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PROGRESS IN BRAIN RESEARCH
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TOPICS IN NEUROENDOCRINOLOGY

EDITED BY

J. ARIËNS KAPPERS

AND

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Introduction

For the VIIth Summer School for Brain Research, organized by the Netherlands Central Institute for Brain Research, Amsterdam, the topic Neuroendocrinology was chosen, partly because this is a rather complicated interdisciplinary subject which is rarely taught at any University as a theme in its own right, and partly because not a few workers at the Institute are especially interested in this field.

It is, perhaps, not entirely out of place to say a few words about this topic by way of general introduction giving a definition of the concept "neuroendocrinology" and, very briefly and schematically, some broad outlines concerning the nature of neuroendocrinological connections and the chemical agents involved. In the first paper, Professor Scharrer will give much more detailed information on these latter subjects.

As for a definition one may say that neuroendocrinology is that branch of the Neurosciences investigating the morphological and functional connections between the central nervous system and the endocrine systems. It is evident that several different disciplines such as anatomy, physiology, biochemistry and pharmacology should cooperate in elucidating these connections, their details as well as their more general implications which are also of great clinical importance.

The definition given indicates that neuroendocrinology is concerned with the functional interdependence between those two systems of the organism, the central nervous system and the endocrine apparatus, which are of the utmost importance for the functional coordination and integration of its many parts. The systems mentioned do not only ensure homeostasis by dealing adequately with the many and various stimuli, both from the milieu externe and interne, with which the organism has to cope at any moment during its existence, but they also ensure its growth and development, its reproduction and its faculties of communication.

The definition, moreover, includes that the central nervous system regulates the function of the different endocrine systems while, on the other hand, these systems influence the function of the nervous system. Endocrine systems often consist of chains constituted of several endocrine centres or organs which are functionally interrelated, the last organ of this chain or axis exerting the final effect on specific target or receptor cells.

Accepting that the central nervous system contains the primary centres involved in neuroendocrinological relationship, the pathways leading from this system to endocrine organs can be termed efferent pathways. They will be dealt with later. Afferent pathways, then, are those along which the products of the peripheral endocrine organs, hormones, are transported to the central nervous system. These latter pathways will mainly, although perhaps not exclusively, consist of blood vascular channels. Such hormones do not only regulate the function of centres, situated in the central nervous system and primarily involved in neuroendocrinological regulation, by way of their feedback activity. Hormones, produced in one endocrine organ, can also regulate the function of other peripheral endocrine organs by way of short feedback vascular connections. It should, moreover, be realized that hormones produced in peripheral endocrine organs do not only exert, in the central nervous system, an either excitatory or inhibitory influence on the centres from which the efferent pathways originate, but also on other neural centres such as, for instance, on those involved in behavioural mechanisms concomitant with neuroendocrinological events.

Most of the papers in this volume are especially concerned with the efferent links in neuroendocrinological relationship, dealing with the neural centres in which effector substances are produced, the nature and origin of these products, the pathways along which they are transported to their target cells, the way in which they are released, and their mode of action on endocrine and non-endocrine effector cells. It goes without saying that the field of neuroendocrinology is so large and deep that it was impossible to dig it up entirely in the single short week during which the Summer School was held.

We shall now deal briefly with the efferent connections. The primary neural centres from which they originate are all situated in parts of the brain and of the spinal cord which belong to the central part of the autonomic nervous system which is involved in the functional regulation of organs in general. Thus, these primary centres are to be found in the hypothalamus, that most important integration centre of autonomic functions, and in parasympathetic and orthosympathetic nuclei of the brain stem and cord.

