

Insect Neuropeptides and their Potential Application for Pest Control

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Our current knowledge regarding primary structure, synthesis, release, receptor-binding, structure-activity relationship and mode of action of insect neuropeptides has increased dramatically during the past decade. Thanks to the development of insect neuroendocrinology – in parallel to this – an even increasing need for modern, yet environmentally sound strategies of plant protection has arisen, becoming a driving force for insect physiologists to concentrate their efforts to combat pests more efficiently. The ultimate aim of these researchers is, however, not the total eradication of harmful insects, but rather, selective targeting by using species- or group-specific control strategies which can only be achieved by taking note of recent results in insect physiology, endocrinology, biochemistry and ecology. The rationale behind this approach is, that, since neuropeptides regulate key biological processes, these “special agents” or their synthetic analogues, mimetics, agonists or antagonists may be effective tools in combating insect pests in an environmentally more sound manner than with conventional pesticides. In this review, taking into account possible practical aspects, some representative insect neuropeptides/groups have been selected, which may be important due to their characteristic structure and/or physiological action, and could be used for the design of novel, safe and selective compounds to control pests.

Keywords: insect neuropeptides, mode of action, safe and selective insecticides, biorational planning of compounds, agonists, antagonists, pseudo-peptide analogues.

All living organisms tend to establish and maintain their internal equilibrium, homeostasis. The continuous interaction of two superimposed control systems – the neural (short term) and endocrine (long-term) – acting concertedly and individually as well, result in the harmony characteristic for multicellular animal systems, including insects. All basic biochemical and physiological processes in insects like growth, development, reproduction, metabolism, ion and water balance, the regulation of typical insect hormones, etc. – otherwise essential for homeostasis – are controlled by the neural system in close relation with (neuro)hormones. The elements of insect neuroendocrine system, its similarities and analogies to higher organisms (e.g. vertebrates), locations of cerebral neurosecretory (NS) centres and circumstances how the active materials are synthesized, the most well-known neuropeptides – grouped into four major classes according to function and/or structure – including mode-of-action with relevance to practical application will be presented.

Basic insect neuroendocrinology

Kopeč (1922) was the first to conclude – based on his studies on the control of moult in the gypsy moth, *Lymantria dispar* – that the brain contains a “factor” responsible for the metamorphosis of the final instar into the pupa. This “factor” is released into the haemolymph (insect “blood”), since severing the neuronal connections between the brain and body does not inhibit pupation, however, removal of brain does. The “brain hormone” is now known as *prothoracicotropic hormone* (PTTH). Since the early thirties classical insect endocrinology studies (including extirpation, organ and tissue transplantations, ligations, transfusions, etc.) revealed that many physiological events are controlled by hormones. Works by Wigglesworth (1935) lead to the discovery of *Corpus allatum* (CA) in the bug, *Rhodnius prolixus*, where another “factor”, later defined as *juvenile hormone* (JH) – responsible for maintaining juvenile characters – is produced (Williams, 1956).

Neuropeptides originating from the brain are produced by NS cells, the latter bearing all morphological and functional characteristics of a nerve cell, but at the same time synthesize hormones, like gland cells. The role of these neurons in communication with the endocrine system was first suggested by Ernst Scharer (1928) when he discovered such cells in the teleost fish, *Phoxinus laevis*. The dawn of insect neuroendocrinology – from cellular aspect – was in 1937, when Berta Scharer described neural cells with glandular function in the brain and *Corpora cardiaca* (CC) of the blattid, *Leucophaea maderae*. Thee Scharers were the first to characterize the structural and functional similarities between the vertebrate hypothalamo(nervous)-hypophyseal system and the insect brain-CC-CA complex.

As presented in Fig. 1. insects share with vertebrates the use of two integrative control systems, the neural and endocrine. The cerebral NS centers (*pars intercerebralis*) and/or *suboesophageal ganglion* (SOG) where the respective active materials are synthesized represent an essential component of a neuroendocrine organ complex showing similarity, structurally and functionally in both insects and vertebrates. The second element is an extra-cerebral neurohemal structure where the secretory material is stored and released, while the third is a non-neural gland of internal secretion with a close spatial relationship with the former. The NS cells in the *protocerebrum* of insects are analogous to the hypothalamic NS centres of vertebrates. The axon extends through the central nervous system, but at some point, characteristic for the particular NS cell, it penetrates the blood-brain barrier, so the product is released into the haemolymph. Axon bundles may enter the CC – a well defined neurohemal organ –, corresponding to the posterior lobe of the pituitary gland, or the *perisymphathetic organ* (PSO) located near abdominal ganglia. The third part of the system is the non-neural endocrine insect gland – considered as the analog of vertebrate adenohypophysis – the CA or the *prothoracic gland* (PTG), where the moult hormone *ecdysone* is produced (Butenandt and Karlson, 1954). A number of well-defined cells are scattered amongst the principal midgut epithelium cells, their function may be closely related to digestion and adsorption, muscle movement as well as diuresis. *Epitracheal gland* cells were found in larvae of Lepidoptera attached to a trachea near each spiracle, their product, the *ecdysis triggering hormone* (ETH) being essential during moult. It is important to note that in the larvae of some Diptera a ring gland is formed by the fusion of CA-CC and PTG.

