New Research Horizons in Insects Control

Julius J. Menn
U.S.D.A.

INTRODUCTION

Japanese pesticide science occupies a pivotal position in crop protection in the world today.

Ten years ago Professor John E. Casida in his inaugural address to the Pesticide Science Society of Japan already referred to Japanese preeminence in this field. The remarkable growth of this society is a clear result of the dedication, perseverance, and brilliance of numerous, and too many to mention, Japanese scientists who have made remarkable discoveries in pesticide chemistry leading to the commercial introduction of highly active, and in many instances also highly selective, pesticide chemicals.

Your success is also a tribute to the Japanese pesticide chemical industry, which has displayed great vision, patience, and support in dedicating major resources to the development of these discoveries. It is appropriate also to honor on this occasion the memory of two founding fathers of this society, Professor Sankichi Takei, a major contributor to our knowledge of rotenone chemistry, and Professor Ryo Yamamoto, an early pioneer in the chemistry of pyrethrins.

There is a continuing thread in this field of natural products research. Starting with the structural studies on pyrethrins by Yamamoto and Staudinger and Ruzicka, followed by synthesis of the first synthetic pyrethroids by Schechter and coworkers at the United States Department of Agriculture and synthesis of the first photostable synthetic pyrethroids by Elliott and coworkers at the Rothamsted Experiment Station and by chemists in the Sumitomo Chemical Company laboratories. These discoveries have been amply documented by Elliott (1977) and by others.

The dynamic chemical evolution of this field is continuing and has recently received further impetus from the discovery of MTI-800 by chemists at the Mitsui Toatsu Chemicals Company, who described this fish-selective pyrethroid, which is devoid of either ester or ether bridging (Udagawa et al., 1985).

Most synthetic chemical insecticides are nerve poisons, including chlorinated hydrocarbons, carbamate, and organophosphorus ester insecticides. They have the same or similar mode of action in invertebrates and vertebrates. Selectivity with these insecticides is usually a function of penetration, transport, and metabolism. Several insect-selective chemicals have also been developed, such as a few juvenoids (Menn and Henrick, 1981) and chitin synthesis inhibitors (Verloop and Ferrill, 1977; Haga et al., 1982). There also appear to be potential prospects for discovery and development of juvenile hormone antagonists (Menn, 1985).

Rapid scientific developments in neuroregulation and molecular biology coupled with environmental and societal demands will undoubtedly lead researchers to concentrate on more and more selective but highly potent control chemicals compatible with modern strategies of integrated pest management to achieve insect control with the least disharmony to nature.

In the remainder of this talk, I will direct my remarks toward insect neurobiology and neuroregulation, more specifically to biologically active neuropeptides, which are becoming available for physiological, biochemical, and chemical exploration, and show potential promise for discovery of a new generation of control agents based on the insect’s own natural products.
VERTEBRATE PEPTIDES

The field of neurosecretion had its genesis in the first two decades of this century as a result of the fundamental observations on fish neurosecretion made independently by Speidel and Ernst and Berta Scharrer (Pickering, 1981). The discovery of a myotropic neuropeptide/neurotransmitter from equine brain and intestine by Von Euler and Gaddum (1931) provided further major impetus to modern concepts of neuroregulation.

Presently, 33 neuroregulatory peptides have been isolated in neurons and nerve terminals in the mammalian central nervous system (Iversen, 1983). These neuropeptides function as neurohormones or neurotransmitters, and they orchestrate a vast array of myotropic, metabolic, and behavioral activities. No doubt, with the advent of advanced microanalytical techniques, sequencing instrumentation, immunoassay, and radioimmunoassay, many more neuropeptides will be characterized and identified in the near future.

These neuropeptides have already led to major developments in pharmacology and medicine, notably in the development of specific inhibitors of peptide processing and degrading enzymes. Notable examples include the drug captopril, which inhibits the conversion of angiotensin I, an inactive decapeptide, to angiotensin II, an octapeptide implicated in hypertension (Ondetti et al., 1977). Especially significant is the high specific inhibition by captopril \( K_1 = 1.7 \times 10^{-9} \, \text{M} \) with very low affinity for several nontarget enzymes.