The nature of the neurochemical effector substances and the pathways along which they are transported from the central nervous system to the endocrine effector organs vary a great deal. Giving first some examples of neural pathways originating from the spinal cord and brain stem, it is, for instance, known that the function of the adrenal medulla is regulated by preganglionic orthosympathetic nerve fibres originating in the intermediate lateral nucleus of the cord and coursing in the splanchnic nerves. The function of the pinealocytes in the mammalian pineal gland, an endocrine organ of neural origin, is, however, regulated by postganglionic noradrenergic orthosympathetic fibres and, as has been recently found, by postganglionic cholinergic parasympathetic fibres. The first type of fibres mentioned originates in the superior cervical ganglia and the second type from intramural pineal parasympathetic nerve cells. The central orthosympathetic preganglionic cells innervating the superior cervical ganglia are situated in the rostral part of the intermediate lateral nucleus in the cord while the parasympathetic preganglionic cells innervating the intramural pineal nerve cells lie in one of the parasympathetic nuclei of the brain stem. So far, it is not exactly known in which one.

More examples of endocrine organs, innervated by peripheral autonomic nerve fibres, could be given. The mode of impulse transmission between the endings of these fibres and their target cells varies. The neurochemical mediator substance, released at these endings, is not consistently transmitted to the effector cells by conventional synaptic junctions. A less well-directed and slower transmission of the stimulus is often realized in case the nerve terminals lie at some distance from the receptor cells.

Then, the neurohumor, once released, diffuses slowly in the organ along perivascular or wide intercellular spaces, as is true in the pineal gland, or in stromal tissue. From the above it appears that neural centres in the spinal cord and the brain stem, belonging to the central autonomic nervous system, send impulses regulating the function of endocrine organs along neural pathways belonging exclusively to the peripheral autonomic system, either directly by preganglionic fibres or by both, preganglionic and postganglionic fibres. The neurochemical transmitter substances involved belong to the category of the neurohumors. In mammals, centres producing neurohormones are, so far, not known to occur in the brain stem and the spinal cord. Such a centre is, however, present in the caudal end of the spinal cord of lower vertebrates as will be shown by Professor Bern in his paper.

The way in which neural centres in the hypothalamus are linked to endocrine organs by efferent pathways is much more complicated while, moreover, the nature of the neurochemical mediator substances involved is more varied. Neural centres in the hypothalamus produce neurohumors, although of a somewhat special kind, as well as neurohormones which are transported to their endocrine target cells along pathways which may consist of more than one link, these links being sometimes of a different nature.

The term "neurohormones" applies to all chemical mediators which are produced by neurosecretory cells, this production being the principal if not the only task of this cell type. Neurosecretory cells show morphological and physiological features characteristic of both, nerve cells and secretory cells. Neurohormones finally reach their target cells by vascular pathways. The term "neurohormones" does not imply anything about the chemical composition of these substances. They may, for instance, be of a proteinaceous or of an aminergic nature. For their definition as neurohormones it is only of importance that they are produced by neuro(secretory) cells and reach their target cells finally along vascular pathways. That this definition sometimes causes difficulties will be clear, especially when aminergic neurohormones are concerned.

As I am sure that Professor Scharrer will go deeper into this question, I will only mention here that three different kinds of "neurohormones" can be distinguished:

(1) Neurohormones of which the polypeptide nature has been well-established for some time: vasopressin and oxytocin. These neurohormones are produced in the hypothalamic magnocellular supraoptic and paraventricular nuclei. The axons of the cells constituting these nuclei form the supraoptico-paraventriculo-hypophysial tract. Most, but not all fibres of this tract end in the infundibular process of the neurohypophysis.

(2) Neurohormones of which the proteinaceous chemical composition, at least of part of them, has been established quite recently. These are the so-called releasing, or better regulating (B. Scharrer) or hypophysiotropic factors. These neurohormones are produced in the parvocellular arcuate and tuberal hypothalamic nuclei situated in the baso-medial part of the hypothalamus. The axons of these cells form the tubero-infundibular tract. Their terminals are situated in the median eminence.

(3) Aminergic neurohormone(s) which is probably produced by nerve cells lying in the arcuate nucleus of the hypothalamus. Their axons join the tubero-infundibular tract to end in the median eminence. The question whether the aminergic compound (*e.g.*, dopamine) produced in this third neuronal system should be termed either a neurohormone or a neurohumor depends on the different opinions existing on its target, and, in connection therewith, on the pathway along which this target is reached (see below).