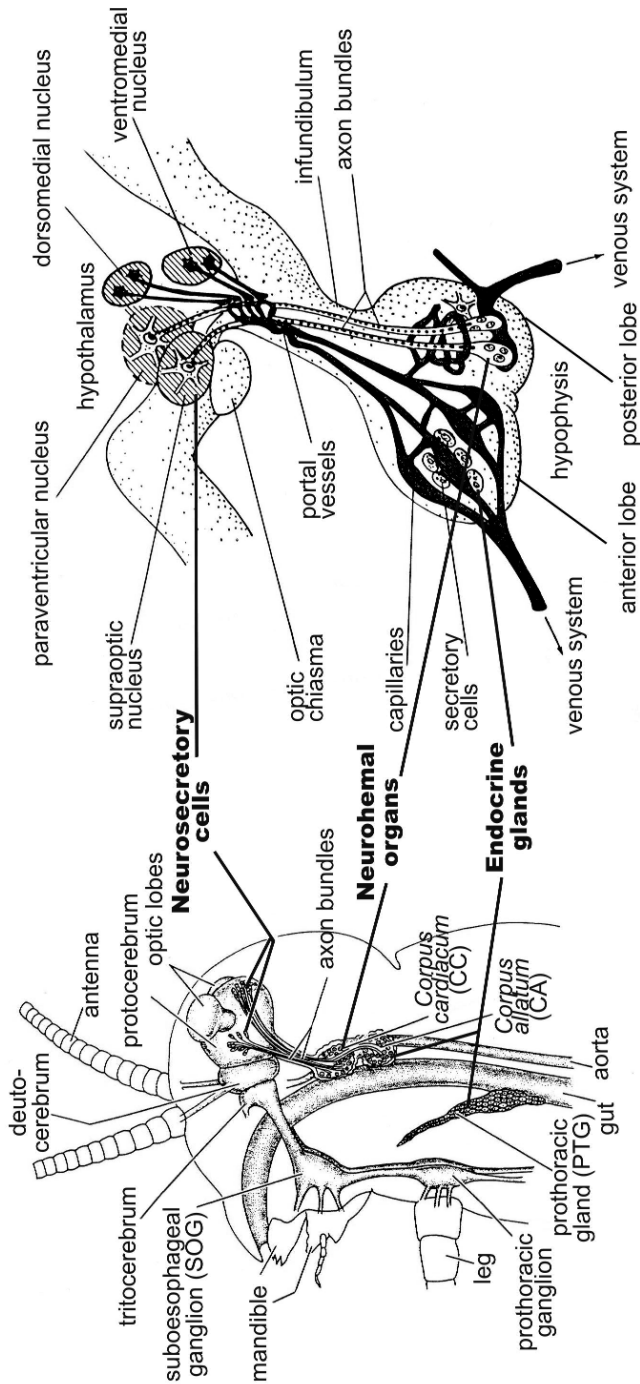


Fig. 1. Neuroendocrine system of an insect (left side) and vertebrate (right side).

On the comparative drawing the most important neurosecretory cells (producing regulatory peptides), neurohemal organs (storage and release sites of neuropeptides) and closely related non-neural endocrine glands (hormone producing glands) are represented

In insects, the multilevel neuroendocrine regulatory model, well known in vertebrates or humans, has been described in its totality in two cases: First distinct regulatory peptides are produced in the NS cells of *pars intercerebralis* which are then stored and released from neurohemal CC to stimulate CA and PTG, respectively. The synthesized hormones, JH and *ecdysone* reach their final target cells via the haemolymph. In conclusion, however, similar to vertebrates in insects there are many neuropeptides (or even more!) but basically, the majority exert their effect in one step or directly on target cell/organ.

It was assumed *a priori* that chemical nature of insect hormones is totally different from those present in vertebrates. Today we have evidence that the endocrine system of insects is as complex as that of vertebrates and that all animals share a common evolutionary descent and the basic principles of the endocrine system are supposed to have originated before the vertebrates and invertebrates started to diverge. Similarities and/or differences therefore have to be dealt carefully and *en place* taking into account possible advantages arising from continuously growing information.

Since the successful structural identification of the *myotropic* pentapeptide *proctolin* (also functioning as a neurotransmitter and modulator) from the cockroach, *Periplaneta americana* (Starratt and Brown, 1975) and the discovery of the first real insect neuropeptide the *adipokinetic hormone* (AKH) from the locust, *Locusta migratoria* (Stone et al., 1976) by nowadays close to thousand various insect neuropeptides have been identified and characterized or predicted with the development of separation and identification methods (high performance liquid chromatography, amino acid sequencing, different mass spectrometries, recombinant DNA technology and polymerase chain reaction, etc.) (Gäde, 1997; Gäde et al., 1997). The various neuropeptides are basically categorized by their (primarily discovered) action which generally implies structural similarity, but not necessarily. At present they are grouped into four main categories which classification will be followed below (*Table 1*; for more information please refer to respective reviews): Growth and development (Gäde and Hoffmann, 2005); Reproduction (De Loof et al., 2001; Gäde and Hoffmann, 2005); Metabolism and homeostasis (Gäde, 2004) and Muscle movement (Schoofs et al., 1994). Since it is now known that quite a few of discovered peptides elicit more than one biological action, they are declared *pleiotropic*. In general they are discussed with respect to their main action (or they are best known for) which has a bearing on their nomenclature too. According to Raina and Gäde (1988) usually the abbreviation of species name (source), followed by primary action/activity extended with Roman number(s) is the most used, but in some instances alternative names are included as well (Mas-AST: *Manduca sexta allatostatin*; Bom-PBAN I and II: *Bombyx mori pheromone biosynthesis activating neuropeptide*).

