degraded. Thus, [^{14}C]MPPA must undergo degradation in the soil with release of [^{14}C]carbon dioxide.

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REVIEW

Insect Neuropeptides: Potential New Insect Control Agents

Julius J. Menn* and Alexej B. Bořkovec

Insect neuropeptides provide exciting new approaches to insect management. The last two decades have seen the emergence of the first chemically identified insect neurohormones, their synthesis, and beginning understanding of their mode of action. The identification of many additional neuropeptides that regulate all aspects of insect development and reproduction can be expected in the near future. Identification of functionally critical neuropeptides and their precursors (proneurohormones) may provide models for designing highly selective insect control agents; these agents may act as antagonists of proneurohormone-processing enzymes or inhibitors of neuropeptide-degrading enzymes. It is also anticipated that through genetic engineering manipulations it will become possible to deliver neuropeptide genes into host cells with successful transformation and subsequent expression via potent expression vectors such as the insect baculoviruses.

EVOLUTION OF NEUROPEPTIDE RESEARCH IN INSECTS

The proposition that neurons, the cellular components of the animal nervous system, produce substances that control the biochemical and physiological processes at distant parts of the body appeared revolutionary when it was first made 70 years ago (Kopec, 1917, 1922). Nevertheless, the hypothesis was confirmed (Scharrer, 1928), and its gradual acceptance in vertebrate as well as invertebrate physiology opened a new area of investigation concerned with the origin, nature, and function of chemical messengers used by the central nervous system to communicate with its subordinate organs. In terms of their chemical structures, most, if not all of these messengers are now believed to be oligopeptides or small protein molecules; but the exact mechanisms by which they function are not known. The generic designation of neuropeptides as "neurohormones" may not always be accurate but will be used here interchangeably as is the common practice.

Kopec formulated his great hypothesis on the basis of laboratory experiments with insects (gypsy moth, *Lymantria dispar*), so it may seem ironic that more than 50 years elapsed before the first insect-derived neuropeptide, proctolin, was isolated and identified (Starratt and Brown, 1975) and that only now are attempts being made, in our laboratory (Masler et al., 1986), to characterize Kopec's original "brain hormone", the gypsy moth prothoracicotropic hormone (PTTH).

The reasons for the slow progress in isolating insect neuropeptides are primarily twofold: They are present in insects in almost unimaginably small quantities that must be measured in femtomoles (1×10^{-15} mol), and they are often highly labile to one or more of the conditions or substances used in the isolation process (e.g., heat, freezing, and organic solvents). Moreover, the richest source of neuropeptides, the neuroendocrine organs, are often microscopic so that thousands or even millions of insects have to be used in the initial extraction. The advent of high-

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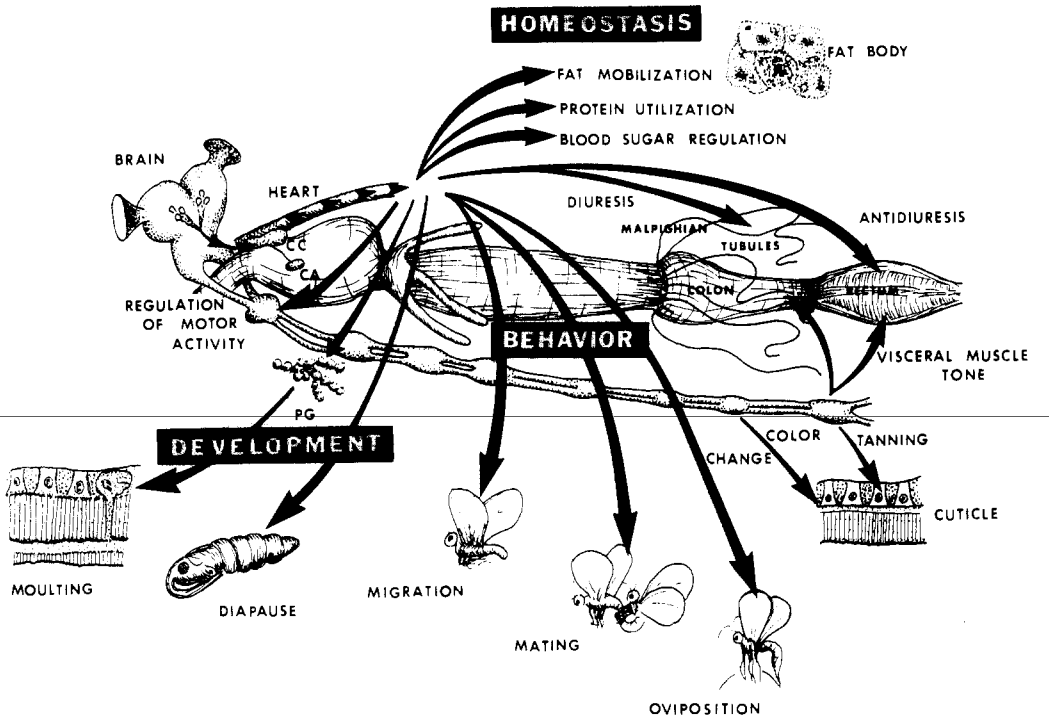


