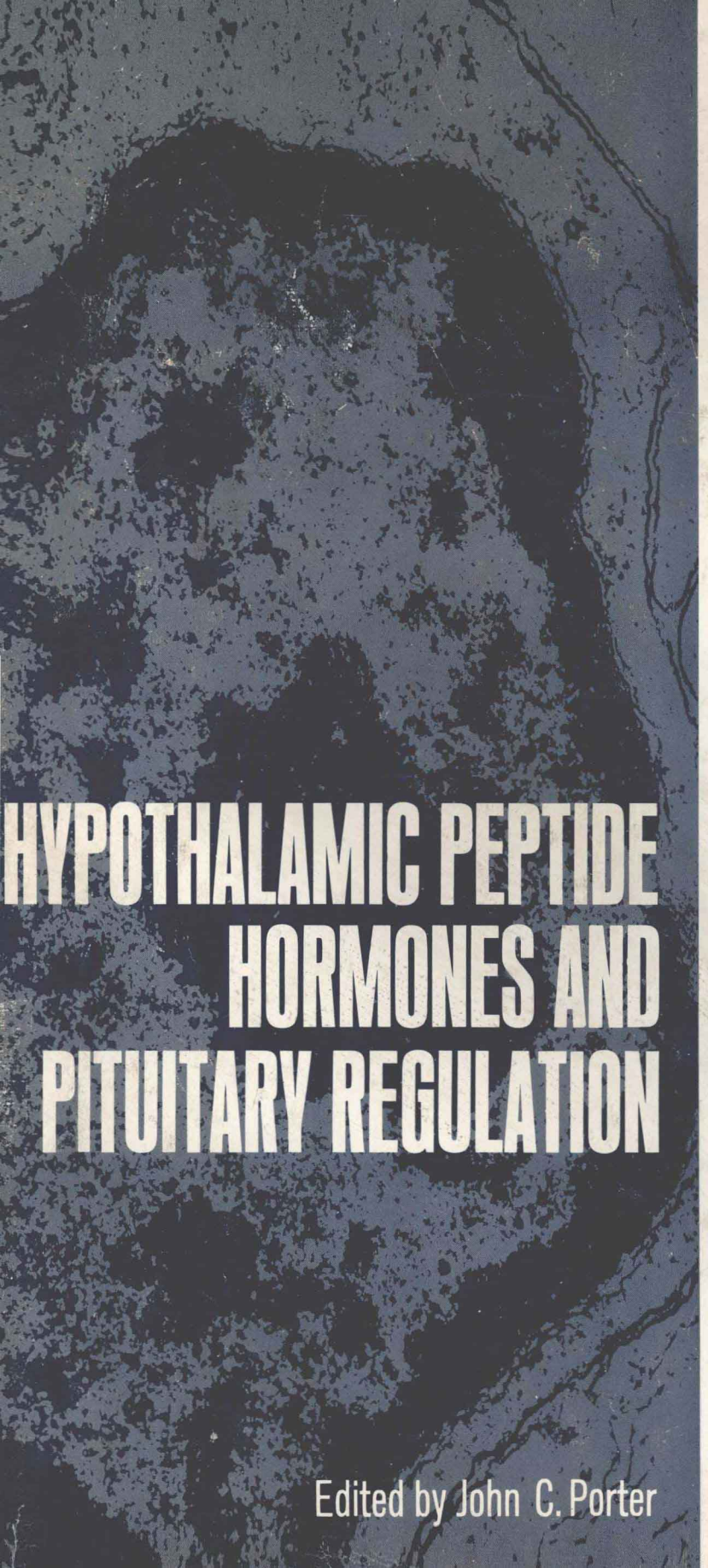

ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Volume 87

HYPOTHALAMIC PEPTIDE HORMONES AND PITUITARY REGULATION

Edited by John C. Porter

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John C. Porter

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PREFACE

The proceedings of a workshop conference are presented in this volume entitled Hypothalamic Peptide Hormones and Pituitary Regulation. The workshop was held in Wilson Hall on the campus of the National Institutes of Health, Bethesda, Maryland, during the days of November 1 – 2, 1976, and is the most recent of three symposia on neuroendocrinology that have been sponsored by the National Institutes of Health. The first one was held on December 6 – 8, 1961, in the New Everglades Hotel at Miami, Florida. During the first meeting, much emphasis was given to the anatomical and physiological basis for the fledgling science of neuroendocrinology. The proceedings of that symposium were published under the title of Advances in Neuroendocrinology, A. V. Nalbandov (ed.), University of Illinois Press, Urbana, Illinois, 1963.

