Insect Hormones and Bioanalogues

By

K. Sláma

M. Romaňuk

F. Šorm

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Dr. K. Sláma
Institute of Entomology
Ing. Dr. M. Romaňuk
Prof. Ing. Dr. F. Šorm
Institute of Organic Chemistry and Biochemistry
Czechoslovak Academy of Sciences, Prague

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Introduction

The application of organic insecticides in the agrotechnical praxis resulted in a great and unexpected progress in the control of insect pests, and was of a great economical value all over the world. The widespread application of these agents, however, is also accompanied by negative effects. The principal drawback of classical insecticides consists in the lack of their specificity, the useful insects being killed together with insect pests. Furthermore, the broad-scale application for many years led to the formation of more resistant insect strains requiring higher and higher doses of insecticides. The residues of the mostly used chlorinated compounds accumulate in human and animal foods producing directly or indirectly harmful effects in human subjects. The critical situation led in many developed countries to the restriction in the usage of some types of classical insecticides.

Under these circumstances it is quite natural that novel routes for the control of insect pests are investigated. In this connection, attention has been paid especially to the insect endocrinology and insect hormones which regulate the admirable and in many regards specific development from the egg to the adult insect. The recent successful discoveries in this field are thus in close relation to the practical requirements. Isolation and identification of moulting hormones and juvenile-hormone-like naturally occurring substances not only made possible an exact investigation of their physiological effects but also stimulated the chemical research. In a short period of time numerous biologically active synthetic compounds have been prepared, many of them possessing an enhanced or selective activity. As shown repeatedly by laboratory assays, many of these compounds and especially the juvenile hormone bioanalogues may be used to interfere with the insect development and consequently they are of value as agents acting against some insect pests. The juvenile-hormone-like substances promise to provide the so called "third generation" pesticides which would operate on the physiological basis.

The literature on the insect physiology as well as on the chemistry

and biochemistry of insect hormones has undergone very considerable expansion in recent years. We have felt therefore desirable to write this book which should summarize in brief form the present state of knowledge in this field with special respect to the potential use of juvenile-hormone-like compounds as insecticides. The scope of the book is somewhat under influence of our own papers which are completely reviewed here. As far as the patent literature, a selection has been made.

We present this book in the hope that it will stimulate attention in the interesting and gratifying field of insect hormones and their analogues. It is a pleasure to acknowledge the encouragement and help given to us by Springer-Verlag Wien. Furthermore we extend our thanks to all coworkers for their valuable assistance in helping us to prepare this book.

Prague, December 1973 The Authors

Contents

I. Brief Survey of the Neuroendocrine System in Insects

This introductory chapter represents a condensed review of the components of the neuroendocrine system of insects and the effects of their extirpation or transplantation on development. We have separated these effects from those of plant and animal extracts, other isolation products, or synthetic compounds, which will be described in the following chapters. The recent literature pertaining to insect hormones has been reviewed in several monographs. The results obtained by classical endocrinological techniques are described in detail in the book by PFLUGFELDER [237] which contains detailed descriptions of insect endocrines from the period when morphological studies predominated. An extensive list of the literature pertaining to the endocrine glands of insects with discussions on developmental theories in insect endocrinology is contained in a book by NOVÁK [218]. Comparative data on insects and other invertebrates, especially crustaceans, may be found in a monograph by GERSCH [90] and a brief but very instructive book of comparative endocrinology of invertebrates containing numerous illustrations has been prepared by HIGHNAM and HILL [126]. Comparative data on the structure and function of neurosecretory system may be found in an extensive work by GABE [88]. The basic physiological data for understanding the mode of action of insect hormones are contained in a book by WIGGLESWORTH [370], and a monograph by WIGGLESWORTH [371] on the physiology of metamorphosis of *Rhodnius*. WIGGLESWORTHS most recent review on insect hormones [379] is also highly recommended. Finally, problems concerned with the hormonal control of reproduction in insects have recently been summarized by ENGELMANN [70].