Figure 1. Major physiological processes regulated by neurohormones in insects. Key: CC, corpora cardiaca; CA, corpora allata; PG, prothoracic gland. Reprinted with permission from Cook and Holman. Copyright 1985, Pergamon.

performance liquid chromatography (HPLC), particularly reversed-phase HPLC, has considerably improved the outlook for isolating new insect neuropeptides, and the development of powerful analytical tools such as fast-atom bombardment/tandem mass spectrometry (Bieman and Scoble, 1987) has significantly increased both the speed and accuracy with which structural determinations can be made and decreased the required sample size.

Despite these advances facilitating the isolation of neuropeptides, the problems facing insect neuroscientists remain formidable. At the First International Conference on Insect Neurochemistry and Neurophysiology (Bořkovec and Kelly, 1984), the number of insect neuropeptides was estimated to be well over 100 on the basis of accumulating reports on species specificity. At the second conference, 3 years later (Bořkovec and Gelman, 1986), it became clear that the occurrence of neuropeptides in groups or families of related materials with the same or similar biological activity was quite common and that, therefore, the number of insect neuropeptides may be considerably larger than previously estimated. Although it would hardly be possible or even necessary to isolate and characterize all neuropeptides from thousands of insect species, the availability of those that affect vital processes in some of the important species is essential if we are to progress in understanding neuroregulation and devise ways of interfering with it.

NEUROPEPTIDES AND THEIR FUNCTION

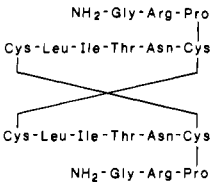
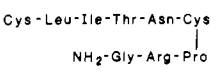
In the hierarchy of entities that regulate endogenous biochemical control functions, the central nervous system and its neuropeptide messengers rank the highest. Figure 1 shows schematically the diversity of some of the more important functions that are known to be under neurohormonal control (for more detailed reviews of these and other neurohormonally controlled functions, including those that have been proposed but not yet confirmed, see Raabe (1982), Downer and Laufer (1983), Bořkovec and Kelly (1984), Kerkut and Gilbert (1985), and Bořkovec and Gelman (1986). Table I lists some of the better known neurohormones, most of which are currently under intensive investigation. Although all neuropeptides are important because they regulate functions necessary for the survival of individual insects or for propagation of the species, some are of particular interest because they affect the titer of other endocrine horomones and, thus, the outcome of a multitude of vital processes that are hormonally controlled. These neurohormones, which have been aptly called glandotropic (Sehnal, 1979), are represented in Table I by allatotropin and allatostatin, which regulate the synthesis of juvenile hormones (JH) by the endocrine glands, corpora allata, and by four ecdysiotropins, which affect the synthesis of ecdysteroids (molting hormones) by the prothoracic gland or other endocrine systems. Whether other neurohormones are glandotropic is a question that must be considered separately in each case. At least two of the ecdysiotropins, PTTH and the egg development neurosecretory hormone (EDNH), are now known to be complex mixtures of active components differing in molecular weight and probably also in some structural features and biological activity. So, for example, PTTH from the head of the silkworm, *Bombyx mori*, was found to consist of two groups of peptides (Nagasawa et al., 1986): the 4K PTTH, with molecular weight of about 4400 and the 22K PTTH, with molecular weight of about 22 000. The 4K PTTH consists of at least three peptides, 4K PTTH I, 4K PTTH II, and 4K PTTH III; but the structural differences between them are not known because only 4K PTTH II has been structurally identified (Table II). The 4K PTTH and 22K PTTH differ strikingly in biological activity. The former is inactive in a bioassay employing *B. mori*, the insect from which the peptides were isolated, but it is active in a similar bioassay employing a related silkworm species, *Samia cynthia ricini*. The reverse is true of the 22K PTTH. Reasons for this perplexing difference in activity are still being investigated.

