Neurotransmitters and Anterior Pituitary Function

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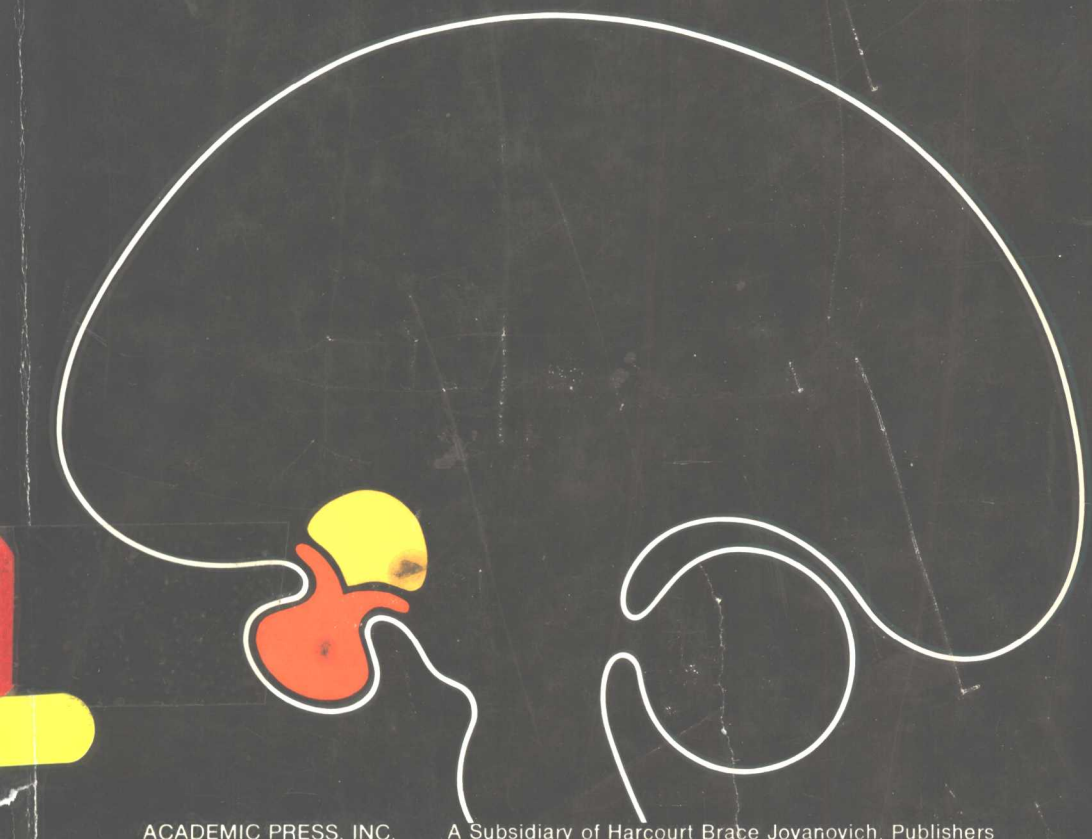

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Preface

Since the report based on a Neuroscience Research Program Work Session, held May 1968 in Cambridge, Massachusetts appeared (see Wurtman, 1971), no complete survey on the biochemistry and physiology of brain neurotransmitters in relation to anterior pituitary function has been published, and in the last few years there has been a tremendous surge of activity in this field. This book presents the first thorough survey of the most salient constituents of the neural–endocrine communication system in mammals, and represents the first detailed attempt to integrate research findings on neurotransmitter–neurohormone interactions in the control of the anterior pituitary.