Insects are characterized by an enormous diversity of morphological forms and living habits. Their indirect form of development is manifested by the occurrence of immature larval stages, which in many insect groups live in a completely different environment and possess an entirely different form than those of the adults. The indirect development requires precise regulation of developmental processes and their

synchronization with the changing environmental conditions. The same precise regulation occurs during metamorphosis when the transformation of the larva into the mature adult takes place.

The regulation and synchronization of insect development is controled by the neuroendocrine system. It records stimuli from the environment such as photoperiod, temperature, humidity, availability of

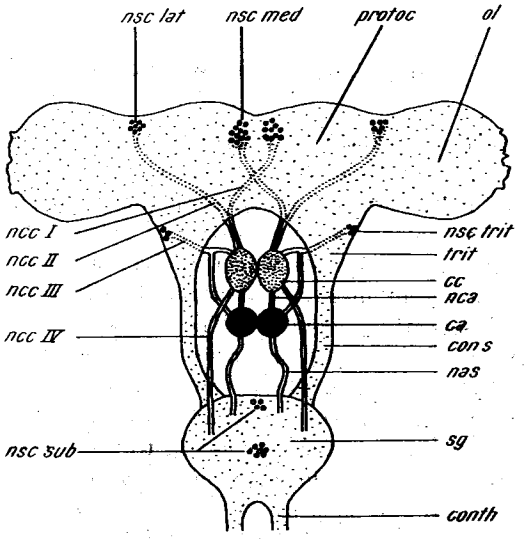

Fig. 1. Schematic illustration of the main neuroendocrine system of an insect. *ca* = corpora allata, *cc* = corpora cardiaca, *con s* = suboesophageal connectives, *con th* = thoracic connectives, *nas* = nervus allatosuboesophagealis, *nca* = nervus corporis allati, *ncc* I–IV = nervi corporis cardiaci, *nsc lat* = neurosecretory cells (nsc) of the lateral part of protocerebrum, *nsc med* = nsc of the pars intercerebralis protocerebri, *nsc sub* = nsc of the suboesophageal ganglion, *nsc trit* = nsc of the tritocerebrum, *ol* = optic lobe, *protoc* = protocerebrum, *sg* = *suboesophageal* ganglion, *trit* = tritocerebrum

food, etc., and transforms these impulses into chemical messengers or hormones which in turn cause functional or developmental changes among various effector organs according to a genetically determined programme. The neuroendocrine system represents a functional unit consisting of the nervous system and the endocrine glands. Its basic structure in insects has many features which are common to higher animals including mammals [289]. The most important part of the system are the neurosecretory cells located in the central nervous system. They are responsible for transmission of neural messages to the endocrine glands or other tissues. Direct effects of their secretions on "target"

tissues have the character of first order neuroendocrine functions since they stimulate secretion of hormones by other endocrine glands such as the corpora allata or prothoracic glands. The hormones secreted by these latter endocrine glands represent neuroendocrine functions of the second order [294]. The neurohormones affecting endocrine glands have thus superior position in the whole hierarchy of hormonal regulations. A schematic illustration of the basic neuroendocrine centers in the insect body is given in Fig. 1.

Growth and developmental processes connected with physiological changes in insect tissues are regulated by three hormones collectively referred to as developmental or metamorphosis hormones. One of them is a neurohormone released by the neurosecretory system while the second is released by the corpora allata and the third by the prothoracic glands. It appears difficult to comprehend that the great diversity of developmental patterns in insects is produced by modulating the activities of these three hormones. It is becoming increasingly evident that the responsibility for such diversity depends on inherited genetic factors which determine for a particular species whether at a certain developmental stage individual cells will be dependent or independent of the hormones. Our intention is to summarize the basic facts concerning the insect endocrine system. These facts are important in understanding the mode of action of natural and synthetic compounds possessing hormone activity.