Another group of neuropeptides constitute the adipokinetic hormone (AKH) family. They are structurally related and are also related to the red pigment concentrating hormone (RPCH), derived from the shrimp, *Pandalus sp.* (Fernlund and Josefsson, 1972). Hormones of the AKH

Table I. Selected Neuropeptides or Neuropeptide Families and Their Functions

neuropeptide	function regulated	references
	Development and Reproduction	
prothoracicotropic hormone (PTTH)	ecdysteroid synthesis	Bollenbacher and Granger, 1985 (review); Nagasawa et al., 1986
oostatic hormone (OH)	ecdysteroid synthesis	Kelly et al., 1984; Borovsky, 1985
egg development neurosecretory hormone (EDNH)	ecdysteroid synthesis	Masler et al., 1984; Borovsky and Thomas, 1985
testis ecdysiotropin	ecdysteroid synthesis	Loeb et al., 1986
allatotropin	juvenille hormone synthesis	Bhaskaran et al., 1980; Tobe and Stay, 1980
allatostatin	juvenile hormone synthesis	Paulson and Stay, 1987, Granger and Janzen, 1987
diapause hormone	diapause	Isobe and Goto, 1980 (review)
bursicon	cuticular tanning	Reynolds, 1983 (review)
	Behavior	
ecolsion hormone (EH)	eclosion behavior	Truman et al., 1981
pheromone biosynthesis activating neuropeptide (PBAN)	pheromone synthesis	Raina et al., 1986; Raina and Menn, 1987 (review)
	Homeostasis and Metabolism	
adipokinetic hormone (AKH)	lipid metabolism	Orchard, 1987 (review)
hypertrehalosemic hormone (HTH)	sugar metabolism	Orchard, 1987 (review)
periplanetin (HGH, CC, K)	sugar metabolism	Orchard, 1987 (review)
diuretic hormone	fluid secretion	Phillips, 1983 (review)
antidiuretic hormone	fluid secretion	Phillips, 1983 (review)
	Muscle Function	
proctolin	muscle contraction	Cook and Holman, 1985 (review)

