Mammalian Neuroendocrinology

Paul V. Malven

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Paul V. Malven, Ph.D.
Department of Animal Sciences
Purdue University
West Lafayette, Indiana

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PREFACE

Neuroendocrinology is the scientific discipline devoted to the interaction between the two major integrative organ systems of the body. It began as a branch of endocrinology and continues today as an important element of that field. As a result of increased knowledge about the nervous system in the last few decades, neuroendocrinology has also become one of the essential components of neuroscience.

The present book was designed as text for a graduate course in neuroendocrinology such as has been taught by the author for the past 20 years. Because of the interests of the author and the population of graduate students taking his course, neuroendocrinology in this book was limited to mammalian species. A great deal of neuroendocrine information exists for invertebrate species as well as for non-mammalian vertebrates, and readers interested in the neuroendocrinology of these species will have to find it elsewhere. The author has sought to provide a comparative mammalian approach to many of the topics in this book. Whenever possible, this comparative approach involves (1) rodents reared for laboratory research, (2) domesticated ungulates reared for agricultural production, and (3) primates studied either clinically (humans) or in the laboratory (monkeys). At the conclusion of many chapters, a section is included that discusses applications of the neuroendocrine information to clinical medicine and/or to agricultural production.

Because this book is intended as a graduate text, the author has tried to explain the bases on which many well-established concepts are founded. Therefore, enough details are provided so that students might be better able to integrate future discoveries that might challenge these well-established concepts. The author begins each semester of his graduate course with the admonition to the students that much of what he will tell them may eventually turn out to be incorrect, but that he does not know what topics fall into that category. Certainly, that admonition holds for this book. To enable readers to evaluate the specific conclusions made in this book, many original references were cited, especially in Chapters 5 through 15. References of a more general type were used in Chapters 1 through 4 because the subjects covered seemed to the author to be more stable, and the concepts were less likely to change.

The author expresses his gratitude to Purdue University for granting him a sabbatical leave to write this book and to the University of South Florida for appointing him a Visiting Professor of Physiology and Biophysics. Sincere appreciation is also extended to the following current and former colleagues who made valuable suggestions on one or more chapters: J. Albright, H. Bryant, M. Diekman, and H. Head. Special appreciation is also extended to S. Grabowski and D. Rasmussen who carefully reviewed all the chapters. Of course, none of these people are responsible for the shortcomings of the book which rest solely with the author.

The scientist/philosopher, Sir Isaac Newton, wrote "*If I have seen further it is by standing on the shoulders of Giants.*" The present author believes strongly in the sentiment of that opinion. Researchers in neuroendocrinology, as in all other fields of scientific endeavor, must begin their quest for new knowledge at the point at which their predecessors brought the field. Although this author appreciates all of his neuroendocrine predecessors, he is additionally appreciative of two neuroendocrine "*giants*" who were also scientific mentors to him. Professor William Hansel helped kindle an early interest in comparative neuroendocrinology involving the domestic ungulates. Professor Charles H. Sawyer, through his personal counsel and by providing an exciting interdisciplinary neuroscience environment, contributed greatly to development of neuroendocrine skills and confidence. The author is most grateful for the graduate and postdoctoral training provided by these two outstanding scientists and for their continuing friendship. Therefore, this book is dedicated to these two scientific mentors and also to the author's wife, without whose love and support neither his research career nor this book would have been possible.

Paul V. Malven
Purdue University
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Chapter 1

PRINCIPLES OF NEUROENDOCRINOLOGY

The discipline of Neuroendocrinology examines the interactions between the nervous system and the endocrine system. During the development of Endocrine Physiology and Neurophysiology as scientific disciplines, the distinction between neural and endocrine systems was very clear, but in the last 30 years the clearcut differences have become less apparent. The scientific discipline of Neuroendocrinology has developed in the interface between strictly endocrine and strictly neural mechanisms. The origin of the field was the discovery over 30 years ago that certain neurons secreted chemical messengers into blood (Scharrer and Scharrer, 1963). This characteristic was previously reserved for hormones secreted into blood by endocrine glands. The term ***neurohormone*** was coined to describe a hormone produced by a neuron. Restrictions on the use of that term have become less stringent in recent years. In current usage, the proof that a chemical messenger produced by neurons acts as a true hormone (i.e., is secreted into blood) has not always been rigorously enforced. In the opinion of this author, to qualify as a hormone or neurohormone a chemical messenger ***must*** have an endocrine mode of action. With such a strict definition, the chemical messengers produced by neurons can be described as having one or more of the following types of action on other cells:

1. **Endocrine action:** Enter the blood stream to reach and alter activity of distant target cells.

2. **Paracrine action:** Diffuse locally through interstitial spaces to reach and influence neighboring cells.

3. **Neurocrine action:** Cross a synaptic junction to either activate or inhibit the postsynaptic cell.

Chemical messengers that act in an endocrine manner are generally known as hormones or neurohormones. Chemical messengers that act in a neurocrine manner are generally known as neurotransmitters. Descriptive terms for chemical messengers produced by neurons and that act in a paracrine manner include (1) neuromodulator and (2) localized hormone, but there is no generally accepted term. Moreover, it is not always known whether a particular chemical messenger acts in a paracrine or neurocrine manner, and some compounds can act in more than one manner.

The chemical structures of neuronal products known as chemical messengers can vary considerably. Peptides constitute a large class of chemical messengers produced by neurons. The size of these neuropeptides is almost always much

smaller than peptides produced by the endocrine system. Some of the greatest advances in Neuroendocrinology have involved these neuropeptides, including their discovery as secretory products of neurons and emerging knowledge about their physiological functions within the nervous system and elsewhere in the body. The other major type of chemical messenger produced by neurons consists of modified amino acids and includes many of the aminergic neurotransmitters that were discovered during the early days of Neurophysiology. Examples of modified amino acids produced by neural elements are catecholamines (norepinephrine, dopamine), indolamines (serotonin), acetylcholine, and others. Some neuronal amino acids do not seem to require modification to function as chemical messengers (e.g., gamma aminobutyric acid, glycine, glutamate, and aspartate).