The neurohormones mentioned are transported along the axons of the hypothalamic nerve cells in which they are produced to the axon terminals in which they are stored and from which they are released. In mammals, axons along which neurohormones are transported do not leave the central nervous system. They end either in the infundibular process of the neurohypophysis or in the median eminence. Here, the axon terminals are in close contact with the outer basement membrane of perivascular spaces surrounding capillary plexuses. Together with these plexuses the axon terminals form a so-called "neurohemal organ".

The neurohormones stored in the axon terminals of the supraoptico-paraventriculo-hypophysial tract fibres, present in the infundibular process of the neurohypophysis as mentioned sub (1), are depleted into capillaries which drain directly into the systemic circulation by which these neurohormones finally reach their target or receptor cells which are not necessarily endocrine cells exclusively. The neurohormones mentioned sub (2) which are stored in axon terminals situated in the median eminence are depleted into the primary capillary plexus of the hypophysial portal system by which they are conveyed, probably exclusively, to their different and specific endocrine target cells in the pars distalis of the hypophysis or adenohypophysis. According to some authors, the aminergic compound, mentioned sub (3), is likewise depleted in the primary capillary plexus of the hypophysial portal system to reach also the endocrine cells in the adenohypophysis supposedly cooperating with the hypophysiotropic factors in regulating the release and possibly the synthesis of the hormones, produced by these cells. Other authors, however, hold that this compound is not depleted in any vascular pathway, but acts, in the median eminence, on the terminals containing the hypophysiotropic factors regulating the depletion of these factors into the primary capillary plexus. In the latter case this aminergic compound should not be called a neurohormone because it is not being depleted into the blood.

From the foregoing it appears that mammalian neurohormones reach their elements of destination first along a neural pathway, the axons of the neurosecretory cells in which they are produced, and then along a post-linked vascular pathway, both pathways together constituting a neuro-vascular pathway.

Some of the axons of peptidergic neurosecretory cells of the magnocellular hypothalamic nuclei do not terminate in the infundibular process of the neurohypophysis, but on pituicytes and on endocrine cells of the intermediate part of the hypophysis. Because, ontogenetically, this pars intermedia originates from the posterior wall of Rathke's pouch, it does not belong to the central nervous system. Therefore, the axons ending in close contact to the endocrine cells of the pars intermedia have to leave the brain. As the proteinaceous mediators stored in the axon endings are not depleted into capillaries but act directly on the pars intermedia cells, they do not qualify as neurohormones although they are produced by neurosecretory cells. Due to various reasons these special mediators are classed in a category in between neurohormones and neurohumors. Similarly, some fibres transporting biogenic amines do not terminate in the median eminence, but in close contact to cells of the pars intermedia. Both, the peptidergic and the aminergic fibres ending on endocrine cells of the pars intermedia, are sometimes termed "neurosecretomotor fibres".

In some non-mammalian vertebrates, a caudal neurosecretory system showing features comparable to that of the hypothalamic magnocellular neurosecretory system, is present in the caudal end of the spinal cord. The axons of its neurosecretory cells terminate in a storage organ, the urophysis, from which the neurohormone is depleted into a capillary vascular system. In the present volume, this caudal secretory system will also be dealt with.

As is quite evident and has also been demonstrated by many authors, the function of the primary hypothalamic centres involved in the regulation of endocrine systems not only depends on the feedback activity of hormones produced in endocrine organs, reaching these centres by afferent vascular pathways, but also on neural stimuli contacting the nerve cells which constitute these primary centres. These stimuli are either excitatory or inhibitory. The axon terminals being in synaptic contact with the dendrites or the soma of the neurosecretory cells contain either biogenic amines, such as noradrenaline or serotonin, or acetylcholine. It can only be indicated here that these axons originate from nerve cells which are situated in various and quite different parts of the brain, such as for instance telencephalic limbic centres and the brain stem reticular formation. These centres, again, receive many impulses from various sources, such as from the sense organs, mostly via relay centres, and from the ascending multisynaptic pathway in cord and brain stem. It should, therefore, be clear that the function of the cells constituting the primary hypothalamic centres from which the efferent pathways for the regulation of endocrine systems originate, are, themselves, modulated by impulses from a multitude of various sources. To use a term, coined by Ernst Scharrer, the neurosecretory cells form a final common pathway for all stimuli reaching them.