Table II. Insect Neuropeptides, Their Sources, and Primary Structures

name	source	structure	references
proctolin	*Periplaneta americana*	Arg-Tyr-Leu-Pro-Thr	Starratt and Brown, 1975
adipokinetic hormone I (AKH I)	*Locusta migratoria*	pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2	Stone et al., 1976
adipokinetic hormone II-L (AKH II-L)	*Locusta migratoria*	pGlu-Leu-Asn-Phe-Ser-Ala-Gly-Trp-NH_2	Siegert et al., 1985
adipokinetic hormone II-S (AKH II-S)	*Schistocerca gregaria*	pGlu-Leu-Asn-Phe-Ser-Thr-Gly-Trp-NH_2	Siegert and Mordue, 1986
adipokinetic hormone (M-AKH, H-AKH)	*Manduca sexta*, *Heliothis zea*	pGlu-Leu-Thr-Phe-Thr-Ser-Ser-Trp-Gly-NH_2	Ziegler et al., 1985; Jaffe et al., 1986
periplanetin I, neurohormone D (CC-I, M-I, HGH-I)	*Periplaneta americana*	pGlu-Val-Asn-Phe-Ser-Pro-Asn-Trp-NH_2	Scarborough et al., 1984; Witten et al., 1984; Baumann and Penzlin, 1984
periplanetin II (CC-II, M-II, HGH-II)	*Periplaneta americana*	pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-NH_2	Scarborough et al., 1984; Witten et al., 1984
hypertrehalosemic hormone (HTH)	*Blaberus discoidalis*, *Nauphoeta cinerea*	pGlu-Val-Asn-Phe-Ser-Pro-Gly-Trp-Gly-Thr-NH_2	Hayes and Keeley, 1986; Gäde and Rinehart, 1986
hypertrehalosemic hormone II (HTH-II)	*Carausius morosus*	pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2	Gäde, 1985
arginine vasopressin-like diuretic hormone, F_2 (AVP-like IDH)	*Locusta migratoria*		Proux et al., 1987
arginine vasopressin-like factor F_1			Proux et al., 1987
leucosulfakinin (LSK)	*Leucophaea maderae*	Glu-Gln-Phe-Glu-Asp-Tyr(SO_3H)-Gly-His-Met-Arg-Phe-NH_2	Nachman et al., 1986a
leucosulfakinin II (LSK II)	*Leucophaea maderae*	pGlu-Ser-Asp-Asp-Tyr(SO_3H)-Gly-His-Met-Arg-Phe-NH_2	Nachman et al., 1986b
prothoracicotropic hormone, bombyxin (4K PTTH II)	*Bomyx mori*	A chain:[a] H-Gly-Ile-Val-Glu-Asp-Glu-Cys-Cys-Leu-Arg-Pro-Cys-Ser-Val-Asp-Val-Leu-Leu-Ser-Tyr-Cys-OH B chain:[a] pGlu-Gln-Pro-Gln-Ala-Val-His-Thr-Tyr-Cys-Gly-Arg-His-Leu-Ala-Arg-Thr-Leu-Ala-Asp-Leu-Cys-Trp-Glu-Ala-Gly-Val-Asp-OH	Nagasawa et al., 1986
eclosion hormone (EH)	*Manduca sexta*	H-Asn-Pro-Ala-Ile-Ala-Thr-Gly-Tyr-Asp-Pro-Met-Glu-Ile-Cys-Ile-Glu-Asn-Cys-Ala-Gln-Cys-Lys-Lys-Met-Lev-Gly-Ala-Trp-Phe-Glu-Gly-Pro-Lev-Cys-Ala-Glu-Ser-Cys-Ile-Lys-Phe-Lys-Gly-Lys-Lue-Ile-Pro-Glu-Cys-Glu-Asp-Phe-Ala-Ser-Ile-Ala-Pro-Phe-Leu-Asn-Lys-Leu-OH	Marti et al., 1987; Kataoka et al., 1987

[a] Chains A and B are connected with disulfide bridges.

family were isolated from the corpora cardiaca of various insects and were found to regulate the metabolism of lipids and sugars. Their structure–activity relationships as well as modes of action have been extensively investigated (Orchard, 1987). Their chemical compositions are shown in Table II. Because of their relative simplicity, the AKH family and proctolin, as well as many of their analogues and homologues, have been synthesized and have provided an indication that the biological activity of natural neurohormones may be increased by structural manipulation. Thus far, however, enhancement of activity has been only modest and achieved only with analogues of proctolin (Starratt and Brown, 1979); it cannot yet be predicted in other neuropeptide families. Table II also contains the structures of two much larger neuropeptides, PTTH and the eclosion hormone (EH). The purification and sequencing of these materials represent more than 10 years of research by teams of scientists in the United States and Japan. The most recent addition to Table II is the arginine vasopressin-like insect diuretic hormone (AVP-like IDH) F_2, which was isolated from the suboesophageal and thoracic ganglia of the locust, *Locusta migratoria*, together with its nonapeptide monomer F_1 (Schooley et al. 1987a; Proux et al., 1987). The structures of F_1 and F_2 were confirmed by synthesis, but at physiological doses, F_1 was inactive in the in vitro bioassay with isolated Malpighian tubules. Although, as will be discussed later, the precursors of active neurohormones are usually thought to be larger molecular entities (proneurohormones), the polymerization of an active monomer may be an alternate mechanism. Undoubtedly, the increasing availability of synthetic insect neurohormones and their antibodies will greatly advance the still only elementary understanding of their biosynthesis, distribution, and mode of action.