It is not possible to make generalizations about the type of action that a chemical messenger may exert based on its chemical structure. For example, both the neuropeptide somatostatin and the catecholamine dopamine appear to exert all three types of actions depending on the target tissue. Somatostatin acts in an endocrine manner to inhibit secretion of somatotropin by the pituitary gland and in a paracrine manner to inhibit secretion of insulin and glucagon by cells of the pancreatic islets. Somatostatin also acts in the central nervous system (CNS) on adjacent neurons (either paracrine or neurocrine action). Dopamine acts in an endocrine way to inhibit secretion of prolactin by the pituitary gland. It also acts within the CNS in a neurocrine manner, and within the mediobasal hypothalamus, dopamine may act in paracrine manner on adjacent neural elements.

Neuroendocrine Transduction

One critical process in the discipline of Neuroendocrinology is called ***neuroendocrine transduction***. This process transforms neural information (i.e., action potentials) into chemical messengers secreted into blood (i.e., hormones) where they exert endocrine effects. The small number of identified neuroendocrine transducers have been studied in detail. The diagrams in Figure 1-1 illustrate the two general categories and four specific types of neuroendocrine transducers. Neuron A in Figure 1-1 typifies the simplest form of ***secretomotor innervation*** in which a single CNS neuron innervates a secretory cell. One example is found in the adrenal medulla where neurally derived chromaffin cells are innervated by axons of the sympathetic nervous system. In response to synaptic release of the neurotransmitter acetylcholine, the chromaffin cells discharge epinephrine and norepinephrine into blood. Another less well-known example represented by neuron A in Figure 1-1 involves hypothalamic neurons sending axons to innervate non-neural cells of the pars intermedia of the hypophysis. The chemical messenger released by these axons (probably dopamine) inhibits the release of pars intermedia products. Neuron B of Figure 1-1 represents a modified secretomotor innervation involving a two-neuron chain in which the axon of the second neuron innervates the secretory cell. Innervation of the pineal gland by

the sympathetic neurons typifies this situation. Postganglionic neurons originating in the superior cervical ganglion release norepinephrine at their secretomotor terminals adjacent to the pinealocyte and this activates the release of melatonin into blood and cerebrospinal fluid (CSF).

Neurosecretory neurons depicted as C and D in Figure 1-1 release their neuronal products into blood. The two types differ only in the type of blood vessel into which they secrete. Hypothalamic neurons, which send axons into the pars nervosa of the hypophysis, release chemical messengers (e.g., vasopressin, oxytocin and others) into the general circulation (neuron C). Other neurons in the hypothalamus and adjacent regions have axons that extend to the median eminence where they discharge their chemical messengers into the capillaries of the hypophysial portal veins. These chemical messengers travel in the portal blood a few millimeters to the capillaries of the hypophysis where most of them probably act in an endocrine manner to stimulate or inhibit the secretion of adenohypophysial hormones into the general circulation. Of course, the hypophysial portal blood then enters the general circulation and any neurohormones that remain also enter that circulation. There is no clear evidence that neurohormones secreted into hypophysial portal blood reach the general circulation in

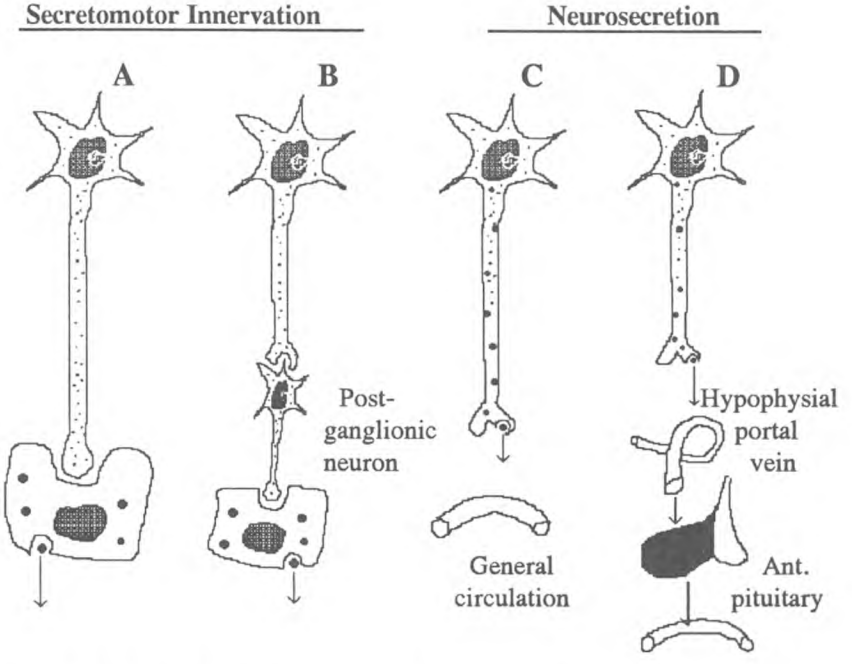

Figure 1-1. Types of neuroendocrine transducers.

Neuroendocrine transduction is depicted in diagrams which illustrate the secretory principles for two types of secretomotor innervation (A and B) and two types of neurosecretion (C and D).

physiologically relevant concentrations, but the possibility remains. In summary, neuroendocrine transduction can involve either secretomotor innervation or neurosecretory neurons, but in both types the transduction from neural to hormonal signal involves substantial amplification of that signal as well as more sustained generalized actions than are possible within the nervous system.

