) contains in addition to PBAN the DiaH[11], a pyrokinin, too. Whereas in the silk moth diapause hormone and PBAN are coded for by the same gene, *D. melanogaster* pyrokinins are found within the *hugin*, the *capa* and the cardioacceleratory peptide *Cap2b* genes (Kean et al. 2002; Baggerman et al. 2002; Meng et al. 2002). In the mosquitoes *A. aegyptii* and *A. gambiae*[12] there are three short FxPRL-amide and a single PRL-amide peptide found in a pyrokinin precursor (GenBank Q16N80 and Q7PTL2). The PBAN neuropeptide proprotein of the moth *Agrotis ipsilon* includes a diapause hormone, where the leucine-amide is replaced by isoleucine-amide, PBAN, and two additional pyrokinins; in the oriental tobacco budworm (*Helicoverpa assulta*) DiaH and PBAN and two further pyrokinins are present in the precursor.

5.3.1.3 Physiology

Originally pyrokinins were found due to their myotropic activity. They further influence contractions of the locust oviduct, diapause of the eggs of the silk moth (diapause hormone; Fig. 5.8), acceleration of pupation in larvae of flesh flies, or melanization and reddening in larvae of moths (Torfs et al. 2001). Ectopic (over-) expression of the *hugin* gene in *D. melanogaster* resulted in over 50 % of the larvae in lethality during the second pupation; only 5 % of the flies reached the adult stage.

[11] Diapause hormone.

[12] Anopheles gambiae.

There were unreparable damages and erratic behavior during the molt (Meng et al. 2002).

PBAN stimulates via the PBAN receptor, a heptahelical GPCR; calcium influx and cAMP increase in cells of the pheromone gland. Thus the acetyl CoA carboxylase activity is enhanced in some species, and in other ones the reduction of fatty acids to aldehydes or alcohols is stimulated, which in turn induced pheromone biosynthesis and release (Tillman et al. 1999). The pyrokinin-1 receptor of the fruit fly is a heptahelical GPCR as well (Cazzamali et al. 2005).

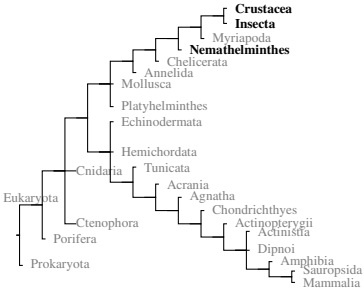
5.3.1.4 Phylogeny

Although PBAN thus far[13] has been observed in insects, homologous genes were found in ticks and spiders as well as in echinoderms without proof of their expression. Pyrokinins were detected in insects, but also in crabs (Torfs et al. 2001).

5.3.2 Orcokinins

Fact sheet 5.10: Orcokinins

Sequence	Fig. 5.10.
Synthesis and target	Orcokinins are neuropeptides, for example, from the stomatogastric ganglion; they act as neurotransmitters and hormones.
Function	Orcokinins contract the gut muscles and regulate the rhythm in the pylorus. They participate in the circadian regulation of motor activity.
Receptor	(not yet known).



5.3.2.1 Introduction

From *O. limosus*[14] Stangier et al. (1992) reported a gut-active neuropeptide: orcokinin. Later they identified similar peptides in other crabs. Orcokinins have now been found in crustaceans, insects, and nematodes; potential mussel or snail orcokinins are not specified as sequences in GenBank.

[13](in Sept 2014)

[14]Orconectes limosus.

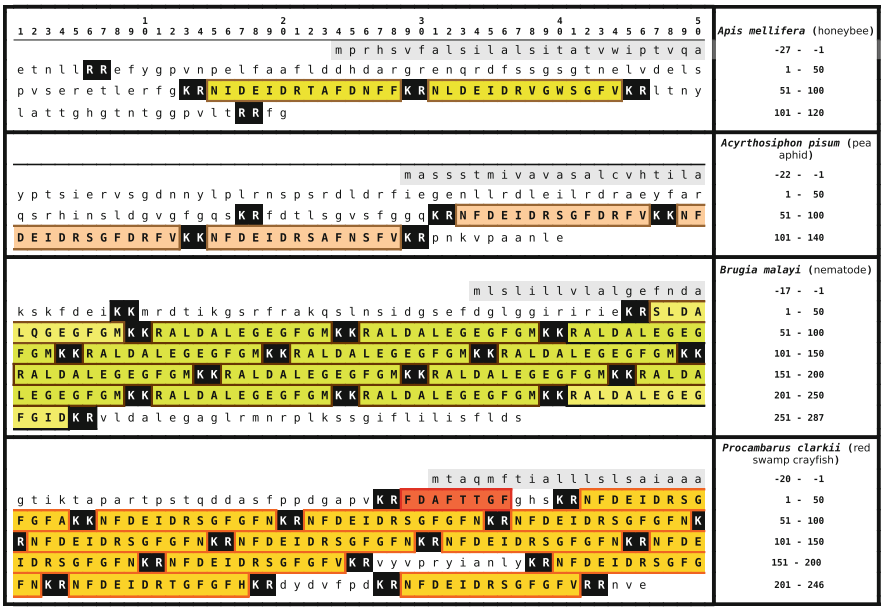


Fig. 5.10 Primary sequences of orcokinins from the arthropods (honeybee), pea aphid (*Acyrthosiphon pisum*), of the nematode *Brugia malayi*, and the red swamp crayfish *Procambarus clarkii*. Signal peptides are on *gray background*, orcokinins are *boxed* and on *colored background*. Dibasic peptide motifs are shown *white on black* (Origin: GenBank P85832, XP_001947462, EDP37605, Q9NL82)

5.3.2.2 Biochemistry and Structure

In some crustaceans and nematodes there are 10 and more identical or very similar neuropeptides on the same orcokinin precursor to be cleaved and released by prohormone convertase 1 or prohormone convertase 2 (**KR** or **KK** motif). In insects there are only few orcokinins on the precursor, however, the N-terminal propeptide sequences are longer (Fig. 5.10). Bees only need PC1; the pea aphid (*Acyrthosiphon pisum*) has to use PC1 and PC2 for orcokinin release (Fig. 5.10).

The *red boxed* **FDAFTTGF**amide peptide in Fig. 5.10 from the red swamp crayfish (*P. clarkii*[15]) was first isolated and analyzed as orcomyotropin from *O. limosus*. That precursor of *O. limosus* has not been sequenced.

Until now no orcokinin receptor has been described. Because in *C. elegans* orcokinins and all the GPCR are known due to total genome sequencing, an orcokinin receptor might most probably be found in this species.

[15]Procambarus clarkii.

5.3.2.3 Physiology

The evolutionary conservation of the neuropeptide structure within the prepropolypeptide in nematodes and crustaceans may point to essential functions of orcokinins. Orcokinin from *O. limosus* acts on gut contractions: contraction amplitude and frequency are enhanced. Orcokinin forming neurons were detected in the stomatogastric ganglion and other ganglia. They exhibit stimulatory activity on rhythm and activity of the pylorus. Whether orcokinins act as neurotransmitters or in an endocrine fashion via the hemolymph is not yet decided. In the hemolymph constant concentrations of orcokinins were observed. On the other hand the middle gut is innervated by several orcokinin neurons.

Finally another orcokinin function in the circadian control of locomotor activity in cockroaches has recently been found. Injections of orcokinin into the accessory medulla of the lobus opticus resulted in stable phase shifts in locomotor activity (Hofer and Homberg 2006).

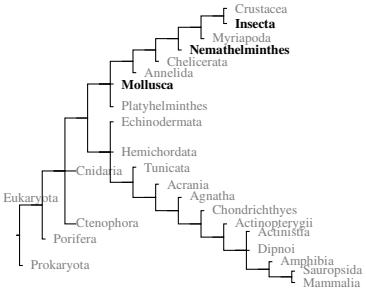
5.3.2.4 Phylogeny

To date, orcokinins were found in insects, crustaceans, and nematodes.

5.3.3 Leucokinins/Lymnokinins

Fact sheet 5.11: Leucokinins, Lymnokinins

Sequence:	Fig. 5.11.
Synthesis and target:	Leucokinins are brain neuropeptides that act via GPCR on muscle cells as well as on malpighian tubules.
Function:	Leucokinins stimulate gut musculature, but most of all they stimulate diuresis.
Receptor:	The lymnokinin receptor belongs to the heptahelical GPCR family.



5.3.3.1 Introduction

Leucokinins, locustakinins, and lymnokinins constitute the **FxxWx**amide neuropeptide family, found with similar structure in insects, molluscs, and nematodes. At first leucokinins were identified due to their gut contracting activity. In the meanwhile regulation of diuresis in malpighian tubules appears as the characteristic feature of leucokinins and related peptides.

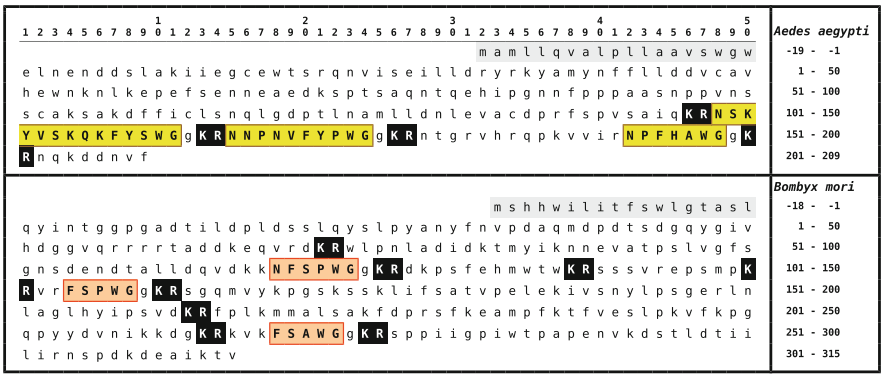


Fig. 5.11 Primary sequence of leucokinins from arthropods: silk moth (*lower*) and yellow-fever mosquito (*upper panel*). The signal peptide is shown on *gray background*, leucokinins are *boxed* and on *yellow* or *red background*. Dibasic peptide motifs are shown *white on black* (Origin: GenBank O02036, BAG50367)

Table 5.6 Kinins from the cockroach *Leucophaea maderae*

	Sequence
I	**DPA F NS W** G-NH_2
II	**DPG F SS W** G-NH_2
III	**DQG F NS W** G-NH_2
IV	**DAS F HS W** G-NH_2
V	**GSG F SS W** G-NH_2
VI	p**ESS F HS W** G-NH_2
VII	**DPA F SS W** G-NH_2
VIII	**GAD F YS W** G-NH_2

5.3.3.2 Biochemistry and Structure

Leucokinins were originally isolated by Holman et al. (1986b,a) in the cockroach *L. maderae*[16] (Table 5.6). Precursor proteins have been identified in the silk moth, the yellow-fever mosquito, and *D. melanogaster*. The three peptide sequences of the yellow-fever mosquito precursor (Fig. 5.11) have been isolated. In the silk moth the precursor has been determined by genome sequencing; the kinins themselves are not yet proven. They might be N-terminally prolonged. The leucokinin of *D. melanogaster*, **NSVVLGKKQR F HS W G**-NH_2 isolated by Baggerman et al. (2002), contains a dibasic peptide motif where prohormone convertase might cleave the precursor.

The lymnokinin from *L. stagnalis*[17] bears a C-terminal serine-amide: **PS F HS W S**-NH_2 (Cox et al. 1997). In *C. elegans* a leucokinin-like precursor

[16] Leucophaea madera.

[17] Lymnaea stagnalis.

protein has been identified (GenBank AAM22049) containing twice the sequence **QFYAWAG** wherefrom a p**E F YA W A**-NH_2 might result.

The lymnokinin receptor has been first characterized by Cox et al. (1997). Other GPCR were later observed in insects: honeybees, fruit flies, and yellow fever mosquitoes. In ticks (i.e., in chelicerates) a very similar receptor was sequenced although a leucokinin has not been found in ticks. In the placozoan *Trichoplax adhaerens*, thus in an very basic metazoan species, a leucokinin-like receptor was sequenced together with the entire genome. Three *A. aegyptii* leucokinins (Fig. 5.11) were found to bind to the identical receptor, but with different affinities (Pietrantonio et al. 2005).