EXPLOITATION OF INSECT NEUROPEPTIDES FOR NEW CONTROL STRATEGIES

Economic insects are still controlled mainly with synthetic organic compounds (Menn and Christy, 1988). These organics, which include chlorinated hydrocarbons, carbamates, organophosphorus esters (OPs), and the synthetic pyrethroids, are neurotoxic insecticides, interfering with both electrical and chemical signals in the nervous system (Lund, 1985). Selectivity of these insecticides is a function of penetration, transport, and metabolism. That is, these insecticides can be highly toxic to mammals, including humans, birds, and fish. Due to its growing awareness of health and ecological considerations, society is increasingly demanding the development of insect-specific control measures that are nonpolluting, safe, and compatible with integrated pest management (IPM) systems. Research in the field of insect neuropeptides holds promise to provide just such control measures.

As shown in Tables I and II, several neuropeptides have already been characterized with respect to function and chemical structure. It is evident now that these oligopeptides are a most versatile and diverse group of small regulatory molecules that govern virtually all functions in insects (Figure 1). Knowledge, therefore, of their functions, modes of action, and structures should enable us to synthesize neuropeptide agonists or antagonists that may be used either as insect control agents in their own right or as starting materials to be modified for greater potency.

Earlier discoveries in human neuropeptide pharmacology have led to the development of promising drugs that are either agonists or inhibitors of neuropeptide expression. Notable among these drugs is the protease inhibitor captopril, a proline derivative substituted with a sulfhydroketoalkyl group on the amino nitrogen. As shown in Figure 2, captopril inhibits the conversion of angiotensin I to angiotensin II. Angiotensin I is an inactive neuropeptide precursor (proneurohormone); and angiotensin II is an octapeptide implicated as the causative agent in essential hypertension in humans (Ondetti et al., 1977). Another promising drug is thiorphan, a potent inhibitor of enkephalinase ($k_1 = 4 \times 10^{-9}$ M) (Roques et al., 1980) and, thus, an agonist for the neuropeptide enkephalin. Enkephalinase is a membrane-bound dipeptidylcarboxypeptidase that inactivates the native opioid enkephalin, thus terminating its pain-killing action. The inactivation is due to cleavage of the opioid to form an inactive metabolite (Schwartz et al., 1981), and the cleavage is initiated when the enzyme orients itself, via the zinc ion at its active site, on the carbonyl group of the opioid. Thiorphan prevents this cleavage—and thus prolongs the pain-killing action of the opioid—by reacting with enkephalinase to form a thiohemiketal. This reaction takes place between the mercapto group of thiorphan and the zinc ion of enkephalinase.

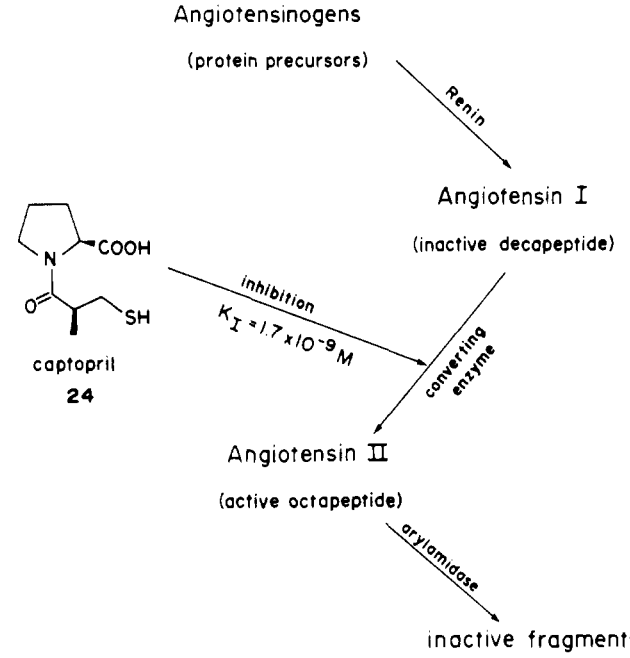


Figure 2. Formation of a neuropeptide through mediation by a proteolytic enzyme and blockage of the formation by captopril. Reprinted with permission from Menn and Henrick. Copyright 1985, Rowan & Allanheld.