Neuroendocrine Integration

In addition to the transduction of neural signals into endocrine signals as just described, the neuroendocrine system mediates the cooperation between the nervous and endocrine systems to regulate in an optimum manner the physiological functions of the organism. This function can be described as ***neuroendocrine integration*** and is illustrated diagrammatically in Figure 1-2. In addition to a neurosecretory neuron that transduces the information, other ordinary (i.e.,

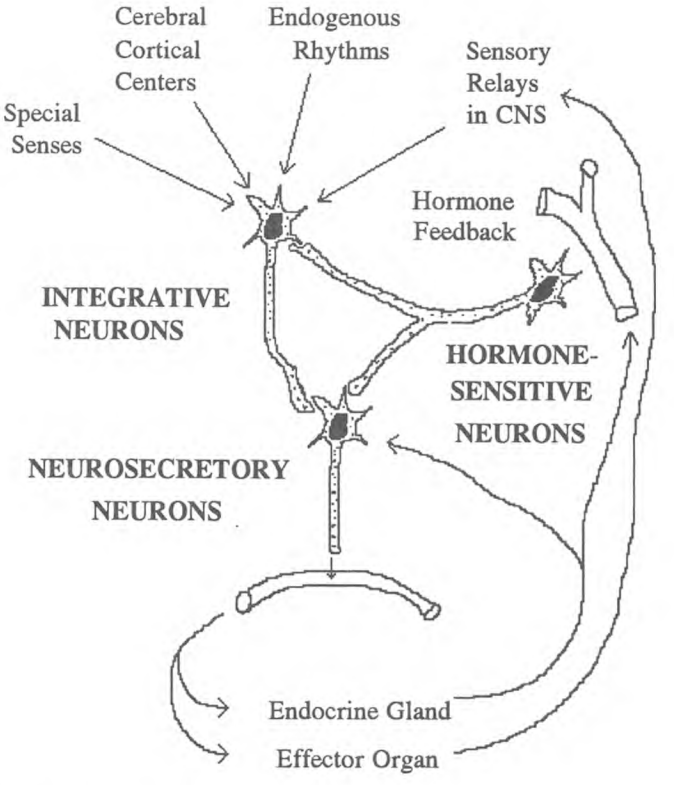

Figure 1-2. Neuroendocrine integration.

Schematic representation of the various elements involved in neuroendocrine integration.

non-neurosecretory) neurons play important roles in the integration of information. Two such neurons represented in Figure 1-2 are (1) integrative neuron and (2) hormone-sensitive neuron that each have direct input to neurosecretory neurons. Figure 1-2 also illustrates that integrative neurons of the neuroendocrine system receive a variety of inputs. These may include (1) information about the ambient environment obtained through the special senses, (2) integration of current inputs with the learned or conditioned information stored in higher cortical centers, (3) endogenous free-running rhythms (e.g., circadian or ultradian), (4) neurally mediated sensory information from internal organs (e.g., reproductive tract) and sensors (e.g., blood osmolarity, pH and pressure), and (5) neural signals from specific hormone-sensitive neurons (e.g., feedback from endocrine glands).

Hormonal Products of Neurosecretory Neurons

The number of chemical messengers of neurosecretory neurons for which endocrine actions are proved is relatively low (Table 1-1). Small peptides

Table 1-1. Specific neurohormones.

Neuro-hormone	*Species Distribution*	*Site of Synthesis*	*Site of Release Into Blood*
Arginine vasopressin	Most mammals	Hypothalamus	Neurohypophysis
Lysine vasopressin	Pig and hippopotamus	Hypothalamus	Neurohypophysis
Oxytocin	All vertebrates	Hypothalamus	Neurohypophysis
Arginine vasotocin	Most vertebrates except mammals	Hypothalamus	Neurohypophysis
Hypophysiotrophic hormones (i.e., releasing and inhibiting hormones)	Most vertebrates	Hypothalamus and adjacent areas of the diencephalon	Median eminence

secreted by the neurohypophysis following their synthesis in the hypothalamus represent a significant proportion of known neurohormones. The chemical structures of oxytocin and vasopressin were identified many years ago and in recent years details of their biosynthesis and gene structures have been forthcoming. Oxytocin, with some structural modifications, is found in submammalian vertebrates, but vasopressin is not. In these submammalian species, arginine vasotocin appears to functionally replace vasopressin in the control of water balance. More details about these neurohypophysial peptides will be presented in Chapter 3.

Hypophysiotrophic hormones are listed in Table 1-1 as a general class of neurohormones common to most vertebrate species. They are also known as ***releasing hormones (factors)***, but they also include neurohormones which are inhibitory to secretion/release of adenohypophysial hormones. The site of secretion into blood for neurohormones that influence the adenohyphysis is the median eminence where they enter the portal blood vessels (see D in Figure 1-1). Most of the hypophysiotrophic hormones are synthesized within the hypothalamus, but some, such as luteinizing hormone-releasing hormone (LHRH), may be synthesized in regions just rostral to the hypothalamus and still be secreted into portal blood. Hypophysiotrophic hormones will be covered in greater detail in Chapter 4, and readers interested in learning more about the history of their discovery are directed to articles in McCann (1988).

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Chapter 2

NEUROENDOCRINE MORPHOLOGY

The unique morphological aspects of the ***hypophysis*** (also known as the ***pituitary gland***) provided some of the earliest clues to the discipline that would become known as Neuroendocrinology. Moreover, the following descriptions of hypophysial morphology in different species (Purves, 1961) will provide the basis for later understanding of neuroendocrine mechanisms. The hypophysis is divided into neurohypophysis and adenohypophysis based on embryological development from neural and epithelial substrates, respectively. Adenohypophysial tissue (pars tuberalis, pars intermedia, and pars anterior) develops specifically from an outgrowth of ectodermal epithelium of the primitive oral cavity called Rathke's pouch. The neural tissue denoted in Figure 2-1 represents the entire neurohypophysis consisting of pars eminens and pars nervosa. Although these neural tissues could be categorized as either hypothalamus or hypophysis, most modern authors classify pars nervosa as part of the hypophysis and pars eminens as part of the hypothalamic infundibulum, where it is also known as the ***median eminence***. The demarcation between pars eminens and pars nervosa cannot be precisely defined, but it occurs somewhere in the hypophysial stalk.

