Recent Advances in Hormones in Insect Pest Control

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New approaches to the development of insect control agents have been revealed through the description of natural and synthetic compounds capable of interfering with the processes of development and reproduction of the target insects. The review presents information on novel insecticides that mimic the action of two insect growth and developmental hormone classes, the ecdysteroids and the juvenile hormones. Neuropeptide structures, their biogenesis, action, and metabolism also offer the opportunity to exploit novel control agents.
KEY WORDS: Biological control; insect hormones; juvenoids; ecdysteroid agonists; ecdysteroid antagonists; neuropeptides.

INTRODUCTION

Modern insecticide research started almost 60 years ago with the chlorinated hydrocarbons, followed shortly by the organophosphates, methylcarbamates and nitroguanidines. The main target of these synthetic chemical compounds is the insect nervous system. The botanicals came next and may have been more exciting because of their structural complexity, potency and selectivity. Pyrethrum has been the most widely used botanical for almost two centuries. Many botanicals, however, are impractical in commercial agriculture and as a group they are not safer than the synthetics (24). In the search for safer insecticide technologies, *i.e.*, more selective modes of action and reduced risks for non-target organisms and the environment, progress has been made in the last 20 years with the development of natural and synthetic compounds capable of interfering with the processes of growth, development and metamorphosis of the target insects (1). These chemicals have been called insect growth regulators (IGR) or third-generation insecticides (34,37). IGRs differ widely from the commonly used insecticides. As they exert their insecticidal effects through their influence on development, metamorphosis and reproduction of the target insects by disrupting the normal activity of the endocrine system, their action is much slower than that of the synthetic chemical insecticides. This paper presents some newer developments of IGRs and discusses the insect endocrine system as a potential and specific physiological target for pest control. More detailed information on novel insecticides that mimic the action of the two insect growth and developmental hormones, the steroidal 20-hydroxyecdysone and the sesquiterpenoid juvenile hormone, can be found in the recent review of Dhadialla and co-workers (7).

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HORMONAL REGULATION OF INSECT DEVELOPMENT AND REPRODUCTION

The principal hormones involved in these life processes include neurohormones (neuropeptides), ecdysteroids (molting hormones) and the sesquiterpenoid juvenile hormones (JHs). During larval and pupal stages, ecdysteroids and JHs are responsible for the control of molting and metamorphosis. The molting process is initiated by an increase of 20-hydroxyecdysone (20-E) in the hemolymph and completed following a decline of 20-E titer and the release of a peptide eclosion hormone. In insects with partial metamorphosis a gradual reduction in the JH titer allows development of subsequent nymphal stages. At the time of the final molt, JH is absent and the adult emerges. Insect larvae with complete metamorphosis have groups of cells called imaginal disks with the potential of expressing adult characters. JH inhibits the growth of these disks. During the last larval instar, the commitment to undergo a pupal molt requires the action of ecdysteroids in the absence of JH, whereas the larval–pupal molt is then induced in the presence of JH again. Finally, pupal–adult transformation occurs at increased ecdysteroid titers but with very low levels of JH. In the adult stage, however, both of these hormones are involved in the regulation of reproductive maturation (13). Recent studies indicate that the molecular target for ecdysteroids consists of at least two proteins, the ecdysteroid receptor (EcR) and the product of another gene, *ultraspiracle*. However, additional factors may also be involved for ligand binding and ecdysteroid-dependent gene regulation. The activity of JH at the molecular level is not well understood (7).

Any interference in the homeostasis of these hormones with exogenous sources of the hormones or with synthetic analogs (agonist or antagonist) would result in the disruption or abnormal course of development and reproduction of the target insect.

A great variety of processes in insects are known to be regulated by peptides originating from various parts of the nervous system. These processes include stimulation of molting by initiating ecdysteroid biosynthesis, initiation of behavioral patterns associated with ecdysis and its timing, control of JH biosynthesis, water and ion balance, influence of contractions of visceral muscles and regulation of energy metabolism (10). Recently, considerable progress has been made in the characterization of these neuropeptides and their genes, owing to the development and refinement of new technologies for isolation, structure elucidation and molecular genetics (10). In addition to the peptides themselves, the mechanisms responsible for their synthesis, maturation, transport, secretion, binding, action, and inactivation offer numerous targets for novel insecticide development.