A. The Neurosecretory System and the Activation Hormone (AH)

The neurosecretory system of insects is comprised of a complex of neurosecretory cells located in the cerebral nervous system and in the ganglia of the ventral nerve cord, their axons and special neurohaemal organs engaged in the storage and release of the neurosecretory material into the haemolymph. Differences in histological and anatomical structure, variability in size, location and staining properties as well as specific functional differences suggest that the neurosecretory system may produce several compounds differing in physiological functions and chemical structures. For the regulation of insect development the most important neurosecretory product, or products, of the system appears to be the hormone which stimulates the secretory activity of other endocrine glands. It is assumed that this hormone is secreted by neurosecretory cells of the protocerebrum and released into the haemolymph by the corpora cardiaca. The hormone is often referred to as brain hormone, prothoracotropic hormone or allatotropic hormone but we shall use the term activation hormone which is considered more appropriate [379].

1. Structure of the Neurosecretory Cells and Neurohaemal Organs

The basic unit of the neurosecretory system is the neurosecretory cell. It represents a modified neurone with dual characteristics possessing many features characteristic of a true neurone and also having typical features of an endocrine cell capable of producing secretions which are then released into the haemolymph [88, 285]. In contrast to typical neurones, neurosecretory cells do not establish normal synaptic contacts with other neurones. However, they form vesicular structures (synaptoids) at axon terminals which resemble presynaptic structures and are probably engaged in the release of neurosecretory material [285, 286, 293].

The neurosecretory cells may occur individually or in small groups, e.g. in the ganglia of the ventral nerve cord or may form aggregates as is observed in the central nervous system of some insects. The cells are always situated at determined loci in the nervous system and are sometimes visible under the dissecting microscope or with dark field illumination where they appear as a bluish white reflection.

Each neurosecretory cell (n. s. c.) has an apparent axon and the axons of groups of these cells form bundles leading to neurohaemal organs. Other axons lead to peripheral organs where they may contact parenchymal cells (Fig. 2). There are many types of n. s. c. which are classified mainly according to their histochemical properties, shape, size, and location in the nervous system [88]. The most common n. s. c. which include some of the largest neurosecretory neurones are the so called A type n. s. c. whose granules stain dark purple with paraldehyde fuchsin and deep blue with chrome-haematoxyline-phloxin. The B type n. s. c. contain granules which are phloxinophilic and stain green or bluish-green with paraldehyde-fuchsin and red with chrome-haematoxylin. Finally, it is possible to distinguish cell types which can be classified as C and D on the basis of their staining properties [106, 108, 140, 245, 254, 301]. Some authors have distinguished 8 or more types of n. s. c. by such tinctorial affinities as well as by special functional differences. However, any sort of general description of all neurosecretory cells contained within an insect body is impossible and we must consider individual situation within a particular species [293]. This conclusion also applies to the more or less generalized distinction between the A and B type n. s. c.

The number of n. s. c. in the protocerebrum may be relatively small, i.e. 8 to 50, but very often there are over 100 such cells; in some cases there may be close to 1000 [88, 90, 126, 140, 218, 379]. Distinctive n. s. c. occur also in the tritocerebral part of the brain [250], in the suboesophageal ganglion and in both the thoracic and abdominal

ganglia [55, 80, 140, 251, 254]. The most intensively studied n. s. c. are the groups of cells in the pars intercerebralis in each of the hemispheres of the protocerebrum [88]. The axons of these cells form the *nervi corporis cardiaci interni* (NCC I) most of which cross and lead to the corpus cardiacum on the contralateral part of the body. The n. s. c. of the lateral part of the protocerebrum send their axons *(nervi corporis*