Since knowledge of neurotransmitter biochemistry and physiology is essential for an understanding of their role in regulating anterior pituitary secretion, this book presents, initially, a detailed analysis of the biochemistry, physiology, and pharmacology of proved or putative neurotransmitters and describes some of the techniques utilized for determining their synthesis or turnover in the CNS of experimental animals or in man. The principal functions of the most well-known neurotransmitter-containing neurons are also considered, based on up-to-date sophisticated techniques for neurotransmitter measurements. Finally, particular attention is given to both traditional and to a vast series of recently developed drugs that affect both neurotransmitter and neuroendocrine function. The experimental evidence that the brain controls pituitary function via releasing and inhibiting hormones secreted by hypothalamic neuroendocrine cells is then concisely reviewed in relation to the secretion of ACTH, GH, LH-FSH, PRL, TSH, and MSH. Studies on the chemical isolation, identification, and synthesis of hypothalamic neurohormones are reported, as well as the most recent concepts of their mechanism of action at the level of the pituitary cells. Space is also allotted to recent findings in the field of

neuroendocrine communication, e.g., radioimmunoassay techniques for neurohormone determination in brain and biological fluids, the topography of the neurosecretory pathways—reviewed in relation to the distribution of the principal neurotransmitter pathways—and the recently described extraendocrine actions of hypothalamic neurohormones.

After elucidation of the neurohumoral and the neurohormonal components of the neural–endocrine communication system, research on the role of specific brain neurotransmitters in controlling pituitary hormone secretions in both experimental animals and in man is considered. The evidence available from the different experimental strategies used is discussed and critically evaluated. A separate section deals with the possible CNS site(s) at which neurotransmitters and neurohormones interact for the control of anterior pituitary secretion. The final part of the book describes the actual or potential import of neuropharmacologic approaches to the diagnosis of and therapy for specific disorders of neuroendocrine function in which neurotransmitter dysfunction may play an etiologic role.

Our major aims have been to interpret and clarify theories derived from different disciplines and to provide not merely a compilation of data but primarily a synthesis of information enabling readers to appreciate the significance of the advances in the field.

This comprehensive work (more than 2000 references and 30 exhaustive tables are included), which has been designed to make the topic understandable to the novice and to appeal to the specialist in the field, will be an invaluable aid to students and research workers not only in the field of neuroendocrinology but also neurobiology, neuropharmacology, and neurophysiology. Clinical neuroendocrinologists will find in it the background essential to the rational and safe use of powerful CNS-acting compounds.

We are grateful to Delia Deriu for providing invaluable help with the literature survey and to Giorgio Marcandalli for original drawings.

Eugenio E. Müller
Giuseppe Nisticò
Umberto Scapagnini

List of Abbreviations

(Other abbreviations used are defined in the text.)

A

AAAD	aromatic amino acid decarboxylase
AC	adenylate cyclase
Ac	acetyl
ACh	acetylcholine
AChe	acetylcholinesterase
ACTH	adrenocorticotropic hormone
AHA	anterior hypothalamic area
Ala	alanine
AP	anterior pituitary
APUD cells	amine precursor uptake and decarboxylation cells
ARC n.	arcuate nucleus
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATP	adenosine triphosphate
APTase	adenosine triphosphatase

B

plasma B	plasma corticosterone
BA	bioassay
BAL	2,3-dimercaptopropanol
BBB	blood-brain barrier
BDB	bisdiazotided benzidine
BLA	basolateral amygdala

C

cAMP	cyclic adenosine monophosphate
3′,5′-cAMP	3′,5′-cyclic adenosine monophosphate
CE	cortical extracts
ChAc	choline acetyltransferase
Che	cholinesterase
CMC	carboxymethyl cellulose
CNS	central nervous system
CNV	contingent negative variation
acetyl-CoA	acetyl coenzyme A
COMT	catechol-*O*-methyltransferase
CPZ	chlorpromazine
CRF	corticotropin-releasing factor
CSF	cerebrospinal fluid
CTZ	chemoreceptor trigger zone
Cy	cyproheptadine
Cys	cystine

D

DA	dopamine
DAO	diamine oxidase
db-cAMP	dibutyryl cyclic adenosine monophosphate
D-β-H	dopamine-β-hydroxylase
DDC	dichlorodithiocarbamate

2-DG	2-deoxy-D-glucose
5,6-DHT	5,6-dihydroxytryptamine
5,7-DHT	5,7-dihydroxytryptamine
DMI	desmethylimipramine
dopa	dihydroxyphenylalanine
DOPAC	3,4 dihydroxyphenylacetic acid
DOPS	3,4-dihydroxyphenylserine