ECDYSTEROID AGONISTS AND ANTAGONISTS

In the majority of insects, the prothoracic glands are the major source of ecdysteroids during larval development. The involvement of alternative sites of ecdysteroid production (ovary, testes, abdominal integument) seems to be limited to pupal and adult stages. Ecdysteroids are synthesized from cholesterol or phytosteroids in the diet, because insects cannot synthesize the steroid nucleus. The very early steps in ecdysone biosynthesis from cholesterol are still not fully elucidated. The final steps of ecdysone biosynthesis consist of a sequence of hydroxylations catalyzed by cytochrome P-450-dependent hydroxylases. Several P-450 inhibitors have been assayed and proved to be effective in laboratory experiments but they seem not amenable to field use. The imidazole compound KK 42 (16) inhibits the ecdysteroid biosynthetic pathway in prothoracic glands as well as in alternative

ecdysteroid sources (18). Ketokonazole, another synthetic imidazole derivative, is known to inhibit the ecdysone 20-monooxygenase and was also very effective in inhibiting the terminal hydroxylation steps of ecdysteroid biosynthesis in adult locusts and crickets (15,18). Acetylenic (B1, B6) and allenic (AL2) cholesterol derivatives inhibit ecdysone biosynthesis and were found to be active not only on conventional ecdysteroid sources but also on epidermal ones (18,27).

The search for ecdysteroid agonists has been very successful (38) and the ecdysteroid mimic, RH 5992 (tebufenozide), is currently marketed under the trade names Mimic,® Confirm® and Romdan® (7). RH-2485, the newest member of the bisacylhydrazine class, seems to be even more active than tebufenozide, acting against a wide range of lepidopteran pests. All these non-steroidal ecdysone agonists manifest their effects *via* interaction with the EcR/USP receptor complex. The high degree of safety with respect to non-target organisms is particularly interesting.

The most efficient natural substance with molt-inhibiting activity is azadirachtin, a tetranortriterpenoid plant (neem tree, Meliaceae) limonoid with ecdysteroid-like structure (21). Its strong antifeedant, insect growth regulatory and reproductive effects are well documented, although its biochemical effects at the cellular level are still unclear. In India, neem extracts are traditionally used for their antiseptic, medicinal as well as insecticidal properties (30). A major action of azadirachtin is to modify insect hemolymph ecdysteroid titers due to a blockage of release of prothoracicotropic hormone from the brain–corpus cardiacum complex. Azadirachtin-containing products have been formulated as liquids or dusts from ethanol extracts of neem tree seeds and are being marketed as botanical insecticides.

The family Meliaceae is the source of numerous other insecticidal compounds. Recently, members of the genus *Aglaia* were shown to contain novel insecticidal constituents of the rocaglamide type (benzofurans), which evince strong insecticidal activity towards neonate larvae of *Spodoptera* (11).

Brassinosteroids represent the first true antiecdysteroids observed. They are a family of growth-promoting hormones found in plants and have striking structural similarities with the ecdysteroids. The effect of brassinosteroids on insects may be explained by their competition with molting hormones at the binding site of the hormone receptor which results, for example, in delayed molting (26). Very recently, two triterpenoids isolated from seeds of a cruciferous plant, cucurbitacins B and D, were demonstrated to be insect steroid hormone antagonists acting at the ecdysteroid receptor (8).

Another group of IGRs affects chitin synthesis and cuticle sclerotization during development and reproduction. Exposure of insect larvae to diflubenzuron (Dimilin®), a benzoyl phenyl urea derivative, causes improper attachment of the new cuticle during molting and produces a cuticle that lacks some of the normal layers. However, diflubenzuron with its broad spectrum of activity against insects, may also reduce populations of natural enemies of pests, as would conventional insecticides. Indomethazine, a non-steroidal amino acid decarboxylase inhibitor, inhibits DOPA decarboxylase, thus interfering with cuticle sclerotization. Its biological activity, however, is so far insufficient to justify commercial development (17).