The peptide hormones are generally oligopeptides (~10–20 amino acids) or small proteins (0.5–50 kD). They are derived from protein precursors which are cleaved and modified post-transcriptionally to produce the active peptide. Occasionally just a single peptide is formed from one protein like in case of PTTH or AKH. In other instances, however, several different peptides are produced from a single protein as found in the cockroach, *Diploptera punctata* where 13 different AST peptides were identified. In *B. mori* a single precursor protein is the source of *diapause hormone* (DH) and PBAN, but their production and function is being separated in time and space.

When a hormone reaches a target cell it initiates biochemical changes in the cell, but not all cells respond in the same way, and some do not respond at all. Response specificity is a feature of receptor proteins in the target cell/organ. Cells with appropriate receptors will be only affected by respective hormones and since a receptor may exist in various forms and can

Table 1

Presentation of most important and well-known insect neuropeptides according to grouping used in the text. *Italics* represent neuropeptides from higher animals showing similarity (structurally and/or functionally). **Bold characters** represent similar amino acids, closely related molecules or neuropeptides with similar endings. One letter codes for amino acids: A: Ala; C: Cys; D: Asp; E: Glu; F: Phe; G: Gly; H: His; I: Ile; K: Lys; L: Leu; M: Met; N: Asn; P: Pro; Q: Gln; R: Arg; S: Ser; T: Thr; V: Val; W: Trp; Y: Tyr

	Neuropeptide	Primary effect	Structure
Growth and development	Bom-PTTH (monomer)	Stimulates ecdysone biosynthesis in prothoracic gland	GNIQVENQAIPDPCTCKYKKEIEDLGENSEVPRFIETRN-CNKTQQPTCRPPYICKESLYSITILKRRETKSQESLEIPNELKYRWVAESHPVSVAACLCTRDYQLRYNNN
	A chain:Bombyxin-II <i>A chain:Human insulin</i>	Decreases carbohydrate reserves <i>Decreases blood sugar level</i>	GIVDECCLRPCSVDVLLSYC <i>GIVEQCCTSICSLSYQLENYCN</i>
	Mas-AT	Stimulates JH synthesis	GFKNVEMMTARGFamide
	Mas-AST	Inhibits JH synthesis	pQVRFRQCYFNPISCF
	Dip-AST-1=Pea-AST-1	Inhibits JH synthesis	LYDFGLamide
	Dip-AST-2=Pea-AST-2	Inhibits JH synthesis	AYSIVSEYKRLPVYNFGLamide
	Mas-EH	Stimulates events of ecdysis, cuticle plasticization, ecdysial behaviour	NPAIATGYDPMEICIENCAQCKKMLGAWFEG-PLCAESCIKFKGKLIPECEDFASIAPFLNKL
	Bom-MRCH-I (=PBAN-I)	Stimulates melanin formation	LSEDMPATPADQEMYQPDPEEMESRTRYFSPRLamide
Reproduction	Bom-DH	Induces embryonic diapause	TDMKDESDRGAHSERGAFCFGPRLamide
	Lom-OMP (two isoforms)	Stimulates egg development	YYEAPPDGRHLLQPAAPAVAPA(A/S)PASWPH-QQRRQALDEFAAAAAADAQFQDEEDGGRRV
	Aea-OEH	Stimulates ovarian ecdysone production	QPTNVLEIRCKLYSGPAVQNTGECVHGAEL-NPCGKLSCLKGVG-DKCGESTAGIIMSGKASGLMC-CGGQCVGCKNGICDHRLCPRL
	Lyd-TE	Stimulates ecdysone production in testis	ISDFDEYEPLNDADNNEVLDF
	Aea-TMOF	Inhibits trypsin activity	YDPAPPPPPP
	Neb-colloostatin	Inhibits vitellogenesis	SIVPLGLPVPIGPIVVGPR
	Hez-PBAN	Stimulates pheromone synthesis	LSDDMPATPADQEMYRQDPEQIDSRTKYFSPRLamide

Table 1 (cont.)