The same holds for the centres in the spinal cord regulating the function of endocrine organs. Light stimuli entering the eye are, for instance, conveyed via a multisynaptic neural pathway, the links of which are now known, to that part of the intermediate lateral nucleus in the cord from which the preganglionic fibres originate to innervate the mammalian pineal gland via the superior cervical ganglia and their postganglionic noradrenergic fibres. In this way, the function of this gland depends, *i.a.*, on photic stimuli.

We will end this introduction to the central topic, primarily written from the standpoint of a neuro-anatomist, by pointing out that not only the blood, but also the cerebrospinal fluid probably serves as a vehiculum for the transport of hormones which, by means of special ependymal elements or after stimulating special liquor-contacting neurones, may modulate the function of hypothalamic and other centres involved in neuro-endocrinological regulation. This relatively new and fascinating subject will also be mentioned in the present volume.

The proceedings of the Summer School testify to the industrious efforts of the participants to unravel, by various methods and techniques, the many structural and functional neuroendocrinological problems. I am most grateful to all participants cooperating by contributing so many important papers and taking such a lively and fruitful part in the discussions. The editors being responsible for the final script of the discussions, our apologies are offered in advance to anyone who would not be entirely satisfied with what has been published from his remarks and comments.

My sincere thanks are also due to the staff members of the Netherlands Central Institute for Brain Research, and more especially to Dr. Schadé. Without their assiduous help and experience the organization of the Summer School and the editing of its proceedings would scarcely have been possible.

The fact that, once again, the governing board of the Royal Netherlands Academy of Sciences and Letters has placed the mansion of the Academy at the disposal of the conference is much appreciated.

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Neuroendocrine Communication (Neurohormonal, Neurohumoral, and Intermediate)

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Reports on current advances in neurobiology reflect a growing interest in control mechanisms involving close collaboration between the nervous and the endocrine systems. This trend reaches its culmination in the present program which addresses itself exclusively to Neuroendocrinology. In essence, this topic concerns two channels of communication, one conveying neural directives to the endocrine apparatus, the other providing the nervous system with information on hormonal events. Therefore, the central theme of a progress report on neuroendocrine phenomena will have to be an examination of the nature and mode of operation of these avenues of interaction. But the major share of our attention will be directed to the efferent link, *i.e.*, the varied means of communication between the neural and the endocrine systems, since it is even more important and considerably more complex than the afferent link. An introduction to this topic in general conceptual terms as provided in this first chapter can be concise, since several recent surveys along similar lines are available (see B. Scharrer, 1967–72; E. Scharrer, 1965, 1966).

Efficient operation of the endocrine system requires precise programming of rates of production and release of individual hormones in concert with others; it may be accomplished by active stimulation or inhibition, or by withdrawal of either kind of signal. There also seems to be an interplay among such varied directives. Since the effectors are subject to control by pluralistic and at times conflicting information, there is the possibility of confusion. Therefore, at any given time, instructions for action must be well integrated, *i.e.*, non-contradictory. They should reach endocrine effector cells of the same kind simultaneously, and their effects should be more or less sustained. Amplification of signals seems to be advantageous in cyclic control systems, and there is also an apparent requirement for trophic influences in the maintenance of endocrine organs.

The need for the conversion of a variety of simultaneous conditioning factors into uniform commands is met by the nervous system through which most of the afferent signals are channeled. In turn, the effectiveness of the "final common path" to the first way station in the endocrine system is assured by a multiplicity of means for the transfer of information. Their existence has become apparent as a result of correlative studies in which electron microscopy provided invaluable leads. A broad comparative approach has revealed the general validity of the conclusions to be discussed for all of the neuroendocrine systems thus far investigated.