5.3.3.3 Physiology

Holman et al. (1986b) called the leucokinins originally cephalomyotropins, *that is*, peptides of the brain eliciting muscle contractions. When additional leucokinins were isolated, for example, in locusts, gut contractions served as the bioassay for testing of active fractions.

Leucokinins became important for insect research because they promote diuresis of sodium and potassium chloride. In blood-feeding insects, for example, *A. aegyptii*, a lot of salt and water has to be removed postprandially. This happens in the malpighian tubules. Therein leucokinins stimulate salt export. By inhibiting this export it might be possible to block reproduction of these dangerous disease vectors.

5.3.3.4 Phylogeny

Until now leucokinins and similar peptides of the **FxxFx**amide family have been found in molluscs, insects, and nematodes. A potential receptor might have already existed since the origin of metazoan evolution.

5.3.4 Tachykinin-Related Peptides (TRP)

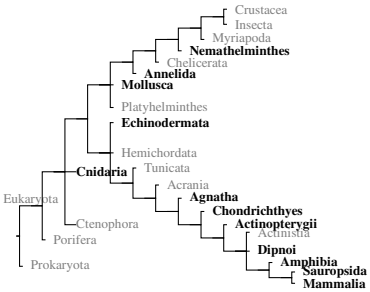
Fact sheet 5.12: Tachykinin-related peptides

Sequence: Fig. 5.12.

Synthesis and target: TRP is found in the brain, in several head ganglia, in the thorax and abdomen, as well as in neuroendocrine cells of the midgut.

Function: TRP activates gut musculature and olfactory nerves.

Receptor: The TRP receptor belongs to the rhodopsin family of heptahelical GPCR.



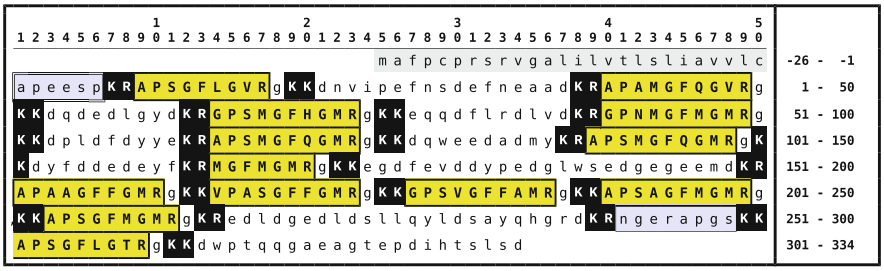


Fig. 5.12 Primary sequences of tachykinin-related peptide from *L. maderae* (LemTRP). The signal peptide is shown on *gray background*, the TRPs are *boxed* on *yellow background*. Dibasic peptide motifs are shown *black on white*. LemTRP-2 and LemTRP-3 have been found as long and short peptides that contain in their long form an intramolecular dibasic peptide motif (Origin: GenBank AAX11211; Nässel 1999; Predel et al. 2005)

5.3.4.1 Introduction

In this book, tachykinins such as substance P have already been described among vertebrate hormones. These peptides bear a common **FxGLM**-NH_2 motif. Invertebrate tachykinin-related peptides belong to the **FxGxR**-NH_2 family (Nässel 1999; Review). They were found in several invertebrate phyla: molluscs, annelids, insects, and crustaceans.

5.3.4.2 Biochemistry and Structure

Up to 14 different tachykinin-related peptide sequences are present in the precursor gene of the TRP in cockroaches, for example, *L. maderae*, where the peptides could equally be identified by chemical analysis. However, in other species, only a few TRP peptides were found.

From different insects and spoon worms (annelids), presumable TRP receptor sequences have been published. All of these proteins belong to the rhodopsin family of GPCR.

5.3.4.3 Physiology

Tachykinin-related peptide (of *L. migratoria*) was found in several brain regions, furthermore in head, thorax, and abdominal ganglia as well as in the midgut. Tachykinin-related peptides stimulate gut musculature as do sulfakinins or allatotropins. When TRP was inactivated by RNA interference in *D. melanogaster*, it could be observed that olfaction was impaired and the locomotion was hyperactive (Winther et al. 2006). Crustaceans form their TRP in endocrine gut cells. These cells secrete TRP when potassium chloride concentrations are increased. A role of TRP in the control of feeding has been assumed (Christie et al. 2007).

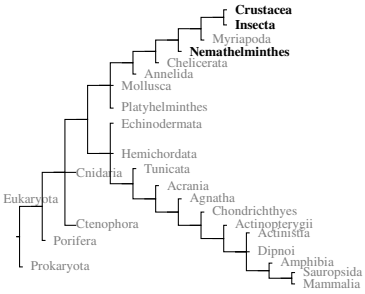
5.3.4.4 Phylogeny

Tachykinins and tachykinin-related peptides might be present in all metazoans. A precursor gene has existed at least before protostomes and deuterostomes developed separately.

5.3.5 Sulfakinins

Fact sheet 5.13: Sulfakinins

Sequence:	Sulfated **DYGHMRF**-NH_2 (Fig. 5.13).
Synthesis and target:	Sulfakinins are formed in individual brain neurons. The molecules act on other neurons and on gut muscle cells.
Function:	Sulfakinins appear to inhibit feeding.
Receptor:	Sulfakinin receptor is a GPCR analogous to gastrin/cholecystokinin receptors.



5.3.5.1 Introduction

The first sulfakinin from the cockroach *L. maderae* was isolated using a bioassay that determined frequency and intensity of gut contractions.

5.3.5.2 Biochemistry and Structure

Sulfakinins are cleaved from a precursor protein. They are characterized by a C-terminal **DYGHMRF**-NH_2 sequence (Fig. 5.13). Some sulfakinins were isolated with a sulfated tyrosine (**Y**); when the sequence has only been identified by RNA/DNA analysis it cannot be assumed that the final peptide is indeed sulfated. Admittedly, nonsulfated sulfakinins were equally active (Nichols 2007, 2003). Tyrosylprotein sulfotransferase (TPST), the enzyme that adds a sulfate group to

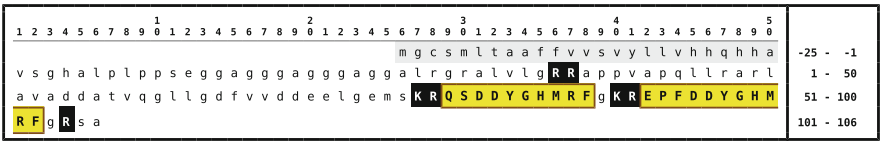


Fig. 5.13 Primary sequence of the sulfakinin from *Grillus bimaculatus*. The signal peptide is shown on a *gray background*; the sulfakinins are *boxed*, *uppercase*, and on *yellow background*. Dibasic or furin peptide motifs are shown *black on white* (Origin: GenBank CAL48349)

tyrosine, has been found in all vertebrates analyzed, in insects, nematodes and annelids, and, too, in several bacteria (for a review, see Moore 2003).

Sulfakinins resemble the equally sulfated C-terminal fragments of gastrin and cholecystokinin. The cionin from *C. intestinalis* also seems to be related.

Sulfakinin receptors of *D. melanogaster* were identified due to their analogy to vertebrate gastrin/CCK receptors and characterized. In other insects and nematodes sulfakinin receptor genes could be established as well.

5.3.5.3 Physiology

Originally sulfakinins were identified for their gut muscle stimulation. In larvae very few brain neurons secrete sulfakinins. Their number increases in pupae and adults. Parallel to their regulation of muscle contractions it was recently found that sulfakinins inhibit food intake (Wei et al. 2000). Thus sulfakinins not only act as neurotransmitters, but as hormones in sensu stricto.

5.3.5.4 Phylogeny

Sulfakinins have been observed in insects and crustaceans. Their relation to gastrin and cholecystokinin, to caerulein, and to cionin as well, argues for the presence of such substances already in early metazoans. The homology of the sulfotransferases strengthens this argument.

5.4 Neuropeptides of Reproduction

5.4.1 PTTH

5.4.1.1 Introduction

Prothoracicotropic hormone (PTTH) is formed in a few neurosecretory cells of the insect brain. It is secreted into the corpora allata and stimulates in the prothoracic gland the synthesis of ecdysteroids. These are indispensable for growth and development.

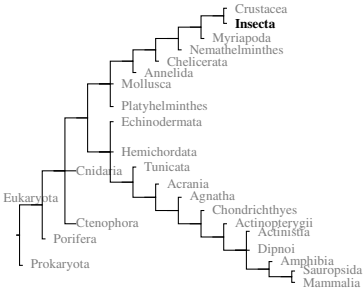
Fact sheet 5.14: Prothoracicotropic hormone (PTTH)

Sequence: Fig. 5.14.

Synthesis and target: PTTH is formed in a few brain neurons and acts on the prothoracic gland.

Function: PTTH stimulates ecdysteroid biosynthesis in the prothoracic gland.

Receptor: Tyrosine kinase receptor *torso*.



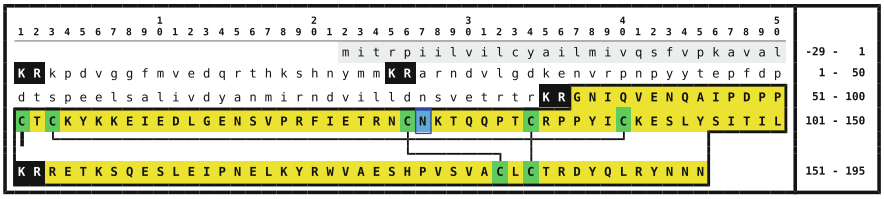


Fig. 5.14 Primary sequence of the prothoracicotropic hormone (PTTH) from silk moth. The signal peptide is shown on a *gray background*, the PTTH is *boxed* on *yellow background*, and the N-glycosylated arginine (**N**) is boxed, too. Dibasic peptide motifs are shown *black on white* (Origin: P17219)

5.4.1.2 Biochemistry and Structure

PTTH is built as a homodimer from two identical peptide chains (Ishizaki and Suzuki 1994 Fig. 5.14); the subunits are linked by a disulfide bridge between the two cysteine residues at position 130 (in Fig. 5.14 indicated by a *gray bar*).

The singular genes (or cDNAs) for PTTH from *B. mori*, *D. melanogaster* and from several *Helicoverpa* species (Noctuidae) have been cloned. Characteristic features are the dibasic peptide motif in front of the PTTH peptide, the positioning of seven cysteine residues, and a glycosylated arginine. Sequence homology is between 40 and 98 % of amino acids (Sauman and Reppert 1996).

A PC1 that recognizes **KR** would internally cleave PTTH again, thus the recognition site might be **RKR** or **RxRK**. The latter motif would also be cleaved by furin. The PTTH-releasing endopeptidase has not been described in the literature thus far.

In 2009, the torso receptor was identified as the PTTH receptor. Its role in early fly embryo-genesis had already been found some time ago (for a review, see Li 2005). The early ligand for torso is trunc. Later in larval development, torso expression is restricted to the prothoracic gland where PTTH exerts its effects (Rewitz et al. 2009)

5.4.1.3 Physiology

PTTH triggers ecdysone biosynthesis. The torso receptor triggers a cAMP- and calcium-mediated stimulation in cells of the prothoracic gland. The restricted expression to a few laterodorsal neurosecretory cells in the insect's brain points to a very specific function of PTTH.

PTTH is released in a circadian rhythm. The pacemaker neurons (expressing *per*) are placed in close vicinity to the PTTH neurons and connected to these synaptically (Sauman and Reppert 1996).

The amount of PTTH mRNA has been found to be constant during larvae development, however, PTTH pulses were secreted into the hemolymph before eclosion indicating a regulation of the secretion and not of the synthesis of PTTH. One transcription factor necessary for expression of PTTH is myocyte enhancer factor 2 (MEF2) which binds to the PTTH promoter.

