Prospects and Promises of Endocrine Biopesticides

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ABSTRACT

The environment hazards resulting from intensive use of synthetic organic crop protection agents, demands that the pest management studies should be biointensive. Environment friendly, safe and compatible approaches paved the way to develop biopesticides. The multiple functional capacities of insect neuropeptides, based on intervention on this system at any level provide opportunities for new insect control strategies. Potential biochemical for insecticidal action like Juvenile hormone, Juvenile hormone esterase, ecdysone, acetyl choline, ion channels and new peptides are discussed in detail.

INTRODUCTION

Biological control of agricultural pests has gained importance in recent years primarily due to increased pressure to reduce the use of agrochemicals and their residues in the environment and food. Publication of *Silent Spring* (Carson, 1962) evoked an increase in public concern of toxic chemicals over the welfare of the environment, especially the detrimental effects associated with pesticide use that heightened in the 1960's. The environmental hazards resulting from intensive use of synthetic organic crop protection agents make it imperative to consider alternative or complementary approaches to sustainable agricultural development and integrated pest management. The current millennium demands that the pest management studies should be bio-intensive. One of the methods that emerged in recent years gaining increased attention is the use of biopesticides in order to develop environment friendly, safe and compatible approaches and tactics for pest management. Most of these attributes do not harm the natural enemies and play a significant role in pest management systems that seek to reduce pesticide inputs and conserve our natural fauna.

Biopesticides are certain types of pesticides derived from natural materials as animals, plants, bacteria, or certain minerals. The different classes of biopesticide include biochemical pesticides, microbial pesticides and plant-incorporated protectants. They are usually inherently less toxic than the conventional pesticides and generally affect only the target pest and closely related organisms, in contrast to a broad spectrum, conventional pesticides that may affect other organisms as well. Biopesticides are often effective in very small quantities and often decompose quickly (Ware,1994), thereby resulting in lower exposures and largely avoiding pollution problems caused by the conventional pesticides. When used as a component of Integrated Pest Management (IPM) programs, biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high.

In the recent decades a variety of biocontrol methods employing peptidic or proteinaceous insect-specific toxins derived from microbes, plants and animals have been examined both in the laboratory and field with varying results. Interdependent factors involved with biopesticides are the production of a cost-effective, pesticide-production expense, kill efficiency, environmental persistence, pest-specificity, pest resistance-development, public perception and ease of delivery-sprayable biopesticides. Several approaches are being investigated for the design of new (bio) pesticides. These include the development of transgenic plants and recombinant baculoviruses as delivery systems for a variety of insect-selective toxins. The killing efficiency of baculoviruses may be augmented by genetic modifications of the baculovirus genome with the genes of another natural pathogen (Szewczyk, 2006). Baculovirus-insect cell system (BICS), with increased insect toxicity is established by universal gene silencing (UGS) system using RNA interference (RNAi). Bicistronic RNA seems an efficient way to lower both cost and effort of gene silencing approach while maintaining the biological activity of the protein of interest when the RNAi is not in use (Salem and Maruniak, 2007). Additional approaches for the development of foliar sprays include the rational design of peptidomimetics based on the key residues of these toxins that interact with the insect target (Nicholson, 2007).

Multi-level endocrine systems control a wide range of physiological processes like moulting, metamorphosis, diapause and reproduction. The multiple functional capacities of insect neuropeptides, based on intervention on this system at any level provide opportunities for new insect control strategies (Muraleedharen and Devi, 1992). Insect specific applications aiming at the disruption of neuroendocrine dependent processes include interference with neurosecretion, inhibition or induction of biosynthesis (transcription, processing, post-translational modifications), release, transport, and binding, activation of the receptors on the target cell, signal transduction and degradation (Couillaud and Peypelut, 1995). Biochemical sites such as chitin formation, Juvenile hormone, Juvenile hormone esterase, ecdysone,

acetylcholine and GABA receptors, ion channels and neuropeptides are all potential targets for insecticide action (Ishaaya, 2001).

