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Chapter 11

Insect Neuropeptides and Application in Pest Management

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1. Introduction

Neuropeptides are biologically active peptides that are mainly produced in neurosecretory cells of insects constituting a diverse widespread class of signaling substances in the nervous system. Insect neuropeptides function as neurotransmitters, neuromodulators and neurohormones and are therefore called 'master regulators' of metabolic, homeostatic, developmental, reproductive and behavioural events during an insect life (Holman *et al.*, 1990). Neurotransmitters are chemicals responsible for transmitting impulses between nerve cells by transiently altering the electrical excitability of cell membranes. Neuromodulators exert slow modulatory effects and control the level of excitability of whole group of nerve cells. Neurohormones tend to influence slow onset of events and act at a distance from the release site. In insects, a large number of neuropeptides are true neurohormones that regulate the above mentioned processes in a precise and controlled way. To interfere with these developmental processes in insects, and use the neuropeptides in safe and rational manner, it is logically important to characterize various neuropeptides and understand their functioning (Gäde and Goldsworthy, 2003).

In insects, neuropeptides have been extensively studied with respect to their roles as circulating hormones. Although role of neuropeptides in the insect central nervous system (CNS) is less understood, it is commonly considered that they act as neuromodulators or co-transmitters rather than as neurotransmitter (Nässel and Homberg, 2006). The term co-transmitter can be applied to a neuropeptide that is

co-localised with a classical neurotransmitter that acts on an ion-channel type of receptor (Burnstock, 2004). When the neuropeptide is coreleased with the classical neurotransmitter, the activation of the peptide G-Protein Coupled Receptor (GPCR) leads to the modulation of the ion-channel mediated signaling. Neuropeptides can play a multitude of functional roles in the brain and even single neuropeptides are likely to be multifunctional (Nässel and Homberg, 2006).

Neuropeptides have been identified in insect neurons by immunocytochemistry and in several cases by *in situ* hybridization histochemistry. Most of the known insect neuropeptides are present in interneurons and in neurosecretory or endocrine cells (Nässel, 2002; Homberg, 2002). Great diversity exists in the patterns of distribution of the various neuropeptides, and the different peptidergic circuits display various degrees of complexity. In *Drosophila melanogaster*, certain peptidergic systems have been explored with molecular genetics approaches, such as cell specific interference with peptide signaling by using the binary GAL4-UAS system (Duffy, 2002) and in other insects more traditional pharmacological and physiological experiments have been performed. Remarkable advances in the field of neuropeptide research and the comparative approach paved way for the discovery of many novel peptide structures. The total number of peptides isolated from insect nervous system exceeded 200 and are classified into 20 families (Nässel, 1995). The first insect neuropeptide identified is proctolin.

2. Proctolin

Proctolin is the first pentapeptide isolated from the gut of American cockroach, *Periplaneta americana* and was proposed to function as a neurotransmitter with myotropic properties. It produces slow graded contraction of longitudinal muscles of proctodeum and modulates muscle excitability (Starrat & Brown, 1975). Using immunohistochemistry, it was found that proctolin-like immunoreactive neurons and processes are widely distributed throughout the central nervous system, stomatogastric nervous system and peripheral tissues such as oviducts and alimentary canal. Of late, proctolin-like immunoreactive lateral neurosecretory cells in the brain project processes to corpus cardiacum and corpora allata. Proctolin was now designated as a releasing factor capable of stimulating the release of adipokinetic hormone from the corpus cardiacum and of stimulating juvenile hormone production from the corpora allata.

3. Nomenclature

Insect neuropeptides are named after the first three and two letters of the genus and species name of the insect, respectively. Adipokinetic hormone from *Locusta migratoria* is therefore named as *Locmi*-AKH (Raina and Gäde, 1988).

This mini review focuses on few neuropeptides of potential application into a number of promising insect model system.

4. Adipokinetic Hormone (AKH)

AKH produced by corpora cardiac, mobilizes carbohydrates and lipids from insect fat body during extreme physical activities such as flight and locomotion.