Another example involves the inhibition of enkephalinase A, a membrane-bound dipeptidylcarboxypeptidase that acts as a synaptic deactivator for the enkephalins, thus terminating the painkilling action of the latter (Schwartz et al., 1981). Roques et al. (1980) discovered that the drug thiorphan effectively inhibited the action of enkephalinase A \( K_1 = 4 \times 10^{-9} \, \text{M} \), thus prolonging the painkilling action of the enkephalins. This inhibition results from binding of a zinc ion in the enzyme-active site to a mercapto group in the inhibitor, where normally the metal group orients the enzyme on the carbonyl group of the enkephalins (Schwartz et al., 1981) (Fig. 1). An analogy can be made here to hydrolysis of acetylcholine by acetylcholinesterase and the inhibition of the latter by a cholinesterase inhibitor such as an organophosphorus (OP) ester insecticide.

These examples show that the action of neuropeptides can be greatly modified by inhibition of either processing or degrading enzymes. Another approach to increase the desired properties of certain neuropeptides involves synthesis of analogs containing backbone modifications. Numerous, more stable analogs of the enkephalins have been synthesized; several of these have shown vastly increased painkilling activity (Smith and Wilkinson, 1982). Undoubtedly, synthetic and metabolic studies will yield improved analogs with enhanced therapeutic utility.

INSECT NEUROPEPTIDES

Rapid advances in vertebrate peptides has also stimulated new research approaches in insect neurohormones and neurotransmitters. In fact, exploitative strategies set forth for mammalian neuropeptides have application to potential development of novel approaches to insect control. Insect neurohormone research has its beginning when Kopeč (1922) first established a connection between brain neurosecretion and initiation of metamorphosis in the gypsy moth, Lymantria dispar.

This early lead lay dormant for several
decades. However, by 1982, 15 to 20 biologically active neuropeptides had been reported to occur in insects acting as neurohormones and/or neurotransmitters (O'Shea, 1982; Truman and Taghert, 1983). These neuropeptides can be classified as having myotropic, metabolic, developmental, and behavioral regulatory activity. The precise chemical structure of only two of these neuropeptides were known in 1982: proctolin, a pentapeptide (Starratt and Brown, 1975), and adipokinetic hormone (AKH), a blocked decapeptide (Stone et al., 1976) (Fig. 2). Presently, the list of identified and sequenced peptides has grown to include periplanetins cc-1 and cc-2 (Scarborough et al., 1984) (Fig. 2). The periplanetins were also independently identified but not sequenced by O'Shea et al. (1984). The latter authors described these myoactive factors as MI and MIT, which are synthesized in the corpus cardiacum and whose release into the blood is calcium-dependent. These workers also found that MI and MIT are present in the CNS and in the gut, indicating transmitter and neurohormonal function in the American cockroach. Periplanetin cc-1 is apparently the same cardioexcitatory neurohormone-D identified but not sequenced by Baumann and Gersch (1982).

The periplanetins cc-1 and cc-2 (MI and MII) are related functionally and structurally to AKH and the red-pigment-concentrating hormone RPCH from the prawn *Pandalus borealis* (Carlsen et al., 1976). In addition to this family of invertebrate neuropeptides, several additional neuropeptides that are being studied in several laboratories are in the final stages of structure elucidation, including the published sequence of 19 amino acid residues in the amino terminus of the prothoracicotrophic hormone (PTTH) of the adult silkworm, *Bombyx mori*, which triggers the prothoracic glands to release ecdysone (Nagasawa et al., 1984).

According to O'Shea (1985), based on immunohistochemical staining, fluorescent immunoassay, radioimmunoassay, and other techniques to localize neurosecretory cells in insect nervous tissue, there are likely to be 100 to 200 neuropeptide neurotransmitters in the insect nervous system. In line with this prediction, there should be great opportunities emerging to isolate, identify, sequence, synthesize, and determine the biosynthetic and degradative pathways for those neuropeptides that regulate the most critical functions in the insect, including such events as metamorphosis, diuresis, reproduction, and communication.