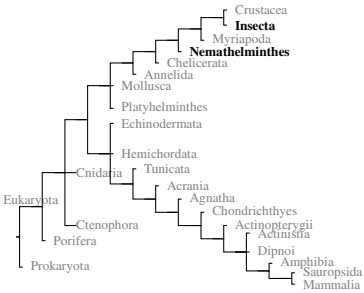
5.4.1.4 Phylogeny

Thus far PTTH has only been described for insects.

5.4.2 PTSH; MIP

Fact sheet 5.15: Prothoracicostatic Hormone (PTSH); Myoinhibitory Peptide (MIP)

Sequence:	Fig. 5.15.
Synthesis and target:	PTSH is synthesized in the brain of larvae and acts on the prothoracic gland.
Function:	PTSH inhibits ecdysone synthesis in the prothoracic gland.
Receptor:	The sex peptide receptor is also the receptor for PTSH.



5.4.2.1 Introduction

Although PTTH stimulates ecdysone biosynthesis in the prothoracic gland, the hormone inhibiting this biosynthesis had not been identified for some time. Either Neb-TMOF or several allatostatins had been described as prothoracicostatic; finally, Hua et al. (1999) identified an additional neuropeptide capable of inhibiting PTTH stimulation.

5.4.2.2 Biochemistry and Structure

The consensus sequence of *B. mori* PTSH contains six invariable amino acids (Fig. 5.16). Known PTSHs all possess an allatostatin type B motif **Wx$_6$W**-NH$_2$.

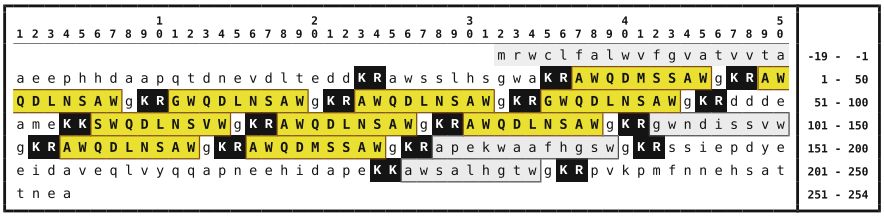


Fig. 5.15 Primary sequence of prothoracicostatic hormone (PTSH) from the silk moth. The signal peptide is shown on a *gray background*; the PTSH is *boxed*, in *uppercase* on *yellow background*. Dibasic peptide PC1 motifs are shown *black on white*. The peptide amino acids 25–33 cannot be amidated due to a G→A substitution and might thus be inactive (Source: NP_00103689)

Fig. 5.16 Consensus sequence of prothoracicostatic hormone (PTSH) from the silk moth. The larger the letter the more frequent the respective amino acid (Max. = 10/10; Min. = 1/10) (Origin: NP_001036890)

In addition, Hua et al. (1999) observed that the PTSH consensus sequence **AWQDLNSAW**-NH_2 is identical to the sequence of myoinhibitory peptide 1 (MIP1) from *M. sexta* where a prothoracicostatic activity had not been found before.

5.4.2.3 Physiology

MIP/PTSH peptides not only inhibit ecdysone biosynthesis, but also block gut peristaltic and possibly juvenile hormone biosynthesis . However, since the discovery of a potential B-type allatostatin receptor in the fruit fly (Johnson et al. 2003) no other study in particular about topological and temporal expression of this protein in the course of fly development has been published.

Apart from MIP/PTSH peptides FRMF-like peptides additionally regulate the prothoracic gland and its ecdysone synthesis (Yamanaka et al. 2006). The questions of the influence exerted by the combination of different peptides together with PTTH on the hormone production, how the regulation is progressing in the course of development, and how gene defects influence this development cannot be answered yet. In 2010 the sex peptide receptor was identiefied by Yamanaka et al. (2010).

5.4.2.4 Phylogeny

Myoinhibitory peptides have only been found in flies thus far, prothoracicostatic peptides, however, are found in moths (Lepidoptera) and beetles (Coleoptera). In addition to insects, *C. elegans* possess a protein homologous to the PTSH precursor with a **W**x_6**W**-NH_2-allatostatin-type-B peptide/PTSH to be cleaved by PC1.

5.4.3 Pheromonostatic Peptide, Sex Peptides

5.4.3.1 Introduction

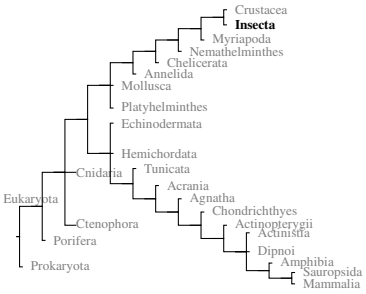
With the release of pheromones female insects signal a sexual mature state and mating eagerness. During copulation the females receive together with the sperms peptide hormones inhibiting pheromone synthesis and release. Thus these females become temporarily unattractive to other males. After egg positioning pheromones are again released and further copulation enabled.

5.4.3.2 Biochemistry and Structure

The pheromone synthesis inhibiting peptide from *Helicoverpa zea* (Fig. 5.17) is a relatively long peptide compared to the sex peptides from the accessory gland of

Fact sheet 5.16: Pheromone synthesis inhibiting (pheromonostatic) peptide (PSP); sex peptide (SP)

Sequence:	Fig. 5.17.
Synthesis and target:	PSP/SP are formed in the male genitalia and are transferred together with the sperms into the female genitalia.
Function:	These peptides inhibit pheromone biosynthesis.
Receptor:	The receptor is a heptahelical membrane GPCR.



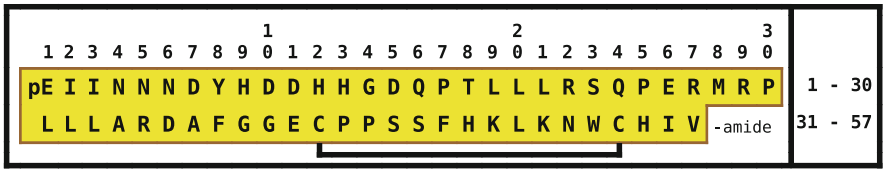


Fig. 5.17 Peptide sequence of pheromonostatic peptide-1 (PSP-1) from *Helicoverpa zea* (cotton bollworm aka corn earworm aka tomato fruitworm). PSP-1 shown *boxed*, *on yellow background* and in *uppercase*. It is N-terminally modified to pyroglutamate and C-terminally amidated. The intramolecular disulfide bridge is indicated (Origin: AAB35024)

fruit flies which are much shorter. A common feature of both types of molecules is a disulfide bridge close to the C-terminus.

5.4.3.3 Physiology

Formation of sex peptides/PSP in the male genitals and their reception by the female are a most interesting neuropeptide transmission. This is neither a neurotransmission, nor an endocrine interaction via the hemolymph. It is pheromone-like inasmuch as it happens between two individuals. The closest relationship appears to be feeding of royal jelly to the larvae creating bee queens and thus influencing ILP1 expression.

PSP/SP are synthesized in the male genitalia more precisely in the ejaculatory duct; there is, however, an expression in the heart in both sexes.. With the seminal fluid as vector they are transferred into the female. Shortly after copulation a reduction of PBAN synthesis in brain neurons could be observed. Whether PSP/SP receptors on cells of the female genitalia and neuronal contacts to the brain or a transport of PSP/SP into the brain are required has been analyzed in only a few examples. In female *D. melanogaster* increasing amounts of SP could be found in the brain after copulation (Nagalakshmi et al. 2004); and still, in order to respond to SP functional neurons are necessary that should express the egghead protein (Soller et al. 2006).

PSP/SP not only influences the PBAN synthesis and release; they additionally stimulate egg deposition and juvenile hormone release. The male thus ensures that its sperms and not those of a competitor are fertilizing the eggs. In several species the PSP/SP action is reduced after several days. The duration of effects is dependent on the presence of sperms onto which the PSP/SP adhere with their N-terminal end and from where they diffuse after fertilization has occurred (Kubli 2003).

When the PSP receptor from the *D. melanogaster* (a GPCR) had been cloned its expression in the genital tract and the CNS of females fruit flies was observed (Yapici et al. 2008). In flies with receptor mutants egg disposition was not seen and these flies copulated again as virgin females.

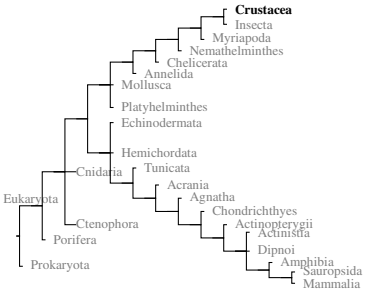
5.4.3.4 Phylogeny

PSP/sex peptides have only been found in insects.

5.4.4 GIH, VIH

Fact sheet 5.17: Gonad-inhibiting hormone (GIH); vitellogenesis inhibiting hormone (VIH)

Sequence:	Fig. 5.18.
Synthesis and target:	GIH are synthesized in sinus glands in the crustacean eye stalks. They act on the gonads.
Function:	GIH inhibits molting and facilitates sexual maturation.
Receptor:	(not yet identified).



5.4.4.1 Introduction

As with the related CHH GIH/VIH is a peptide from crab synthesized in the X organs in eye stalks and released in the nearby sinus gland.

5.4.4.2 Biochemistry and Structure

The GIH precursor has the signal peptide and the GIH peptide with a C-terminal **GKR** to be oxidized to amide. Six cysteines are similarly spaced as in CHH. In contrast to CHH there is no associated N-terminal peptide in GIH/VIH.

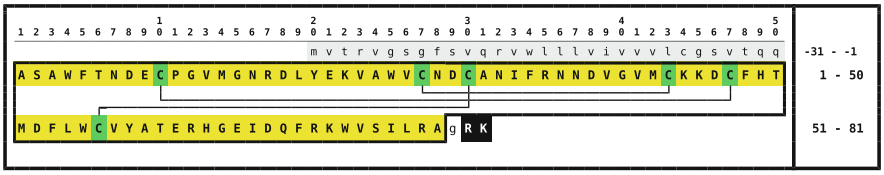


Fig. 5.18 Peptide sequence of gonad-inhibiting hormone (GIH) from the American lobster (*H. americanus* [Homarus americanus]). GIH is *boxed*, shown on *yellow background* and in *uppercase*. It is C-terminally modified to amide. The intramolecular cysteine bridges are indicated (Origin: P55320)

5.4.4.3 Physiology

GIH is a hormone controlling the life cycle of the crustacean. As long as GIH is present, molting is inhibited. After a decrease of GIH levels maturation occurs and vitellogenesis is activated. Finally molting occurs.

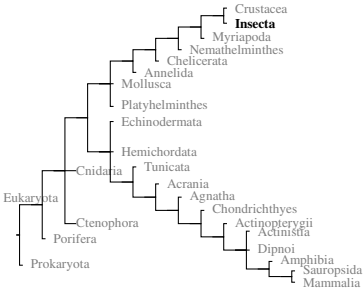
5.4.4.4 Phylogeny

GIH/VIH are thus far only known in crustaceans.

5.4.5 TMOF

Fact sheet 5.18: Trypsin-modulating oostatic factor (TMOF)

Sequence:	Fig. 5.19.
Synthesis and target:	TMOF is released by gonads and acts on gut enzymes.
Function:	TMOF inactivates enzymes.
Receptor:	TMOF binds to an unknown receptor in the gut epithelium and inhibits trypsin generation.



5.4.5.1 Introduction

A trypsin-modulating oostatic principle was first observed 75 years ago (Iwanov and Mescherskaya 1935, additional references at Borovsky 2003). With the sequence yet unknown it had already been found that an ovarian extract could inhibit vitellogenesis and digestion of blood meal in *A. aegyptii*. Borovsky et al. (1990) identified the sequence of TMOF. TMOF might be a pesticide against the yellow fever mosquito.