The last decades have brought remarkable advances in the field of comparative neuropeptide research and this comparative approach paved the way for the discovery of many novel peptide structures, as well as a few of their receptors . Exposure of insects to genetically-engineered agonists or antagonists is generated either by transgenic plants or insect control enhanced by insect specific vectors by a combination of biological control contributed by microorganisms and neurochemical control contributed by neuropeptides.

Disruption of metamorphosis

Endocrine approaches to insect control have focused largely on hormones involved in regulating insect growth and metamorphosis. The two principal players towards this are the sesquiterpenoid Juvenile hormone (JH) and the steroid, molting hormone Ecdysone. Ecdysteriods are secreted from the prothoracic glands (PTG) that lies on the dorsal surface of the lateral tracheal trunks in the prothoracic segment. Upon stimulation by prothoraci cotropic hormone (PTTH) the glands secrete ecdysone into hemolymph, which is hydroxylated to the active hormone, 20-hydroxy ecdysone.

Juvenile hormone is being secreted by a pair of tiny glands the corpora allata (CA), attached to the base of brain, and is controlled by the neuropeptides, allatostatins and allatotropins secreted by the neurosecretory cells in the brain (Sindhu *et al*.,2001).Several hemolymph, cytosolic and nuclear JH binding proteins have been identified and characterized from insects (Mohan *et al*., 2005).JH rece ptors, enzymes involved in biosynthesis and degra dation of JH have also been characterized (Gilbert *et al*., 2000).

JH Endocrine System

The first attempt to utilize JH endocrine system as a pesticide was to develop Juvenoids or JH analogues. These are substances which elicit hormonal imbalance by disrupting vital functions of the insect resulting in arrested development, suppression of reproduction or other lethal morphogenetic effects (Muraleedharan and Devi, 1992). As a biopesticide, they induce adult sterility, physical body changes, water loss and premature death in insects. Methoprene, Hydroprene, Kinoprene, Juvocimen, Juvabione, R-394 are glaring examples of JH analogues that attracted the attention as insecticidal agents. Juvenogen, a fatty acid ester of a juvenoid alcohol, induced greatest soldier differentiation in representatives of three Reticulitermes species tested, showing the potential of juvenile hormone analogues in termite control (Hrdý *et al*., 2006).The biological activity of the juveno gens was also studied against the red firebug *Pyrrhocoris apterus*, termites *Reticulitermes santonensis* and *Prorhinotermes simplex*, and the blowfly *Neobellieria bullata* (Wimmer *et al*., 2007). Methoprene acts on *Aedes aegypti* by interfering with the expression of genes involved in 20E action, resulting in a block in midgut remodeling and death during pupal stage (Wu *et al* , 2006). Pyriproxyfen and Fenoxycab are the most important components in IRM strategy in cotton fields (Horowitz *et al*., 1999).

The discovery and subsequent development of effective Juvanoids, functional mimics of endogenous juvenile hormones, inspired that the reverse principle, Anti-Juvenile hormone action could be explored to compliment the use of Juvanoids. Precocene II (anti- JH) showed a very strong antifeedant effect against *Tribolium confusum*, the granary weevil beetle *Sitophilus granarius*, and the khapra beetle *Trogoderma granarium*, larvae and also against the herbivorous pest insects, Colorado potato beetle *Leptinotarsa decemlineata* and aphid *Myzus persicae* (Szczepanik *et al*., 2005). Alternatively, because many juvenoids including JH III itself have been isolated from plants (Bede *et al*., 2001), genetic engineering of plants could force them to produce Juvenoids for phytophagous insects. Common limitation of Juvenoids as pesticides is that they prolong the destructive instar of many pest insects, acting at specific periods of development.