JUVENILE HORMONE ANALOGS AND INHIBITION OF JH BIOSYNTHESIS

Since the early 1970s, numerous analogs of JH (juvenoids) have been tested for insecticidal activity (31). Most of the early analogs resemble JH in their basic terpenoid structure. The first juvenoids were farnesol and farnesal isolated from insects themselves (29). The 'paper factor' (32), now called juvabione, represents a group of hormone mimics present in a variety of plants where they may function as defensive mechanisms against herbivorous insects. Two very active JH analogs, methoprene (Altosid®) and hydroprene (Altozar®), lack the epoxide function present in JH. They are used in controlling many dipteran and household pests, *e.g.* flea larvae, Homoptera on houseplants, and pharaoh ants. Use of a third registered juvenoid, kinoprene, was discontinued in 1985. More recently, several highly active compounds with less apparent similarity to JH (aromatic non-terpenoidal JH analogs) like fenoxycarb, pyriproxifen and diofenolan, have been synthesized (7). JH analogs have a relatively broad spectrum of insect toxicity. Different formulations of fenoxycarb have been used for the control of a number of coleopteran and lepidopteran pests. Pyriproxifen is active against mosquitoes, various other dipterans and some insects of other orders, *e.g.* whiteflies. Diofenolan has good activity against lepidopteran pests and scale insects in orchards. JH analogs, like the natural JHs, can restore physiological and biochemical processes that are dependent upon the presence of JH, such as maintenance of a juvenile status, synthesis of egg yolk proteins, and their uptake by the developing oocytes. The reason for the increasing popularity of juvenoids includes their short residual effects in the field and the few and short sensitive phases of the target insects (5). However, since juvenoids act only on certain stages, their application must be timed precisely and, owing to their low field stability, it ought to be repeated several times. In other words, JH analogs are presently most practical where instantaneous control is not needed.

Since JH analogs may keep the insects in an immature and potentially injurious stage longer than normal, a chemical which would shut off JH production could be more useful. Such a chemical should cause premature metamorphosis of larvae and sterilization of adult insects. Two such anti-JH substances were discovered in the garden plant *Ageratum houstonianum* and named precocene I and II. Precocenes appear to exert a cytotoxic action on the corpora allata, mediated by an oxidative bioactivation *in vivo* (3). Interspecific differences in sensitivity of the corpora allata and/or in precocene pharmacokinetics may explain the observed differences in sensitivity to these compounds.

Several other materials also have been studied as potential inhibitors of JH biosynthesis. These include fluoromevalonate, mevinolin and fluvastatin. Fluvastatin, a synthetic 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor, inhibited JH biosynthesis by locust corpora allata *in vitro* but exhibited only a weak effect *in vivo* (6). A new inhibitor of JH biosynthesis has been isolated from the entomopathogenic fungus *Penicillium brevicompactum.* The substance, a disubstituted heterocyclic oxime, was named brevioxime (22). It clearly inhibits the final methylation/epoxidation steps of JH hormone biosynthesis. In our laboratory we recently demonstrated that the natural plant alkaloid arborine has an allatostatic potential (23). This alkaloid was shown to inhibit JH biosynthesis *in vitro* in the corpora allata from adult female crickets in a dose-dependent manner. Arborine also showed a larvicidal activity against the mosquito *Culex quinquefasciatus.* Its mechanism of action, however, is still unknown.

Stimulation or inhibition of JH degradation will also modify JH titer and thereby disturb the physiology of an insect. JH catabolism involves mainly epoxide hydrolases and esterases. Hammock and co-workers (12) identified the coding region for JH esterase from the genome of *Heliothis virescens,* introduced it into the nuclear polyhedrosis virus *Autographa californica,* and expressed it under the control of polyhedrin and p10 promoters. However, when recombinant JH esterase viruses were tested *in vivo*, only a few larvae exhibited increased levels of JH esterase in the hemolymph. Although the results demonstrated the feasibility of such an endocrine-based strategy, the effect was too unreliable to be of current value for practical insect control (12). A recombinant baculovirus expressing a modified form of JH esterase has enhanced activity against lepidopteran larvae (2). This virus, however, does not kill the larvae by an anti-JH mechanism but apparently by contraction-paralysis, or disruption of the normal sequence of events at the molt.

INSECT NEUROPEPTIDES AND THEIR APPLICATION IN PEST CONTROL

Peptides released by specialized neurosecretory cells of the insect's central nervous system (neuropeptides) may function as neurotransmitters, neuromodulators and as hormones and have been called the 'master regulators' of development, behavior, metabolism, homeostasis and reproduction. Several hundred novel insect neuropeptides have been sequenced to date, numerous peptide analogs have been synthesized, a large number of gene sequences have been determined and neuropeptide genes have been expressed in vector systems. It is impractical to consider neuropeptides themselves as pest control agents since, for example, their physico-chemical properties would render them susceptible to degradation under field conditions and to be digested after feeding; their polarity would make their uptake through the cuticle most difficult. What is important about neuropeptides as potential leads to control agents is the structural information included within the molecules, which provides clues to the manner in which the active neuropeptide is synthesized, processed, addressed, prompted to act upon a target tissue, and degraded (20). The disruption of any step leading to biosynthesis of neuropeptides, their modification during storage, their release into the hemolymph as well as their interaction with the target-cell membrane-bound receptors offer multiple modes of action for a novel neuropeptide-based insect control strategy (fourth generation insecticides). Of course not all biochemical mechanisms will be worth exploiting, nor will all neuropeptides be of equal importance with regard to pest control.