E

E	epinephrine
EEG	electroencephalogram
EM	electron microscopy
EP	estrogen-progesterone

F

plasma F	plasma cortisol
FA	fusaric acid or butylpicolinic acid
FSH	follicle-stimulating hormone
FSH-RH	follicle-stimulating hormone-releasing factor

G

GABA	γ-aminobutyric acid
GABA-T	γ-aminobutyric acid transaminase
GAD	glutamate decarboxylase
GAD-I	glutamate decarboxylase I
GAD-II	glutamate decarboxylase II
GH	growth hormone
GH-IF, GIF or GH-RIH	growth hormone-inhibiting factor or somatotropin-release inhibiting factor or somatostatin
GMP	guanosine monophosphate
GRF	growth hormone-releasing factor
Glu	glutamic acid
Gln	glutamine
Gly	glycine

H

H	histamine
hGH	human growth hormone
HHAA	hypothalamohypophyseal-adrenal axis
HHTA	hypothalamohypophyseal-thyroidal axis
5-HIAA	5-hydroxyindoleacetic acid
His	histidine
HIOMT	hydroxyindole-*O*-methyltransferase
5-HT	serotonin
5-HTP	5-hydroxytryptophan
HVA	homovanillic acid

I

IC	intracisternal
IgG	immunoglobulin
INI	isoniazid
IP nucleus	interpeduncular nucleus
ip	intraperitoneally
IR	immunoreactive
Ile	isoleucine
ISO	isoproterenol
iv	intravenous
IVT	intraventricular

L

L-AAAD	aromatic L-amino-acid decarboxylase
L-dopa	L-dihydroxyphenyl-alanine
Leu	leucine
LH	luteinizing hormone
LHA	lateral hypothalamic area
LH-RH	luteinizing hormone-releasing hormone
LH-RH/FSH-RH	luteinizing hormone-releasing hormone/ follicle-stimulating hormone-releasing hormone
L-5-HTP	L-5-hydroxytryptophan
L-TH	L-tyrosine hydroxylase
L-Trp	L-tryptophan
L-Tyr	L-tyrosine
LVP	lysine vasopressin

M

MA's	monoamines
MAO	monoamine oxidase
MAOA	monoamine oxidase A
MAOI	monoamine oxidase inhibitors
MBH	medial basal hypothalamus
α-MD	α-methyldopa
ME	median eminence

Met methionine
MFB medial forebrain bundle
MI melanophore index
MIF melanocyte-inhibiting factor
MIF-I melanocyte-inhibiting factor I
MIF-II melanocyte-inhibiting factor II
α-MmT α-methyl-*m*-tyrosine
α-MNE α-methyl norepinephrine
MPOA medial preoptic area
α-MpT α-methyl *p*-tyrosine
MRF melanocyte-releasing factor
MSH melanocyte-stimulating hormone

N

n. nucleus
NE norepinephrine
NAD nicotinamide-adenine dinucleotide
NAS *N*-acetyl serotonin
NAT *N*-acetyltransferase
NEFA nonesterified fatty acids

O

17-OHCS 17-hydroxycorticosteroids
6-OHDA 6-hydroxydopamine
5-OHDA 5-hydroxydopamine
6-OH-dopa 6-hydroxydopa

P

pCA *p*-chloroamphetamine
pCMA *p*-chloro-*N*-methylamphetamine
pCPA *p*-chlorophenylalanine
PG's prostaglandins
PGDH prostaglandin dehydrogenase
pGlu pyroglutamic acid
PGO pontogeniculooccipital
Phe phenylalanine
Phe-H phenylalanine hydroxylase
PI pars intermedia
PIF prolactin-inhibiting factor
PMS pregnant mare serum
PNMT phenylethanolamine-*N*-methyltransferase
PO preoptic
POA preoptic area
PON preoptic nucleus
PPP polyphloretin phosphate
PRA prolactin-releasing activity
PRL prolactin
Pro proline
PRF prolactin-releasing factor
PS paradoxical sleep
PVN paraventricular nuclei