Changes in the rate of biosynthesis are of considerable importance in determining the physiology of insect. Identification of allatomodulatory neuropeptides (Sheng *et al* , 2007; Noriega *et al* 2006; Sindhu *et al*., 2001) toge ther with their cDNA (Abdel-latief *et al*., 2003) opens new avenues for the practical use of these neuropeptides to disrupt JH biosynthesis. Furthermore, identification of allatomodulatory peptide receptors at the level of the allatal cells, elucidation of the mechanisms of signal transduction and identification of their mode of action on the activity of enzymes of the JH biosynthetic pathway will certainly provide new targets for the design of JH biosynthesis inhibitors.

There has been considerable interest in the discovery of chemical inhibitors of JH biosynthesis. Juvenile hormone acid methyl transferase (JHAMT) is an enzyme that converts JH acids or inactive precursors of JH to active JHs at the final step of JH biosynthesis pathway in insects. It has been indicated that JHAMT enzyme is develop mentally regulated in a few lepidopteran insect species (Bhaskaran *et al*., 1990). Correlation of JHAMT gene expre ssion and the biosynthetic activity in the CA suggests that the transcriptional suppression of the JHAMT gene is crucial for the termination of JH biosynthesis in the CA, which is a prerequisite for the initiation of metamo rphosis (Shinoda and Itoyama, 2003). Deeper insight into insect metamorphosis and its endocrine mechanism at

the molecular level especially in the field of developmental Insect Endocrinology should be of fundamental value in developing such newer strategies for disrupting insect life cycle and reproductive potential that are destructive to crops and hence of immense economic importance.

The reduction in JH titer is a key event in insect develo pment that leads ultimately to inhibition of JH biosyn thesis, causing premature metamorphosis, termination of feeding and adult sterilization (Kamita *et al*., 2003). This reduction is associated with dramatic increase in the levels of very active Juvenile Hormone esterase (JHE) (Gilbert *et al*., 2000, Wogulis *et al*.,2006). Because of its pivotal role in insect development, JHE have been targeted for use as a biopesticide. Hammock *et al* (1990) constructed a homology based molecular model of JHE from *Heliothis virescens*. This model is being used as a predictive basis to design such biopesticides. The expressed recombinant JHE acting as an anti-JH is potentially used for Mosquito control (Harshman *et al*., 1991).Thomas *et al* (1999) have constructed a homology-based molecular model of JHE from the agricultural crop pest, *Heliothis virescens* and also have identified a site on the protein surface that is suggestive of a recognition site for the putative JHE receptor. A fast acting recombinant baculovirus, that expre sses a modified form of JHE, which is inactive with respect to its function of JH catalysis, was developed by Bonning *et al*. (1995). The alteration of specific residues of JHE that disrupted lysosomal targeting dramatically increased the insecticidal activity of this enzyme. The insect growth regulator (IGR) imidazole KK-42 induces hemolymph juvenile hormone esterase activity and precocious metamorphosis in *Bombyx mori* (Hirai *et al*., 2002)

Ecdysone Endocrine System

Ecdysteroids do not apparently play physiological roles in vertebrates and hence offer a complete panel of potential targets for insect pest management strategies which have the advantage of being unlikely to affect vertebrates. The heterodimer of the ecdysone receptor (EcR) and ultraspiracle protein (Usp), members of the nuclear receptors superfamily, is considered the functional receptor for ecdysteroids initiating molting and metamorphosis in insects. The ligand binding domains of EcR and Usp from insects belonging to different orders fall into two separate groups and this can be exploited to discover order-specific insecticides (Palli and Retnakaren, 2001).Two compounds, tebufenozide and methoxy fenoxide, which bind to ecdysone receptor have been commercialized and used to control lepidopteran pests. These compounds are considered highly selective doing no harm to parasitoids and predators and fit well in IPM and insect resistance management programs (Dhadialla *et al*., 1998). Enzymes involved in biosynthesis and degrada tion of ecdysteriods as well as proteins that are critical for secretion and uptake of ecdysteriods into cells can be used as targets for developing newer insecticides.