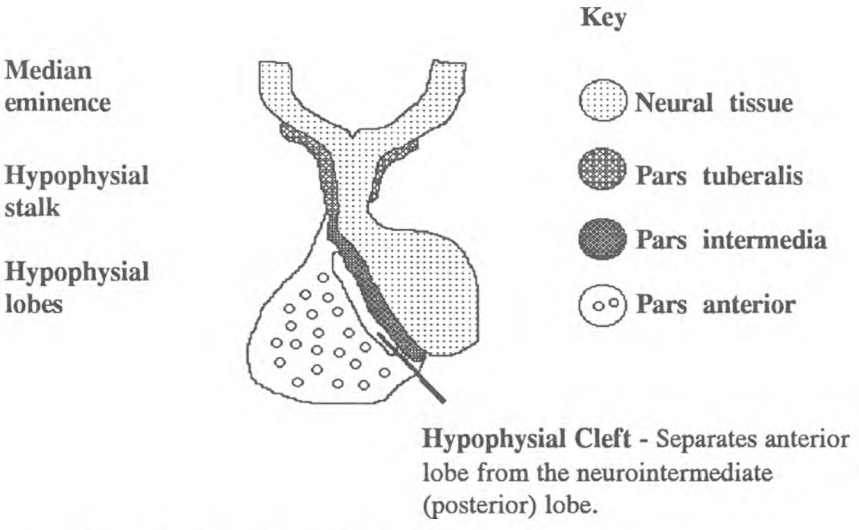

Figure 2-1. Divisions of the hypophysis.

This generic diagram illustrates various tissue types (key on right) and regional names (listed on left) for the hypophysis.

Subdivisions of adenohypophysial tissue are the pars tuberalis, the pars intermedia and the pars anterior. A thin rim of pars tuberalis tissue adheres to and encircles the neurohypophysial tissue of the pars eminens and the neurohypophysial stalk. Pars intermedia tissue is located adjacent to the pars nervosa in the lobular part of the hypophysis and is separated from the pars anterior tissue by the hypophysial cleft which derives from the lumen of Rathke's pouch. The commonly used terms, ***anterior pituitary*** and ***posterior pituitary***, refer to tissues anterior (i.e., rostral or ventral) or posterior (i.e., caudal or dorsal) to the hypophysial cleft. The term anterior pituitary always includes pars anterior and may also include the pars tuberalis. The term posterior pituitary includes the pars nervosa and the pars intermedia (when it exists as separate entity). A more appropriate term for the posterior pituitary is neurointermediate lobe (NIL) because this name reflects the combined presence of pars nervosa and pars intermedia tissue.

Hypophysial morphology differs among species and Figure 2-2 illustrates some examples of this diversity. The hypophysis of the rat represents the conventional morphological divisions as shown generically in Figure 2-1, but the axis of the rat hypophysis is rotated so that the neurointermediate lobe lies dorsal to the pars anterior. The hypophysial cleft in the rat is much narrower *in vivo* than in the diagram, but upon postmortem dissection the tissue will readily separate along the hypophysial cleft. The hypophysis of the cow (B in Figure 2-2) is somewhat unique because it usually contains pars anterior tissue known as the Cone of Wulzen located on the pars intermedia side of the hypophysial cleft in the caudal part of the hypophysis. Therefore, bovine tissue of the neurointermediate lobe will contain variable amounts of pars anterior tissue which would complicate the interpretation of some types of *in vitro* studies. Only some sheep hypophyses have a Cone of Wulzen, whereas another ungulate species, the pig, does not possess this unique feature. The diagram of the cow hypophysis in Figure 2-2 also illustrates that the bovine median eminence is a thin-walled tubular structure with a lumen continuous with the 3rd ventricle of the brain.

Figure 2-2 also illustrates the human hypophysis as an example of species that lack a well-defined hypophysial cleft. Although the human hypophysis may sometimes contain colloid-filled remnants of an embryonic cleft, a variety of mammals (blue whale, porpoise, elephant, beaver) completely lack a hypophysial cleft, and in some there may also be physical separation of the neurohypophysial and adenohypophysial lobes. In species that lack a hypophysial cleft, pars intermedia cells mix together with pars anterior cells in what is then appropriately called the ***pars distalis***. Sometimes the term pars distalis is misused to describe the pars anterior tissue in those species such as the rat which do have a hypophysial cleft. Colloid-filled remnants of the hypophysial cleft are quite variable in the human hypophysis, and in their absence, the cells of the pars distalis and pars nervosa are closely adhered and the border is often less smooth than shown in Figure 2-2.

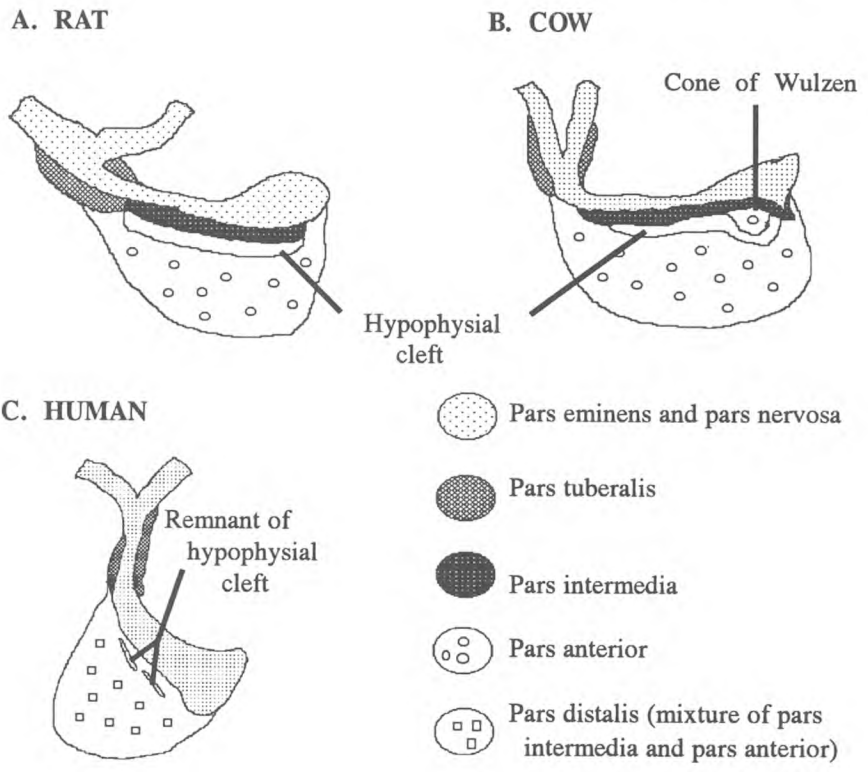

Figure 2-2. Examples of hypophysial morphology.

