SUPPL. NO. 5 THE SOUTHWESTERN ENTOMOLOGIST MARCH 1983

INSECT NEUROPEPTIDES - AN OVERVIEW

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ABSTRACT

Ordinary neurons, neurosecretory cells, and endocrine cells mediate the adaptive properties of the living state in insects. These cells release specific chemicals - in many instances peptides which selectively alter the normal channels of reception and command. Thus they regulate both behavior and metabolism.

Guiding Principles. Biologist have recognized for some time that multicellular organisms tend to preserve a uniform internal environment despite external changes. This steady state or homeostatis in and around cells and tissues depends on a complex coordination of many physiological and biochemical processes (Cannon 1929). Basically, each process contributing to homeostasis is regulated by a series of functional elements known as receptors, regulators, and effectors. These elements are classically represented in ther nervous system by the reflex arc, and equivalent units can now be recognized in many other cellular mechanisms.

In insects, homeostasis is accomplished by the joint action of the nervous and endocrine system. The principle types of cells that transmit information to target organs and tissues are illustrated in Fig. 1. In each instance information is conveyed by the release of a specific chemical. Neurons that regulate rapid control mechanisms like muscular contraction require compounds that can be quickly inactivated. However, other processes such as growth, development, and homeostasis need more stable substances to insure an appropriate response duration. Various hormones regulate these slower mechanisms in insects, and a specialized group of neurons in the central nervous system synthesizes a number of them. Although these neurosecretory cells can conduct electrical signals like ordinary neurons, they generally release their secretory products into the circulatory system like endocrine cells. The release occurs at specialized end structures called neurohemal organs. These organs are formed by a profuse branching of terminal axons, the swollen ends of which are separated from hemolymph only by an acellular basement membrane (Maddrell 1974). Since the hemolymph serves as a medium for transport, the sites of neuroendocrine action are often at a considerable distance from the point of release. However, certain tissues and organs are directly innervated by neurosecretory cells (Hoyle et al. 1980 and O'Shea and Adams 1981). In these instances the terminal axons lose their envesting glial sheaths as they penetrate the basement membrane of an organ and come into close proximity with other cells. Their mode of innervation is termed neurosecretomotor (Fig 1).

All insect neurohormones isolated thus far are either polypeptides or proteins that can be inactivated by proteases. Obviously the sequence of amino acids in a peptide can provide more informational signals for transfer than can a single amino acid or small organic molecule. Not only can a complex message be sent to a single target organ, but there is also the possibility of multitarget action in various tissues and organs. The wealth of pharmacological information implicit in these possibilities remains largely unexplored because as yet so few insect neurohormones have been structurally identified. Neverthe-