	Neuropeptide	Primary effect	Structure
Metabolism and homeostasis	Lom-AKH-I	Stimulates lipid mobilization	pQLNFTPNWG Tamide
	Mas-AKH	Stimulates lipid mobilization	pQLTFTSSWG Amide
	Pea-CAH-I	Stimulates heart beat frequency	pQVNFSPNWamide
	Hez-HrTH	Increases "blood" trehalose level	pQLTFSSGWG Namide
	Mas-DP-I	Stimulates excretion	RMPSLSIDLPMSVLRQKLSLEK ERKVHALRAAANRNFLNDIamide
	<i>Rat-CRF</i>	<i>Stimulates ACTH production</i>	EPPISLDLTFHLLREVLEMARA EQLAQQAHNSNRKLMELI amide
	Lom-AVP-DP	Stimulates diuresis	CLITNCPRG amide CLITNCPRG amide
	<i>Vertebrate-AVP</i>	<i>Stimulates blood-brain barrier permeability</i>	CYFQNCPRG amide
	Lom-neuroparsin	Antidiuretic in hindgut	NPISRSCEGANCVDLTRCEYGDVTDFFGRKV- CAKGPGDKC-GGPYELHGKCGVGMDCRCGLCSGC- SLHNLQCFFFEGLPSSC
Muscle movements	(Pea)-proctolin	General muscle stimulator	RYLPT
	Mas-CAP	Stimulates heart beat	pQLYAFPAVamide
	Lom-TK-I	Stimulates visceral muscles	GPSGFYGV Ramide
	<i>Mammalian Substance P</i>	<i>Stimulates gut smooth muscles</i>	RPKPQQFFGLM amide
	Lem-MK-I	Stimulates visceral muscles	DPAFNWSWamide
	Lem-PK	Stimulates visceral muscles	pQTSFTPRLamide
	Lem-SK-I	Stimulates visceral muscles	EQFEDY(SO ₃ H)GHMRFamide
	<i>Vertebrate-CCK</i>	<i>Stimulates gut movements and secretion by pancreas</i>	DY(SO₃H)MGWMD Famide
	Lom-MIP	Myoinhibitory effect	AWQDLNAGWamide
	Lom-FaRP	Myoinhibitory effect	GQERNFLRFamide
	Neb-MS (FaRP)	Myosuppressin effect	TDVDHVFLRFamide
	<i>Mollusk-FMRF</i>	<i>Inhibition of motoneurons</i>	FMRF amide

be expressed at distinct times, the hormone may have different effects in different tissues. Depending on the nature of the hormone, the receptors are in the cell membrane (for peptides inducing signal transduction cascade) or within the cell/nucleus (for JH/*ecdysone*). This review is, however, concentrating on information regarding neuropeptide hormones only.

When alternatives are searched for interfering with insect regulatory peptides or their effects a number of possibilities should be considered. The synthesis of neuropeptides generally results first in a larger pre-prohormone, which is processed to a smaller bioactive peptide. Various processes may serve as potential targets in this series of events like blocking of expression of antisense RNA or inhibition of specific processing enzymes. Theoretically secretion or release from CC may also be an alternative, but inactivation of circulating neuropeptides by binding with specific antibodies or degradation by specific peptidases could be taken into account. Last but not least, inhibition of neuropeptide binding to receptors or disruption of signal transduction cascade also offers a possibility. Recently besides *in vivo* and *in vitro* structure-effect and mode-of-action studies the discovery of true neuropeptide receptors also provides perspectives in this area. The most important identified and described receptors according to publication dates are: *Drosophila melanogaster* tachykinin-like peptide receptor (TKr) (Li et al., 1991); *Manduca sexta* diuretic peptide receptor (DPr) (Reagan, 1994); *Drosophila*-ASTr (Birgül et al., 1999), all identified by reverse physiology approach. A real breakthrough was achieved with the cloning of AKHr from *D. melanogaster* and *B. mori* (Staubli et al., 2002). The identification of moth, *Helicoverpa zea* PBANr (Choi et al., 2003) and *B. mori*-PBANr (Hull et al., 2004), is of similar importance since it is likely that they are structurally and functionally different, which may provide a plausible explanation for the different modes of PBAN signal transduction cascade and mode-of-action in pheromone synthesis in the two species, despite the fact that their PBAN molecules are rather similar and their C terminal active sequence is also identical (FXPRLamid, X=T;V;S) (see below).

The most potent rationally designed agonists, superagonists, antagonists however, provide the most likely and realistic solution from which a couple of possibilities will be explained based on the wealth of primary structural information providing an opportunity for successful neuropeptide-receptor interaction disruptions encoded in the amino acid sequences (Gäde and Goldsworthy, 2003). Information about both chemical and conformational requirements for insect neuropeptide activity can constitute a template from which agonist/antagonist peptide mimetics might be fashioned. Consequently such mimetics would be capable of disrupting the delicate physiological balances neuropeptides help maintain. This concept raises the possibility that the listed "rational molecules" may represent new, selective, and environmentally safe insect pest control agents. The most important requirements which are to be achieved when a molecule is designed are as follows: An exogenously applied "peptide" should be able to reach the haemolymph compartment unaltered, either via the cuticle or, upon ingestion by crossing intact epithelium of the gut. At present, the large scale synthesis of such "peptides" is still very expensive therefore price reduction is of prime importance, although the minute amount required for necessary effect is, however, a big advantage. The best alternatives are the specially designed "pseudopeptides" nowadays, where the planned and synthesized molecules bear the crucial active sequence (core, or a mimetic) but three dimensional steric properties may be still maintained despite that "non-peptidic" elements are introduced like rings, special bonds, or other stabilizing solutions are incorporated.