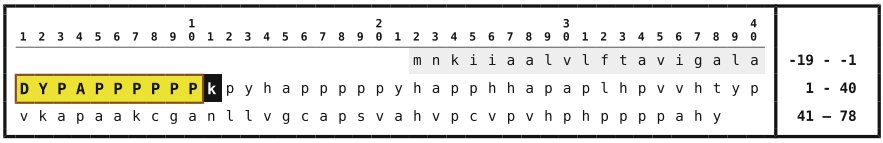


Fig. 5.19 Peptide sequence of trypsin-modulating oostatic factor (TMOF) from yellow fever mosquito (*A. aegyptii*). TMOF is *framed*, *on gray background* and in *uppercase letters* (Source: P19425; Borovsky et al. 1990)

5.4.5.2 Biochemistry and Structure

With its seven proline residues TMOF is an unusual peptide. For TMOF receptor activity the N-terminal four amino acid residues are critical. An **DYPR** analogue was found to be fourfold more active than TMOF or natural **DYPA**. TMOF is cleaved from the precursor protein by the signal peptidase and another endopeptidase cleaving after lysine, that is, a trypsin or chymotrypsin-like enzyme.

From the grey flesh fly, a similarly active TMOF was isolated whose sequence **NPTNLH** differs strongly from the one from *A. aegyptii* (Bylemans et al. 1994).

5.4.5.3 Physiology

The name "oostatic factor" is misleading. TMOF inhibits synthesis and release of gut enzymes. Thus it inhibits gonads only in an indirect way. Released from gonads, TMOF can diffuse without any transporter protein across the gut wall. In adult mosquitoes TMOF is released about 30 h after a blood meal. At this time the blood is digested and TMOF might inhibit excess digestion enzymes. Larvae treated with TMOF die from starvation. TMOF might also influence the prothoracic gland (Gilbert et al. 2002)

5.4.5.4 Phylogeny

The original findings of oostatic or antigonadotropic factors include as a source a decapod crustacean (compare Borovsky 2003), but a sequence has not been published. Only in insects has TMOF been cloned and sequenced.

5.4.6 Nebcolloostatin

5.4.6.1 Introduction

While isolating TMOF a second peptide was identified with oostatic activity which was named colloostatin due to its closeness to collagen. It has not been analyzed thus far in great detail.

5.4.6.2 Biochemistry and Function

The sequence **SIVPLGLPVP IGPIVVGPR** of colloostatin resembles preprocollagen-IV from fruit flies, however, the collagen-IV from the grey flesh fly has not yet been sequenced. The enzymes releasing colloostatin are not known.

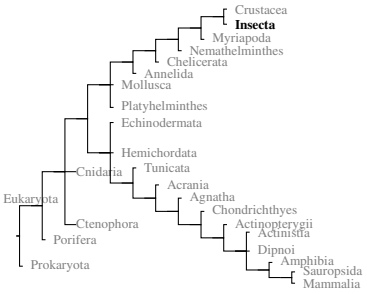
Fact sheet 5.19: Collagen-like oostatin from the grey flesh fly *Neobellieria bullata* (Neb-Colloostatin)

Sequence: `SIVPLGLPVP IGPIVVGPR`.

Synthesis and target: Potentially from preprocollagen IV by unknown endopeptidase(s).

Function: Blocks vitellogenesis.

Receptor: (not yet known).



The peptide has been isolated from the abdomen of *Neobellieria bullata* and inhibits vitellogenesis at nanomolar concentrations.

5.4.6.3 Physiology

Recently Wasielewski and Rosinski (2007) observed that in the mealworm (*Tenebrio molitor*) colloostatin as well as TMOF inhibit vitellogenesis, slows down ovary development, delays ovulation and egg deposition, and finally reduces the number of eggs deposited.

5.4.6.4 Phylogeny

Colloostatins have only been found in insects.

5.5 Peptide Hormone of Metamorphosis and Molting

5.5.1 Molt-Inhibiting Hormone (MIH)

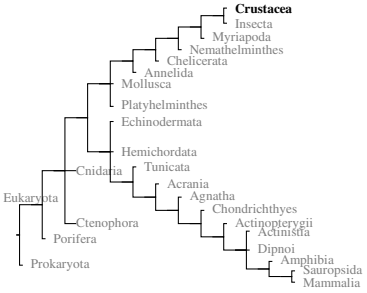
Fact sheet 5.20: Molt-inhibiting hormone (MIH)

Sequence: See, for example, Fig. 5.20.

Synthesis and target: MIH is synthesized in the X organ of crustaceans and released in the sinus gland.

Function: MIH delays molting (this view is challenged).

Receptor: Possibly a membrane guanylate cyclase.



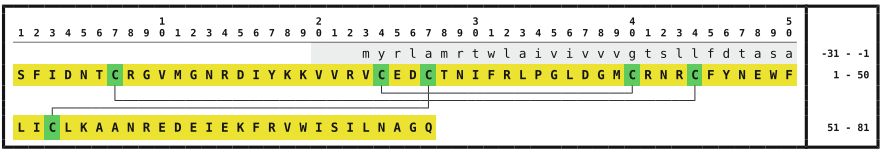


Fig. 5.20 Peptide sequence of molt-inhibiting hormone (MIH) from the Kuruma prawn (*Marsupenaeus japonicus*). The signal peptide is *highlighted gray*; MIH is *boxed, highlighted yellow and in uppercase*. The intramolecular cysteine bridges are indicated (Source: P55847)

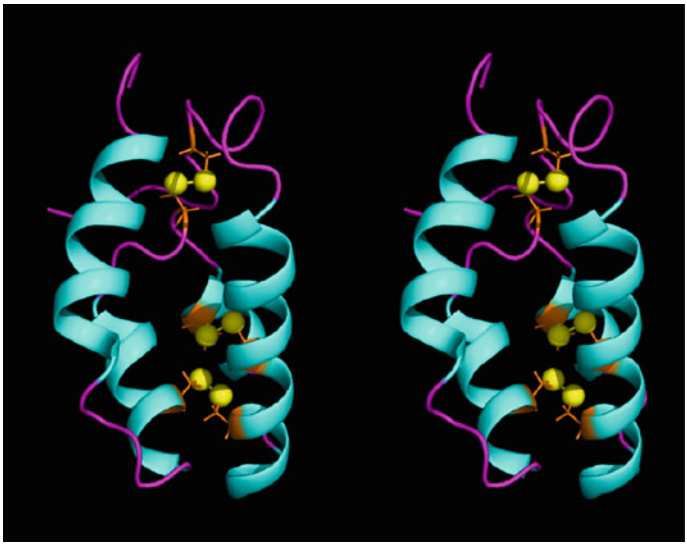


Fig. 5.21 Stereo view of the structure of molt-inhibiting hormone (MIH) from the Kuruma prawn (*Marsupenaeus japonicus*). Helices are shown in *light blue*, and other sequence parts in *pink*; cysteines are in *orange* and disulfide bridges drawn *yellow* (Source: Katayama et al. 2003)

5.5.1.1 Introduction

MIH belongs to the family of CHH/GIH/VIH peptides. Like those it is made in a few neurosecretory cells of the crustacean eye stalks and released in the sinus gland therein.

5.5.1.2 Biochemistry and Structure

As with GIH/VIH the MIH precursor protein consists of the signal peptide and the hormone alone (Fig. 5.20). The cysteine positions and the three disulfide bridges are similar to CHH and GIH (Fig. 5.21).

5.5.1.3 Physiology

MIH is like CHH a hormone controlling ecdysone biosynthesis via the sinus gland. The actions of MIH are also mediated by guanylate cyclase. NO synthase seems to play a role. A specific receptor has not yet been found, only one candidate

gene (Zheng et al. 2008, and an unpublished sequence of a guanylate cyclase (AGB51125)).

The standing hypothesis that MIH expression decreases before a molt to enhance ecdysone levels has been challenged because any significant difference in ecdysone concentrations before and after molting could not be observed in the hemolymph or the eye stalks (Chung and Webster 2005).

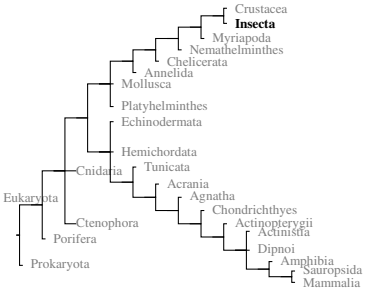
5.5.1.4 Phylogeny

MIH is a crustacean hormone.

5.5.2 Corazonin

Fact sheet 5.21: Corazonin

Sequence:	p**ETFTYSHGWTN**-NH_2.
Synthesis and target:	Corazonin is released in the CNS and acts on abdominal Inka cells.
Function:	Corazonin triggers in Inka cells release of ecdysis-triggering hormone (ETH).
Receptor:	An heptahelical GPCR.



5.5.2.1 Introduction

Corazonin was originally found and characterized by Veenstra in cockroaches and thereafter in other insects (Veenstra 1989). A corazonin gene in beetles could not be found. Corazonin's role has only recently been identified by Kim et al. (2004b). It is the earliest peptide acting in the molting cascade.

5.5.2.2 Biochemistry and Structure

The corazonin peptide has 11 amino acids, a N-terminal pyroglutamate, and a C-terminal amide. Variations between different insects are mostly due to residue 7: histidine in bees and arginine in cockroaches and in flies (Fig. 5.22). The corazonin receptor is a GPCR of the rhodopsin family (Cazzamali et al. 2002).

5.5.2.3 Physiology

Corazonin is a brain neuropeptide. It is synthesized by one pair of dorsomedial neurons, three pairs of dorsolateral cells in the brain, and by eight pairs of cells in the ventral cord. The dorsolateral corazonin neurons survive metamorphosis, however,

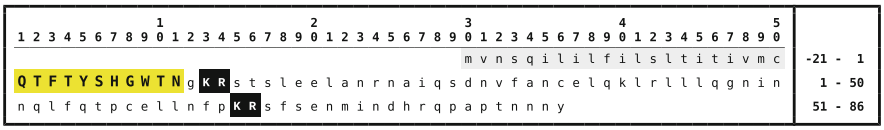


Fig. 5.22 Peptide sequence of corazonin from the honeybee (*A. mellifera*). The signal peptide is highlighted *gray*, and the corazonin peptide *blocked, highlighted yellow and in uppercase*. Dibasic motifs are *inverted* (Source: Q5DW47)

the others undergo apoptosis therein effected by ecdysone. After the decay of the pre-ecdysial ecdysone peak corazonin neurons secrete corazonin which triggers pre-ecdysis-triggering hormone (PETH) and ETH in abdominal Inka cells.

Corazonin has originally been described as a cardioacceleratory peptide. This function appears of minor importance now. Additionally corazonin acts as dark color inducing hormone (DCIN) because it induces dark pigmentation in certain albino locusts.

5.5.2.4 Phylogeny

As of today, corazonin has been found in insects, but not in every species analyzed.

5.5.3 Ecdysis-Triggering Hormone (ETH)

5.5.3.1 Introduction

More than 100 years ago, Ikeda postulated a role of the silk moth's epitracheal glands in air uptake of tracheae (Ikeda 1913). Zitnan et al. (1996) isolated ETH from Inka cells and demonstrated in additional studies its role in ecdysis and metamorphosis.

Fact sheet 5.22: Ecdysis-triggering hormone (ETH)

Sequence: Table 5.7.

Synthesis and target: ETH is the product of epitracheal Inka cells. It acts on the CNS.

Function: By ETH pre-ecdysis and ecdysis are triggered. Moreover stimulated by ETH tracheae fill with air.

Receptor: An heptahelical GPCR.