TABLE I

MODES OF COMMUNICATION BETWEEN NEURONS AND NON-NEURAL EFFECTORS

			Endocrine effectors	*Nonendocrine effectors*
Conventional neuroeffector junctions	Contiguous	Neurohumoral	Pars intermedia Endopancreas Adrenal cortex Ultimobranchial body Parafollicular cells Leydig cells, testis Interstitial cells, ovary Corpus allatum	Neuromuscular junctions Secretomotor junctions (exocrine cells)
Nonconventional mechanisms	Noncontiguous	Neurohormonal	Anterior pituitary (higher vertebrates) Prothoracic gland (insects)	Kidney tubule cells Uterine muscle Myoepithelial cells
		Nonhormonal (stromal pathway)	Some fish pituitaries Insect endocrines	Smooth muscle Some striated muscle Regeneration blastema (NTS)
	Contiguous (nonconventional)		Pars intermedia Corpus allatum	Muscle, *etc.* (insects)

The various modes of operation differ with respect to the type and amount of the neurochemical mediator in use, the manner in which it reaches its destination, and the duration of the signal. These parameters are related to the nature of the extracellular pathway. In addition, there are diagnostically useful ultrastructural and histochemical differences among the respective intracellular storage sites of the active principles.

The existing possibilities for neuroendocrine communication are listed in Table I which, for the sake of comparison, also includes non-neuronal effectors other than endocrine. Special adaptations to the needs of the endocrine system take precedence over control by conventional innervation, but it is difficult to decide which factors determine the choice of "language" used in different situations, and whether or not there are sharp lines of demarcation.

I. Conventional control mechanism

The presence of synaptic or synapse-like (synaptoid) configurations in electron micrographs of a variety of endocrine organs leads to the conclusion that at least some of the neurochemical signals in operation are the same as those in regular neuron-to-neuron communication. Quite often these junctional complexes resemble adrenergic elements, with the exception that some of the characteristics of conventional chemically transmitting synapses tend to be absent. Aside from those in the adrenal medulla and the pineal gland, putative secretomotor junctions also occur in a number of endocrine structures of non-neural derivation. Examples are the pars intermedia of the pituitary (Bargmann *et al.*, 1967; Follenius, 1968, 1970; Meurling and Björklund, 1970, and others); various components of the pancreatic islets (Legg, 1967; Esterhuizen *et al.*, 1968; Watari, 1968; Shorr and Bloom, 1970; Kern *et al.*, 1971), the adrenal cortex (Unsicker, 1969; Alvarez, 1970), various calcitonin producing cells (Robertson, 1967; Stoeckel and Porte, 1967; Young and Harrison, 1969), and interstitial elements in both male and female gonads (Baumgarten and Holstein, 1968; Unsicker, 1970; Dahl, 1970). Analogous observations obtain for invertebrates, *e.g.*, those reported for the corpus allatum of insects (for further details see Scharrer, 1970; Weitzman, 1964–71).

This kind of morphological information is substantiated by physiological data, for example in the case of the beta cells of the endocrine pancreas. The adrenergic nature of the transmitter operating at neuron terminals that are in contact with some of these glandular elements is suggested by their ultrastructure. The specific fluorescence for catecholamine displayed at these sites increases after the administration of monoamine oxidase inhibitors, and the measurable rise in catecholamine content is accompanied by a stimulation of insulin secretion (Gagliardino *et al.*, 1970; see also Tjälve, 1971; Tjälve and Slanina, 1971). Furthermore, alloxan-treated animals show fine structural alterations in the terminals that are indicative of neuronal hyperactivity in conjunction with stimulation of insulin secretion (Shorr and Bloom, 1970).

Another example is seen in the pars intermedia. The adrenergic nature of one class of fibers among those supplying this part of the adenohypophysis has been conclusively established by their uptake of adrenaline-^{3}H, as demonstrated by ultraautoradiography, and by their selective destruction with 6-hydroxydopamine (Doerr–Schott and Follenius, 1969; Hopkins, 1971).

The question may be raised as to how much control over endocrine systems can be accomplished by neurohumoral signals at such secretomotor junctions. It need hardly be stressed that the special features of synaptic transmission (strict localization of signal, high speed, rapid turnoff) which are so crucial in regular interneuronal communication, represent a disadvantage in the case of endocrine receptors. The efficiency of their operation requires types of signals that cannot be provided by the activity of neurotransmitters alone. As will become evident in the next section, the special requirements of endocrine receptors are in fact largely met by other than synaptic types of neurochemical input.