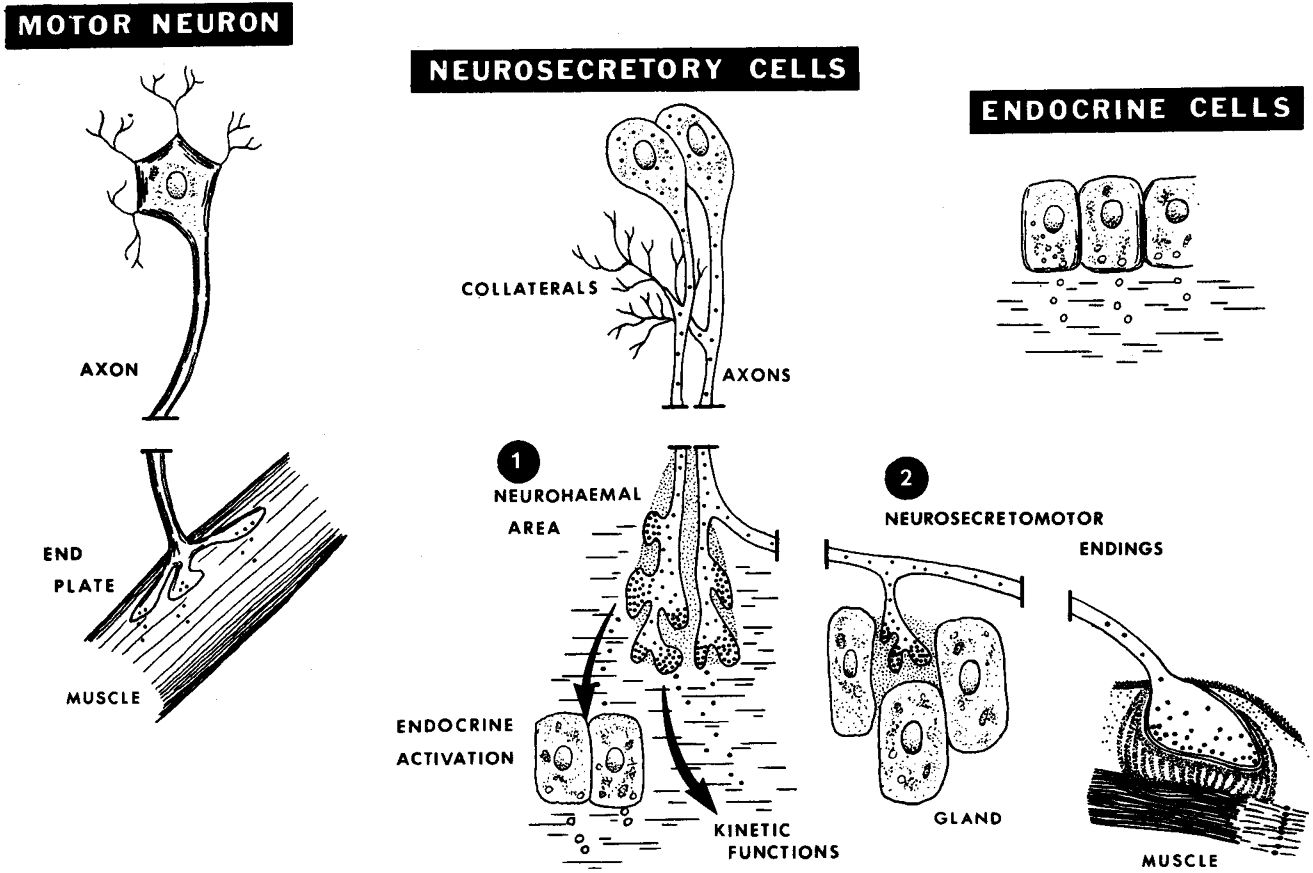

FIG. 1. Principle systems of intercellular communication in insects.