The second workshop was held on January 8 – 11, 1969, in the Arizona Inn at Tucson, Arizona, and was unique in several respects. It was evident to the participants that definitive identification and the determination of the chemical structure of at least one hypothalamic releasing factor was at hand (see Workshop Conference on Bioassay and Chemistry of the Hypophysiotropic Hormones of the Hypothalamus: A Critical Evaluation, J. Meites, ed., The Williams and Wilkins Co., Baltimore, Maryland, 1970). Much of what was presented at the second workshop was dedicated to methods of bioassay of the various releasing factors. With the advent of immunoassays, several of these bioassays, especially those for releasing factors which have been subsequently characterized chemically and synthesized, have been largely superseded by more precise procedures of quantification. It is worth noting that by the time of the second workshop, in contradistinction to the first one, the phrase, hypothalamic releasing factor, was well entrenched in the scientific lexicon.

During the present and third workshop, the phrase hypothalamic releasing hormone and hypothalamic releasing factor were used synonomously. However, the demonstration of hypothalamic releasing hormones or factors in extrahypothalamic regions of the brain as well as in some non-neural tissues may denote a deficiency in the present nomenclature of this class of substances. This deficiency not withstanding, it was evident from the presentations of the participants that the progress in neuroendocrinology in the interval between the second and third workshops had been substantial if

not extraordinary. Moreover, the accomplishments of the research endeavors of the experimental laboratories are now finding use in clinical settings. And, it is reassuring to see that what is learned in one species is so generally applicable to another, even man. Such findings increase confidence in our generalizations. Yet, those who prefer to dwell on the promises of the future rather than the bones of the past, will note that much remains to be done, as the participants stressed repeatedly.

The program was organized jointly by Drs. D. S. Dhindsa, R. A. Gorski, J. D. Neill, J. C. Porter, and S. S. C. Yen with the assistance and advice of the Reproductive Biology Study Section and other interested scientists to whom the Organizing Committee is grateful. The Organizing Committee thanks the Reproductive Biology Study Section of the Division of Research Grants of the National Institutes of Health for sponsoring the workshop. The Editor wishes to acknowledge especially the extraordinary assistance of Judy Wagers in the planning and organizing phases of the workshop and in the preparation of the manuscripts for publication. Her dedication has been unstinting, and her contributions have been of inestimable value.

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April 20, 1977

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THE ENDOCRINOLOGY OF THE NEURON AND THE NEURAL ORIGIN OF ENDOCRINE CELLS

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I understand that this meeting is somehow a sequence to that memorable gathering that took place in Tucson, Arizona, in January of 1969. We all knew at that time that the Tucson meeting had been called by our colleagues of the Division of Research Grants of the NIH. The NIH had been generously funding research in the field of neuroendocrinology with an enormous share of that research money going to those who were involved one way or another in the characterization of "the elusive hypothalamic hypophysiotropic hormones." Concern had been expressed that after about ten years of supporting these efforts and in spite of a fair number of papers claiming "isolation and structure at hand" to paraphrase a famous choice of words, the discoveries expected, namely a structure for any one of the hypothalamic releasing factors, had just not been forthcoming. The Division of Research Grants wanted to have a hard look at the status of the field at that time. Knowing what was going on in my own laboratory at Baylor on the isolation of the thyrotropin releasing factor (TRF), I did the best I could to have the meeting postponed, even by only a couple of months; but the meeting was held on the appointed date.

What took place at that meeting and what followed is now part of the history of science. Within two months of the Tucson meeting, Burgus, Dunn, Vale, and I had synthetic tripeptides of known molecular structure with biological activity qualitatively indistinguishable from that of the native TRF which we had described in Tucson. A few months later the primary structure of ovine TRF was established by high resolution mass spectrometry. Characterization of TRF was the turning point which separated doubt, and often confusion, from unquestionable knowledge in the field of neuroendocrinology. It was of such heuristic significance, that I can say, and few will disagree, that neuroendocrinology became a true science on that event. Then followed the structure of porcine TRF; the following year, the isolation and characterization of porcine luteinizing hormone-releasing

factor (LRF), then ovine LRF. A year later, synthesis was achieved of the first analogs of LRF with partial agonist and antagonist activities as well as the first analogs of TRF having potencies greater than that of TRF. One more year, and we isolated, characterized, and synthesized somatostatin.