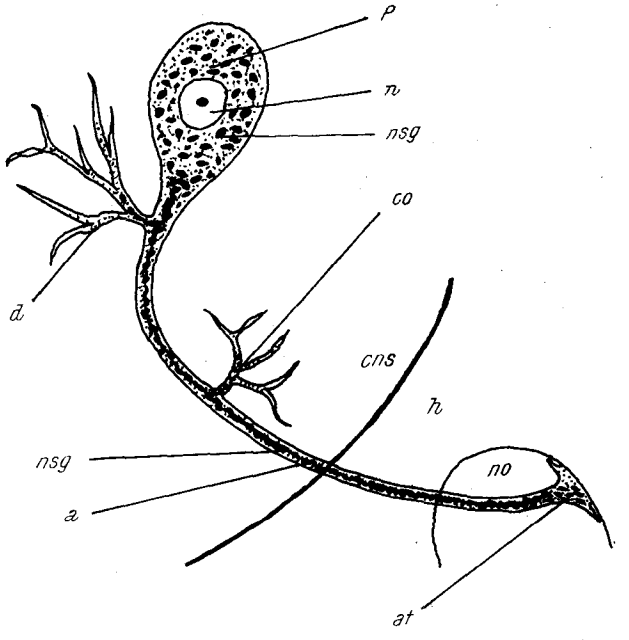

Fig. 2. Schematic illustration of a neurosecretory neuron in the pars intercerebralis of an insect. a = axon, *at* = axon terminal, *cns* = central nervous system, *co* = collaterals, d = dendrite, h = haemolymph, n = nucleus, *no* = neurohaemal organ, *nsg* = *neurosecretory granules*, p = pericaryon

cardiaci externi) (NCC II) to the ipsilateral corpus cardiacum. The axons of the tritocerebral n. s. c. form the *nervi corporis cardiaci III* leading also to the corpus cardiacum, but in several insect species, these nerves have not been found [301].

The protocerebral n. s. c. occur in all insects including the most primitive Apterygota and the most specialized groups of Endopterygotes [140]. The importance of n. s. c. in the phylogeny of the animals is evident from a fact that similar cells occur in all phyla of animals beginning with primitive worms, the Turbellaria [40, 88, 90, 140, 285, 293].

The main neurohaemal organ in insects is the corpus cardiacum. It is of ectodermal origin and has the character of both nervous tissue and endocrine gland [285, 286]. Macroscopically the corpora cardiaca appear as paired rounded or elongated bluish-white organs located posterior to the brain and usually attached to the wall of the aorta. The morphology of the corpora cardiaca has been modified in different groups of insects. For example in higher Diptera the corpora cardiaca are not distinct paired organs but are fused into the ring gland. The corpora cardiaca form a close connexion with the brain by the cardiacal nerves I to III and also connect via nerves to the hypocerebral ganglion and with the suboesophageal ganglion (*nervus cardiaco-suboesophagealis* or *nervus corporis cardiaci* IV). From the distal end of the corpora cardiaca, nerves lead to the corpora allata *(nervus corporis allati)*. In certain insects it is possible to distinguish more or less separated nervous and glandular parts in the corpora cardiaca [42, 218, 249, 301] (see Figs. 1 to 4).

The corpora cardiaca are surrounded by a connective tissue sheath consisting of acellular stroma. While the surface of the gland may be smoth in some insects it more often shows deep invaginations. The internal structure of the gland is complex and individual structural elements may differ in different species [32, 88, 286]. A large part of the corpora cardiaca consists of nerve bundles and enlarged axon terminals of neurosecretory cells which contain accumulated neurosecretory granules. Many of these axon terminals occur near the outer surface but some are also observed on the stromal invaginations of the organ (Fig. 3). Near the surface it is possible to observe numerous chromophobic interstitial or glial cells having a small nucleus, dense chromatin pattern, little perinuclear cytoplasm and numerous cytoplasmic processes [140, 285, 286, 301, 342]. The glial cells are the main component of the connective tissue inside the gland [42, 280]. Another type of cells present in the corpora cardiaca are the parenchymal cells or intrinsic secretory cells [140, 218, 279, 280, 286, 301, 342] which have a large nucleolus, abundant cytoplasm with short thick process and numerous cytoplasmic granules of Golgi origin. These cells are probably identical with the "chromophilic cells" of the light microscope and are thought to be modified neurons with particularly expressed secretory function [40, 41, 285]. Their axon-like processes contain neurosecretory granules and terminate presumably inside the gland [280, 301, 342]. In addition to these internal structural elements, muscle fibres are also part of the corpora cardiaca in some insects [301].