Over the years, several attempts have been made to use ecdysone analogs for insect control. Diacyl hydrazines (RH) compounds bind to ecdysone receptor/ultraspiracle heretodimer that leads to incomplete precocious molt resu lting in the mortality of the larva due to persistence of the RH compounds (Dhadialla, 1998). Characterization of a non-steroidal, ecdysone agonist, bisacylhydrazine com pound, RH-131039 was carried out by Dhadialla *et al* (2007).

Genetic engineering of plants using constitutive promoters are presently the primary means used to express transgenes in plants. The ecdysone receptor gene switch is one of the best inducible gene regulation systems available, because the chemical, methoxyfenozide, required for its regulation is registered for field use. An EcR gene switch with a potential for use in large-scale field applications has been developed by adopting a two-hybrid format CfEcR:LmRXR for regulating the expression of a Superman-like single zinc finger protein 11 (ZFP11) gene in both Arabidopsis and tobacco transgenic plants(Tavva *et al*., 2007). Kojima *et al*. (2007) is the first to report of an ecdysone response element in a baculoviral gene promoter. These results also suggested that the regulation of the immediate-early gene baculovirus *ie1* by ecdysone may militate viral replication at least under certain conditions during natural infections *in vivo*.

Neuropeptide antagonists

A new integrated approach is the generation of a novel type of insect neuropeptide (Np) antagonists and putative insect control agents based on conformationally-constrained compounds .Antagonists which are selective inhibitors of the neuropeptide receptors may disrupt and interfere with the normal growth, development, homeostasis and behavior of the insect by blocking the receptor of the neuropeptide; therefore, they can form receptor-selective, insect-specific insecticides.

The approach, termed "Insect Np-based Antagonist *i*nsecticide (INAI)", was applied to the insect pyrokinin (PK)/pheromone biosynthesis-activating Np (PBAN) family as a model (Altstein and Gilon, 2001). In the noctuid moth, sex-pheromone biosynthesis follows a circadian cycle, which is cued by the release of the neurohormone pyrokinin/pheromone biosynthesis activating neurop eptide (PK/PBAN family) to the hemolymph, which also mediates a variety of other functions in moths and other insects (Ajitha and Muraleedharan, 2005). These neuropeptides exert their functions through activation of the G protein-coupled receptor PBAN receptor (PBAN-R), in pheromone glands (Zheng *et al*., 2007).

This new approach led to the discovery of a potent linear lead antagonist and several highly potent, metabolically

stable backbone cyclic (BBC) conformationally constrained antagonists (BBC antagonists), devoid of agonistic activity, which inhibited PBAN-mediated activities in moths *in vivo* antagonists and acted as the basis for the design of insect control agents (Altstein 2001,2004). Ben-Aziz *et al*., (2006) showed that Backbone cyclic pheromone biosynthesis activating neuropeptide (PBAN) antagonists inhibit melanization in the moth *Spodoptera littoralis*. Beyond the immediate benefits introduced by the cyclic peptides as selective antagonists, the information on the bioactive conformations may serve as a basis for the design of improved nonpeptide, mimetic antagonists. Such compounds are potential candidates for agrochemical applications and could serve as prototypes for the development of a novel group of highly effective, insect-specific and environmentally friendly insecticides.

Systematic replacement of the naturally occurring L-ami noacids by their non natural D-isomers or replacement of aminoacid residues, such as D-Phe or D-Trp will convert agonist to antagonist (Altstein and Gilon, 2001).The D-Phe approach to proctolin resulted in the discovery of a few peptides with antagonistic activity (Kuczer *et al*., 1999).

Chitin synthesis inhibitors

The insect cuticle serves as an interface between the living animal and its environment forming the exoskeleton and supporting the lining of the gut, respiratory systems, reproductive ducts and some gland ducts (Tunaz and Uygun, 2004).The first chitin synthesis inhibitor was benzoylphenylurea, diflubenuron (Miyamoto *et al*., 1993). Benzoyl phenyl ureas affect ecdysone-dependent biochemical sites which lead to chitin inhibition. The chitin synthesis inhibitors were quite effective against multi resistant *Musca domestica* strains (Pospiscil *et al*.,1997). The chitin synthesis inhibition site has proved to be important for the development of control agents which act selectively on important groups of insect pests.