Analogs of LRF are now available with increased potency and extended duration of activity as well as powerful analogs of LRF with antagonist properties. TRF has been demonstrated in parts of the brain other than the hypothalamus and found to exert profound effects in the central nervous system. Somatostatin has been shown to inhibit not only the secretion of growth hormone and thyrotropin by the pituitary but also that of glucagon, insulin, gastrin, and acetylcholine by direct peripheral actions. Analogs of somatostatin have recently been described which have increased potency at the level of all its target organs; others have been prepared with remarkably dissociated activities at the level of several of the target tissues mentioned above. Somatostatin has been found not only in the extrahypothalamic central nervous system, like TRF, but also in specialized cells of the endocrine pancreas, gastric mucosa, and intestinal mucosa as well as in neurons of the myenteric plexus.

The still unresolved question of the growth hormone releasing factor led me about a year ago to look into the question of the nature of the endogenous ligand for the opiate receptors known to exist on brain synaptosomes. It is possible that an endogenous peptide having a morphine-like activity might be involved in the release of growth hormone (GH) since morphine releases GH. Less than a year ago the enkephalins were isolated in Scotland, while at the Salk Institute, I isolated several endorphins which Ling, Burgus, and I characterized and found to be identical to several fragments of the β-lipotropin, as are the enkephalins.

The clinical significance of these several discoveries I enumerated above has also become apparent, both in fields that were obvious from the start and in others that were not so obvious. Let me mention the use of TRF in classical endocrinology for the study of pituitary-thyroid functions; the use of LRF for the treatment of some types of infertility such as the induction of ovulation; the use of somatostatin to show the role of glucagon in juvenile diabetes with the possibility of a role of somatostatin or one of its analogs in the treatment of that disease. The latest of the clinically significant developments in this area of research include (a) the proposal that newly recognized endorphins along with their likely precursor, β-lipotropin, may play a role in the pathogenesis of certain mental illnesses in man (1) and (b) the observation that naloxone, a morphine antagonist, reportedly eliminates in minutes some of the symptoms of schizophrenia.

This meeting, the Bethesda meeting, is thus held in a spirit of much elation. I would like to occupy this opening lecture not so much by discussing in detail new technical achievements, but rather in presenting a few new concepts, as they pertain to the overall subject of this meeting. While already obvious to many of us, they are still not generally widely recognized.

One of these concepts I will call the "low voltage processing of information by brain cells." The other I will call the "neural origin of endocrine glands" or "is endocrinology a branch of neuroendocrinology." Both concepts have remarkable implications which, in my own mind, foretell what the major areas of research in this field will be for the next few years. What we will see also is that these two concepts will lead us to consider a remarkable unity of the mechanisms involved in physiological phenomena as widely separated as the stimulation of the secretion of adrenocorticotropin (ACTH) or growth hormone by pituitary cells and the inhibition by β-endorphin of the firing pattern of a neuron in the cerebral cortex.

Until recently the neuron has been seen primarily as a one-way communication system with a central processor for proximally received inputs and a one-way cable for output, the axon. The axon is characterized by its self-regenerating ability to conduct waves of high voltage depolarization for rapid transmission of an essentially binary type of information, expressed at the axon terminal. The axon is usually of considerable length, many times the average diameter of the cell body. While the dendritic surface has long been recognized morphologically and its vastness well documented, it was not granted much of an active role in the performance of the neuron, principally because experimental evidence of such activity was simply lacking. Any integrative ability or capability of the system was located at the axon hillock. One of the major characteristics of this view of the projection neuron, well studied for over fifty years, is the high voltage action potential ranging from a few millivolts to as much as 100 millivolts. Classically, such a neuron will deliver its ultimate message at essentially a single address in the form of packets of a discrete neurotransmitter. For most projection neurons, we still do not know whether norepinephrine, acetylcholine, dopamine, and in a few instances serotonin are involved. No consensus exists as to the ultimate significance of such substances as certain amino acids, γ-aminobutyric acid, substance P, and lately, small peptides like somatostatin, the hypothalamic releasing factors, and the endorphins, many of which have been traced by immunoassays or immunocytochemistry to increasing numbers of neuronal fibers and neuronal bodies far removed from the ventral hypothalamus and the pituitary.