Growth and development

Two major groups of hormones regulate growth and development in insects, the steroid *ecdysteroids* and the acyclic *sesquiterpenoid epoxides* the JHs. Both are produced in endocrine glands of epithelial origin (i.e. PTG and CA) their synthesis being strictly controlled by PTTH and *allatoregulatory* peptides, respectively. PTTH was first identified in *B. mori* as a 109 amino acid long polypeptide (Ishizaki et al., 1983) which is originally synthesized as 224 amino acid precursor peptide. This peptide (30 kD) is the true steroidogenic hormone in this species while the 4kD PTTH – also known as *bombyxin* – is built up from 5 further fragments (PTTH-I-V) and was found to be responsible in reducing carbohydrate reserves (*hypoglycaemic* and *-trehalosemic* activity). The PTTH-II is composed of an A and B chain connected by disulfide bonds and the former shows considerable similarity to vertebrate insulin and is known to exist in two different molecular sizes. Though the mode-of-action of PTTH via cAMP signal transduction cascade and the *in vitro* bioassay system of PTG is well established (Smith, 1995) there is no substantial evidence whatsoever using this peptide in developing new active compounds in preventing the production or release of moulting hormone, *ecdysone*, while *allatoregulatory* (*allatotropins* /AT/ and *allatostatins* /AST/) peptides are of great potential, therefore they will be discussed in more detail.

The endocrine gland CA does not store JHs in appreciable amounts and the titre of the hormone in the haemolymph is determined by the rate of biosynthesis or inhibition in CA, along with the activity of JH-esterases (JHE). Since JHs are not only crucial during the fine tuning of development, but in adult females they are responsible for vitellogenesis for example, therefore allatoregulation is important throughout insect life. To date only few ATs, compared with several ASTs have been isolated from nervous tissues of various species. The Mas-AT isolated from tobacco hornworm pharate adults is a 13 amino acid peptide, but had no effect on CA activity of larvae (Kataoka et al., 1989). Furthermore CA of the beetle, *Tenebrio molitor*, the locust, *Schistocerca gregaria* and the cockroach *P. americana* were not activated by synthetic AT, whereas the CA of the moth, *H. virescens* were stimulated, suggesting order specificity. Studies on the amino-terminal truncated fragments of synthetic peptide indicate that the 6–13 amino acids constitute the biological active core. It was also found that isolated peptides structurally belonging to ATs show other *pleiotropic* effects like *myotropic* activity.

The ASTs are structurally diverse peptides and were originally shown to inhibit (*in vitro*) biosynthesis of JH in the CA of a variety of insects (Stay et al., 1994). Some confusion has arisen, however, in the terminology of these peptides due to their (various) bioactivities, but still three families of peptides are referred to as ASTs: the “A” or “cockroach” type (characterized by the C terminus FGL/I/Vamide; the “B” or “cricket” type (W residues at positions 2 and 9) and “C” or “moth” type (N terminal pQ, a free C terminus and two C residues apparently forming an intramolecular disulfide bridge).

The Dip-ASTs, belonging to group “A” possess a common C terminal pentapeptide Y/FXFGL/Iamide representing the active core region or minimum sequence capable of eliciting inhibition of JH production *in vitro*. Chemists have formulated special pseudo-peptides with introducing sterically bulky components like indane ring or cyclopropyl into the active core region to render them resistant to both haemolymph and tissue bound catabolic peptidases, while maintaining biological activity (Nachman et al., 1999).

A novel transportation method for the Mas-AST has been reported, which may have a general influence in developing methods of delivery for insect neuropeptides as potential agents in pest management. The tomato moth, *Lacanobia oleracea* AST is identical to that of *M. sexta*. The injection of this peptide does not inhibit JH synthesis of *L. oleracea* larvae but reduces feeding, retards growth, resulting in up to 80% mortality. If it is administered in the diet – most likely due to the activity of proteases – the effects are not observed. If, however, a fusion protein of snowdrop, *Galanthus nivalis* agglutinin (GNA) and Mas-AST is made, expressed in *Escherichia coli* and then added as diet supplement thus feeding and consequently their growth may be strongly inhibited (Fitches et al., 2002).

Growth and metamorphosis are characterized by a number of moults in the course of which a new cuticle is produced. The ETH responsible for the pre-ecdysial behaviour stimulates moto-neurons of muscles and is synthesized in the epitracheal *Inka* cells. A neuropeptide that is secreted by NS cells in the brain and stored in and released from the CC stimulates the shedding of the old cuticle therefore it is referred to as *eclosion hormone* (EH). The cellular targets and action of these large hormones are diverse and does not only stimulates ecdysial behaviour, but also causes cuticle plasticization before moult or initiates programmed degeneration of certain intersegmental muscles which are not needed by the adult. *Melanization and reddish colouration hormone* (MRCH) is responsible for melanin synthesis following moult. In *B. mori* MRCH-I and PBAN-I (see below) are structurally similar but are produced at different stages bearing different functions and both belong to the big family of *pyrokinins* (PK) (see below) bearing the common C terminal pentapeptide FXPRLamide. *Bursicon* is another – very large in size – hormone which is released from the abdominal ganglia and is primarily responsible for the sclerotization of the fresh cuticle.