These diagrams illustrate the location of various hypophysial tissues in three different species (key in lower right). Pars anterior and pars intermedia tissues occur only in rat and cow whereas the pars distalis of humans combines these two tissues.

Vasculature of the Hypophysis

The unique blood supply of the hypophysis provided early indications for hypothalamic control of the adenohypophysis via blood-borne compounds. Blood vessels now known as ***long*** portal veins were described as connecting the pars eminens/pars tuberalis tissue of the median eminence with the pars anterior tissue of the hypophysial lobes in a large number of vertebrate species (Green, 1951). In the absence of a direct arterial supply to pars anterior tissue, the concept arose that all the blood supply of the adenohypophysis passed through two capillary networks, one conventional network in the median eminence and a second sinusoidal network in the adenohypophysis. The primary portal capillaries

in the median eminence possess a fenestrated endothelium similar to peripheral capillaries but different from most brain capillaries. The fenestrated endothelium of the capillaries in the median eminence is responsible for this tissue being outside the blood-brain barrier.

Another type of hypophysial portal blood vessel was discovered during the 1950s, and these vessels connected pars nervosa with pars anterior. Because the distance between the two capillary networks of these portal veins was so much less than the one discovered earlier, these blood vessels were called ***short*** portal veins. Their discovery was probably delayed because they are not nearly as visible as the long portal veins. The functional significance of short portal veins was emphasized by the results of surgical transection of the hypophysial stalk in various species. In all species, tissue of the pars anterior or pars distalis that is adjacent to the pars nervosa survives much better than other adenohypophysial tissue, and it is precisely this tissue that would be supplied by short portal veins after transection of the long portal veins which traverse the hypophysial stalk.

Venous outflow from the adenohypophysis and neurohypophysis occurs through conventional veins similar to those draining the brain and other cranial structures, with one possible exception. Several authors have suggested that some portion of hypophysial outflow may ascend the hypophysial stalk and reach the hypothalamus. There is both morphological and hormonal evidence to support delivery of hypophysial hormones to the brain without dilution in the systemic circulation. This hypothesis is sometimes called ***retrograde flow*** of hypophysial hormones to the hypothalamus where they might participate in feedback regulation of factors that influence hypophysial secretion. This postulated feedback is referred to as ***short*** feedback in contrast to ordinary feedback by hormones produced in the periphery and delivered to the hypothalamus in systemic blood.

The morphological evidence consistent with retrograde flow is as follows: (1) confluent capillary network of the pars nervosa and pars eminens which could shift venous outflow, as well as reversed flow in the short portal veins, toward the hypothalamus if vascular pressures were appropriate, and (2) subependymal veins located on the ventricular wall of the median eminence into which injected dye was observed to ascend subsequent to its downward flow in the long portal veins of a anesthetized preparation.

The hormonal evidence in support of retrograde flow was provided by concurrent quantification of hypophysial hormone concentrations in cannulated long portal veins and the systemic vasculature (Oliver et al., 1977). Portal concentrations of several hypophysial hormones were up to 100-fold greater than in the systemic circulation where they are diluted by blood from other sources. If all hypophysial secretions drain directly into the systemic circulation, it is very difficult to explain the concentration gradient between portal and systemic blood without retrograde flow to deliver enriched levels of hypophysial secretions to the hypothalamus for subsequent entry into the long portal veins. Some authors have suggested that the technique of collecting blood from long portal veins may create an unphysiological pressure which would abnormally draw hypophysial

effluent back up the cannulated vein. Others have suggested that certain hypothalamic neurons may secrete small quantities of hypophysial hormones, and this could explain the concentration differential between portal and systemic blood. In summary, the hypothesis of retrograde flow to deliver enriched levels of hypophysial hormones to the hypothalamus should be considered unproven but still tenable.

Species Differences in Cranial Vasculature

The arterial supply to the brain and hypophysis differs among species. Some differences involve only the nomenclature of arteries that are homologous between species (e.g., anterior hypophysial in rat versus superior hypophysial in human). The arterial supply to the pars nervosa and par eminens region of the hypophysial stalk in various species is known by different names. It is known as peduncular artery in rat, artery of lower infundibular stalk in sheep, and artery of the trabecula (also middle hypophysial) in human. Despite species differences in nomenclature, arteries that supply blood to the hypophysis in most species originate from the arterial arrangement known as the Circle of Willis that also supplies blood to the brain. The manner in which blood is supplied to the Circle of Willis represents the greatest difference in cranial vasculature among species.

Selected species of ungulates (cow, pig, sheep, camel) and carnivores (cat, dog) have been shown to possess a unique arterial vasculature called a ***carotid rete*** (sometimes also called rete mirabile) that is located at the base of the brain and provides blood for the Circle of Willis (Daniel et al., 1953). The carotid rete consists of a dense network of many small arterioles, but no capillaries, and the network is located within the venous sinus known as the cavernous sinus. The arterioles of the carotid rete subsequently reunite into larger arteries which supply the Circle of Willis. Because the carotid rete lacks capillaries for exchange with tissue fluid or venous blood, early theories regarding its function emphasized blood pressure modulation. However, it is now clear that one very important function of the carotid rete is temperature exchange between the venous blood in the cavernous sinus and arterial blood in the carotid rete (Hayward and Baker, 1969). Because much of the venous blood in the cavernous sinus drains nasal structures and other peripherally located tissues, the temperature of that venous blood is less than core body temperature. The arterial blood entering the carotid rete reflects core body temperature, and during transit through the carotid rete, it is cooled by heat exchange with the cooler venous blood of the cavernous sinus. Through this heat exchange, animals that possess a carotid rete have the ability to maintain the temperature of their brain lower than core body temperature. This ability is used primarily during situations of ambient heat stress or exercise when maintenance of brain temperature lower than core body temperature represents a distinct biologic advantage. During situations of exercise or heat stress, evaporative cooling in nasal structures becomes

very important, and the venous drainage from nasal structures into the cavernous sinus may become cooled even more. Laburn et al. (1988) demonstrated in hyperthermic sheep that experimental deflection of airflow away from the upper respiratory tract increased brain temperature abruptly to that of core body temperature.

