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Pesticide and Venom Neurotoxicity

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Plenum Press · New York and London

Library of Congress Cataloging in Publication Data

Main entry under title:

Pesticide and venom neurotoxicity.

Includex index.
1. Insecticides—Physiological effect—Congresses. 2. Neurotoxic agents—Physiological effect—Congresses. 3. Nervous system—Insects—Congresses. 4. Miticides—Physiological effect—Congresses. 5. Nervous system—Mites—Congresses. 7. Insects—Physiology—Congresses. 8. Mites—Physiology—Congresses. I. Shankland, Daniel Leslie, 1924-
SB951.5.P47 632'.951 77-25006
ISBN 0-306-31123-2

Selected papers from the Fifteenth International Congress of Entomology held in Washington, D.C., August 19-27, 1976

A Division of Plenum Publishing Corporation
227 West 17th Street, New York, N.Y. 10011

Printed in the United States of America

Preface

The development of the modern organic insecticides has contributed a major chapter to the history of neurotoxicants. From their roots in the organochlorines and organophosphates discovered prior to and during the Second World War, to the carbamates developed in the 1950's, and most recently to the extremely potent and promising synthetic pyrethroids, the most important organic insecticides have been those whose wite of action lies within the nervous system. In this regard, man is only mimicking nature in attacking the nervous system with lethal intent since potent neurotoxins are common components of the venoms with which animals of all types defend themselves and subdue their prey.

This central role of the nervous sytem as a broad target for pesticides may also be projected into the future - a prediction to which this volume is devoted. The nervous system in its diversity is likely to be of central concern to those charged with discovering novel pesticides whether they be modifications of familiar chemical groups or structurally novel neurotoxicants such as the nitromethylene insecticides described here. On a second front, the ability to influence insect behavior through the nervous system will become increasingly important in pest management. Pheromones represent one obvious example of this; recent work described in this volume indicates that the formamidine pesticides may represent another.

It is unfortunate but true that we cannot yet hope to design pesticidal molecules ab initio, but there is an increasing interest in developing a more rational basis for pesticide discovery than has been typical in the past. Such a rational approach depends in part on the investigation of possible critical sites and modes of action for chemicals in the nervous system, including comparative aspects which can lead to more selective and safer compounds; study of the mode of action and structure-activity relations of known neurotoxicants; and development of model assay systems for screening purposes. These are the major topics addressed in this book.

Perhaps a word of justification is needed concerning the inclusion here of papers on arthropod venoms. In addition to their very real ecological, medical, and evolutionary interest, venoms and toxins have potential as chemical models for novel control agents as witnessed by the development of the insecticide cartap from the marine annelid toxin, nereistoxin. Even more significantly, they are vital aids for the exploration of the biochemistry and physiology of nervous function upon which rational development and the future understanding of the actions of neurotoxicants depends. Thus, for example, the observation presented here by Piek and Spanjer regarding the presynaptic blockade of neuromuscular transmission in insects by certain wasp venoms provides a challenging model for insecticide development in addition to its intrinsic significance for insect biology and neurophysiology.

With these themes in mind, several symposia were arranged for the 15th International Congress of Entomology held in August, 1976, in Washington, D.C. Many of the world's leaders in the study of pesticide and venom neurotoxicity participated. It is from these presentations that the current volume is drawn.

We were fortunate to persuade Dr. Clyde W. Kearns to introduce both our symposium on insecticide neurotoxicity and this volume. We would like to dedicate the book to him in recognition of the key role which he has played in the study of organic insecticides and their activity as neurotoxicants.

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GENERAL INTRODUCTION

C. W. Kearns

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The need for a better understanding of the neurotoxic action of pesticides and arthropod venoms has been apparent for many years. This publication presents clear evidence that the general subject is today being given some of the attention which it has long deserved. It presents a representative sample of some of the most interesting research which is being undertaken by workers in various parts of the world. The diversity of the subject matter is a reflection of the many different approaches which may be taken to contribute to a better understanding of the functioning of the arthropod nervous system.

It has long been known that the conduction processes of the arthropod nervous system could not be fully equated in terms of vertebrate neuropharmacology. Recognition of the fact that neuroactive pesticides may be useful tools for the elucidation of the arthropod nervous system is evident in these chapters.

The probability seems eminent that a component of arthropod venom will be isolated which will prove to have the specific property of irreversibly inhibiting the cholinergic receptor of arthropods. The advent of this discovery will most certainly lead to an inspired investigation of the nature and properties of this vital process which would appear to be different from that of the vertebrates.

Highly discriminating electrophysiological techniques and novel biochemical approaches reported in these chapters is assurance that efforts to understand the mode of action of pesticides will become increasingly more precise. Hopefully, this in turn will reduce our dependence upon the empirical approaches to the discovery of novel and useful pesticides.