Diapause is defined as a spontaneous developmental arrest which occurs to adapt to changing environments. Insects may enter diapause either in the embryonic, larval, pupal or imaginal stage (e.g. arrest of reproduction). The most well known is the egg diapause of *B. mori* which is caused by the DH produced by the egg laying female due to deterioration of environmental conditions.

Reproduction

In case of vertebrates gonadotropins are quite uniform in their structure and effects. Opposite to this, reproduction of insects involve a number of consecutive events beginning from sex determination ending up with egg deposition which are all influenced by humoral factors like JHs, *ecdysone* (both are produced in adults as well; in CA or in ovaries/testis and oenocytes, respectively) and many various regulatory neuropeptides which processes altogether may be even species specific (De Loof et al., 2001).

Among hormones influencing development of ovaries *ovarian maturing peptide* (OMP) responsible for maturation, the *ovarian ecdysteroidogenic hormone* (OEH) (or *egg development neurosecretory hormone* /EDNH/) acting on egg development are the most well known. The OEH by stimulating *ecdysone* synthesis in ovaries enhances vitellogenesis (production of vitellogenins and incorporation) by positive feedback mechanism. *Ecdysone* is said to be gonadotropic in many species (estrogen equivalent). The *testis ecdysiotropin* (TE) stimulates *ecdysone* production in testis (testosterone equivalent).

The *colloostatins* inhibiting incorporation of yolk material into developing eggs are acting as true antigonadotropins. The *trypsin modulating oostatic factor* (TMOF) is also declared as antigonadotropic due to its secondary effect. Since it is produced in the ovaries it is not a neuropeptide. Research of this peptide is, however, of prime importance since its use as a biorational insecticide against mosquito and other aquatic insect larvae and even against terrestrial larvae is rather promising (Borovsky et al., 1998; Borovsky, 2003). In brief, the yellow fever mosquito, *Aedes aegypti* requires the digestion of blood meal to stimulate the process of vitellogenesis and egg development. Feeding stimulates the synthesis of trypsin (and chymotrypsin) being responsible for the digestion of the blood. Free amino acids liberated following digestion are transported to the fat body, where vitellogenin biosynthesis occurs, which are in turn transported to ovaries. About a day later following the blood meal, the follicular epithelial cells of the ovaries begin to synthesize the TMOF which is then released into the haemolymph where it binds to specific receptors on the epithelial cells of the midgut. As a result trypsin biosynthesis ceases in the gut and consequently no more amino acids are released, finally leading to the arrest of egg development. If synthetic TMOF is administered to adult mosquitos via their diet, the peptide is not inactivated and exerts its effect, which is remarkable. For lethal effect (via inhibition of digestion) high doses have to be administered, however, if TMOF gene is fused into the coat protein gene of *Tobacco Mosaic Virus* together with the proper cleavage site for trypsin (to release TMOF) significantly lower amount is enough to inhibit proteases and induce high mortality among larvae. According to another alternative the gene of TMOF was incorporated into the alga *Chlorella*. Upon feeding this alga to mosquito, larval death occurred within 3 days. The administration of such genetically modified “agent”, however, raises serious environmental issues.

Insects depend heavily on semiochemicals for communication and the co-ordination of many activities, therefore this area is intensively researched from both basic and applied aspects. Female Lepidoptera are well known to produce species sex pheromones (C_{10–18} unsaturated acyclic aliphatic compounds bearing aldehyde, alcohol and acetate ester functional group) to attract conspecific males (Blomquist and Vogt, 2003). The volatile sex pheromones are synthesized and secreted from specific glandular cells of the *pheromone gland* located in the abdomen. The *de novo* biosynthesis of pheromones are primarily regulated by the neuropeptide PBAN, the first being isolated from corn ear moth, *H. zea* (Raina et al., 1989). They are produced in the SOG and are released periodically (daily, depending on species) from CC and/or ventral nerve cord. All PBANs isolated and identified so far are 33–34 amino acid long, amidated at C terminus and bear the well-known FXPRL active core. Based on the above not only the use of synthetic species specific blends of several hundred known pheromones may be effective in agriculture (forecasting, reduction by trapping, etc.), but the employment of latest results regarding the delicate mechanisms controlling pheromone production (by PBAN, occasionally supplemented by JHs and *ecdysone*) has great potential. The interest towards PBANs and other neuropeptides bearing the common C terminal pentapeptide FXPRLamide (active sequence) like DH or *myotropic* PKs is well established. Synthetic PBAN (or other PKs) injected *in vivo* into females maintain their activity and induce intensive pheromone production, therefore designing and production of PBAN/PK analogs, antagonists/agonists may be practically very promising.

Choi et al. (2003) identified and fully cloned the Hez-PBANr from the *pheromone* gland which is a G protein-coupled receptor (GPCr) built up from 346 amino acids. The PBAN following coupling to the GPCr opens up the Ca^{++} channel, the influx of Ca^{++} occurs first, the activation of adenylate cyclase and the subsequent increase of cAMP follows. In theory the next step would be the activation of a cAMP-dependent protein kinase, which in turn would phosphorylate one or more rate determining enzymes in the biosynthesis of pheromones. In some *Helicoverpa* species (Rafaeli, 2002), or in the cabbage moth, *Mamestra brassicae* (Fónagy, 1999) the involvement of cAMP in inducing the rate limiting enzyme prior to fatty acid synthesis is strongly supported. To date, however, no convincing data on cAMP dependent protein kinases have been presented, therefore the complete model has not yet been established.