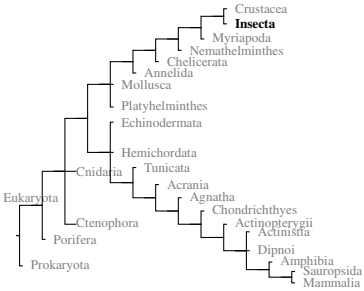


Table 5.7 Ecdysis-triggering hormones

Species	Short name	Sequence
B. mori, *M. sexta*	**PETH**	**SFIKPN.NVPRV**-NH_2
B. mori	**ETH**	**SNEA FDEDVMGYVIKSNKNIPRM**-NH_2
M. sexta	**ETH**	**SNEAISPFDQGMMGYVIKTNKNIPRM**-NH_2
D. melanogaster	**ETH1**	**DDSSPGFFLKITKNVPRL**-NH_2
D. melanogaster	**ETH2**	**GE..NFAIKNLKTIPRI**-NH_2
A. aegyptii	**ETH1**	**DETPGFFIKLSKSVPRI**-NH_2
A. aegyptii	**ETH2**	**GDFENFFLKQSKSVPRI**-NH_2
B. mori	**ETH-AP**	**NYDSGNHFDIPKVYSLPFEFYGDNEKSLNNDDAEE...YYAKKMGSM**-OH
M. sexta	**ETH-AP**	**NYDSENRFDIPKLYPWRAENTELYEDDAQPTNGEEINGFYGKQRENM**-OH

5.5.3.2 Biochemistry and Structure

The silk moth (*B. mori*) precursor bears—as in the tobacco hornworm—three different peptides: the pre-ecdysis—triggering hormone (PETH), ETH, and associated peptide (ETH-AP). In fruit flies (*D. melanogaster*) and in the yellow fever mosquito (*A. aegyptii*) there are two ETH peptides. The structural motif is **I/LKxxKxI/VPRx**amide.

5.5.3.3 Physiology

ETH and PETH are hormones of the molting cascade: In Inka cells the neuropeptide corazonin stimulates an increase of intracellular cGMP. This induces ETH synthesis and release. ETH in turn acts locally on tracheae which fill with air. ETH also acts in an endocrine way on EH and CAP neurons and together with these neurons and their hormones induce the behavioral patterns of ecdysis.

5.5.3.4 Phylogeny

ETH has only been found in insects. An ETH receptor like protein was identified in echinoderms

5.5.4 Eclosion Hormone (EH)

5.5.4.1 Introduction

As the isolation of TRH from thousands of sheep hypothalami before, the isolation of EH from 1,700 tobacco hornworm larvae was a heroic task. This isolation resulted in 3.5 μg available hormone for chemical analysis. Marti et al. (1987) determined thereof the sequence of the 62 amino acids of EH. Following PTTH which stimulates ecdysone biosynthesis, EH was the second hormone of the molting cascade to be identified.

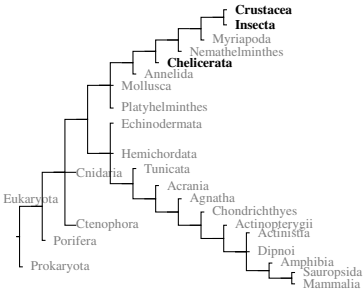
Fact sheet 5.23: Eclosion hormone (EH)

Sequence: Fig. 5.23.

Synthesis and target: EH is the product of ventromedial neurons that acts on Inka cells.

Function: EH stimulated the ecdysis program *i.a.* by activation of CAP release.

Receptor: EH receptor is a receptor guanylate cyclase.



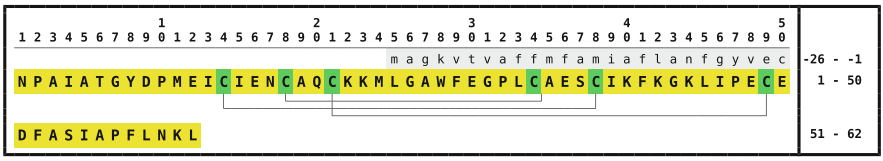


Fig. 5.23 Primary sequence of the eclosion hormone (EH) of the tobacco hornworm. The signal peptide is shown on *gray background*, and the EH polypeptide in *bold* and *uppercase* letters. Disulfide bridges are indicated (Source: P11919)

5.5.4.2 Biochemistry and Structure

Eclosion hormone is cleaved from the precursor by the signal peptidase. The characteristic features are three disulfide bridges which let the peptide resemble the CHH/MIH/ion transport protein family although any significant sequence homology has not been established. Very recently Chang et al. (2009) identified in the *Bactrocera dorsalis* (oriental fruit fly) the membrane guanylate cycles BdmGC-1 as the receptor for EH.

5.5.4.3 Physiology

EH is made in ventromedial neurosecretory cells of the brain and released from neurosecretory cells of the CNS. It is released either centrally or in the periphery: for the latter release axons of EH neurons project to the abdominal proctodeal nerve and EH is released within a neurohemal organ there. Central release takes place in the corpora cardiaca. For the ecdysis program central release is required. The abdominal release stimulates several skin glands (Copenhaver and Truman 1986a,b; Hewes and Truman 1991; Truman 1992, 2005; Gammie and Truman 1999; Truman and Copenhaver 1989).

As shown in the section on ETH EH participates in the ecdysis program. It acts *i.a.* on tracheal filling with air, stimulates ETH release in Inka cells and activates CAP release. EH-defective mutants are unable to perform ecdysis in the normal pattern, but sometimes they eventually escape the old skin.

5.5.4.4 Phylogeny

In GenBank there are insect and crustacean EH sequences and one from a spider.

5.5.5 Bursicon

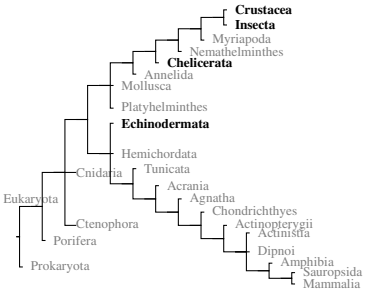
Fact sheet 5.24: Bursicon

Sequence: Fig. 5.24.

Synthesis and target: The bursicon dimer is made in a few neurosecretory cells of thoracic and abdominal ganglia. It acts on epidermal and wing cells.

Function: Bursicon enables hardening of skin and wings after ecdysis.

Receptor: A heptahelical GPCR.



5.5.5.1 Introduction

Some decades ago, it had already become obvious that to finish a molt a longtime unknown hormone stimulated hardening and coloring of the coat. This hormone was called bursicon. Only recently in 2005, the complete bursicon molecule as a dimer of

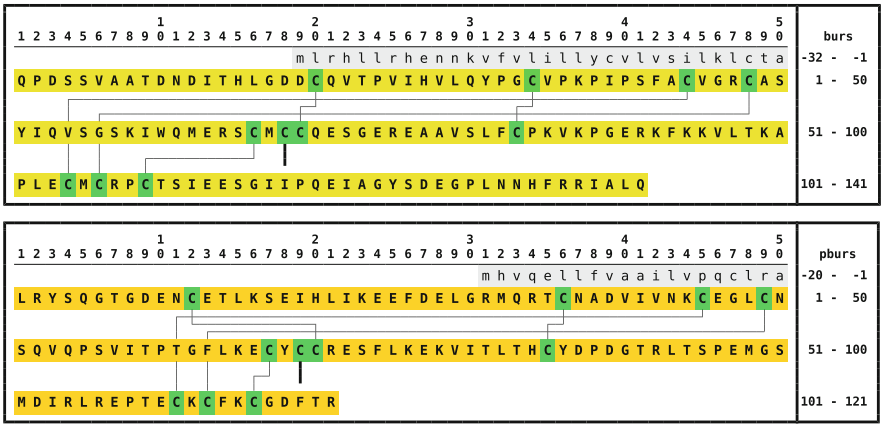


Fig. 5.24 Peptide sequences of the two bursicon subunits from the fruit fly (*D. melanogaster*). The signal peptide is highlighted *gray*; the peptides are in *uppercase*. The intramolecular disulfide bridges taken from the source are indicated. The intermolecular disulfide bridge coupling the two subunits is indicated by *short bold lines* (Source: Q9VD83 (burs) and Q9VJS7 (pburs))

two cysteine knot polypeptides was identified, together with the GPCR specifically binding the dimer of both proteins (Luo et al. 2005).

5.5.5.2 Biochemistry and Structure

Bursicon is formed by the two subunits burs and pburs (partner of burs), with similar composition: after the signal peptide follows a cysteine knot polypeptide. The organization of the cysteine bridges is the same: Cys1 → Cys7[18]; Cys2 → Cys8; Cys3 → Cys9; Cys4 → Cys10; Cys5 intermolecular; and Cys6 → Cys11. Homology exists between the respective subunits in different insect species analyzed. In the fruit fly and the red flour beetle the receptor for the bursicon heterodimer (burs/pburs) is a GPCR with characteristic analogies to the glycoprotein hormone receptors of vertebrates, the glycoprotein hormones equally heterodimers of cysteine knot proteins.

Burs and pburs have been found expressed in the same and in different cells (Honegger et al. 2008). A homodimer has been shown to be active different from bursicon to activate immune and stress genes (An et al. 2012).

5.5.5.3 Physiology

The known functions of bursicon are the induction of the stabilization of wings and coat coloring. The bursicon dimer is synthesized in some cells of the subesophageal ganglion, and thoracic and abdominal ganglia (SEG, TG, AG), and released by neurosecretion. Pburs is additionally expressed in an intrinsic cell of corpora cardiaca. For the tobacco hornworm, this has been recently analyzed in detail (Table 5.8; Dai et al. 2008).

During larval stages, bursicon is expressed in the SEG and TG; in the pupa all CNS ganglia bear some bursicon-expressing neurons, in the adult animal a pair of

Table 5.8 Differential expression of bursicon in the nerve system of the tobacco hornworm (abbrev.: CAP: cardioaccelatory peptide; CC: corpora cardiaca; SEG: subesophageal ganglion; TG: thoracic ganglion; AG: abdominal ganglion; TAG: terminal abdominal ganglion; St-3 larvae: third instar larvae (Source Dai et al. 2008)

	3rd instar larvae	Cells	Cotranslated	Pupae	Cells	Cotranslated	Adult	Cells	Cotranslated
CC	pburs	›10		Not shown			pburs	5–7	AKH
SEG	Bursicon	2 × 2	CAP 1 pair	Bursicon	2 × 2		None		
TG1	Bursicon	2 × 2	CAP 1 pair	Bursicon	2 × 2		None		
TG2/3	Bursicon	1 × 2		Bursicon	2 × 2		None		
AG1	Bursicon	1 × 2		Bursicon	2 × 2	CAP 1 × 2	Bursicon	3 × 2	CAP 1 × 2
AG2	Bursicon	1 × 2		Bursicon	2 × 2	CAP 1 × 2	Bursicon	3 × 2	CAP 1 × 2
AG3-5	None			Bursicon	2 × 2	CAP 1 × 2	Bursicon	2 × 2	CAP 1 × 2
AG6	None			Bursicon	1 × 2		Bursicon	3 × 2	CAP 1 × 2
TAG	None			Bursicon	1 × 2		Bursicon	3 × 2	CAP 1 × 2

[18]Counted from the N-terminus: Cys1 first cysteine, Cys2 second cysteine . . .

cells in the SEG and in seven pairs of cells in abdominal ganglia still maintain some bursicon expression. Bursicon's role has been reviewed very recently (White and Ewer 2013): it is responsible for postecdysial behavior which is governed by a pair of subesophageal cells and for wing expansion and hardening under the endocrine control of seven pairs of abdominal neurosecretory bursicon cells.

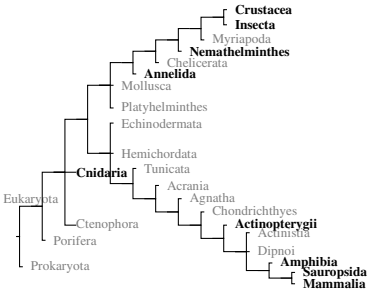
5.5.5.4 Phylogeny

Bursicon dimers have been found in insects, crustaceans, and chelicerates. A bursicon-like protein has been sequenced in echinoderms.

5.6 Regulators of Food Intake: RFamide and FMRFamide

Fact sheet 5.25: FMRFamides

Sequence:	**FMRF**-NH_2 and N-terminally extended analogues.
Synthesis and target:	FMRFamides originate in neurons and act via synapses on GPCR or ion channels.
Function:	FMRFamides were identified for their muscle contraction activity.
Receptor:	FMRF receptors in insects and nematodes are heptahelical GPCR.