Energy demand is profusely accelerated during high intensity muscular work like flight, where 100-fold enhancement is documented. Hence, flight muscles are depended on aerobic energy metabolism and oxidation of lipids, carbohydrates and amino acids (Gade and Auerswald, 1998). Intermediary metabolism is orchestrated by endocrine glands releasing short peptides from paired neurohemal glands, corpora cardiaca (Gade, 1996). Such metabolic regulators are termed adipokinetic, leading to higher levels in lipids, trehalose and proline in insect haemolymph as and when the demand is on (Gade and Goldsworthy, 2003).

4.1 AKH-Structure

AKH was purified and sequenced from the migratory locust, *Locusta migratoria* with 8, 9, or 10 amino acid residues having pQ group at N-terminus, aliphatic or amino acid residue at position 2 and amide-group at C-terminus position. AKH was identified in all insect orders, certain annelids, mollusks and nematodes and nearly thirty-six different isoforms of insect AKH are reported (Gade *et al.*, 1997). Recently, "AKH-like" and "proto-AKH" with more than 10 amino acid residues are also characterized (Li *et* al., 2016). In addition to the mobilization of energy substrates during high demand phase of flight and stress-shooting mechanism, AKH is also involved in inhibition of RNA synthesis of lipids and proteins in fat body, activities linked to cardio-excitatory properties in *Pyrrhocoris apterous*, accelerated long-term locomotion as well as insect immunity engulfing entomopathogens (Gade, 1996; Gade *et al.*, 1997).

5. Locomotion

It was well known and demonstrated that the availability of energy substrates are crucial for sustained flights in locusts rather than extracts from corpora cardiaca. Substantial availability of lipids in synergy with the extracts from corpora cardiac induced higher flight speeds in locusts, and that the flight speed was tremendously diminished under reduced titre of lipids even with the injection of corpora cardiaca extracts. Thus, the flight dynamics in locusts is influenced by the availability of lipids and regulated by AKH (Goldsworthy *et al.*, 1979). In *P. apterous*, AKH enhanced the mobilization of lipids and accelerated locomotion under its influence (Kodrik *et al.*, 2002). Topical application of con-specific AKH admixed with organic solvents penetrated the cuticle of the cricket, *Gryllus bimaculatus* and elicited flight-induced responses. Though structurally unrelated, AKH has similar function in insects as glucagon and adrenalin have in mammals.

6. Insect Immunity

A defensive response is exhibited by an insect when their tissues are invaded by entomopathogens. The immune response is elicited at both cellular and humoral level to counter the aggression but not akin to antibody production mechanism in vertebrates. Proteins in insect haemolymph recognize glucans in fungal cell walls and peptidoglycans in bacterial cell wall stimulating cellular mechanism involving phagocytosis and encapsulation. In certain cases, invading pathogens are engulfed by haemocytes to form nodules. Activation of prophenoloxidase cascade resulting

in the synthesis of antimicrobial peptides to neutralize infection in heamolymph indicates the humoral defense mechanism (Lavine and Strand, 2002).

AKH has greater influence in signaling the activation of prophenoloxidase cascade in locusts. Mere injection of lipopolysaccharide and laminarin from infectious bacterium and fungal cells, respectively into the locusts could not trigger the prophenoloxidase cascade unless Locmi-AKH1 is co-injected. Nodule formation as well as increase in phenoloxidase in locusts could be elicited only in the simultaneous presence of AKH and lipopolysaccharide and laminarin (Goldsworthy *et al.*, 2003). Induction of apolipophorin III by the action of AKH was involved in the activation of prophenoloxidase cascade by lipopolysaccharide and laminarin (Dettloff *et al.*, 2001). Deciphering the interaction between the insect immunity and endocrine system has opened out to understand the role of certain eicosanoids on the activation of phenoloxidase and nodule formation in insects.

7. Homeostasis and Feeding Regulation by Neuropeptides

Water and ion balance in insects are well regulated and maintenance of sufficiently stable state of water balance has long been recognized. In the absence of significant blood pressure in the insect circulatory system, primary urine formation is mostly by secretion rather than by filtration. Malpighian tubules located at the junction of midgut and hindgut secreting KCl or NaCl rich solutions which are essentially isoosmotic to haemolymph and water and solutes follow by passive diffusion, with exception of some active transport involved for elimination of toxic substances. The excretory system is primarily responsible for homeostasis, following metabolic modification of toxic compounds to chemicals more readily excreted or could be safely stored (Ramsay, 1954).