NEUROHORMONE MEDIATED FUNCTIONS

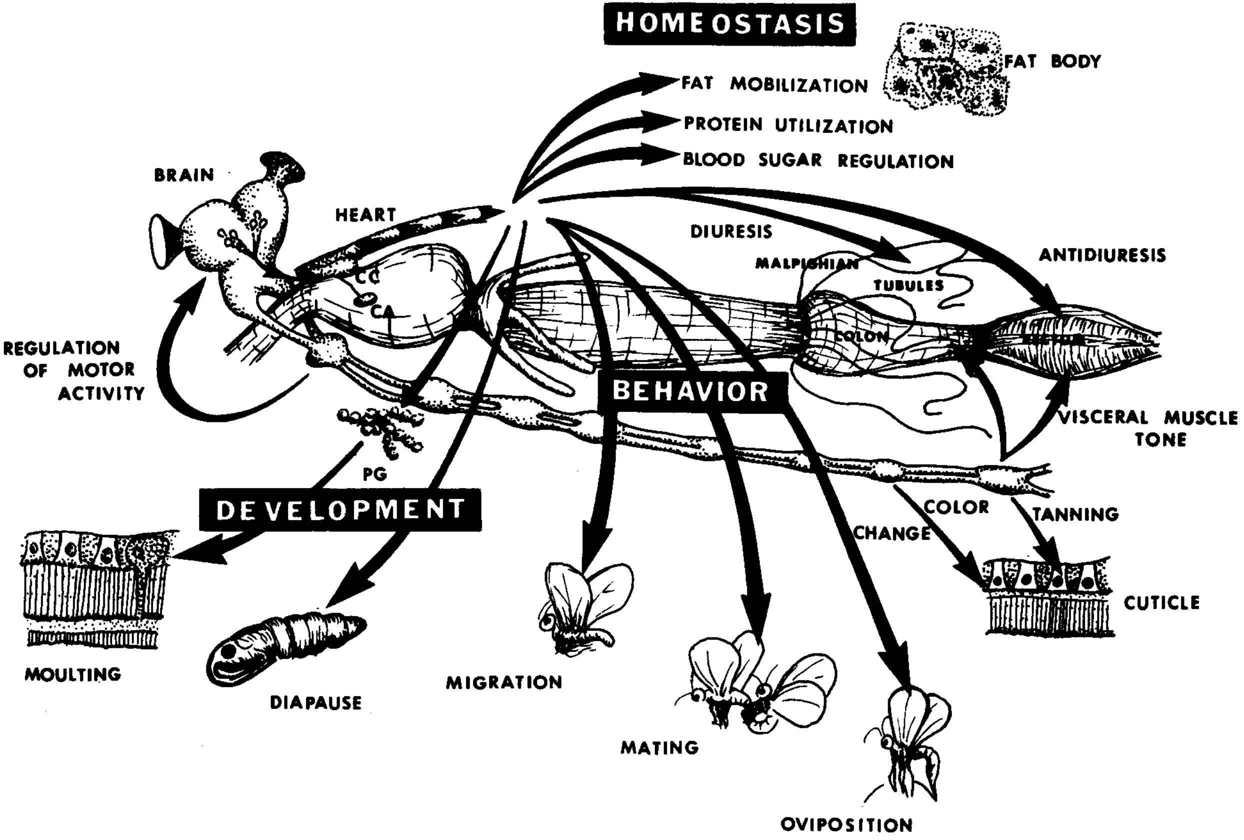

FIG. 2. Major physiological functions regulated by neurohormones in insects. Hormonal distribution is accomplished chiefly by release into the hemolymph, but localized secretion from nerves does occur.
CC - Corpora cardiaca
CA - Corpora allata
PG - Prothoracic gland

less, research aimed at finding a chemical basis for physiological action has not been neglected, and there is now strong evidence of chemical mediation in all of the functions shown in Fig. 2.

This search for endogenous chemical mediators in living systems was originally termed autopharmacology by Sir Henry Dale in 1933. Such studies invariably begin by observing some physiological change caused by the contents of a tissue extract from an experimental animal. Of course, when a biologically active factor is initially discovered there is no certainty that the observed response is caused by a single chemical. Nevertheless, in order to proceed one must assume that the effect does have a single cause. This assumption is strengthened if one selects a simple isolated preparation, which minimizes the number of sites for extraneous chemical-tissue interaction. Although many chemicals that directly affect specific physiological events have now been isolated and identified from biological sources, the effort to isolate the pure chemical often results in a protracted struggle. The fact that such efforts are successful must be attributed not so much to human ingenuity as to the remarkable uniformity and order present in living systems. The problems encountered in the study of insect autopharmacology are formidable, and in order to achieve success the following difficulties must be resolved: 1) Generally only minute quantities of the active compound are found in each insect. Thus, large numbers of individuals (from ten thousand to several hundred thousand) are required as starting material. 2) A rapid, sensitive, and dependable bioassay is essential. Failure to meet this criterion can make it impossible to follow almost any plan for purification. 3) Some peptides and proteins may become unstable during the process of purification, which always complicates a structural determination. 4) Careless transfer procedures can cause contamination of a nearly pure material. Of the difficulties mentioned thus far, 1 and 3 can be minimized by the development of ultramicro analytical techniques. For example, during the past year amino acid analyzers have appeared that can detect and quantitate amino acids from the hydrolysate of a 100 picomole sample. A vapor-phase sequencer that can make determinations on the same sample size is now available.

Behavior and Neurophysiology. Animal behavior, like most phenomena in the sensible world, can be described by experiment and observation. However, these methods can generate two rather distinct perspectives.

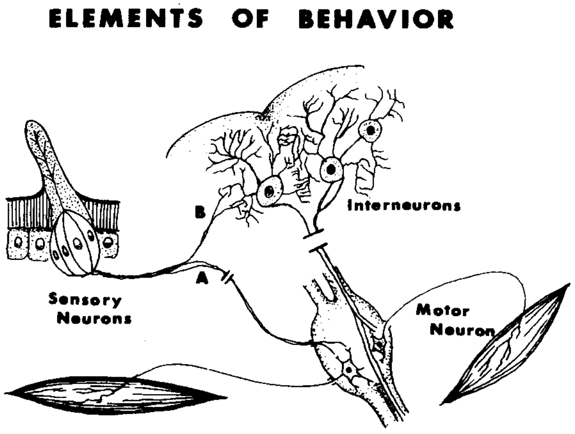

FIG. 3. The reflex (a) and the integrative (b) pathways for sensory reception.

The neurophysiologist begins his experimental study by examining the factors that affect single stimulus-response sequences in the animal. Once he understands these elementary units or reflexes, he can attempt to describe behavior in a simple mechanistic fashion by a summation of units. The analysis appears simple enough when a direct relationship exists between sensory and motor neurons (Fig. 3a), but such an ideal arrangement rarely occurs in nature. Moreover, neurons do not exist in isolation. They are constantly interacting with one another and exchanging information. Furthermore, a transfer function or interneuron is often interposed between motor and sensory units (Fig. 3b). Physiologists, of course, hoped to resolve these complex interactions into chain reflexes. However, as the importance and diversity of the third variable in neural networks became more apparent over the years, attempts to explain behavior in such rigid terms seemed less and less realistic.