R

REMs rapid eye movement sleep
RF's releasing factors
RIA radioimmunoassay
RNA ribonucleic acid
mRNA messenger ribonucleic acid
RNase ribonuclease

S

SCN suprachiasmatic nucleus
Ser serine
SLI somatostatin-like immunoreactivity
SME stalk median eminence
SON supraopticus nucleus
SR-IF growth hormone-inhibiting factor
SSA succinic semialdehyde
SWS slow wave sleep

T

$t_{1/2}$ half life
T_3 triiodothyronine
T_4 thyroxine
TH tyrosine hydroxylase
Thr threonine
TIDA tuberoinfundibular dopamine
Trp tryptophan
TPO tryptophan pyrrolase
TRH,TRF thyrotropin-releasing factor
Trp-H tryptophan hydroxylase
TSH thyroid-stimulating hormone
TYA 5,8,11,14-tetraynoic acid
Tyr tyrosine

U

UV ultraviolet

V

Val valine
VMA vanillylmandelic acid
VMN ventromedial nucleus
vs versus
VTP ventral tegmental pathway

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I

Neurotransmitters and Neurohormones

A. NEUROCHEMICAL MEDIATION: CURRENT CONCEPTS

The stability of the internal environment, i.e., the plasma and extracellular fluids bathing the cells, depends on the coordinated activity of two major regulatory systems: the endocrine system and the nervous system. The manner in which the two integrative systems communicate with one another in coordinating effectively the body's regulatory activity and the results of this combined activity constitute neuroendocrinology (E. Scharrer, 1966; B. Scharrer, 1967). Communication between neurons and endocrine cells in mammals is usually mediated by two types of chemical signals: neurotransmitters and neurohormones.

The distinction between neurotransmitter and neurohormonal activities is not always clear. Neurotransmitters characteristically are low molecular weight, water-soluble compounds that are charged at physiological pH. These messenger substances elicit strictly localized short-lived responses at an easily identifiable locus, the synapse. Since the effector cells are in close proximity to the respective presynaptic terminals, and a presynaptic neuron makes contact with few cells, minute amounts of neurotransmitters are effectively directed at the appropriate receptor sites and the contacts are well insulated from other cells. A feature peculiar to neurotransmitter substances is the rapidity with which they are inactivated following the completion of the signal, either by return of the active principle to its presynaptic storage site or by enzymatic degradation (see Chapter II, Sections B,9 and 10). Substances of this kind [those most generally responsible for brain function include acetylcholine (ACh) and three monoamines, i.e., dopamine (DA), norepinephrine (NE), and seroto-

nin (5-HT)] lack the essential capabilities of endocrine factors, particularly access to and use of vascular pathways. Further properties of this class of neurochemical mediators will be described later (see Chapter II).

In addition to this conventional type of communication, neural elements can influence effector cells by means of a second class of chemical mediators. The principal feature of these compounds is that they act as blood-borne neurochemical messengers which, in sharp contrast to those characteristic of synaptic function, generate more prolonged signals since they are not as speedily inactivated. Being disseminated by a vascular route, these neurochemical messengers are available simultaneously to multiple effector cells in appropriate concentrations. The specificity of the signal derives from the fact that it is in code, and only few cells are capable of translating the code into understandable "information." This group of compounds can be classified as neurohormones (B. Scharrer, 1969). Typically, neurohormones reach their destination in effective concentration, being delivered from sender to receiver cell either by way of the general circulation or by a more restricted vascular route (see Section B,2). It should be noted that the effector sites of neurohormones are not necessarily always endocrine cells. These hormones may, in fact, act directly on target nonendocrine tissue such as the uterus, mammary gland, or kidney tubules (Fig. 1). A neurohormonal mechanism of this type is that controlled by the posterior lobe hormones, vasopressin and oxytocin.