In some other species, especially in *B. mori* the situation is quite different. Hull et al. (2004) recently identified and cloned Bom-PBANr, which is 67 amino acid longer than Hez-PBANr, and this portion seems to be crucial for receptor internalization following PBAN coupling. This difference may explain why signal transduction cascade alters in *B. mori* and most likely also in corn borer, *Ostrinia nubilalis* or *Spodoptera littoralis* although structurally closely related PBANs are responsible for pheromone biosynthesis stimulation. After coupling of PBAN to receptor, Ca^{++} influx also occurs which is followed by the formation of Ca^{++} -Calmodulin complex, which in turn activates phosphoprotein-phosphatase 2B (Calcineurin) enzyme system stimulating the rate limiting enzyme the acyl-CoA-reductase. PBAN also activates the cytosolic lipases inducing lipolysis in the lipid droplets (showing daily fluctuation in size and number) to liberate the stored pheromone precursor fatty-acyl and to provide it for reduction resulting in the bombykol (main component of *B. mori* pheromone). This full description of pheromone production (very characteristic physiological event in insects) including signal transduction, biochemical events along with morphological evidences is unique and may also serve as a springboard for establishing more complete mode-of-action models for other neuropeptides (Iwanaga et al., 1998; Fónagy et al., 1999; Fónagy et al., 2000; Matsumoto et al., 2002).

Based on recent achievements there are two major concepts in targeting the development of analogs or mimetics of PBANs for insect control. The first strategy is based on the analogy of *Substance P* where the backbone cyclisation to impose conformational constraint on peptide was implemented (Gilon et al., 1991). The method involves the cyclisation of the active hexapeptide of Hez-PBAN_{28–33} (YFSPRLamide) called Backbone cyclic neuropeptide-based antagonist (BBC-NBA) approach (Altstein et al., 2000). The below steps may as well be useful for other neuropeptides too:

1. Identification of the neuropeptide that controls the required function; 2. Elucidation of the shortest sequence which constitutes the active site; 3. Discovery of peptidic linear lead agonist on the basis of the sequence found in (2); 4. Discovery of a potent BBC antagonist devoid of agonistic activity based on (3); 5. Determination of the structural requirements for antagonistic activities as determined in (4); 6. Designing and synthesizing an insecticide prototype (i.e. small, metabolically and structurally stable, with selective bioavailability, cost-effective peptidomimetic compound).

According to the second strategy the development of PBAN/PK pseudopeptides is a good alternative (Nachman et al., 1996a). The basic concept is to provide a protection

for the synthetic active core peptide, which allows easy penetration of the polar molecule through the apolar matrix of the cuticle, while it resists attacks of endopeptidases in the gut and haemolymph. According to one trial the FTPRLamide otherwise moderately active molecule was used as a starting compound where the phenyl ring of the side chain of F is replaced with a hydrophobic carboranyl moiety (2-carborane-ethanoic acid; Cbe). The Cbe-TPRLamide has an even greater (10-fold) *pheromonotropic* and/or *myotropic* activity when injected or *in vitro* tested on hindgut muscles. Further experiments with other amphiphilic analogues showed that the hydrocinnamyl-TPRLamide is a superagonist, the 9-fluoreneacetyl-FTPRLamide and 1-pyrenebutyryl-FTPRLamide induced prolonged *pheromonotropic* response that lasted up to 20 h following topical application (Teal and Nachman, 1997).

Metabolism and homeostasis

The main site of intermediary metabolism in insects is the fat body composed of lobes and bands and fills out body cavity. It has a major role in accumulating energy, lipid and carbohydrate metabolism, synthesizing egg yolk (vitellogenins) or diapause proteins, moreover in detoxification and excretion of certain compounds. Intermediary metabolism of insects is under endocrine control as well. The peptides that control metabolic functions are *hyperlipaemic* (AKHs), *hypertrehalosaemic*, *hypotrehalosaemic* (HrTHs, HoTH) or *hyperprolinaemic* according to their major actions when injected into insects, *in vivo* (Gäde, 2004). They are produced in retrocerebral cells of brain and/or most likely in the glandular intrinsic cells of CC, where they are stored and accumulated, then periodically released. AKHs increase the concentration of energy substrates in the haemolymph (diacylglycerols, and proline) whereas HrTH increases trehalose concentration, the major circulating sugar in insects, while HoTH decreases that. *Ecdysone* and JHs have also determining effects on above processes during metamorphosis or reproduction.