7.1 Osmoregulation

Excretion and water balance are under neuroendocrine control. The hormone involved in the process of diuresis are called diuretic hormone (DH). They are responsible for the secretion of urine by the Malpighian tubules. Although, a number of diuretic hormones have been identified in insects, 5-hydroxytryptamine is the major diuretic hormone of *Rhodnius prolixus* which could consume 10-20 folds their body weight in a single meal. There are three primary diuretic hormones found in insects: Corticotropin Releasing Factor (CRF) and a family of smaller kinins, and serotonin. CRF-like DH and kinins are classified as neuropeptides and the serotonin, 5-hydroxytryptamine is classified as a neurotransmitter (Coast *et al.*, 2002). Diuretic hormones act in synergism to enhance the process of diuresis.

The most studied CRF-like DH are Manse-DH, Locmi-DH and Achdo-DH. CRF-like DH is similar to vertebrate-CRF that mediates the secretion of urine through the production of cyclic-AMP. Active transport of Na^+ and K^+ into the Malpighian tubules could be accomplished by the CRF-like DH through cAMP. In general, transport of ions from the haemolymph into the Malpighian tubules leads to the secretion of urine. Insect CRF-like diuretic peptides adopt a folded helix-loop conformation bringing C-terminal amide close to N-terminus whereas human CRF assumed rod-like ?-helical conformation (Coast *et al.*, 2002).

7.2 Feeding Regulation by DH

Sensitivity of a given species of an insect herbivore is dependent upon the quantity and chemical structure of the feeding deterrent. Food as well as non-food stimuli both from within and outside the animal is involved in regulation of feeding, amply modulated by feedback mechanism from stretch receptors of gut wall, hormones and composition of blood (Simpson and Bernays, 1983). Tobacco leaf painted with Manse-DH-11 and fed to first-instar larvae of *Manduca sexta* reduced food consumption and caused high mortality. Injection of diuretic peptides induced antifeedant activity for Manse-DH in *Heliothis viresencs* larvae and for Locmi-DH in *L. migratoria* (Coast *et al.*, 2002). In locusts, filling of foregut during the feeding process caused closure of the pores at the tip of the taste sensilla on the mouth by release of a factor from corpora cardiaca into haemolymph. It was therefore indicated that the normal release of endogenous CRF-like DH signals the end of the meal in locusts and thus altered the feeding behavior in *L. migratoria*. Development of analogues for CRF-like DH could interrupt the feeding behaviour in insects.

7.3 Feeding Regulation by Sulfakinins

Sulfakinins are myostimulatory peptides partially similar to gastrin in mammals. Sulfakinins cause muscle contraction on insect gut and occasionally releases amylase that is involved in digestion. By inducing muscle contraction, sulfakinins cause irregular peristaltic movement of gut wall and may interrupt the movement of food ingested from crop to midgut. Injection of sulfakinin into locust and cockroaches diminished the food intake in these insects by altering muscular contraction in gut (Wei *et al.*, 2000).

8. Disrupting Sex Attraction

Sex pheromones are intra-specific semiochemicals that attract conspecific males/females from over great distance. Basic chemistry of these pheromones is well understood in certain of the Lepidopteran and Coleoptera insects; however, research on regulation of pheromone biosynthesis has been initiated. In corn earworm moth, a unique factor from brain was found responsible for the biosynthesis of pheromones (Raina and Klun, 1984). This factor was later identified as a peptide containing 33 amino acid residues that stimulates the biosynthesis of moth's sex pheromone termed as Pheromone Biosynthesis Activating Neuropeptide (PBAN), which was later characterized by a common amino acid sequence FXPRLamide motif in the C-terminus (Raina *et al.*, 1989). In flesh flies, these peptides are pyrokinins and myotropins that stimulate the hind gut and oviduct *in vitro* accelerating puparium formation. PBAN is released into the hemolymph of females during the scotophase and is drastically reduced after mating, contributing to the loss in female receptivity. Pheromone production is age-dependent and Juvenile Hormone is involved in its regulation. It was determined that substitution of T at X-position of PBAN was more biologically active in pheromone bioassay than analogues containing V, S or G (Abernathy *et al.*, 1995).