The extensive work of RAABE and her co-workers has shown that the neurosecretory material of the neurosecretory cells located in the ganglia of the ventral nerve cord is released from the segmentally arranged

neurohaemal organs. These organs occur in diverse groups of insects. The neurohaemal organs appear as swellings on the median or transversal visceral nerves and are called perivisceral or perisympathetic neurohaemal organs [251, 253, 254]. Histologically the neurohaemal organs appear similar to the corpus cardiacum. A thin cellular stroma covers

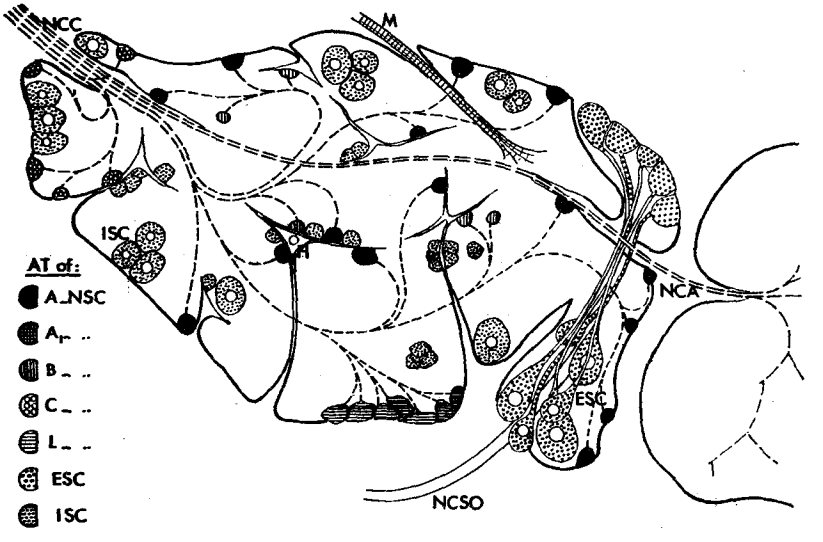

Fig. 3. Diagram of the corpus cardiacum of adult *Leptinotarsa* showing the course of the axons of different neurosecretory cells. AT = axon terminals, ESC = extrinsic secretory cells, H = haemocyte, ISC = intrinsic secretory cells, M = muscle, NCA = nervus corpori allati, NCC = nervi corporis cardiaci, NCSO = nervus cardiaco-suboesophagealis, NSC = neurosecretory cells. From SCHOONEVELD [301]

many axons some of which contain neurosecretory granules. In addition, there are glial cells as in the corpora cardiaca and a well developed lacunar system, but modified neurons comparable to the intrinsic secretory cells of corpus cardiacum with neurosecretory axons have not been found [110, 248, 253, 254, 257].

2. Formation and Release of Neurosecretory Material

The presence of neurosecretory material can be demonstrated in the light microscope by histochemical techniques [245] and selective staining procedures which have already been mentioned. The neurosecretory material of the peptidergic A-type neurosecretory cells can be demonstrated histochemically by the presence of disulphide or sulphydryl groups in polypeptides rich in cysteine or cystine. By means of sensitive

fluorescence methods it is possible to demonstrate in other types of neurosecretory cells the presence of biologically active amines such as noradrenaline, serotonine [293]. According to RAABE and MONJO [256] the granules contained in C-type neurosecretory cells situated in the ventral nerve cord and in the lateral part of the pars intercerebralis do not contain catecholamines or polypeptides rich in disulphide or sulphhydryl groups. They contain basic proteins with indol or pyrrol residues.