The side effects of neuroactive pesticides have not been disregarded. The fact that such agents may induce the untimely release of neurohormones and other compartmentalized biogenic agents has been clearly demonstrated. The relevance of these findings remains to be discovered.

Section I

Sites of Neurotoxic Action in Insects

SITES OF NEUROTOXIC ACTION IN INSECTS

INTRODUCTION

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Insect nervous systems are relatively simple compared with those in higher animals. Nevertheless, they contain tens of thousands of cells in exquisitely integrated communication networks which insure functional integrity in every activity. Motor activity is of course under direct control, but through the secretion of neuroendocrine products the regulatory role of the nervous system is extended to growth and development, diapause, digestion, metabolism, excretion, reproduction, and other processes. Normal nervous function depends on a great variety of precisely timed events, and alterations in any one of them may so disrupt nervous integrity as to have lethal consequences. Many natural and synthetic poisons owe their effects to interference with one or more of these events. Some effects have unknown bases, while others can be ascribed to discrete action on biochemical entities such as receptor or enzyme molecules.

DDT, pyrethrins, tetrodotoxin, saxitoxin, and batrachotoxin, for example, interfere by unknown mechanisms with important transient changes in Na^+ permeability of axonal membrane. In so doing they produce aberrent axonal activity or block transmission entirely. On the other hand, the organophosphate and carbamate insecticides act primarily, if not solely, to inhibit acetylcholinesterase. In the same vein, nicotine mimics the action of acetylcholine on the acetylcholine receptor, while nereistoxin and the related synthetic insecticide cartap block the same receptor. Receptors of other kinds, transmitter synthesis and release, presynaptic vesicle formation,and various electrogenic processes are potential or actual targets for natural and synthetic neurotoxicants.

The first of the following chapters discusses the structure, penetrability and normal function of the insect central nervous system to provide a basis of understanding for detailed discussions of selected targets and specific kinds of neurotoxic action in this and other sections of the book. In this first series of chapters, the detailed treatment of several cholinergic elements reflects the importance of that system. However, important secondary effects of neurotoxic action mediated through the neuroendocrine system are also considered, and these may well relate more directly than the primary lesion to the cause of death. Finally in this section, a chapter is devoted to neuromuscular transmission as a potential insecticide target, with an examination of the current status of the debate on glutamate as an excitatory transmitter.

THE INSECT CENTRAL NERVOUS SYSTEM AS A SITE OF ACTION OF NEUROTOXICANTS

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INTRODUCTION

For safe and efficient progress in the development of chemicals for the control of insect pests it is essential to improve our understanding of the cellular and molecular mechanisms of action of these compounds. Most insecticides are potent central nervous toxins (O'Brien, 1967; Corbett, 1974). A prerequisite to the identification of the central sites of action of insecticides is a detailed knowledge of the normal function of the major cell types which constitute the central nervous system. Recent advances in neuroanatomy, neurophysiology and neurochemistry have led to the notion of the insect central nervous system as a target organ for insecticide action within which there are several potential target sites. In this chapter some of the fundamental aspects of central nervous function are discussed in order to provide a basis for interpretation of the central actions of insecticides which will be described in later chapters.

The insect central nervous system consists of paired connectives linking paired segmentally arranged ganglia. A ganglion (Fig. 1) consists of an outer connective tissue envelope (the neural lamella) overlying a modified glial layer, the perineurium. Intercellular channels can be seen between adjacent perineurial cells but towards their inner ends they are at least partially occluded by tight junctions. Beneath the perineurium is located an outer rind of nerve cell bodies. Glial cells are also prevalent in this region. Processes from the cell bodies pass into the neuropile. This region which forms the central core of the ganglion contains numerous axons (the conducting elements of the nerve cells) some glial cells and many synapses - the points at which nerve cells make functional