**EXPLORATION OF INSECT NEUROPEPTIDES IN CONTROL STRATEGIES**

A major difficulty confronting researchers in this field is the present lack of bioassays that can demonstrate in vivo activity of any of the known insect neuropeptides with the exception of the eclosion hormone (EH), which induces ecysis behavior in lepidopterous insects (Truman, 1980). However, this assay is laborious, the structure of EH is still unknown, and brain extracts have to serve as the reference standard. Nevertheless, numerous specialized assays have already been developed for assaying the action of neuropeptides in insect nerve/muscle preparations, organ culture, and metabolic assays. These have been amply described (Borkovec and Kelly, 1984).

We are still in the early learning mode in this field. Consider just a few of the problems to be solved: (1) How can a hydrophilic peptide penetrate the lipophilic cuticle of insects? (2) Do peptides possess sufficient stability to be transported to the site of action? (3) What are the receptors for these peptides? (4) What is the nature of the peptidases that degrade these peptides? (5) How are these peptides processed? (6) Are there larger precursors or prohormones which truncate to the smaller peptides?

Few answers exist presently to the above
questions. Nevertheless viable strategies are emerging based in part on pharmacological approaches that were developed in conjunction with mammalian neuropeptide pharmacology and from a variety of biochemical studies.

Quistad et al. (1984) studied the in vitro metabolism of [tyrosyl-3,5-3H] proctolin in various proctolinergic tissues from the American cockroach. Results of these studies showed that [3H] proctolin was degraded by proteolytic enzymes within minutes. Brain tissue and proctodeum degraded proctolin most rapidly. Major cleavage yielded the Tyr-Leu fragment with lesser cleavage at the Arg-Tyr linkage. Preliminary experiments indicated that this endopeptidase (proctolinase) was a membrane-bound enzyme. In further experiments it was shown that [3H] proctolin did not penetrate the cuticle of topically treated Manduca sexta. Furthermore, when ingested by M. sexta larvae (215 ppm in the synthetic diet), only 5% of the consumed diet was recovered as proctolin 2-5 hr after treatment. These range-finding studies provided useful information explaining why certain small myotropic peptides fail to show activity in vivo. However, studies of this type also suggest meaningful new avenues of research, including research on the nature of the degrading enzymes; are they membrane bound, are they metalloc enzymes, and how specific are they? Answers to these questions could provide meaningful leads in developing enzyme inhibitors that could prolong the action in vivo and provide agonist models for further research. Another approach would involve synthesis of analogs as agonists and antagonists with improved stability. The latter can theoretically be achieved by altering metabolically vulnerable peptide linkages.

Attempts at analog synthesis to increase bioactivity were reported by Starratt and Brown (1975). Of 15 synthetic pentapeptide analogs of proctolin, one peptide, [Phe (-OMe)²]-proctolin, was approximately three times more potent than the native peptide, as determined by assays on the isolated proctodeum of the American cockroach. Sullivan and Newcomb (1982) have shown that single amino acid substitutions in the proctolin backbone result in loss in activity or in binding affinity. Undoubtedly, such studies will continue with a greater diversity of peptide models and more precise knowledge of the associated enzymology. It took 20 years of research to design and develop captopril. Perhaps in another decade or two, potent insect models will be in hand to devise selective insect-control strategies.

THE FUTURE

This brief discourse deals with an evolving new chapter in insect neurobiology and biochemistry. Potentially we now have an opportunity to design selective control chemicals based on the insect’s own natural products. We can already visualize developments in conjunction with the soon-to-come identification of a diuretic hormone (Proux and Girardie, 1982) and a brain hormone controlling sex pheromone production in the female corn earworm moth, Helothis zea, and in other moth species (Raina and Klun, 1984). It is exciting to speculate that the latter finding may hold the key to the development of antipheromones, an ultimate goal in insect-control strategies of the future.

LITERATURE CITED

1) E. Bauman & M. Gersch: Purification and identification of neurohormone-D, a heart accelerating peptide from the corpora cardiaca of the cockroach, Periplaneta americana, Insect Biochem. 12, 7 (1982)
7) S. Kopeč: Studies on the necessity of the brain


12) M. O' Shea: Peptide neurobiology an identified neurone approach with special reference to Proctolin, TINS (March 1982), pp. 69–73, 1982