II. Nonconventional control mechanisms

(a) Neurohormonal mediation. Among the special means of neurochemical instruction available to endocrine cells, those conveyed by either a general or a limited (portal) circulatory pathway predominate. They seem to be made to order for maximum efficiency of the neuroendocrine axis. Here the mediator is not classified as a neurotransmitter but as a neurohormone. It is derived not from a conventional neuronal element but from a special "neurosecretory" neuron. The main distinction of the latter is that its capacity for the synthesis of specific secretory products is so highly developed as to overshadow all other neuronal functions (for more detailed information on the phenomenon of neurosecretion see Bargmann and Scharrer, 1970; Gabe, 1966; B. Scharrer, 1967, 1969a, c; 1970; B. Scharrer and Weitzman, 1970; E. Scharrer, 1965, 1966; E. Scharrer and B. Scharrer, 1963).

The material released from classical (A type) neurosecretory neurons is proteinaceous, but aside from these "peptidergic" elements there are aminergic (B type) fibers which also seem to manufacture enough active material to reach threshold concentrations sufficient for hormonal interaction. But here the mode of operation may be somewhat different from that of peptidergic mediators. This specialization in synthetic activity expresses itself morphologically by the presence of distinctive types of glandular products in considerable quantity.

The prolonged signals generated by blood-borne neurochemical messengers are in sharp contrast to those characteristic of synaptic function. A further advantage of neurohormones is their simultaneous availability to multiple effector cells. An A type neurohormone released into the general circulation by neurosecretory cell groups in the insect brain reaches the prothoracic gland, a receptor organ controlling postembryonic development. An analogous group of neurohormones in vertebrates is represented by the well known hypophysiotropic (releasing or regulating) factors of the hypothalamus that control adenohypophysial function via the portal circulation. As has been shown in several mammalian species, these neurohormonal factors originate in hypothalamic centers such as the arcuate nucleus and enter their restricted vascular route at the level of the median eminence. The thyrotropin releasing factor

(TRF) which may serve as a prototype has been identified as a tripeptide (see Meites, 1970a). LH release and synthesis, as well as FSH release, are stimulated by another hypothalamic hormone which seems to be an octapeptide (Schally *et al.*, 1971; Redding *et al.*, 1971). In addition to small peptides, aminergic elements may participate in the control of hypophysial cells that furnish adenotropic hormones. Cytological and cytochemical data indicate the presence of both peptidergic and aminergic fibers in the median eminence (see, for example, Konstantinova, 1970; Oksche *et al.*, 1970; Sharp and Follett, 1970). The possibility that the two types of neurochemical mediators act synergistically has been discussed (see Scott and Knigge, 1970). Experimental data from *in vivo* and *in vitro* tests suggest aminergic (especially dopaminergic) synaptic control over neurosecretory neurons that furnish various hypophysiotropic factors (Barry, 1970; Fuxe and Hökfelt, 1970; Müller, 1970; Schneider and McCann, 1970), but there is some disagreement as to which type of synapse is responsible for stimulatory and which for inhibitory signals.

In view of the known relationship between photoperiod and gonadotropic function, it is of interest that the activation of the hypothalamic-hypophysial system in frogs maintained on a long-day regime involves only the neurosecretory material released from the median eminence, and not that destined for the posterior lobe (Vullings, 1971). Comparable effects of relevant exteroceptive and interoceptive factors on the synthesis and release of hypophysiotropic factors have been demonstrated in mammals (Meites, 1970b).

(b) Intermediate mechanisms. Several types of nonconventional mediation operate without the intervention of neurohormones. It appears that in the dissemination of neurosecretory messengers vascular channels can be circumvented altogether. An interesting variant of such "directed delivery" occurs, for example in the adenohypophysis of some teleost fishes where axon terminals carrying neurosecretory material abut on areas of intercellular stroma that separate the release sites from the receptor cells (Vollrath, 1967).