A primary aim is the synthesis of stable and more lipophilic agonists or antagonists of natural neuropeptides by molecular modelling. Here, neuropeptides regulating JH biosynthesis are discussed according to the aspects outlined above. Brain neuropeptides that either stimulate (allatotropin) or inhibit (allatostatin) JH biosynthesis by the corpora allata have been described for several insect species (moths, cockroaches, locusts, crickets, flies and bees). Except for one allatotropin and one allatostatin from *Manduca sexta,* all the other allato-regulating peptides belong to the so-called allatostatin superfamily, having a FGL-amide C-terminus (10). In some insect species these allatostatins do not act upon JH biosynthesis but have myo-inhibiting properties. Recently, we have identified a family of neuropeptides (B-allatostatins) from crickets and stick insects, which had been purified on the basis of their ability to inhibit JH biosynthesis, but which show high sequence homology with a class of myo-inhibiting peptides from locusts and moths (19). In addition to their

potent inhibition of JH biosynthesis in crickets, the B-allatostatins exerted myo-inhibiting activity in a cockroach hindgut bioassay, and effectively inhibited ovarian ecdysteroid biosynthesis in crickets.

A first step towards designing more potent neuropeptide analogs or true non-peptide agonists has recently been taken by Bellés and co-workers when they replaced the Leu^3-Tyr^4 peptide bond of Blg-AST 2 (BLAST 2) with the methyleneamino Ψ [CH_2NH] and ketomethylene Ψ [$COCH_2$] surrogates, respectively, with the aim of increasing the resistance of this octapeptide to degradation by endopeptidases (25).

Another effective strategy may be to design specific inhibitors of the enzymes involved in processing of peptide precursor proteins. In the cockroaches *Diploptera punctata* and *Periplaneta americana,* the desert locust *Schistocerca gregaria* (for review see 35), the blowflies *Calliphora vomitoria* and *Lucilia cuprina* (9), the mosquito *Aedes aegypti* (36), and the field cricket *Gryllus bimaculatus* (our unpublished results), the gene that encodes a precursor polypeptide containing 5 to 15 allatostatins has been isolated. Any of the three identified enzymatic steps in prohormone processing (endoproteolysis, carboxypeptidase trimming or amidation) could be targeted. The use of recombinant viruses for expression of specific neuropeptide genes might combine viral effects with an endocrine imbalance. A third emerging technology focuses on the identification of receptor proteins as potential insecticidal targets. These new approaches can be combined with computer-assisted modelling and lead to a reduction of *in vivo* experiments.

CONCLUDING REMARKS

When, 30 years ago, compounds with insect hormonal activity were first proposed to be used as insecticides, insects were believed to be unable to develop resistance to molecules that mimic their own hormones. This presumption, however, has not proved true. Several IGRs recently have been added to the list of insecticides to which insects are resistant. Included in the list of IGR compounds are methoprene, hydroprene, kinoprene, pyriproxifen, RH 5992, and diflubenzuron (14,28,33). Presently, at least 13 insect species, representing the Diptera, Coleoptera, Homoptera and Lepidoptera, show cross-resistance to IGRs (24). Although resistance mechanisms are still under investigation, it seems as if most IGR resistance results from reduced penetration and increased metabolism of the compounds.

Development and open-field application of a potential new insecticide requires evaluation of its efficacy, selectivity and specificity, stability under outside conditions, compatibility with other pest management tactics, and innocuousness for vertebrates. For example, molecules based on the JH system are more or less common to all insect species and will affect not only the pest insect under attack, but also other species not currently monitored for their biological life cycle (5). Chitin synthesis inhibitors may be toxic to other arthropods such as Crustacea, and IGR metabolites may have adverse effects on vertebrates because of their ability to bind to certain members of the nuclear hormone receptor family.

Certainly, strategies based on effects on the insect endocrine system will help to find new, promising substances that can be used in agricultural applications together with other approaches, based, for example, on insect chemical communication, use of natural insect parasites, and plant genetic engineering. Insecticide research is now in a renaissance of integrating chemicals and biologicals for sustainable pest control with human safety, but it

is still unlikely that the 'golden age of genetic engineering' will eliminate synthetic organic insecticides in the foreseeable future (4).

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