5.6.1 Introduction

Thirty years ago Price and Greenberg (1977) described the isolation of **FMRF**-NH_2 as a cardiostimulatory peptide from the sunray Venus clam (*Macrocallista nimbosa*), equally active inducing contraction of the radula muscle of the lightning whelk *Busycon contrarium*. In the meantime many vertebrate and invertebrate RFamide peptides have been isolated, that is, kisspeptins, prolactin releasing peptide. Neuropeptide FF (NPFF) (**FLFPQPQRF**-NH_2) and the cotranslated NPAF or NPSF have a role on nociception in human and other mammals (Vilim et al. 1999 and references). Other RFamide peptides have been found expressed in the mammalian and human brain, for example, the RFamide-like peptide in the hypothalamus, but their function has escaped elucidation thus far (Bechtold and Luckman 2007).

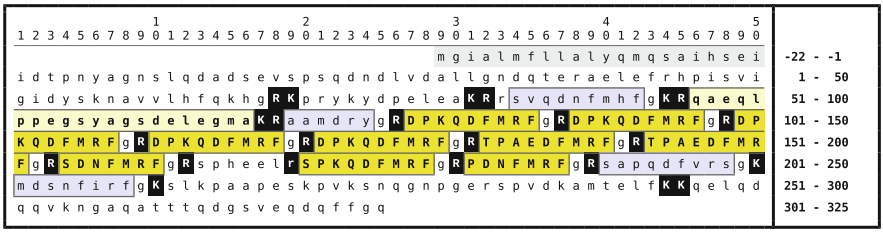


Fig. 5.25 Primary sequence of the FMRF precursor from the fruit fly. The signal peptide is highlighted *gray*, peptides discovered chemically are *boxed*, FMRF peptide *bold*, on *yellow background* and in *uppercase*, and additional peptides in *lowercase*; within the precursor a CRH-like peptide is found: 96–116. Monobasic and dibasic peptide motifs for prohormone convertase are shown *inverted*; all those glycine residues in front of these monobasic or dibasic motifs are oxidized to NH_2 by PHM (Source: P10552)

In invertebrates some species have multiple FMRFamide-like genes; for example, in *C. elegans* there are 22 RFamide genes (*flp-1* to *flp-22*) that can be translated into 50 peptides.[19]

5.6.2 Biochemistry and Structure

Whereas in molluscs the tetrapeptide **FMRF**-NH_2 is found, in insects the FMRF-amides are N-terminally extended, for example, in *D. melanogaster* (Fig. 5.25).

In *C. elegans* only FLP have been found (see Table 5.9), in toto 63 different RFamides in 23 genes whereas these genes, however, code for additional neuropeptide without RFamide C-termini. Mostly the precursor proteins contain a single FLP type, however, in multiple copies: flp1, flp3, flp7, or flp18. Sometimes a precursor harbors different FLP types: flp14 or flp17.

In addition to PC1 or PC2 motifs, there are **RxxR**, **RxxK**, and monobasic cleavage sites for prohormone convertases, for example, in the great pond snail *L. stagnalis* (Fig. 5.26). A furin-like enzyme (cleaving **RxxR** or **RxxK**) has been described in *L. stagnalis* (Smit et al. 1994). Southey et al. (2008) evaluated the usage of potential cleavage sites in insects and showed that in those organisms analyzed, an endopeptidase cutting after **RxxK** or after a single **K** was not observed. Cleavage required either dibasic **RK** or **KK** or **RxKK** motifs. A similar analysis in molluscs had been published before by Spijker et al. (2004). It appears, however, fairly impossible with eight FMRFamides at hand to identify those that had not been liberated. Three of seven FMRFamides are cleaved by an RxxR recognizing PC, for example, furin. One of the seven by PC1 (**KR**) and three others by an

[19]For reasons of conciseness we call FMRFamides those peptides with N-terminally extended **FMRF**amide, and variants, for example, **FIRF**amide are labeled FLP for FMRFamide-like peptides

Table 5.9 FMRFamide-like peptides in *C. elegans*

Gene	Example	Consensus	Peptide count
flp-1	**SADPNFLRF**-NH_2	PNFLRF	6
flp-2	**SPREPIRF**-NH_2	EPIRF	2
flp-3	**SAEPFGTMRF**-NH_2	GTRMRF	9
flp-4	**PTFIRF**-NH_2	FIRF	2
flp-5	**AKFIRF**-NH_2	KFIRF	3
flp-6	**KSAYMRF**-NH_2	YMRF	1
flp-7	**SPMQRSSMVRF**-NH_2	MVRF	4
flp-8	**KNEFIRF**-NH_2	FIRF	1
flp-9	**KPSFVRF**-NH_2	FVRF	1
flp-10	**QPKARSGYIRF**-NH_2	YIRF	1
flp-11	**AMRNALVRF**-NH_2	L/FVRF	2/1
flp-12	**RNKFEFIRF**-NH_2	FIRF	1
flp-13	**APEASPFIRF**-NH_2	PFIRF	6
flp-14	**KHEYLRF**-NH_2	YLRF	1
flp-14	**SLLDYRF**-NH_2	DYRF	1
flp-14	**EIVFHQISPIFFRF**-NH_2	FFRF	1
flp-15	**GGPQGPLRF**-NH_2	GPLRF	2
flp-16	**AQTFVRF**-NH_2	FVRF	2
flp-17	**KSQYIRF**-NH_2	YIRF	1
flp-17	**KSAFVRF**-NH_2	FVRF	1
flp-18	**EMPGVLRF**-NH_2	PGVLRF	6
flp-19	**WANQVRF**-NH_2	Q/SVRF	1/1
flp-20	**AMMVRF**-NH_2	MVRF	1
flp-21	**GLGPRPLRF**-NH_2	PLRF	1
flp-22	**SPSAKWMRF**-NH_2	WMRF	1
flp-23	**TKFQDFLRF**-NH_2	FLRF	3

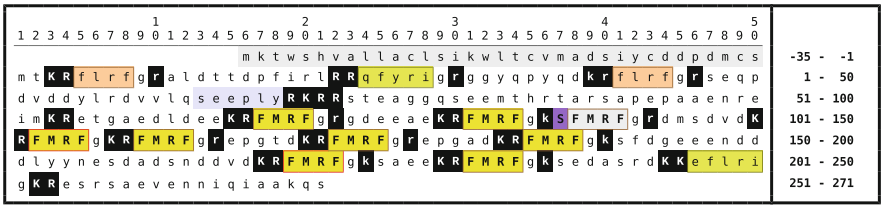


Fig. 5.26 Primary sequence of the FMRF precursor from the great pond snail (*L. stagnalis*). The signal peptide is highlighted *gray*. FMR- like peptides are *bold* and in *uppercase*; additional peptides are in *lowercase*. Monobasic and dibasic peptide motifs for prohormone convertase are shown *inverted*; all those glycine residues in front of these monobasic or dibasic motifs are oxidized to NH_2 by PHM. Whether a **SFMRF**amide (137–141) has ever been isolated is not described (Source: P19802)

unknown RxxK recognizing PC. In flies, in contrast, all 10 FLP can be cleaved by RxxR- recognizing furin, whereas the associated peptides exhibit a **RxxK** motif. It might well be that the degree of FMRF release and of associated peptides is regulated by the differential expression of prohormone convertase, or that although the FMRFamides are conserved, they are not released.

The thus far identified receptors for FMRFamides are GPCR: for example, in *D. melanogaster* (genbank AAF47700) or in *C. elegans* (GenBank ACG61342). Of the many FLP in *C. elegans* only *flp10* and *flp17* coded peptides bind to this receptor.

5.6.3 Physiology

As far as can be said today, (FM)RFamides act preferentially as neurotransmitters, and less as hormones *in sensu proprio*. Immunohistology in vertebrates with fluorescent antibodies most often generates an image of the entire nervous system, because in some areas more than 40 % of all neurons are labeled (Pernet et al. 2004). There is, however, for example, in *C. elegans* differential expression of different *flp* genes pointing to special functions of individual peptides or peptide types. Only for a few of them have specific receptors been discovered. Some hints to flp functions may come from the analysis of defect mutants: *flp-1* defect nematodes do not sense hyperosmolarity any further; they do not react to contacts with the nose, but react to contacts with the remaining body. They exhibit a variant movement coordination compared to wildtypes (Li et al. 1999).

FMRFamide was originally found as a peptide inducing muscle contraction in snails. A similar role has been observed in earthworms (*Eisenia fetida*, *Lumbricus terrestris*; Csoknya et al. 2005). In anthozoans (Cnidaria), too, muscle cells were contracted by antho-RFamide neurons (Pernet et al. 2004). It has been supposed that food intake and reproduction are controlled by antho-RFamides. Tessmar-Raible et al. (2007) have argued that RFamide neurons are multifunctional sensory cells that present an ancient developmental pattern in invertebrates (annelids, molluscs, hemichordates, and cephalochordates) as well as in fish (at least in teleosts).

Lingueglia et al. (1995) identified apart from the GPCR in garden snail *Helix aspersa*, a FMRF-controlled sodium channel. This amiloride sensitive channel assembled by four identical subunits is largely analogous to the renal human epithelial sodium channel sodium channel (eNaC) required for sodium resorption. This molluscan channel is preferentially opened by the tetrapeptide **FMRF**amide. **FLRF**amide is partial agonist and **FKRF**amide is an antagonist. N-terminally elongated peptides are less active than the original tetrapeptide (Cottrell 1997).

Similar sodium channels have been found in *C. elegans*; they were called degenerines because single point mutation led to neuronal degeneration. In this species these channels have a role in tactile sensing; they do not act as FMRFamide receptors.

5.6.4 Phylogeny

RFamides and FMRFamides have been found in many eumetazoans. Although in protostomes peptides are present in multiple copies on the precursor, the neuropeptide FF in humans exists as a single copy. Phylogenetic evaluations have not been performed; some vertebrate RFamides have been listed by Osugi et al. (2006). Apart from neuropeptide FF, kisspeptin and prolactin-releasing peptide are among the RFamides.

5.7 Neuropeptide Regulators of Juvenile Hormone Metabolism

5.7.1 Allatotropins

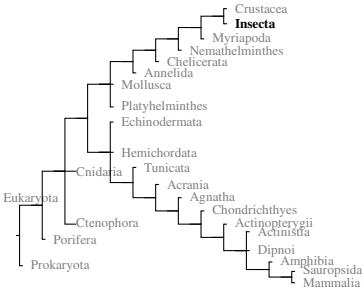
Fact sheet 5.26: Allatotropin

Sequence: **GFKNVEMMTARGF**-NH_2.

Synthesis and target: Allatotropins are released by neurosecretory brain neurons in the corpora cardiaca and act on the corpora allata.

Function: Allatotropins are stimulators of juvenile hormone synthesis.

Receptor: The allatotropin receptor is a heptahelical GPCR.



5.7.1.1 Introduction

Allatotropin (Fig. 5.27) is produced in neurosecretory brain cells, released in the corpora cardiaca and acts via the hemolymph in the corpora allata stimulating juvenile hormone biosynthesis.

5.7.1.2 Biochemistry and Genes

The *M. sexta* allatotropin (MAS-AT) is generated by PC1 and a furin-like protease from the precursor. It is C-terminally amidated.

The allatotropin receptor has recently been discovered as GPCR (Yamanaka et al. 2008).

5.7.1.3 Physiology

The balance between ecdysone and juvenile hormone is critical for the outcome of molting. If JH is missing, the imago develops. An additional dose of JH, in contrast,

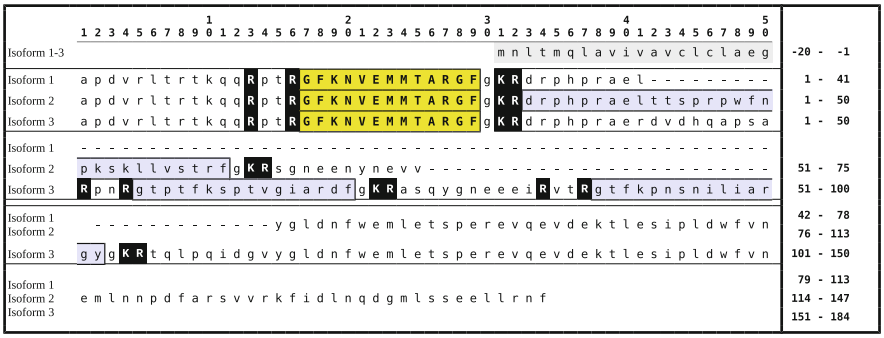


Fig. 5.27 Primary sequence of the allatotropin precursor of the tobacco hornworm (*M. sexta*). Three isoforms derived by alternative splicing give rise to the allatotropin and to additional peptides. The signal peptide is highlighted *light gray*, allatotropin *boxed, bold* and in *uppercase*, and other peptides are *boxed*. RxxR- and dibasic peptide motifs for prohormone convertases are *inverted*; glycine residues in front of **KR** motifs are oxidized to amides (Source: P21786)

blocks metamorphosis and leads to an additional instar. Allatotropin regulates the JH production in a thus far unknown way.