The remarkable inhibitory activity of captopril and thiorphan has opened new vistas in pharmaceutical research: Scientists can now look for nonpeptidic, relatively simple, and metabolically stable compounds to inhibit enzymes that mediate neuropeptide expression in humans. By analogy, such compounds can also be sought to inhibit neuropeptide expression in insects. The relatively simple chemical structures of captopril and thiorphan suggest a good likelihood that the inhibitors may be modified peptides, pseudopeptides, or nonpeptides. For insect control, the desired attributes of such inhibitors are that they be peptidomimetic, stable, and lipophilic enough to penetrate the cuticle and exert their action in vivo. The last attribute would allow them to be effective whether sprayed directly on the insects or onto surfaces for contact action.

By inference from the action of the myotropic neuropeptide proctolin (Table II), which is active on proctolinergic tissues and organs (muscle) at concentrations below 1×10^{-10} M (O'Shea and Adams, 1981), inhibitors of enzymes involved in the formation of neuropeptides (i.e., neuropeptide-processing enzymes) in insects would also

Table III. Selected Neuropeptide Targets for Disruption of Function in Insects

neuropeptide	function regulated	end point resulting from disruption
PTTH	in concert with ecdysone initiates molting	failure to develop into next stadium
EDNH	ecdysteroid synthesis	inhibition of oogenesis
OH	vitellogenesis	inhibition of oogenesis
allatotropin	juvenile hormone synthesis	disrupts development in all stages
allatostatin	juvenile hormone synthesis	disrupts development in all stages
EH	initiates larval, pupal and adult ecdysis	disrupts development in all stages
diuretic hormone	fluid secretion	fluid loss, dehydration
antidiuretic hormone	fluid secretion	fluid retention, toxemia
PBAN	pheromone biosynthesis	disruption of mating

have to be active in this range. Thus, as regards level of bioactivity, they would be in the same class as the most potent OP insecticides, which phosphorylate acetylcholinesterase and block the hydrolysis of the neurotransmitter acetylcholine (Menn, 1985). Our knowledge of neuropeptide-processing enzymes in insects and, hence, their inhibitors is still rudimentary, since no insect neuropeptide precursors have yet been fully characterized (Raina and Menn, 1987). Also, we still lack information on the regulation of neuropeptide biosynthesis; identities of processing enzymes and target receptors; and the role of neurotransmitters, which likely are the initiators of neuropeptide release from storage sites in glandular and nervous tissues. Raina and Menn (1987) described a stochastic model that incorporates the external cues influencing production of PBAN (Table I) in the suboesophageal gland. Storage of PBAN in the corpus cardiacum and its release into the hemolymph are believed to be under the influence of a putative neurotransmitter. Upon release, PBAN circulates in the hemolymph; then, by a yet unknown mechanism most likely involving its action on a neuroreceptor in association with the pheromone biosynthesis gland, PBAN initiates pheromone biosynthesis in *Heliothis zea*. The major elements in the foregoing model may also apply to the biosynthesis, processing, storage, release, and action of other circulating neuropeptides. However, almost nothing is known about the type of neurotransmitters that might be involved in the release of neuropeptides; and, similarly, nothing is known about the nature of receptors that might activate organs or glands whose function it is to initiate or terminate the biosynthesis of a given regulatory product.