Cellular Components of the Hypophysis

Neurohypophysis. The cytology of the neurohypophysial tissue consists only of axons, axon terminals, and neuroglial cells because there are no neuronal cell bodies (perikarya). The axons arise from very large (i.e., magnocellular) perikarya located in the adjacent hypothalamus. It has been estmated that about 10,000 perikarya in the rat hypothalamus project their axons into the pars nervosa, and the estimate for the human is much larger. Each axon gives rise to multiple branches, each with a terminal for release of neuronal products into blood (see Figure 1-1.C). There are also numerous capillaries with especially large perivascular spaces, which is consistent with the high levels of blood flow and the secretion of neuronal products into blood.

The neuroglial cells of the neurohypophysis (also known as ***pituicytes***) share many characteristics with neuroglia known as astrocytes elsewhere in the brain, but recent observations suggest that they may serve unique functions in the neurohypophysis. For example, the physical relationship among axon terminals, capillaries, and pituicytes changes during extreme states of altered neurohypophysial secretion (Hatton et al., 1988). Second, receptors for opioid peptides exist in pituicytes rather than in neurohypophysial axons (Bicknell et al., 1989). Third, mRNA transcripts that encode the synthesis of neurohypophysial secretory products continue to exist in the isolated pars nervosa (Murphy et al., 1989), and the pituicyte is the only cell type thought to be present in the isolated pars nervosa that would be capable of gene transcription. In summary, the pituicyte of the mammalian neurohypophysis functions as a neuroglial cell in support of axons, but it may also have unique functions that are not fully understood.

Adenohypophysis. Great progress has been made in understanding the cytology of the adenohypophysis due in large part to the following three techniques: ***immunocytochemistry*** (Polak and Van Noorden, 1986); ***in situ hybridization*** (Morrell, 1989); and ***reverse hemolytic plaque assay*** (Frawley et al., 1985). Space limitations do not permit detailed discussion of these important techniques, but the interested reader is directed to the cited references. However, the type of knowledge derived from each technique applied to adenohypophysial cytology will be summarized. Immunocytochemical analysis, using both light and electron microscopy, permits the definitive identification of intracellular (or intragranular) peptides against which the antibodies used were directed. *In situ* hybridization allows the identification of cells containing mRNA transcripts of

specific peptide-producing genes, and specificity depends upon the nucleic acid probe used for hybridization. The intracellular presence of the mRNA capable of encoding a peptide represents strong evidence that the hybridized cell actually synthesizes the peptide. For secretory cells of the adenohypophysis, intracellular content of the peptide as determined by immunocytochemistry and of the mRNA as determined by *in situ* hybridization almost always provide confirmatory results. In other tissues where cells may take up the secretory product of other cells as well as synthesize their own products, application of both techniques provides insight into which intracellular peptides are synthesized and which are taken up.

The reverse hemolytic plaque assay is performed under slightly less physiological conditions than the techniques described above because the secretory cells of the adenohypophysis must be enzymatically dispersed prior to *in vitro* culture. If one assumes that this dispersion and culture do not alter the cellular function, this technique can determine which cell types actually release specific peptides. When combined with immunocytochemistry of the dispersed and cultured cells, it is possible to determine what proportion of peptide-containing cells actually release that peptide (Kineman et al., 1990). When the hemolytic plaque assay is combined with *in situ* hybridization, it is possible to quantify gene transcription in cells that release a specific peptide (Scarbrough et al., 1991).

Pars Intermedia. This tissue is present as a distinct structure in only some species, but its secretory cells are present within the pars distalis of other species. When present as a separate tissue, the pars intermedia is innervated by secretomotor axons from hypothalamic perikarya (see Figure 1-1.A) and perhaps from the pars nervosa. Pars intermedia tissue is very poorly vascularized, and the sources of its blood-borne nutrients are not fully understood. The secretory cells of the pars intermedia appear to all synthesize the precursor protein known as ***pro-opiomelanocortin*** which can be proteolytically cleaved to yield many hormonal peptides (to be discussed in Chapters 5 and 6).

Pars Tuberalis. The vasculature of the pars tuberalis is not well understood, but there may be diffusion of secretory products and nutrients to and from the adjacent neural tissue of the pars eminens of the median eminence and hypophysial stalk. Although there are fewer cell types, pars tuberalis cytology is similar in some ways to that of the pars anterior. Relatively few stainable secretory vesicles are seen with light microscopy, but ultrastructural analysis does reveal the presence of secretory vesicles. Pars tuberalis cells that contain stainable luteinizing hormone (LH) and thyrotropin (TSH) have been observed in several species. There is even some evidence for secretion of these hormones from the pars tuberalis into blood. Many cells of the pars tuberalis do not stain for any known adenohypophysial hormones. Recent evidence that almost all cells of the pars tuberalis cells contain receptors for the pineal gland secretory product, melatonin, has raised the possibility of some unique function for the pars tuberalis in relation to pineal-mediated processes (de Reviers et al., 1989). It has been suggested that melatonin regulation of LH secretion by the pars tuberalis modulates release of LHRH in the adjacent pars eminens by diffusion

(short feedback) of LH produced by pars tuberalis (Nadazawa et al., 1991). In this regard, it should be noted that almost all cells of the ovine pars tuberalis contain mRNA encoding the synthesis of LH, whereas only a fraction of the cells contain stainable quantities of LH (Pelletier et al., 1992).

It should also be noted that ***hypophysectomy*** (surgical removal of the hypophysis), as it is commonly performed in rats and other species, does not remove pars tuberalis tissue from the animal. Therefore, small quantities of adenohypophysial hormones that are sometimes detected in the blood of hypophysectomized animals could potentially have come from pars tuberalis tissue. Moreover, hypophysectomy may stimulate hormone synthesis in cells of the pars tuberalis (Ordronneau and Petrusz, 1980; Gross, 1983).