Although this simplified picture of the projection neuron is still correct, a recent view of other types of neurons which seem to be in the majority appears to welcome this multiplicity of effectors and may well in the next few years permit satisfactory integration of these multiple effectors, with specific functional processes of individual neurons as members of a neuronal network. This new view of the neuron is based on new morphology elucidated by means of the electron microscope.

Much of the discussion that follows is based on a recent review by Schmitt, Dev, and Smith (2) and on the text of the proceedings of a Meeting of the Neurosciences Research Program devoted to local circuits (3). The dendrite is no longer seen as a "passive receptor surface" but rather as a locus for transmitting as well as receiving information in traffic with

dentrites of other neurons or with extracellular compartments, including capillaries. The means of such communications are seen in the release or uptake of diverse small or large molecules. The electric phenomena involved are those of pin-point depolarizations and are measured in a few microvolts. Such electrotonic currents spread only over distances measured in microns, not millimeters or centimeters. This type of extremely low voltage communication constitutes the so-called local circuits. The local circuit neuron can also modify the ultimate behavior of one or more projection neurons which can send responses to a remote contact by a high voltage, long-axon pathway. Such systems have been well studied in the retina, the olfactory bulb, and the lateral geniculate body. There is increasing evidence that such local circuitry is actually present in all parts of the central nervous system (CNS). It actually represents the structure of the greatest mass or volume of the CNS, with the projection neurons in their classical anatomical arrangements, probably a minority in number as well as in occupied space.

These multiple dendritic connections have been observed using the electron microscope. A diagram of possible connections between one axon terminal and two dendrites, one of which also is in contact with three other dendrites at about ten other locations is presented in Fig. 1. The diagram conveys the observation that dendrites may be both presynaptic and post-

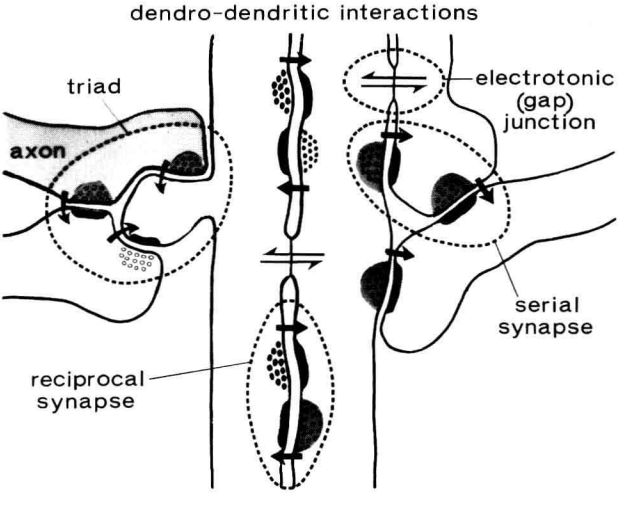

Figure 1. Diagram illustrating the proposal that dendrites in their reciprocal interactions can be pre- and post-synaptic to one another. One axon only is shown in the diagram; all other connections between dendrites. The hypothesis is that several neurochemicals may be involved at the several junction points (peptides, enzymes, catecholamines etc.) with either inhibitory or excitatory activity. Redrawn from Schmitt, Dev, and Smith (2).

synaptic to each other as in reciprocal synapses. There is also electron microscope evidence of gap junctions between dendrites. Such electronic coupling has been demonstrated in the CNS. Several neurons so coupled will respond synchronically with extremely low voltages required. Oscillatory behavior has been observed in such populations of neurons in some invertebrates (4) when electrically coupled. Such electronic junctions are frequently observed in immediate proximity to chemical synapses (2). None of these phenomena requires and none of these structures produces high voltage spikes. Information transfer by such mechanisms is relatively slow, in seconds or longer, not milliseconds.