A basic difference between conventional synaptic transmission and neurohormonal mediation lies in the type of neuronal element from which neurohumoral and neurohormonal substances are derived. The source of neurohormones is not, in fact, a conventional neuronal element but a special "neurosecretory" neuron. This is a neuron which is engaged in secretory activity to a degree which exceeds that of the conventional neuron (see B. Scharrer, 1969). Their capacity for the synthesis of specific secretory products is so highly developed as to dominate all other neuronal functions and to impose a prominent glandular appearance to the neuron. The proteinaceous nature of the secretory products—another characteristic distinguishing them from the classic neurotransmitters—allows for their identification with light and electron microscopy. Characteristic membrane-bound granules of several sizes have been observed in many ultrastructural studies (for further information on the phenomenon of neurosecretion, see B. Scharrer, 1969).

The neurosecretory neurons are usually not so ubiquitous within nervous tissue as are more conventional neurons. They tend to gather in specialized neuronal groups and terminate in close proximity to a general or limited circulatory pathway thus frequently forming neurohemal or-

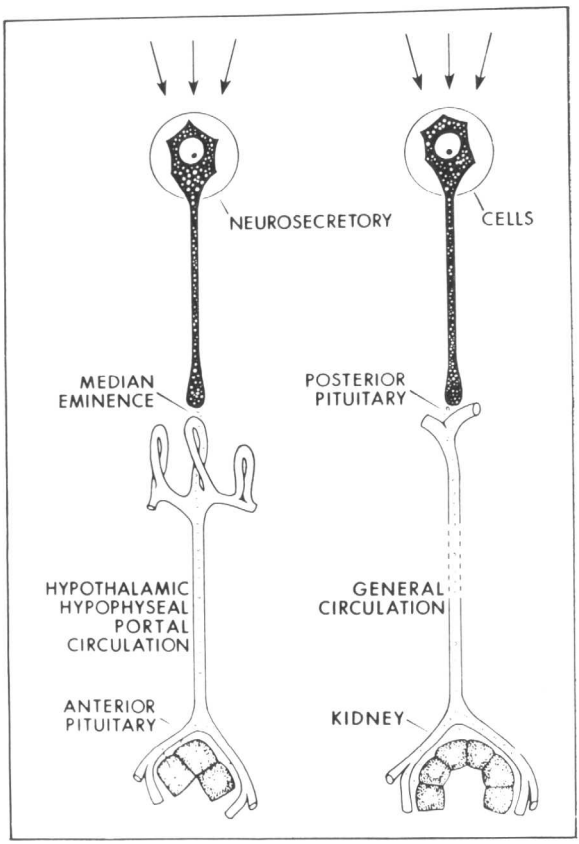

Fig. 1 Release of neurohormones from hypothalamic neurosecretory neurons occurs either in the posterior pituitary, from which they reach terminal target cells by way of the general circulation (right diagram), or in the median eminence of the neurohypophysis, where a special portal system carries neurohormonal signals to endocrine way stations in the adenohypophysis (left diagram). (Adapted from E. Scharrer, 1966.)

gans. Examples of this are the neurohypophysis of vertebrates, the corpus cardiacum of insects, and the sinus gland of crustaceans. These neurohemal organs and the corresponding group of neurosecretory perikarya in which the active material is synthesized constitute neurosecretory systems.

It must be noted, however, that because of the additional knowledge gained in the last few years, the dividing line between conventional and neurosecretory neurons is not so distinct as it appeared to be in the past:

(1) new fibers have been discovered (B fibers), in addition to the "peptidergic" neurosecretory A fibers, in which the secretory product to be discharged into the circulation is of nonpeptide character (see Knowles, 1965; Björklund *et al.*, 1968; B. Scharrer and Weitzman, 1970; these elements will be further discussed below); (2) A and B fibers have been found that do not necessarily end at or near a vascular route since they may establish direct contact with neuronal or endocrine elements or with neither. In the pars intermedia of vertebrates or in the corpus allatum or prothoracic gland of insects (Bargmann *et al.*, 1967; B. Scharrer, 1964a,b) sites exist of these direct contacts (neurosecretomotor junctions) (Bern, 1966). Since the transfer of the chemical message does not involve, in this instance, a vascular route and the site of release is close to the site of action of the messenger substance the latter is not a neurohormone. On the other hand, due to its peptidergic nature, this mediator can be distinguished from conventional neurotransmitters. These peptidergic neurosecretomotor junctions do not end in a synapse, but rather make synaptoid contacts.