Acetyl Choline Receptors.

Efforts have been made to develop nicotinyl insecticides with high affinity to insect nicotinic acetyl choline receptors (nAChR) resulting in the development of a new group of neonicotinoid insecticides (Elbert *et al*.,1996).Imidacloprid, acetamiprid and thiamethoxam are potential neonicotinoids used as agro-chemicals. Acetamiprid has been introduced in Israel as a component in the IRM program to control *B. tabaci* in cotton fields, while imidacloprid is used systemically through the soil to control white flies and aphids in vegetable and ornamentals. The neonicotinoids act specifically on sucking pests and have no effect on parasitoids and predators, and as such fit in various IPM programme.

GABA and Glutamate Receptors and Ion Channels.

The ã-aminobutryric acid (GABA) receptor/chloride ionophore complex has been the focus of intense interest as a site of insecticidal action. A Voltage-dependant sodium channel and ã -aminobutyric acid (GABA)-gated chloride channels are primary sites of a number of established insecticides. Abamectin, emamectin, milbemectin and spinosad, which act on GABA receptors and ion channels, have been developed to control mites and other agricultural pests (Bloomquist, 2001).

Insect Gut as a Site for Insecticide Action

Naturally occurring protease inhibitors have been explored since they interact and block the active center of the digestive enzymes, both proteases and amylases,in the gut system. Commonly used inhibitors include are crystalline soybean trypsin inhibitor of Kunitz, Bownman-Birk soybean trypsin inhibitor, lima bean inhibitor, chickpea trypsin inhibitor, chymotrypsin inhibitor and ovomucoid.(Reeck *et al*., 1997). These proteins bind tightly to the active site of the enzyme in the midgut preventing access to normal substrates. Failure by the pest insect to overcome this inhibition of digestion results in death by starvation. This principle of digestive enzyme inhibitors can be exploited with the recombinant DNA-technology in transgenic plants for crop protection (Nathan *et al*., 2006).

CONCLUSIONS

Several of the developed botanical pesticides to-date inculcate repellency, anti-feedancy or often interfere with either growth or reproduction in the specific pest species. Invertebrate hormones like Juvenile hormone, Ecdysone and so many related regulatory neuropeptides; all control several vital physiological processes including morphogenesis or development and reproduction in arthropods. Compounds that interact with JH and ecdysone receptors such as fenoxycarb and pyriproxifen (JHA) and tebufenocide and methoxy fenozide (ecdysone agonists) are being developed as selective insecticides especially for the control of scale insects, white flied or lepidopteran caterpillars. Inhibitors of acetylcholine receptors like Neonicotinoids and imidacloprid acetamiprid and theomethoxam that are more specific to aphids and white flies. Abamecvtin, emamectin, milbermectin and spinosad are inhibitors of GABA receptors and ion channels specific to mires and related agricultural pests. Rational design and selection methods to develop antagonistic cyclic peptides based on their specific insect neuropeptide sequences like PBAN or allatomodulatory neuropeptides is another attractive mode of approach to combat specific arthropod pests. Signal transduction mechanisms of many of these hormones and neuropeptide ligands to their respective target cell and

also their respective gene have been poorly understood. Some of the key enzymes involved in the synthetic cycle of these hormone molecules and even their genes also need to be understood at the nanoscale as manipulations of these are being projected as strategy for effective management of arthropod pests of both agricultural and medicinal importance. Novel approaches to develop arthropod pesticidal agents much more specific to insect biochemical sites that are not active to mammal need to be explored in future for developing safer and efficient pesticidal agents as a principal component of Integrated Pest Management program.

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