8.1 Antagonists of PBAN

A linear lead antagonist that imposed conformational modulation on Helze-PBAN was designed by Alstein *et al.* (2000). It was observed that in Helze-PBAN, the hexapeptide $PBAN_{28-33}$ is as active in stimulating pheromone biosynthesis of *H. zea* as the complete $PBAN_{1-33}$. Replacement of L-amino acids (Ser^{30} or Arg^{32}) by the D-hydrophobic amino acid D-Phe was found to be an effective PBAN antagonist capable of inhibiting sex pheromone biosynthesis in the female moth, *H. zea*. Compounds with antagonistic activity up to 96 per cent could be obtained and the most active peptide had an alkyl chain length of two and a spacer chain length of three carbons (Alstein *et al.*, 2000). Development of non-peptide analogues with insecticidal properties is being attempted.

8.2 Pseudopeptide Analoges of PBAN

Neuropeptides, which are polar in nature could not penetrate the non-polar lipid layer of insect cuticle and these peptides are broken down by peptidases in the gut and haemolymph as well. Nachman *et al.* (2001) developed paseudopeptides that were able to penetrate the hydrophobic cuticle and had enhanced peptidase resistance. Addition of various hydrophobic groups to the N-terminus of the C-terminal pentapeptide active core, which in conjunction with the polar Arg side chain, confer an amphiphilic property. Hydrophobic groups appended to N-terminus included fatty acids of various chain length, cholic acid, aromatic acids and carboranylpropionic acid. Amphiphilic analogues are more resistant to digestion by aminopeptidases and have higher binding affinity for the receptor. Topical application of native PBAN and the pentapeptide fragment had no pheromonotropic activity but the amphiphilic analogues had significant pheromonotropic activity. Higher number of phenyl rings conferred higher resistance to aminopeptidases.

9. Growth and Development

The steroid hormone, ecdysteroids and the terpenoid, juvenile hormone regulate growth and reproduction in insects. Ecdysteroid triggers moulting events and the characteristic of the moult is orchestrated by juvenile hormone. Both the hormones are produced by endocrine glands and their synthesis is regulated by neuropeptides (Gade *et al.*, 1997). The neuropeptide, Prothoracicotropic hormone (PTTH) stimulates the synthesis and release of ecdysteroids. PTTH was first identified in *B. mori* as 109 amino acid residue long, with seven Cys residues forming disulfide bridges and a N-glycosylation site (Gade, 1997). Juvenile hormone is a sesquiterpene produced by the corpora allata. Several slightly different forms known as JH0, JH1, JHII and JHIII containing 19, 18, 17 and 16 carbon atoms, respectively have been isolated. JHIII is the ubiquitous form occurring in most insects. Titre of juvenile hormone in the haemolymph is determined mainly by the rate of biosynthesis from corpora allata (Tobe and Stay, 1985). It was now understood that two types of neuropeptides regulate the JH production *in vitro*, allototropins by stimulation of JH biosynthesis and allotostatins by its inhibition.

9.1 Moth Allatostatin or C-type

The Moth Allatostatin is a 15-residue peptide reported first from horn worm, *Manduca sexta* which is characterized by an N-terminal pGlu residue, a free C-terminus and two cys residues forming an intramolecular disulfide bridge (Kramer *et al.*, 1991). This peptide inhibits JH biosynthesis *in vitro* in several moths but is found cardioinhibitory in fruit fly. It also inhibits the feeding of tomato moth, *Lacanobia oleracea* L. *in vitro* (Audsley *et al.*, 2001).