Under the electron microscope cytoplasmic granules of high electron density surrounded by the membrane can be observed in the n. s. c. Their size ranges usually from 1000 to 3000 Å [279]. The granules are formed in the perikaryon of the neurosecretory cell by the granular endoplasmic reticulum (ergastoplasm) and are then transported to Golgi bodies where they are packaged and budded off to the axons [23, 108, 126, 246, 285, 286]. Under certain circumstances the granules may accumulate in the perikaryon, in the axons or axon terminals.

The main feature of the neurosecretory cells, in addition to mitochondria, neurotubules, cisternal profiles and multivesicular bodies, is the presence of varying amount of vesicles with electron dense material [286]. It is generally agreed that the neurons containing large vesicles (1000–3000 Å) represent "classical" neurosecretory neurones containing proteinaceous secretory material [288] which consists of phospholipoprotein rich in -S-S- or -SH groups [246]. The neurosecretory product of the axons with smaller vesicles may represent material containing monoamines [285, 286].

The enlarged terminus of an axon which is in contact with the matrix of the extracellular stroma is thought to be the site of release of the neurosecretory material. As pointed out by SCHARRER [286], release can take place also at other preterminal parts of the axon. Moreover, the neurohaemal organs are not the sole place of release. Neurosecretory material can be released directly at the parenchymal gland cells such as those of the corpus allatum, or to connective tissue layers, as is also the case in the prothoracic glands, or in the vicinity of other nerve fibres or glial cells, etc. In some insects such as aphids neurosecretory axons terminate in the musculature or in some visceral organs [141]. In *Oncopeltus* and several other Hemiptera neurosecretory products are released through the wall of the aorta and this is also the place where they eventually accumulate [140]. The release sites are characterized by the presence of numerous small vesicles of low electron density which are judged to be residues of fragmental neurosecretory granule membranes [286].

According to the extracellular pathway of the released chemical mediators we can distinguish between neurohumoral and neurohormonal

agents. B. SCHARRER [287, 288] has recently classified neurons along these lines and has proposed a terminology for the biologically important neurosecretions. The neurohumours are engaged in the transfer of informations in the synapses where they cause post-synaptic reactions of very short duration. Hence their action is not of the endocrine type. Various names such as 'chemical transmitters', 'neurotransmitters', or "neurohumours" have been proposed for these materials and typical examples are acetylcholine and noradrenaline. The neurohormonal materials are released by neurosecretory cells without synaptic transmission and are transported by the haemolymph to their target cells where the neurohormones can then act. Their action is therefore very similar to that of other hormones secreted from parenchymal cells of regular endocrine glands. However, there are exceptions. For example, in the case where the secretions reach the peripheral tissues directly via axons, we can not classify this type of activity as neurohormonal since there is no transport of neurosecretory material by the haemolymph.

3. Changes in the Neurosecretory System during Development

Various authors have tried to find some correlations between the amount of stainable material in the neurosecretory system and developmental processes governed by endocrine glands. The scope of this chapter does not permit a detailed analysis of this complicated problem. However, the various aspects of this question are discussed in a number of review articles [8, 66, 67, 88, 90, 92, 121, 123, 124, 126, 140, 190, 218, 303, 379, 384]. Cytological studies have revealed changes in the content of neurosecretory material in the pericarya of neurosecretory cells as well as in the axons, neurohaemal organs, or aorta wall. These changes mainly refer to the relative content of the material in the cells as compared to that in the neurohaemal organ. In many insects it appears that the amount of neurosecretion in the neurohaemal organs is less at the beginning of each developmental cycle. This decrease may occur at the beginning of a larval instar, the onset of metamorphosis, the beginning of a reproduction cycle in adult female, etc. Towards the end of a cycle the neurohaemal organ again accumulates neurosecretory material. There are also instances with no apparent alteration in the amount of neurosecretory material during the developmental period. In addition there are reports suggesting that for some species the relationship described above may actually be reversed [122, 123, 124, 126].

The problem of relationship between the amount of stainable material in the neurosecretory system and the degree of physiological activity of this system has been intensively studied by HIGHNAM [122, 124]. He concluded that the amount of material in the cells was not