Allatotropin is made in a few neurosecretory cells of the insect brain. The control of its release in the corpora cardiaca has not been sufficiently analyzed.

In addition, allatotropin is expressed during the development of the antennal lobe in *M. sexta* (Utz et al. 2008) and further acts on gut contractions.

5.7.1.4 Phylogeny

The known allatotropins were discovered in butterflies, flies, and beetles. The five C-terminal amino acids **TARGF**-NH_2 are conserved, a synthetic **TARGF**-NH_2 has been found to stimulate juvenile hormone biosynthesis, too. In further insects (e.g., in locusts) the allatotropin activity is present: brain peptides stimulate JH synthesis and the peptide has not yet been isolated. The *B. mori* allatotropin is identical to that of *M. sexta*. For *D. melanogaster* an investigation in the protein database looking for the peptide **TARGFGKR** did not succeed. **TAMRGF** could be found, but the prohormone convertase motifs were lacking.

In two annelids allatotropin-like precursors have been found, however, from these precursors no peptides can be cleaved that might resemble allatotropin (GenBank P46978 and P46980).

5.7.2 Allatostatins

5.7.2.1 Introduction

With use of isolated ıcorpora allata in vitro, three peptide types inhibiting JH biosynthesis have been isolated from different insect species: those from cockroaches were labeled type A, those from crickets type B, and lepidopteran allatostatins as type C.

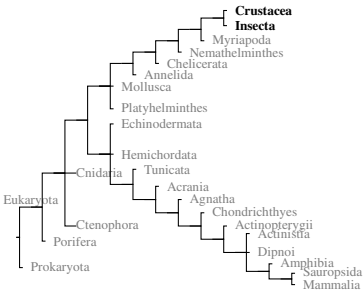
Fact sheet 5.27: Allatostatins (AST)

Sequence: Three types: A-type: Y/F-X-FG-L/Iamide
B-type: WX_6Wamide
C-type:
EVRFRQ C YFNPIS C F-OH
with an intramolecular disulfide bridge.

Synthesis and target: The action of allatostatins on the juvenile hormone biosynthesis takes place at the axon ends of AST neurons in the corpora allata.

Function: AST inhibit juvenile hormone biosynthesis and are involved in additional regulations.

Receptor: Type-A receptors are GPCR of the galanin family, a type-B GPCR belongs to the bombesin family, and type-C receptor are members of the somatostatin/opioid GPCR family.



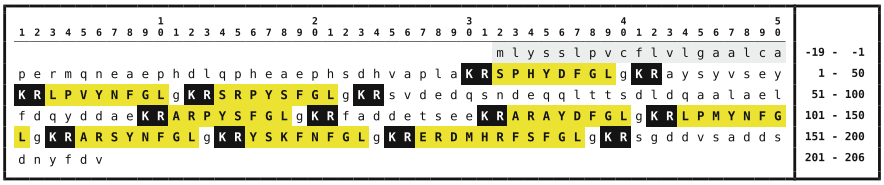


Fig. 5.28 Primary sequence of the *Helicoverpa armigera* allatostatin precursor (type-A): Allatostatins (pattern **F/Y-x-FGL**-NH_2) are on *yellow background* and in *uppercase*; additional peptides are *in lowercase*. Dibasic prohormone convertase peptide motifs are *inverted*; glycines in front of those are all oxidized to amides (Source: O44314)

In vivo, however, not all of these peptides are functional or not always (see Audsley et al. 2008).

5.7.2.2 Biochemistry and Structure

Type A allatostatins are characterized by the *Y/F-X-FGL*-amide motif and by their origin from large precursors with more than 30 single peptides (Fig. 5.28). Only PC1 acts on the (**KR** motif) to release AST, whereas other peptides from the same precursor are cleaved by PC1 plus a furin-like enzyme.

Type B allatostatins with their **Wx$_6$W**-NH_2 have only been found in crickets thus far. Although type A AST act in additional species and insect families, type B AST are only active in crickets. They are related to other, myoinhibitory peptides in other families (Stay and Tobe 2007). It is only in crickets that these type B peptides act allatostatically (Audsley et al. 2008).

Type C allatostatins from different species possess in each case one single sequence: for example, pyro-**EVRYFRQ C YFNPIS C F**-OH in *D. melanogaster* with an intramolecular disulfide bridge and an N-terminal pyroglutamate. Such a peptide is active as allatostatin in moths and mosquitoes.

It is noteworthy that the allatotropin-2 from the moth *Spodoptera frugiperda*, **RVRGNPISCF**, exhibits remarkable C-terminal sequence homology to C-type AST.

The AST receptors are GPCR from different families: type A—galanin receptor family; type B—bombesin receptor family; type C—somatostatin/opioid receptor family.

5.7.2.3 Physiology

In the tobacco hornworm the allatostatic effect has been discovered for a type-C peptide: the JH biosynthesis in isolated corpora allata of fifth instar larvae could be inhibited by the isolated peptide 0–4 h, as well as 24 h after molt and in 3-day-old adults, too (Kramer et al. 1991; Audsley et al. 2008). JH synthesis was inhibited in the same organs of other species, however, not as completely as in *M. sexta*.

The focus on the larval stage where inhibition could be observed is necessary and the pointer to in vivo or in vitro is important because any effect could rarely be observed in vivo. Whereas the *M. sexta* type-C peptide showed activity at concentrations of 10 nM, in the organs of other species levels of 1 μ M were required for inhibition.

It would be relevant, too, to note whether constitutive JH biosynthesis had been inhibited or (only) an experimental increase by allatotropin. In summary, only few species have been analyzed in detail and the findings do not yet appear conclusive (compare the recent reviews by Audsley et al. 2008 and Stay and Tobe 2007).

The immunological characterization of allatostatin receptor is also far from being finished.

5.7.2.4 Phylogeny

Allatostatins have thus far only been found in insects and crayfish, whereas allatostatin receptor-like proteins have been discovered in placozoans, nematodes, and echinoderms.

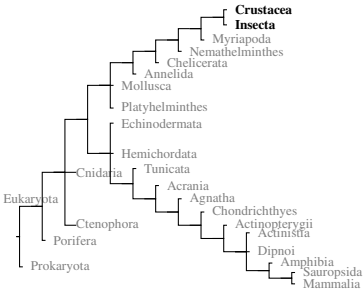
5.8 Peptide Hormones of Skin: Pigment-Dispersing Hormone

5.8.1 Introduction

Adaption to light in eyes and skin is mediated in crustaceans by two antagonistic peptide hormones: red-pigment-concentrating hormone (RPCH/AKH) and pigment-dispersing hormone (PDH) called pigment-dispersing factor (PDF) in insects. The discovery of an endocrine influence on dark/light adaption stems from Koller and Perkins (reviewed by Rao 2001). In insects PDF expression is directly coupled to neurons of the circadian pacemaker.

Fact sheet 5.28: Pigment-dispersing hormone (PDH)

Sequence:	**GFKNVEMMTARGF**-NH_2.
Synthesis and target:	The PDH is a hormone of the eye stalks and acts on the skin of crayfish.
Function:	Influenced by PDH the crustacean skin decolorizes.
Receptor:	The PDH receptor is a GPCR of the secretin family.



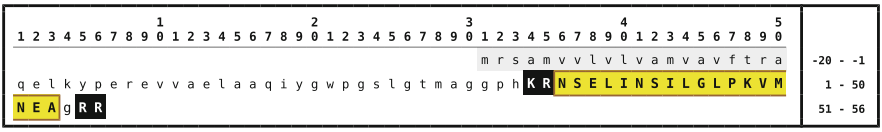


Fig. 5.29 Primary sequence of pigment-dispersing hormone (PDH) from spiny-cheek crayfish *O. limosus*. The PDH is *boxed* and in *uppercase*. Dibasic peptide motifs for prohormone convertase are *inverted*; the glycine placed in front is oxidized to amide (Source: P37085)

5.8.2 Biochemistry and Structure

Pigment-dispersing hormones (PDH; Fig. 5.29) are peptides of 18 amino acids with conserved N- and C-termini as well as other conserved amino acids. The consensus sequence for β-PDH found in crustaceans *and* insects is **NSELINSxLxxSxxxxxA**-NH_2. α-PDH has only been found in a few crustacean species; its consensus sequence is **NSGMINSILGIPxVMxxA**-NH_2. It is noteworthy that in the ocean shrimp *Pandalus jordani* in addition to two different α-PDH a β-PDH had been sequenced, too (see Table 5.10).

PDF receptors in nematodes and insects are GPCR of the secretin family; in crustaceans no receptors have been deposited in GenBank.[20]

5.8.3 Physiology

PDH are products of the eye stalk. Most PDH neurons secrete into the neurohemal organ sinus gland; some are, however, not connected to the sinus gland.

In crustaceans PDH serves light/dark adaption. Influenced by PDH and its antagonist PCH pigments in the eye's ommatides are moved and light adapted.

[20] As of September 2014.

Table 5.10 Sequence comparison of PDH from crustaceans and insects

	Sequence
β-PDH	
Crustaceans:	
Uca puligator, *Cancer magister*, *C. maenas*, *Pastifastacus leniusculus*, *Callinectes sapidus* I	**NSELINSILGLPKVMNDA**-NH_2
Callinectes sapidus II	**-------L--ISAL--E**--NH_2
P. clarkii, *O. immunis*, *O. limosus*	**----------------E**--NH_2
Penaeus aztecus, *Penaeus vannamei* I/II	**----------I--------**-NH_2
Penaeus vannamei III	**-------L----------**-NH_2
Penaeus japonicus I	**----------I----T---**NH_2
Penaeus japonicus II	**-------------F-I---**NH_2
Pandalus jordani I	**---------------T---**NH_2
Armadillidium vulgare	**----------A-R-L-N**--NH_2
PDF	
Insects:	
Periplaneta americana	**--------------L----**NH_2
Acheta domesticus	**---I----------L----**NH_2
Romalea microptera	**---I---------LL----**NH_2
Carausius morosus	**---------A----L----**NH_2
D. melanogaster	**---------S---N-----**NH_2
α-PDH	
Crustaceans:	
Pandalus borealis, *Pandalus jordani* II	**--GM---I--I-R--TE**--NH_2
Pandalus jordani III	**--GM---I--I----A---**NH_2
Macrobrachium rosenbergii	**--GM---I--I----AE**--NH_2

In insects it was shown that PDF neurons are involved in the regulation of the circadian pacemaker. They control daylight-dependent fly activity. PDF has been localized to lateral brain neurons found in larvae in the Bolwig organ and in adult flies in the extraretinal eyelet (Helfrich-Forster et al. 2002).

5.8.4 Phylogeny

GenBank contains PDH/PDF sequences only from crustaceans and insects.

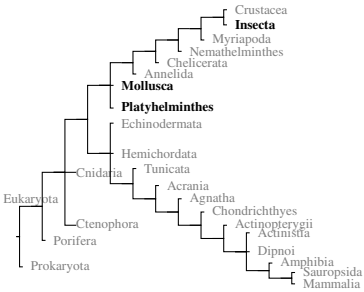
Fact sheet 5.29: Neuropeptides F (NPF)

Sequence: Fig. 5.30.

Synthesis and target: NPF is made in a few neurosecretory cells of the CNS.

Function: NPF is a neurotransmitter and involved in circadian control of foraging in larvae. It plays in additional role in reaction to stressors.