9.2 Snow Drop Lectin Fusion Protein

Topical sprays of neuropeptides were not successful on account of its polar nature and as such peptides are prone to degradation in the environment. In this context, a novel delivery of neuropeptides was attempted. It was observed that the allotostatin identified in *L oleraceae* is identical with that of *M. sexta*. Injection of allotostatin did not inhibit JH biosynthesis in larvae of *L. oleracea* but feeding was reduced and growth was retarded inducing up to 80 per cent mortality. Dietary incorporation of allotostatin was ably inactivated by the proteases in the digestive system. In a classical study it was observed that the mannose-binding plant lectin from snow drop (*Galanthus nivalis* agglutinin, GNA) was not digested by the gut proteases and could be detected in the haemoloymph (Fitches *et al.*, 2001). GNA was found to be potent carrier of peptides into haemolymph as it could cross the gut epithelium without being disintegrated. In order to make the delivery successful, a fusion protein of GNA and Manse-AST was made and expressed in *Escherichia coli*. Feeding of fusion protein by the fifth-instar *M. sexta* larvae drastically affected the feeding and weight gain. Fusion of GNA to Manse-AST protected from proteolytic breakdown in the haemolymph and induced antifeedant effect, which free allatostatin could not exert. It was now observed that the fusion of neuropeptide to GNA could effectively release the peptide into the haemolymph and cause detrimental effect. Fusion protein could be genetically engineered into plants and the bio-engineered product would combat pest invasion (Gade and Goldworthy, 2003).

10. Reproduction

Insect reproduction is well regulated and the hormones ecdysteroids and juvenile hormone along with neuropeptides control adult reproduction with great precision. Juvenile hormone and ovary maturating parsin regulate oocyte maturation in locusts. Peptide hormone that regulates the expression of serine proteases, the key luminal proteases in insects, was identified (Borovsky *et al.*, 1993). These peptides form the core technology upon which Insect Biotechnolgy Inc., California is developing a variety of products.

Digestion of blood meal by mosquitoes is very crucial for ovary maturation and egg development. Gut proteases especially trypsin-like serine proteases are activated after the blood meal and are responsible for the digestion of blood. Oocytes in mosquitoes are developed after the transport of free amino acid from intestine into the fat body leading to the biosynthesis of vitellogenin. Borovsky *et al.* (1993) identified a decapeptide synthesized from the mosquito ovary, 18-24 h

after the blood meal and termed it as Trypsin Modulating Oostatic factor (TMOF). Once TMOF is released into the haemolymph, there is complete stoppage of trypsin biosynthesis and no more egg maturation occurred. Injection of TMOF indicated inhibition of trypsin biosynthesis (translation), however, transcription of mRNA of trypsin is not affected (Borovsky *et al.*, 1993).

It was observed that TMOF was not inactivated in the gut of mosquitoes and subsequently stopped trypsin biosynthesis and egg maturation, when fed. Higher dosages of TMOF @ 1 ng per larva absorbed on yeast and fed to wrigglers caused mortality of mosquitoes in laboratory. It was further proved that TMOF gene could be fused with coat protein gene of tobacco mosaic virus at the restriction site for trypsin and this construct @ 140 pg inhibited proteases and killed mosquito wrigglers in a period of five days (Borovsky *et al.*, 1998). Protease inhibitors through trypsin inhibition are a very important tool employed in pest management in a wide array of crops. Most of lepidopteran insects have protease activity in the mid gut and the key pest of coconut *viz.*, red palm weevil was also found to have trypsin-like protease for protein metabolism. In *H. virescens*, a factor closely associated with Aedae-TMOF was unraveled, which has an important role in biosynthesis of trypsin. This could be reality in coming years of advancement in science in pest management.

Some of the insect neuropeptides have been discussed for a possible utilization as one of the tools in pest management programme. Refinement is further required for commercial exploitation especially against major pests of our tropical zone. Insect neuropeptides isolated on the basis of potential inhibitory control will emerge as a likely antifeedant lead molecule. The disruption of any step leading to biosynthesis of neuropeptides, their modifications during storage, their release into the haemolymph as well as their interaction with the target-cell membrane-bound receptors offer multiple modes of action for a novel-neuropeptide based pest management programme. Indeed, not all biochemical mechanisms will be worth exploiting nor will all neuropeptide be of equal importance with regard to pest control. Science has gone so deep that even evolutionary relationship are being unraveled with the study on neuropeptides in invertebrates and some of them are likely to be potential markers in the near future. Insect pest management is so dynamic and no one tool is likely to eliminate them and we have to learn to live with insects, of course not allowing to cause economic damage to our livelihood. Such novel tools will be very useful in the long run.

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