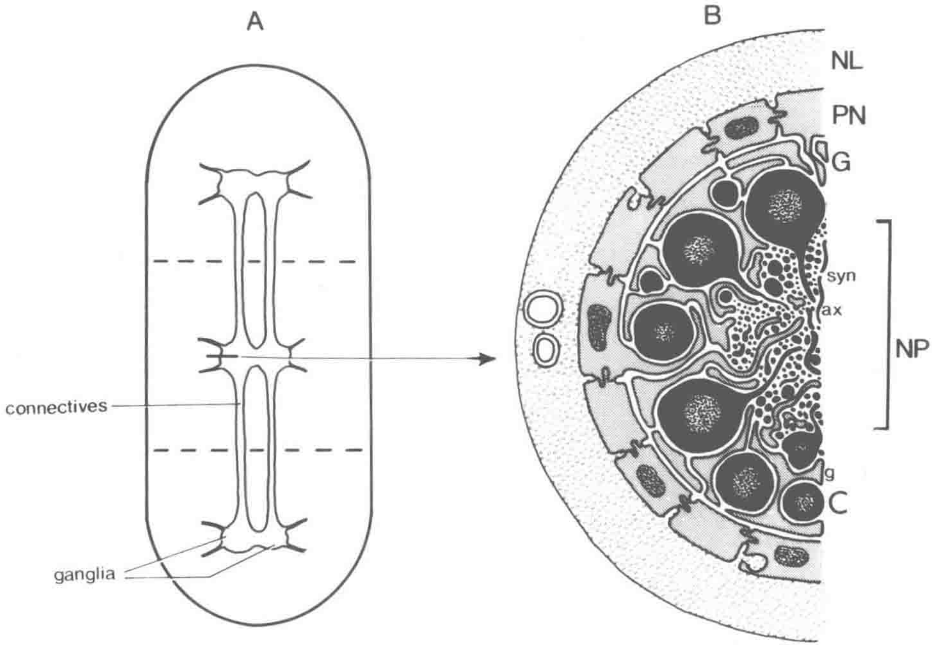

Fig. 1. The insect central nervous system. A. Generalized central nervous system consisting of segmentally arranged ganglia and connectives. B. Schematic hemisection of an insect ganglion showing (not to scale) neural lamella (NL), perineurium (PN), glia (G), nerve cell bodies (C) and the neuropile (NP). Within the neuropile axons (ax) synapses (syn) and glial processes (g) are shown.

connections. In this survey particular attention will be given to synaptic function in view of the fact that many of the current generation of insecticides are anticholinesterases.

BLOOD-BRAIN BARRIER

It is well established that neurons in the central nervous system of insects are relatively insensitive to ions and molecules applied to the fluid bathing the surfaces of the tissue. Early studies established that insect central nervous tissue contained a diffusion barrier or barriers that discriminated against large size, positive charge and polarity (Treherne, 1966; O'Brien, 1967; Pitman 1971). In this section we shall examine recent evidence pertaining to the location of the barrier(s).

A. The Neural Fat Body

Parts of the ganglia and connectives of the central nervous system are surrounded by patches of fat body tissue. Examples of this are reported from Periplaneta americana (Smith & Treherne, 1963; Boulton & Rowell, 1968) and Manduca sexta (Pichon et al., 1972). In the stick insect Carausius morosus the fat body cells form a complete sheath around the ventral nerve cord (Maddrell & Treherne, 1966; Lane & Treherne, 1971; Huddart, 1972). It has been suggested that this fat body sheath regulates the level of sodium ions in the fluid space between the fat body sheath and the surfaces of the central nervous system (Weidler & Diecke, 1969, 1970), However, electrophysiological studies indicate that the neural fat body sheath of Carausius is leaky to ions (Treherne, 1972). This accords with the demonstration that the exogeneous tracer molecule macroperoxidase (MW 40,000) penetrates from the haemolymph into the extraneural space (Lane & Treherne, 1971). It therefore appears that the neural fat body sheath does not constitute a major site of restriction of ion movements between the haemolymph and the neurons within the central nervous system.

B. The Neural Lamella

The neural lamella is the connective tissue layer surrounding both peripheral and central nervous systems. It contains tracheae and tracheoles and in some cases fibroblast-like cells. Neutral mucopolysaccharides and mucoproteins (Ashhurst, 1968; Ashhurst & Costin, 1971a,b) form the ground substance in which collagen-like fibres are embedded (Smith & Treherne, 1963; Locke & Huie, 1972). A number of studies have been performed on the permeability of the insect neural lamella. Electrophysiological studies reveal a rapid access of potassium ions in the bathing medium to the outer membranes of the perineurium (Treherne et al., 1970; Pichon & Treherne, 1970; Pichon et al., 1972). Histological studies have demonstrated that the neural lamella surrounding the nervous system of Periplaneta americana is permeable to water soluble dye molecules (Wigglesworth, 1960; Eldefrawi et al., 1968). Recently molecules as large as macroperoxidase (MW 40,000) have been shown to penetrate the neural lamella and enter the underlying perineurial clefts in Periplaneta americana, Carausius morosus and Manduca sexta (see Lane, 1974). Microperoxidase and colloidal lanthanum penetrate the neural lamella of Periplaneta americana (Lane & Treherne, 1972). It can therefore be concluded that the neural lamella offers no significant restriction to the access of small water soluble molecules to the underlying cellular layer, the perineurium.