Receptor: The NPF receptor is a heptahelical GPCR of the rhodopsin family.



5.9 Other Neuropeptides

5.9.1 Neuropeptide-F—Two Peptide Genes

5.9.1.1 Introduction

Neuropeptide-F is considered as the invertebrate homologue of neuropeptide Y. The sequence homology, however, is five of 36–40 amino acids, one proline residue close to the N-terminus, one or two tyrosines with a conserved spacing, and two arginines at the C-terminal end (highlighted *red* in Fig. 5.30). The analogy, however, extends to NPF/NPY receptor homology and to functional activities. It has been, for example, possible to use anti-PYY antisera (against the vertebrate PYY) to identify neurons that later turned out to express NPF.

There is another "short neuropeptide-F" (sNPF) not to be mixed up with neuropeptide-F (NPF). The four sNPF1..4 peptides in *D. melanogaster* are coded for by a different gene and differentially expressed compared to NPF. Although NPF has been discovered in platyhelminthes, that is, in an original metazoan species, sNPF has only been found in arthropods.

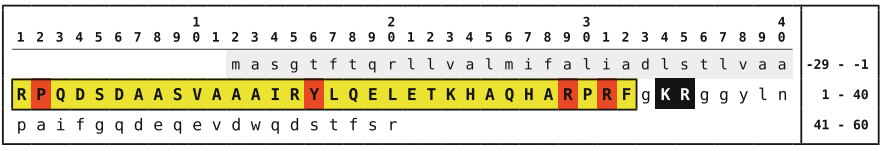


Fig. 5.30 Primary sequences of neuropeptide F from *A. aegyptii*. The signal peptide (−29 to −1) is highlighted *light gray*. NPF (*boxed*) is cleaved by PC1. The mature peptide is C-terminally amidated; the [*red highlighted*] amino acids are identical with mammalian neuropeptide Y

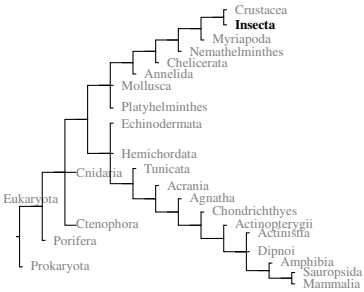
Fact sheet 5.30: Short neuropeptide F (sNPF)

Sequence: Fig. 5.31.

Synthesis and target: sNPF is made in neurosecretory cells of the CNS and acts preferentially as a neurotransmitter.

Function: sNPF is expressed in the olfactory sensory cell and is involved in circadian control of larval foraging.

Receptor: sNPF receptor is a heptahelical GPCR.



5.9.1.2 Structure and Genes

The NPF peptide is cleaved from the precursor by the signal peptidase and a PC1. It is amidated C-terminally by PHM.[21]

sNPF was originally isolated as NPF-like peptide from beetle brain (Led-NPF; Spittaels et al. 1996). Further sNPF have been identified in several other insect families. sNPFf of *A. gambiae* are cleaved from the precursor by the signal peptidase, a PC1/PC2, and a furin-like peptidase (see Fig. 5.31). C-termini are amidated. It is not known which enzyme(s) release sNPF2 or sNPF4. The NPF and the sNPF receptors are heptahelical GPCR of the rhodopsin family with homology to NPY receptors.

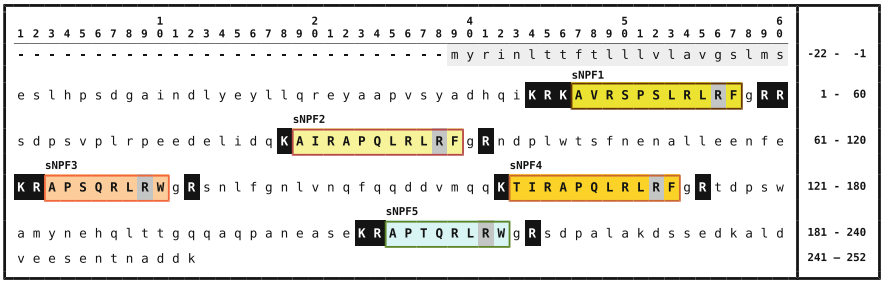


Fig. 5.31 Primary sequence of short neuropeptide F (sNPF) from *A. gambiae*. The signal peptide is highlighted *light gray*. Five sNPF are *boxed*. They are C-terminally cleaved by a furin-like peptidase (motif **RxxR**) and N-terminally by prohormone convertases or other enzymes. Mature peptides are C-terminally amidated

[21] Peptidylglycine alpha-hydroxylating monooxygenase.

5.9.1.3 Physiology

NPF is a neuropeptide found in arthropods, molluscs, and flatworms. In *D. melanogaster* there are four NPF-forming neurons with axons projecting in other brain areas and the ventral nerve cord. Due to its presence in neurohemal organs, for example, in the corpora cardiaca NPF is regarded as a neurohormone. In turbellarians NPF supports organ regeneration: these animals are capable if, for example, the head has been removed to generate fully with the help of NPF (Kreshchenko 2008; Kreshchenko et al. 2008).

The sNPF gene has, however, only been found in insects: flies, beetles, and mosquitoes, for example. It is made in many neurons. A detailed analysis has recently been published (Nässel et al. 2008). Because of its presence in the hemolymph, sNPF is regarded as a neurohormone and as a neurotransmitter, too. The majority of sNPF neurons have been positive for other neurotransmitters. The conclusion thus is that sNPF acts preferentially as a neurotransmitter.

In *D. melanogaster* additional NPF-releasing endocrine cells were detected in the middle gut (Veenstra et al. 2008), sNPF secreting cells, however, could not been found. NPF-secreting cells contained tachykinins, as well; sNPF was discovered in hypocerebral ganglia which innervate the foregut.

NPF as well as sNPF are considered involved in foraging and food intake, in analogy to NPY. sNPF has an additional role in olfaction in flies. NPF and NPF receptor expression studies using transgenes or RNA interference, have demonstrated that without NPF signals flies reject food, whereas an enhancing NPF induced signals resulted in prolonged food intake (Nässel et al. 2008). Yamanaka et al. (2008) have argued that sNPF are coupling food intake to juvenile hormone synthesis. During diapause, NPF had not been expressed in potato beetles (*Leptinotarsa decemlineata*) (Huybrechts et al. 2004).

NPF expression also appears sex dependent: some particular NPF expression in male flies was found regulated by the *transformer* gene with an involvement of *fruitless*. When this sex-dependent NPF expression is disabled, male flies change their courtship behavior. This NPF expression is additionally controlled by genes generating circadian rhythms that are expressed in neurons in close proximity to NPF neurons (Lee et al. 2006). These authors discovered an NPF role in body size regulation: with NPF expression prevented, flies got remarkable larger than those with normal NPF expression.

Looking for additional analogies of NPF and NPY, Dierick and Greenspan (2007) observed that NPF knockout flies were more aggressive than wildtype flies, independently of the effect mediated by an enhanced serotonin level.

5.9.1.4 Phylogeny

NPF has been found in different invertebrates, from flatworms on, in molluscs and in insects. The structural homology, including that of the NPF receptor, to the NPY/PYY proteins and their receptors in vertebrates suggests that this molecule might be present in the entire bilaterian subregnum.

As of today, sNPF is a peptide of insects.

5.9.2 Proctolin

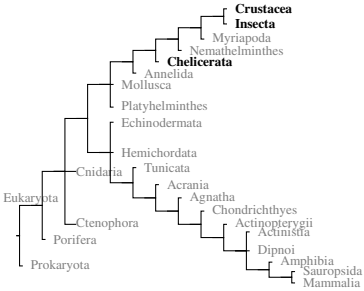
Fact sheet 5.31: Proctolin (PCT)

Sequence: Fig. 5.32.

Synthesis and target: Proctolin is made by neurons in the CNS and other parts of the insect and crustacean nervous system.

Function: Proctolin acts on muscle activity and enhances the frequency of muscle contractions.

Receptor: The *D. melanogaster* proctolin receptor is heptahelical GPCR.

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          1         2         3         4         5
1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0
                    m g v p r s h g t g i g c g s g h r w l l v w m t v l l l v v p p h l v d g   -28 -  -1
RYLPTRs h g d d l d k l r e l m l q i l e l s n e d p q q q q q q q q q q h p q l r l h n e     1 -  60
a t g g s s s s s n i n n p r v s n g n s n a a w l q k l s a m g a l d e l g g d g a r f g p n y g   60 - 102
r y
```

Fig. 5.32 Primary sequence of proctolins from *D. melanogaster*. The signal peptide is highlighted *light gray*. Proctolin (on *yellow* background and in *uppercase*) is cleaved C-terminally by a furin-like peptidase (motif **RxxxxR**) and N-terminally by the signal peptidase. (source: GenBank CAD30643; the cleavage site of the signal-peptidase was estimated using the program Signal 3.0 (http://www.cbs.dtu.dk/services/SignalP/))

5.9.2.1 Introduction

Cockroach proctolin was the first neuropeptide ever sequenced (Starratt and Brown 1975). Using anti-PCT antisera PCT neurons were identified first in insects, later in crustaceans and even in the horseshoe crab *Limulus polyphemus*, a living fossil.

5.9.2.2 Structure and Genes

Proctolin is a simple pentapeptide without protection at the C-terminus or N-terminus. The molecule is instead by itself an inhibitor of enkephalinases. PCT is cleaved from the precursor by the signal peptidase and a furin-like peptidase (recognition motif **RxxxxR**). The last years have seen an increase in sequenced proctolin precursors.

5.9.2.3 Physiology

PCT acts via a GPCR from the rhodopsin family (in *D. melanogaster*). The attribute of PCT most analyzed is its role in muscle contractions. Using PCT and anti-PCT antibodies the immunohistology to identify PCT neurons has been developed (Bishop et al. 1981; Eckert et al. 1981). PCT neurons have been found in the brain and in thoracic and abdominal ganglia. Siwicki et al. (1985) identified more than 1,400 PCT neurons in lobster. Apart from its activity on muscles, PCT act on the oviduct, too.

Studying leg muscles of locusts Evans (1984) observed that PCT enhances the frequency of muscle contractions by a blockage of potassium influx and the thus-enhanced membrane resistance.

5.9.2.4 Phylogeny

GenBank contains PCT sequences from limulus (Chelicerata), crustaceans, and insects.

5.10 Summary and Overview

Table 5.11 summarizes several important features of invertebrate neuropeptides. All these neuropeptides are active as hormones; they are secreted by neurosecretory cells into the hemolymph and act on distant cells. Those neuropeptides synaptically active have at least endocrine active analogues in vertebrates, the reason to present them here.

Table 5.11 Neuropeptides in insects, crustaceans, and other protostomes

Name	Site of synthesis	Synaptically/endocrine	Vertebrate analog
CHH	X organ	Endocrine	
Bombyxin/ILP	CNS/GI tract	Endocrine	Insulin
AKH	CC	Endocrine	
CAP	Neurons	Paracrine/synaptically	Oxytocin/vasopressin
NdWF	Neurons	??	
Enterins	Neurons	Endocrine/synaptically	
MIP/AMRP	Neurons	Synaptically	
DiuH	Neurons	Endocrine	
Pyrokinin/PBAN	Neurons	Endocrine	
Orcokinins	Neurons	??	
Leucokinins	Neurons	Endocrine?	
TRP	Neurons		Tachykinin
Sulfakinins	Neurons	Synaptically/endocrine	Gastrin, CCK
PTTH	Neurons	Endocrine	
PSP/SP	Male accessory gland	Pheromonal	
GIH/VIH			
TMOF	Gonads	Endocrine	
Nebcolloostatin	??	??	
MIH	X organ	Endocrine/paracrine	
Corazonin	CNS		
ETH	Inka cells	Endocrine	
EH	CNS	Endocrine	
Bursicon	Neurons	Endocrine	
RFamide/FMRFamide	Neurons	Synaptically	Neuropeptide FF
Allatotropin	Neurons	Endocrine	
Allatostatin	Neurons	Synaptically/paracrine?	
PDH	Neurons	Endocrine/synaptically	