The ethologist, by comparison, uses unobtrusive observation to obtain his results. He recognizes that animals in their natural habitat often show purposeful activity in the apparent absence of stimuli. Such behavior is generally termed instinctive, because its fixed patterns of movement are not a product of individual experience or learning but are complete when first performed. Insects are endowed with an impressive array of such stereotyped behavior. The honeybee, for example, has an inherent behavioral capacity to seek out flowers and collect pollen and nectar. Ethologists have theorized that these fixed behavioral patterns are programmed from "centers" in the central nervous system, and indeed neurophysiologists have discovered discrete areas in the brains of both the cricket (Huber 1965) and the bee (Vowles 1961) that can produce a patterned sequence of movement when stimulated. Huber, for example, found that courtship song or running behavior could be evoked by electrically stimulating specific regions of the brain of the male cricket. How the electrical stimuli call forth these responses remains obscure, but presumably in the free living state a key stimulus or a specific constellation of stimuli trigger the whole sequence. Hoyle (1964) suggested that the process of releasing these instinctive patterns might be considered analogous to a magnetic tape which plays off the whole set of separate motor acts from a central memory store. In spite of the insights ethologists have offered on endogenous activity, their scheme does not provide the conceptual tools for physiological analysis. Ultimately, the question of behavior must be resolved in neurological terms.

Today neurophysiologists clearly recognize that behavior is essentially an integrative expression of the whole nervous system. Animals do not behave as hard-wired automatons as the mechanists had supposed. Rather, their activities possess adaptive and flexible qualities. The intriguing question, of course, is what imparts this adaptive property to the function of nervous systems? At the moment a number of factors seem important: 1) Each neuron acts as a delicate receiver and filter of information and speaks with a discriminating voice. 2) The contact geometry between interacting neurons expresses a hierarchy of networks which seems to represent the essence of the integrative process that we call behavior. 3) The release of chemical messengers (hormones and transmitters) can selectively alter the normal channels of reception and command. Just as contact geometry may represent the essence of integration in the central nervous system, chemical messengers may impart that flexible or pliant quality to neural function. Hormones and transmitters share two unique features: 1) They can effect a functional change in neural networks without disturbing contact geometry; and 2) Their action can be temporally regulated.

Neurosecretion and Programmed Behavior. Our attention will be focused on the last element in this discussion. The first suspicion that peptides might be involved in regulating insect behavior came from experiments with extracts from the corpora cardiaca (CC) of the cockroaches *Periplaneta americana* and *Blaberus craniifer* (Ozbas and Hodgson 1958). When these extracts were placed on the isolated ventral nerve cord, a decrease in the frequency of spontaneous nerve signals was recorded. Injection of the extracts into intact animals caused a decrease in coordination but an increase in stereotyped locomotor behavior. This study was important because it was the first to illustrate that the products of neurosecretion can affect both the electrical properties of neurons and whole animal behavior.

These results prompted Milburn et al. (1960) to try CC extracts as a means of altering the inhibitory action of the central nervous system on reproductive behavior. From earlier experiments it was recognized that decapitation provoked reproductive movements in the abdomen of the male praying mantis, Mantis religiosa, and the cockroach P. americana (Roeder 1935 and Roeder et al. 1960). Together with these characteristic abdominal movements, a marked increase in motor output along the phallic nerve was observed. Presumably the neurons responsible for inhibition are located in the subesophageal ganglion. Although Milburn and Roeder (1962) showed that CC extracts applied directly to the subesophageal ganglion or the nerve cord caused an increase in the firing frequency of the phallic nerve, they made no attempt to determine the chemical nature of the substance or substances responsible for this activity. Moreover, the large number of CC required to produce the effect made the observation little more than a pharmacological novelty until Gersch and Richter (1963), as well as Unger (1965), were able to show that neurohormone D_1 (a peptide) could stimulate the bursting pattern in the phallic nerve of the cockroach, as described by Milburn and Roeder (1962).

The eclosion hormone offers one of the most dramatic examples of behavioral regulation in insects. This neurosecretory peptide is released from the brain of the fully developed moth toward the end of metamorphosis. It causes the central nervous system to release a long, pre-set pattern of motor events that results in eclosion. This neural program directs a sequence of abdominal movements. The best description of the interaction between the hormone and neural events comes from studies on the silkmoth, Hylophora cecropia (Truman 1978). In this species the hormone triggers the following behavioral program: 1) Fifteen minutes after exposure to the hormone the abdomen of the pupa displays frequent rotary movements for 30 minutes, followed by a quiet period of 30 minutes. 2) Then abdominal peristaltic contractions with wing and leg movements begin, which free the moth from the pupal cuticle. 3) Finally, a sequence of abdomen and wing postures permits the wings to inflate. Although the hormone primarily regulates these motor events during the process of eclosion, other effects have been discovered including selective muscle degeneration, induction of cuticle plasticity, and the activation of dermal glands (Truman 1980). The eclosion hormone is an acidic peptide (pI=4.8) with an estimated molecular weight of 8500, as shown on Sephadex G 50 chromatography (Reynolds and Truman 1980). Incubation with trypsin completely destroyed hormonal activity, and treatment with chymotrypsin caused a 90% reduction in activity.