Although little is known about the residence time of neuropeptides in vivo, Quistad et al. (1984) reported that [3,5-$^3H_2$-Tyr]proctolin was degraded within minutes by proteolytic enzymes when incubated with proctolinergic insect tissues. Because neuropeptides would thus seem to be active for only a short duration and because they are too polar to penetrate the cuticle of insects (Quistad et al., 1984), they would likely never be used directly, as agonists, in insect control. Doubtless, therefore, much research effort will focus on developing inhibitors of enzymes involved in the formation of neuropeptides—specifically, neuropeptides that mediate vital functions in insects. Several such (Table I) are the diuretic neurohormones, behavioral neurohormones (PBAN and EH), reproductive neurohormones (EDNH and OH), and growth and developmental neurohormones (PTTH, allatotropin, allatostatin). Table III shows the likely outcome of inhibition and/or disruption of the normal functions of these neuropeptides. Interference with these functions would invariably result in either direct death or population reduction via reproductive failure.

The most direct way to interfere with the action of the foregoing neuropeptides is to inhibit their precursor molecules, or proneurohormones. We have already discussed the inhibitory action of captopril blocking the proteolytic cleavage of angiotensin I to the hypertensive angiotensin II. The conversion of proneurohormones to neurohormones may involve a variety of reactions, including glycosylation, phosphorylation, amidation, acetylation, derivatization with amino acids, and formation of intramolecular and intermolecular disulfide linkages (Habener et al., 1981). To test and design inhibitors requires specific in vivo and in vitro bioassays that will account for the function or disfunction of the neuropeptide. Currently, the difficulty in characterizing processing enzymes is related to our meager knowledge of insect proneurohormones. Hekimi and O'Shea (1985) and O'Shea (1986) have reported on preliminary work to identify the proneurohormone of AKH by vapor-phase amino acid sequencing, but theirs is the only report on characterization thus far. Recombinant molecular and bioanalytical techniques would be required for positive proneurohormone sequencing.

In theory, inhibitors of those proteolytic enzymes whose physiological function is to degrade neuropeptides and thus terminate their action could also be useful control agents by functioning as agonists for the neuropeptides. However, no effective in vivo inhibitors have been reported thus far. Schooley et al. (1987b) reported that degradation of [3,5-$^3H_2$-Tyr]proctolin (Table II) in cockroach proctolinergic tissue homogenates proceeded mainly via an aminodipeptidase that cleaved proctolin primarily at the Tyr–Leu bond and to lesser extent by an aminopeptidase that cleaved proctolin at the Arg–Tyr bond. These proteases could be inhibited in vitro with 0.2 mM 1,10-phenanthroline, and the inhibition could be reversed by the addition of 0.2 mM $ZnBr_2$ and $CoCl_2$; thus, they were established as metalloproteases. These authors also synthesized a proctolin analogue that was truncated at the carboxyl terminus. Its in vitro assays with the tissue homogenates yielded a dipeptide with a hydroxamic acid functionality, and the proteolytic activity could be reduced 50% with 10^{-7} M 1,10-phenanthroline. In in vivo tests with whole cockroaches, injection of the inhibitor with or without proctolin had no effect on the insects. Subsequent metabolic studies conducted by these workers showed that the inhibitor blocked the action of the aminodipeptidase but not the aminopeptidase. Since even a simple pentapeptide such as proctolin is metabolized by two or more enzymes, more complex neuropeptides are expected to be degraded by a large variety of proteolytic enzymes, and thus it would be most difficult to design a single enzyme inhibitor as a multipurpose agonist control agent, unless the enzyme in question would happen to control the primary degradative steps.

Proctolin also served as a model for the synthesis of several modified peptidomimetic compounds (Starratt and Brown, 1979). However, as already mentioned, they were only modestly more active than proctolin, and they provided no significant increase in agonist activity in the cockroach hindgut bioassay. Despite these disappointing results, further explorations of the agonist approach should continue, considering the remarkble success of highly active insect juvenile hormone agonists (juvenoids) that were synthesized by using the native JH III as the prototype (Siddall, 1977; Masner et al., 1987).

The discovery of compounds that are both chemically unrelated to and several orders of magnitude more active than the native JH in insects suggests the possibility that nonpeptides with potent neurohormonal activity may also be discovered. A cogent analogy to recall involves morphine, which mimics the action of the native opioids in humans.