Pars Anterior. Secretory cells of the pars anterior are very diverse because there are at least six different peptide hormones secreted into blood from this tissue. At one time, it was thought that there was one adenohypophysial cell for each secreted hormone. It is now known that a single cell may secrete more than one hormone. The corticotroph cell synthesizes pro-opiomelanocortin which can be cleaved to yield corticotropin (ACTH), β-lipotropin, β-endorphin, and several forms of melanotropin (MSH). Corticotrophs comprise about 4% of the cells in the rat pars anterior, and their secretory vesicles show diverse sizes and shapes.

Thyrotroph cells that secrete TSH comprise about 3% of pars anterior cells in rats, and their secretory vesicles are very small (i.e., diameters of about 50 nm). Thyrotrophs appear to be strictly monohormonal (i.e., secrete a single hormone). In contrast, gonadotrophs may be either monohormonal or bihormonal, secreting LH and/or follicle-stimulating hormone (FSH), and there are species differences as regards the relative proportions of these monohormonal and bihormonal gonadotrophs. Moreover, the proportions of LH only, FSH only, and LH + FSH gonadotrophs may also depend on physiological status. Secretory vesicles present in gonadotrophs have diameters of intermediate size, and it is unclear whether LH and FSH always exist in separate secretory vesicles within individual gonado-troph cells.

Although the coexistence of LH and FSH in one gonadotroph was initially surprising, each hormone contains one subunit that also occurs in the other hormone. The discovery that the evolutionarily related prolactin (PRL) and somatotropin (GH) could sometimes occur in a single cell (Frawley et al., 1985) was more surprising because these hormones are different molecules arising from different genes. Cells that contain only PRL are known as mammotrophs because PRL has strong actions on mammary function. The secretory vesicles of mammotrophs found in the rat pars anterior are unique because of their irregularly shaped (i.e., pleomorphic) secretory vesicles located peripherally within the cytoplasm. However, pleomorphic secretory vesicles are not a general feature of mammotrophs because the secretory vesicles in sheep mammotrophs are spherical rather than pleomorphic. Cells that contain only GH are known as somatotrophs and usually have spherical secretory vesicles whose size varies with species. Cells that contain both PRL and GH are known as mammo-

somatotrophs, and they have been observed in several species. Rat mammosomatotrophs are quite small cells containing inconspicuous rough endoplasmic reticulum (RER) and Golgi apparatus. Individual secretory vesicles of rat mammosomatotrophs appear to contain both PRL and GH. In contrast to the rat, bovine mammosomatotrophs are not as small and are often binucleated cells (Kineman et al., 1990).

Cytological Features of Adenohypophysial Secretion. Hormones are synthesized, packaged into secretory vesicles, and secreted from adenohypophysial cells in ways that appear to be similar to those of other hormone-secreting cells. Gene transcription and RNA processing occurs in the nucleus followed by transport of the mRNA to the RER where synthesis of the prohormone occurs. The prohormone is cleaved into the products for secretion, and occasionally subunits produced from different gene transcripts are covalently linked. The secretory products are packaged into dense-core secretory vesicles in the Golgi apparatus from which they migrate to a position near the plasma membrane to await release. Release into the extracellular space occurs by exocytosis wherein (1) the membrane of the secretory vesicle fuses with the inner surface of the plasma membrane, (2) the double layer of fused membrane breaks down, and (3) the contents of the secretory vesicle enter the extracellular space. The speed of the entire process of synthesis, packaging, and exocytosis probably depends upon the intensity of stimulation of the adenohypophysial cell. Studies with strongly stimulated mammotrophs from rats suggest that the entire process can occur in as little as 50 min, but this may represent a lower limit.

Brain Morphology Related To Neuroendocrinology

Selected aspects of neuroanatomy necessary to understand neuroendocrinology will be presented here. Most of the important brain regions are part of what is known as the ***limbic system,*** proposed by Papez as being structures that were intimately involved in the control of emotion. It is now clear that limbic system structures of the brain control many processes in addition to emotional responses, but that there are close linkages between emotion and these other physiological processes.

Hippocampus. This large horn-shaped bilateral structure curves laterally and ventrally into the tissue beneath each temporal lobe of the cerebral cortex. The primary connecting fibers between the hippocampus and other limbic structures are contained within the fornix. Established functions of hippocampus include the encoding of memory information as well as mediating some aspects of emotion. In regard to neuroendocrine regulation, electrical stimulation of the hippocampus can modify pituitary secretion of LH, and the morphology of the synaptic input from hippocampus to preoptic area differs between sexes in the rat. Neurons in the hippocampus also contain a very high density of receptors for adrenal steroids which implies that it is a target tissue for blood-borne adrenocortical hormones.

Amygdala. These circular structures are located at the lateral tip of each hippocampal horn beneath the temporal lobe. Each amygdala contains several subdivisions, the morphology of which are beyond the scope of this brief overview. The amygdala receives a sampling of the inputs from all the sensory modalities and is intimately involved with emotional states. Lesions of amygdala tissue in experimental animals can cause behavioral depression followed later by nonselective hyperactive behaviors (sexual and feeding). Electrical stimulation of the amygdala can also induce anxiety and fear, suggesting that this region is very important in the regulation of emotion. Experimental manipulation of the amygdala has also been shown to modulate the secretion of pituitary LH similar to the results described above for hippocampus. The amygdala also contains receptors for adrenal steroids (like hippocampus) and estradiol (unlike hippocampus). These two steroid receptors may mediate the effects of these blood-borne hormones on behavior and/or on feedback effects to influence the adenohypophysial secretion of ACTH and LH.

Other components of the limbic system include the septum, olfactory gray, and parts of the cerebral cortex (i.e., cingulate gyrus and hippocampal gyrus). The epithalamus and its associated pineal gland should probably be considered functionally part of the limbic system although they were not included in the original designation. Finally, the hypothalamus is the part of the limbic system most intimately involved with neuroendocrinology. Not only does it contain perikarya of neurons which participate in neuroendocrine regulation and integration, but it contains many tracts which connect with other parts of the limbic system. The preoptic area is included herein as a functional part of the hypothalamus although morphological classifications often list the preoptic area as a separate entity. The major fiber pathways connecting the hypothalamus with limbic and non-limbic structures are depicted in Figure 2-3 in a schematic and highly simplified diagram.