Invertebrates in which no capillary system exists depend much more on extracellular channels, and are therefore particularly well suited for the exploration of their physiological features. For one, the relative closeness of the effector sites restricts the sphere of dissemination without pinpointing the signal to a single cell. But beyond this merely spatial issue, it is the physico-chemical nature of the pathway which deserves attention in that it may allow for temporary sequestration of the neurochemical mediator and thus determine the time course in this type of information transfer (see also Barer, 1967).

Another mode of action for which physiological correlates are still missing, is suggested by ultrastructural evidence indicating that "peptidergic" neurosecretory terminals can make direct contact with endocrine receptor cells. Examples of such "neurosecretomotor junctions" occur in the pars intermedia of the cat (Bargmann *et al.*, 1967), and the corpus allatum of insects (B. Scharrer, 1964). Although here the spatial relationships are the same as in standard types of synapses, the different nature of the chemical messenger involved sets these junctional configurations apart.

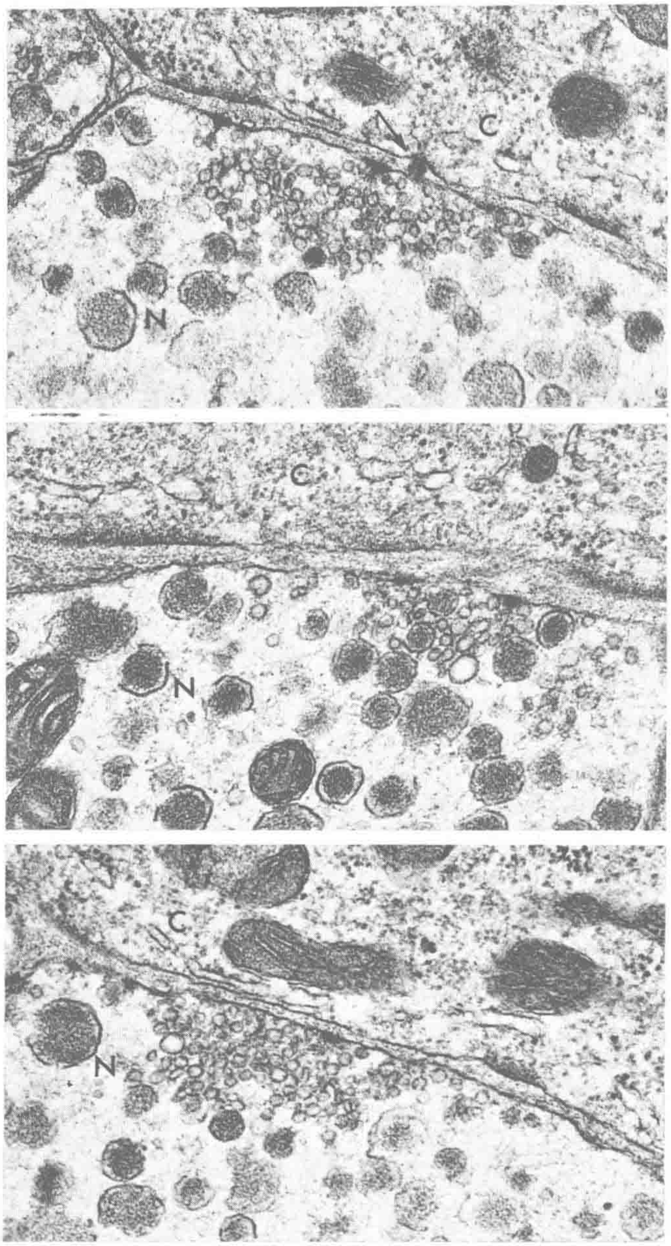

Fig.1. Three examples of neurosecretory fibers with synaptoid configurations in close proximity to endocrine cells (C) in corpus allatum of adult male of insect, *Periplaneta americana*. Note clusters of heterogeneous vesicles and small accumulations of electron dense (neurosecretory?) material close to axolemma or within narrow intercellular space (arrow). N, neurosecretory granules. Prior to fixation in Karnovsky's (1965) solution, the gland had been incubated for 30 min in insect Ringer's with excess of K^+ (*cf.* Sachs and Haller, 1968). Epon, uranyl acetate and lead citrate. × 40,000.