Following the isolation of the first "real" AKH from *L. migratoria* (Stone et al., 1976) dozens of *adipokinetic* peptides were isolated. All members are from 8–10 amino acids long, with a pyroglutamate residue blocking the N-terminus and an amide blocking the C-terminus. At position 4 an F or Y is found and at position 8 the aromatic W residues are present. A major breakthrough is expected in this area after the identification and cloning of the first AKHr (Staubli et al., 2002) although, this group of peptides was always in the focus of interest due to their *pleiotropic*, multifunctional effects like increasing levels of blood trehalose or lipid, stimulation of the oxidation of lipids by the flight muscles, suppressing of lipid synthesis by acetate uptake inhibition, decreases RNA synthesis, moreover may act as a cardiostimulant. The flight muscles are entirely adapted to an aerobic energy metabolism, the mobilization of different fuels and the oxidation of substrates (lipids in locusts, carbohydrates in cockroaches and the amino acid proline in some beetles) are controlled by AKHs (Gäde and Auerswald, 2003). The circulating AKHs bind to a GPCR on the surface of fat body cell resulting in elevated cAMP level. The cAMP and the increased level of Ca^{++} activate via protein kinases the lipases. In locust the produced diacylglycerols enter the haemolymph and are transported by lipophorins to muscle cells, where after β oxidation energy is liberated. In some beetles the free fatty acids undergo β

oxidation (in fat body cells) and the formed acyl-CoA with the alanine entering the cell, the alternative fuel, proline is produced. In species where carbohydrates serve as substrates AKHs (or HrTH) also increase the cAMP level following binding to GPCR. The activated inositol-triphosphate releases internal Ca^{++} stores and along with the entering Ca^{++} the cAMP, via a kinase cascade induces the glycogen phosphorylase and other enzymes, finally resulting in the production of trehalose. Keeping practical aspects in focus a number of different AKH mimetics were designed and synthesized like ones were the C (Lee et al., 1996) or N termini were modified (Lee et al., 1997), or by replacing just one amino acid (Ziegler et al., 1998) and by mathematical means (Lee et al., 2000).

For haemolymph homeostasis, insects must regulate their water and ion balance, thus, interference with these processes is a particularly attractive target. Agonists of *diuretic* peptides (DP) or antagonists of *antidiuretic* hormones could induce dehydration leading to death. Among the DPs the most important ones are the *corticotropin-releasing factor* (CRF)-like peptides (showing 30% similarity to vertebrate CRFs) and the various *kinins* (K; see *myotropic* peptides) (Coast, 1998). Both act on the main excretory organ, the *Malpighian*-tubules producing the primary filtrate. The CRF-DP acts via the cAMP signal transduction cascade stimulating urine secretion, while the Ks coupling to other receptors induce Ca^{++} influx switching on another signal transduction cascade. The latter peptides may act synergistically too due to their *myotropic* activity by stimulating writhing movement of tubules in the body cavity. The Ks are characterized by the C terminal sequence $\text{FX}_1\text{X}_2\text{TGamide}$ where X_2 is replaced with a sterically hindered aminoisobutyric acid (Aib) proved to be resistant to enzymes while showing even higher biological activity (Nachman et al., 1997). The *antidiuretic* hormones (*neuroparsins*) act on the hindgut papillae of insects, since water re-absorption (saving) is of prime importance.

Muscle movement

The majority of insect neuropeptides fully characterized so far have the property of regulating the (spontaneous) contractile activity of visceral and/or skeletal muscles. Structurally quite different peptides showing *myotropic* activity are grouped together, generally due to their primarily described effect (Schoofs et al., 1994).

Proctolin, the first identified neuropeptide (Starratt and Brown, 1975) and the *cardioactivating peptides* (CAP) stimulating heartbeat frequency compose a separate family. Studies related to the pentapeptide *proctolin* and possible analogues are still in the focus of interest and in 1997 Konopińska summarized results derived from the analysis (*in vitro* insect and *in vivo* rat toxicology) of about 80 different molecules in which amino acids were replaced in 1–5 position, cycloanalogues and analogues with a truncated or elongated structure were synthesized. The *proctolin* receptor has been also characterized pharmacologically, but its isolation and cloning is still lacking. All the above results have, however, theoretical significance, since so far suitable, stable, easy penetrating analogues have yet not been found.

The *myotropic* insect TK family characterized by the C-terminal pentapeptide sequence $\text{FX}_1\text{GX}_2\text{Ramide}$ is closely related to vertebrate TKs among which the *Substance P* is the most well known. Other *myokinins* (MK) are also small peptides composed of 6–13

amino acids where the C terminal region is very conservative (FX₁X₂YRamide). The PKs are well known for their FXPRLamide C terminus (including DH, MRCH and PBAN which all show *myotropic* cross reactivity). The *sulfakinins* (SK) belong to the vertebrate *gastrin-cholecystokinin* (CCK) family bearing the C terminal hexamer Y(SO₃H)GHMRamide. From practical aspects, in case of different Ks, due to their *pleiotropic* capacity one should refer to previously mentioned studies (Nachman et al., 1996a; Altstein et al., 2000).

Besides the *myoinhibitory* peptides (MIP) from *Locusta* the majority of inhibitors belong to large family of *FMRamide related peptides* (FaRPs) which is characterized by its C-terminus (Orchard et al., 2001). Peptides with such ending are quite spread in the animal kingdom and from insect species sometimes 6–10 peptides with similar ending are isolated. It is however, questionable that are we really dealing with so many distinct active peptides? It's noteworthy to mention the discovery of a non-peptide *myoinhibitor* agonist, the benzethonium chloride (Bztc) which is capable to mimic physiological activity of *myosuppressins* (MS) belonging to FaRPs (Nachman et al., 1996b).

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