The following three tracts connect the hypothalamus with the midbrain and lower areas: mammillopeduncular tract (labeled H in Figure 2-3), mammillotegmental tract (G), dorsal longitudinal fasciculus (F). The mammillopeduncular tract and mammillotegmental tract both connect the mammillary bodies of the hypothalamus with the midbrain and lower regions. The hippocampus inputs into the hypothalamus pass by way of the fornix (E) to the preoptic area, arcuate nucleus and mammillary bodies of the hypothalamus. The thalamus connection with the mammillary bodies involves the mammillothalamic tract (I). The amygdala connections with the hypothalamus consist of (1) stria terminalis (C) which curves around in parallel with the fornix and (2) the shorter route from amygdala to hypothalamus called the direct amygdalohypothalamic tract (D). A major fiber tract passing through the hypothalamus is the medial forebrain bundle (B) which connects hypothalamus with the septum, rostral structures such as the olfactory gray, and structures caudal to the hypothalamus. The epithalamus sends input to the preoptic area of the hypothalamus via the stria medullaris (A).

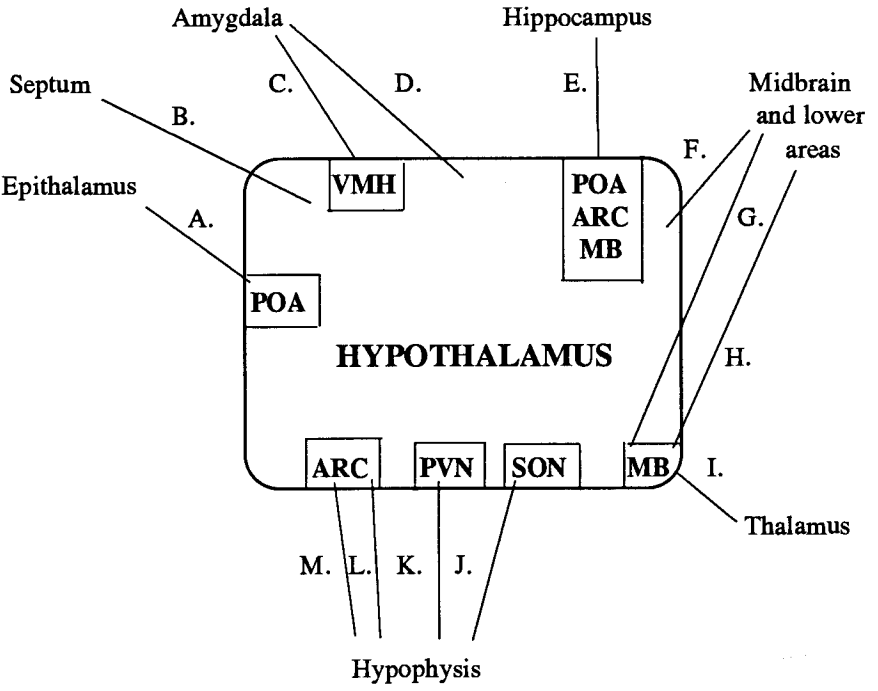

Figure 2-3. Fiber tract connections with the hypothalamus.

Schematic representation of neuroanatomic pathways connecting various hypothalamic areas with extrahypothalamic structures.

Key for hypothalamic areas: ARC = arcuate nucleus; MB = mammillary bodies; PVN = paraventricular nucleus; POA = preoptic area; SON = supraoptic nucleus; VMH = ventromedial hypothalamic nucleus.

Key for pathways: A = stria medullaris; B = medial forebrain bundle; C = stria terminalis; D = direct amygdalohypothalamic tract; E = fornix; F = dorsal longitudinal fasciculus; G = mammillotegmental tract; H = mammillopeduncular tract; I = mammillothalamic tract; J = supraoptic hypophysial tract; K = paraventricular hypophysial tract; L = tuberohypophysial tract; M = tuberoinfundibular tract.

Fiber tracts that connect the hypothalamus with the hypophysis are very important in neuroendocrinology. They include the paraventricular hypophysial tract (labeled K in Figure 2-3) from the paraventricular nucleus to the pars nervosa and the supraoptic hypophysial tract (J) from the supraoptic nucleus to the pars nervosa. These two tracts contain axons of the magnocellular neuronal perikarya, and they transport the neurohypophysial peptides to the pars nervosa for release into blood (like C in Figure 1-1). Another important tract is the tuberoinfundibular tract (M) containing axons that discharge their neuronal products into the primary capillaries of the hypophysial portal veins located in the median eminence of the pars eminens. A final tract from hypothalamus to hypophysis is called the tuberohypophysial tract (L), and its axons extend down the pituitary stalk into the pars nervosa along with axons of the supraoptic hypophysial and paraventricular hypophysial tracts. However, these axons of the tuberohypophysial tract do not originate from magnocellular perikarya.

Because of its intrinsic functions and its many connecting pathways illustrated in Figure 2-3, the hypothalamus is an important component of the limbic system. The hypothalamus also mediates the linkage between neuronal functions of the limbic system and the hormonal functions of the hypophysis.

Neuroendocrine Implications of the Blood-Brain Barrier. Most neuronal tissues of the brain are separated from the vascular compartment of the body by what is called the ***blood-brain barrier*** (Bradbury, 1979). This barrier restricts the movement of blood-borne compounds into the brain as well as the secretion of neuron-derived compounds into blood. The endothelium of brain capillaries may provide the morphological basis for this conceptual barrier. The ventricles of the brain appear to be on the brain side of the blood-brain barrier. Circumventricular organs (Chapter 4) lack the usual blood-brain barrier, and the endothelium of their capillaries is fenestrated. Therefore, circumventricular organs may be the functional link by which some blood-borne molecules affect the brain and by which neurohormones are released into blood (Johansson, 1990). This second function is especially true for the median eminence and pars nervosa where many neurons release their neurohormones into the blood. Perikarya of those neurosecretory neurons in which the axons have such access to the circulation may be localized by their uptake of a blood-borne dye which can later be visualized histochemically together with the neurohormone of interest (Witkin, 1990).

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