A major advance in the search for viable neuropeptide inhibitors was recently reported by Evans et al. (1986), who developed a stable, nonpeptide inhibitor of cholecystokinin. This finding further suggests that the opportunity exists to discover stable nonpeptide antagonists of insect neuropeptides. Such compounds very likely compete effectively for the neurohormone receptor site, thus blocking the cellular response to the neurohormonal message.

Undoubtedly, research will progress in the embryonic but enormously promising field of insect neuropeptides as insect control agents. Paramount to this progress, however, is the need to develop meaningful in vitro and in vivo bioassays to guide biological, biochemical, and synthesis research in this field. Presently, practical in vivo assays have been developed for only a few neuropeptides such as EDNH, EH, PBAN, and PTTH. Research on neurohormone receptors would also be highly useful, helping us to understand the modes of action of neuropeptides and to design and synthesize viable and stable peptidomimetic compounds. We can also anticipate the development of recombinant DNA technology that will enable us to isolate insect genes coding for neuropeptides and peptide neurotransmitters. DNA sequences encoding specific peptides can be obtained from three sources, according to Kaufman and Tobin (1984): (1) chromosomal DNA containing genetic information of an insect, (2) messenger RNA isolated from tissues that synthesize the peptide of interest, and (3) chemical synthesis if the entire amino acid sequence is known.

If, as generally accepted, the biosynthesis of an insect neuropeptide is governed by a single encoding gene, it should be possible to clone a targeted gene and insert it via an appropriate vector for expression in an insect host plant, or microbe (Keeley and Hayes, 1987). Although this is an attractive tactic, major obstacles are likely to be encountered including instability of expression in the host, instability of the site of expression in the host, rapid degradation in the transgenic host, and lack of appropriate processing enzymes in the host cell if the gene message is to synthesize the proneurohormone.

Among the microbials, insect viruses, especially the baculoviruses, have been suggested as the preferred cloning expression vectors for foreign genes (Dougherty, 1987; Keeley and Hayes, 1987). There are efficacious baculoviruses for several economically important insects, including the gypsy moth, *Lymantria dispar*, cotton bollworm and budworm, *Heliothis* species, and members of the looper complex, *Trichoplusia ni*, *Rachiplusia ou*, etc. Several of these viruses have been registered as microbial pesticides and are considered environmentally acceptable because they are nonpolluting and possess a narrow host range that spares beneficial insects and other nontarget organisms. The only drawback of insect viruses is their relatively slow mode of action. Unlike synthetic chemicals and certain toxins that are quick acting, viruses require several rounds of replication before compromising a targeted pest.

Introduction of the recombinant viruses could control insects in two ways—as microbial pesticides and as means for disrupting normal neuropeptide function. Viruses naturally breach the digestive tract, gain entrance into the hemocoel, and replicate in host cells, from which secreted neuropeptides could be delivered directly into the hemocoel. A neuropeptide gene placed behind a strong nonessential viral promoter is capable of turning an infected cell into a neuropeptide factory within the insect if the neuropeptide gene retains stability in the virus vector and if cellular processing and transport will mimic the activities of neuroendocrine tissue. Thus, some of the major obstacles facing the use of neuropeptides as control agents could be overcome. Interference by plant surfaces, digestive enzymes, UV light, and other physical and chemical inactivating agents would also be avoided, and in turn insect virus efficacy would be enhanced. If insect development can be arrested through neurohormonal deregulation, a double return is envisioned. First, early instar larvae could be controlled before they develop into larger insects and cause crop damage; and, second, late instar larvae, which normally exhibit maturational resistance to viruses, could be suspended in development and killed before they pupate.

Undoubtedly, through genetic engineering manipulations, other tactics will come to light. The foregoing provides a sample of the many opportunities that will emerge from the powerful technologies of molecular genetics. Manipulation of the insect neuroendocrine system may eventually lead to the most successful phase in biochemical insect control. Although it will not necessarily solve and remove the vexing problem of resistance development, it should yield new control procedures that are highly specific to pest insects and, consequently, safe for humans and the environment.

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