Continued research on insect neurohormones and peptides will no doubt offer new prospects for insect population management in the years to come through behavior modification. However, our efforts should reflect the precept, that nature is to be commanded only by obeying her, given to us by Francis Bacon. If we stop and think for a moment the whole drama of our professional lives as scientists revolve about this statement. Everything in society today seems to invite us to begin our research on the positive aspect of commanding nature. Certainly the missions and priorities of most research agencies are directed to this end. Nevertheless, our frustrations and failures are a constant reminder of the importance of the conditional. In fact we can only meet the terms of this conditional when we have set aside our social concerns and simply focused our attention on nature. This is especially true when we are searching for new ways of working with nature harmoniously. As we gradually develop our sense of wonder about the world that we live in, it become possible to ask those questions that will uncover the many surprises that still lie just beneath the surface of appearances. Even after discoveries have been made and social and economic interests have pressed for immediate technological application, the scientist must not be greatly distracted. He must continue to test his findings to fathom their full import in nature's terms. This is the surest defense against illusion and the only certain path to prudent action.

LITERATURE CITED

Cannon, W. B. 1929. Bodily changes in pain, hunger, fear and rage. D. Appleton & Co., New York and London.

Gersch, M., and K. Richter. 1963. Auslosung von nervenimpulsen durch ein neurohormon bei Periplaneta americana. Zool. Jb. (Physiol.) 70:301-308.

Hoyle, G. 1964. Exploration of neuronal mechanisms underlying behavior in insects. P. 346-376. In R. F. Reiss (ed.) Neural theory and modeling. Stanford University Press.

Hoyle, G., W. Colquhoun, and M. Williams. 1980. Fine structure of an octopaminergic neuron and its terminals. J. Neurobiol. 11:103-126.

Huber, F. 1965. Brain controlled behaviour in orthopterans. P. 233-246. In J. E. Treherne and J. W. L. Beament (eds.) The physiology of the insect central nervous system. Academic Press, London and New York. 277 pp.

Maddrell, S. H. P. 1974. Neurosecretion. P. 307-357. In J. E. Treherne (ed.) Insect neurobiology. North-Holland Pub. Co., Amsterdam.

Milburn, N., and K. D. Roeder. 1962. Control of efferent activity in the cockroach terminal abdominal ganglion by extracts of corpora cardiaca. J. Gen. Comp. Encocr. 2:70-76.

Milburn, N. S., E. A. Weiant, and K. D. Roeder. 1960. The release of efferent nerve activity in the roach, Periplaneta americana, by means of extracts of the corpus cardiacum. Biol. Bull. Mar. Biol. Lab. Woods Hole 118:111-119.

O'Shea, M., and M. E. Adams. 1981. Pentapeptide (proctolin) associated with an identified neuron. Science 213:567-569.

Reynolds, S. E., and J. W. Truman. 1980. Eclosion hormones. P. 196-215. In T. A. Miller (ed.) Neurohormonal techniques in insects. Springer-Verlag, New York. 282 pp.

Roeder, K. D. 1935. An experimental analysis of the sexual behavior of the praying mantis. Biol. Bull. 69:203-220.

Roeder, K. D., L. Tozian, and E. A. Weiant. 1960. Endogenous nerve activity and behaviour in the mantis and cockroach. J. Insect Physiol. 4:45-62.

Truman, J. W. 1978. Hormonal release of stereotyped motor programmes from the isolated nervous system of the Cecropia silkmoth. J. Exp. Biol. 74:151-173.

Truman, J. W. 1980. Eclosion hormone: Its role in coordinating ecdysial events in insects. P. 385-401. In M. Locke and D. S. Smith (eds.) Insect biology in the future. Academic Press, New York. 977 pp.

Unger, H. 1965. Der Einfluss korpereigener wirkstoffe auf die nerventatigkeit von Periplaneta americana L. Zool. Jb. (Physiol.) 71:727-740.

Vowles, D. M. 1961. Neural mechanisms in insect behaviour. P. 5-29. In W. H. Thorpe and O. L. Zangwill (eds.) Current problems in animal behaviour. Cambridge University Press, Cambridge, England.