What is the nature of the chemicals involved in these dendrodendritic contacts? The fragmentary, emerging picture is most interesting. It has long been considered that dendrites are involved in uptake of necessary metabolites such as sugars, free amino acids, adenosine, etc. Such uptake can proceed from extracellular fluid and also from capillary vessels through endothelial cells. Molecules of much larger size appear similarly to be taken up by dendrites. For instance, an enzyme such as acetylcholine esterase, after being released into extracellular compartments, has been reported (5) to be bound first to the outer surface of the dendritic membrane and later to be taken up by dendrites.

Thus, an interesting hypothesis would be for the release at dendritic points of (still to be characterized) enzymes of neuronal origin that would specifically cleave a biologically inactive precursor, such as β-lipotropin, present in extracellular compartments or perhaps in the axoplasm.

Classical transmitters appear to be released and taken up by the dendrites. Similar release and reciprocal uptake of small peptides such as substance P, neurotensin, somatostatin, TRF, and endorphins has not been demonstrated as yet. That this is possible and actually happens is a working hypothesis worth investigating. If confirmed, it would go a long way in explaining the multiplicity of effects of the polypeptides on the CNS. See for instance (Table 1) the multiplicity of effects of the tripeptide TRF on biological events which have nothing to do with the release of pituitary thyrotropin, the well known hypophysiotropic activity of TRF, for which it was originally named and recognized. The possibility of an enormous number of such transfer sites might also explain the psychotropic effects of some of these peptides. Cajal as early as 1899 made the comment that local circuit neurons may well play an important role as the substrate of complex behavior because of their "prodigious abundance and unaccustomed wealth of forms." In view of such enormous dendritic trees, with each dendrite ending compounded to the description as in Fig. 1, the number of contacts and control points for a single neuron defies imagination. Such cellular anatomy when considered with the hypotheses mentioned above for chemical inputs and outputs shows the considerable possible functional significance of an expanded dendritic connection network.

The multiple terminal network hypothesis, which includes involvement of biologically active peptides, may also be utilized in another way in ex-

Table 1
Central Nervous System Mediated Actions of
Thyrotropin Releasing Factor

1.	Increases spontaneous motor activity
2.	Alters sleep patterns
3.	Produces anorexia
4.	Inhibition of condition-avoidance behavior
5.	Head-to-tail rotation
6.	Opposes actions of barbiturates on sleeping time, hypothermia, lethality
7.	Opposes actions of ethanol, chloral hydrate, chlorpromazine and diazepam on sleeping time and hypothermia
8.	Enhances convulsion time and lethality of strychnine
9.	Increases motor activity in morphine-treated animals
10.	Potentiates DOPA-pargyline effects
11.	Amelioration of human behavioral disorders?
12.	Central inhibition of morphine-mediated secretion of growth hormone and prolactin
13.	Alteration of brain cell membrane electrical activity
14.	Increases norepinephrine turnover
15.	Releases norepinephrine and dopamine from synaptosomal preparations
16.	Enhances disappearance of norepinephrine from nerve terminals
17.	Potentiates excitatory actions of acetylcholine on cerebral cortical neurons

plaining some current data. It has been shown that the total amounts as well as the concentrations of TRF, LRF, and somatostatin in the extrahypothalamic CNS, measured by bioassays or radioimmunoassays, are considerably greater than can be accounted for by the number of cell bodies shown to contain such peptides by immunocytochemistry. A hypothesis to consider is that there may be relatively few neurons synthesizing say TRF, LRF, (primarily) located in the hypophysiotropic area of the hypothalamus with perhaps a few more cells in the amygdala and that these neurons have long axons with multiple axon collaterals, all with peptide containing and secreting bouton terminals.

There is already evidence that the dendritic traffic of chemicals works both ways with release and uptake. Thus, in a reciprocally functioning system, if the endorphins and enkephalins are enzymatically cleaved extracellularly from β-lipotropin as the circulating precursor in a manner reminiscent of the biogenesis of angiotensin, the endorphins and enkephalins could then be picked up by multiple dendritic endings and carried by retrograde axoplasmic flow whatever distance is necessary for their physiological function.

In summary, in this hypothesis we see the small peptides as substances released locally and perhaps produced locally at innumerable source points. The functions they would serve would be dependent upon the effector cell they would be modulating. Not necessarily secreted at classical synapses, they would be truly modulators of neuronal functions rather than true neurotransmitters. They could also be either transmitters or modulators, depending on their locus of release. If the effect of the small peptides is the activation of the adenylcyclase-cAMP system of their effector neurons as they may well do in other target tissues such as the adenohypophysis, their effects in neuronal networks would be amplified, long lasting as well as possibly expanding from their exact source-point. Such a system involving neuronal cAMP has already been demonstrated by Bloom and Siggins for neurons of the locus coeruleus (6).

This concept of a local release and local immediate effect of peptides from multiple sources in the CNS, is a point to remember for future discussion. It does not belong to substances called hormones in the classical definition of the word. This is true even if each local event leads ultimately to widespread effects. Needless to say, the technology involved in exploring such secretory functions of dendrites or of boutons of axon collaterals will be especially challenging.

During or shortly after the time when TRF activity, LRF activity, and somatostatin activity were being demonstrated by bioassay and by radioimmunoassay in the extrahypothalamic CNS, reports appeared showing that somatostatin inhibited the secretion of glucagon and insulin by direct action at the level of the endocrine pancreas. Because somatostatin has a short biological halflife upon injection in peripheral blood, it was unlikely that any physiological effect of endogenous somatostatin on the endocrine pancreas would be due to somatostatin of hypothalamic origin. During attempts to demonstrate the peptide in pancreatic nerve endings, somatostatin was found by immunofluorescence in discrete endocrine cells of the pancreas, now well characterized as the delta or D cells. The same studies showed somatostatin in discrete cells in the jejunum, colon, duodenum, and gastric mucosa. Observations were also made of somatostatin inhibiting the secretion of gastrin, secretin, gastric HCl, and recently acetylcholine from the myenteric plexus. TRF and LRF, though present in the extrahypothalamic CNS, have not been found to my knowledge in extra CNS tissues. This is something to look for.

As early as 1957, I observed corticotropin releasing factor (CRF) activity in extracts of gut tissues also containing substance P (7). Brodish has also described extrahypothalamic CRF. Besides somatostatin, other peptides are now known to be present and most likely synthesized by cellular elements in the central and peripheral nervous system as well as in glandular elements of the gastrointestinal tract. The first peptide so observed was substance P in the remarkable experiments of Ulf von Euler and Gaddum as early as 1936. There is now evidence that neurotensin, gastrin, vaso-intestinal peptide (VIP), gastro-intestinal peptide (GIP), the endorphins, and enkephalin(s) are found both in the brain and in the gastrointestinal tract plus the pancreas. This is also true for several of the small peptides such as bombesin, caerulein, and physalaemin, isolated years ago from extracts of the skin of frogs of several species.

Furthermore, there are remarkable analogies and homologies between the amino acid sequences of several of these peptides of CNS origin and gastrointestinal origin as well as those isolated from the frog skin. These peptides have been found by immunocytochemistry essentially in two types of cells: (a) They are seen in cell bodies and nerve fibers, i.e., neural and dendritic processes of neurons in brain, spinal cord, spinal ganglia, and the myenteric plexus; (b) they are seen also in typical endocrine cells, for instance, the islets of Langerhans, the enterochromaffin cells of the gastro-intestinal tract, and the adrenal medulla. Neuroblastomas contain high levels of VIP (8). An undifferentiated mediastinoma has been reported to contain somatostatin, calcitonin, ACTH, and prolactin.

All these results are based on radioimmunoassays, immunocytochemistry, and in some instances also bioassays, with, in most cases, evidence of parallelism of the responses to the known peptide-reference standard and the crude tissue extracts. In cases involving immunological methods, there is a modicum of specificity of the antibodies utilized. More significant, reports are beginning to appear showing identity of the primary structure of the gastrointestinal variety of a peptide when compared to its CNS variety as for instance neurotensin (9) and substance P. Our laboratory has already reported the complete sequencing of hypothalamic α-melanocyte-stimulating hormone (α-MSH) which is identical to that of pituitary α-MSH (10). Thus, there is every reason to believe that we are dealing with the same peptides regardless of their tissue origin.

What is the message to be read in these observations of startling commonalities between the central nervous system and endocrine tissues, and what does it imply for future research?

There is already an interesting unifying concept. Much credit must go to A. G. E. Pearse for his visionary concept formulated some ten years ago of the APUD cells. Pearse observed that neurons and some endocrine cells which produce polypeptide hormones shared a set of common cytochemical features and ultrastructural characteristics. APUD is an acronym referring to amine content and/or amine precursor uptake and decarboxylation as