The broad spectrum of neuroendocrine mediation and the existence in mammals of peptidergic neurons with neurohormonal and probably neurotransmitter function (see below) create the problem of distinguishing between neurohormones and neurohumors. The two sides of the neurochemical spectrum are neither rigidly uniform nor separated by as clear-cut a demarcation as originally conceived. Data are rapidly emerging for the hypothalamic peptidergic neurons, which control AP function, which show their widespread distribution to extrahypothalamic CNS areas, their localization in nerve terminals and release from nonhypothalamic nerve endings after hypothalamic stimulation and, finally, their important behavioral effects not attributable to action on the pituitary (see Chapter III, Sections C and E). The possibility that the involvement of the hypothalamic peptides in the regulation of the AP function may merely represent a recent evolutionary development of molecules endowed uniquely with neurotransmitter function in the lower phylogenetic species has to be considered (Jackson and Reichlin, 1974; Martin *et al.*, 1975a; Reichlin *et al.*, 1976). The postulated neurotransmitter function of hypothalamic hypophysiotropic peptides seems to have its counterpart in the neurohormone-like behavior of known brain neurotransmitters. Although the evidence shows that catecholamines act predominantly in the CNS (see Chapter IV), several findings suggest that DA, transported by hypophyseal portal blood, may directly affect the AP gland by inhibiting the secretion of prolactin (Chapter IV, Section A,4,a,i).

In summary, the mode of neuroendocrine interaction ranges from conventional information transfer by chemical synaptic transmission to classic

neurohormonal signals, and also deals with several intermediate types in which the neurochemical mediator cannot be classified as either hormonal or neurohumoral.

B. NEUROENDOCRINE TRANSDUCTION IN MAMMALS

The widespread diffusion of neurosecretory neurons in living systems underlines their basic function: that of providing a link between the nervous and the endocrine system for the control of stability of the internal environment. It is then apparent that the "vocabulary" of neuroendocrine communication in the animal kingdom is rich and susceptible to further enrichment by new types of "languages." We will now consider the most common type of neuroendocrine communication in mammals.

Mammalian cells differ from bacterial cells in that they are less equipped to cope with hostile environments, and compensate for this lack through the organization of similarly differentiated elements—specialized cells—which react with other groups of cells to regulate the composition of plasma and extracellular fluid constituents. These communication cells have been classified as "neuroendocrine transducers" because they act to translate brain-type signals (i.e., neurotransmitters acting at synapse) to hormonal outputs (Wurtman, 1970, 1973). Communication, therefore, is usually mediated by two types of chemical signals, neurotransmitters and hormones. In this respect, neuroendocrine transducers are said to differ from "endocrine transducers" (thyroid, ovary, adrenal cortex), whose physiological inputs come only from the circulation, since, in addition to specific humoral signals, they also respond to neuronal inputs.

Many groups of neuroendocrine transducer cells have been recognized in mammals, and their characteristics have been described (see Wurtman, 1973, and Table I). At least six such groups of neuroendocrine transducer cells have been demonstrated; additional candidates include the cells of the rodent thyroid and the "amine precursors uptake and decarboxylation cells" (APUD cells) (Pearse, 1968) for which either monoamines or somatostatin or both may represent the input signal(s) (see also Chapter III, Sections A,3,c, and C). We have also proposed that pituitary lactotrophs may act as transducer cells.

The demonstration that a particular cell type mediates neuroendocrine transduction requires that nerve terminals be in close proximity to the cell and that the capacity of the cell to make a secretory response after an appropriate stimulus be damaged when its innervation is interrupted. As for their embryologic derivation, even though most neuroendocrine transducers are of neuroectodermal origin, others (e.g., β-cells of pancreatic

TABLE I

Neuroendocrine Transducer Cells

Cell type	*Input signal*	*Hormonal output signal*
Hypothalamic neurohormone cells	Monoamines and other neurotransmitters	Releasing and inhibiting neurohormones secreted into pituitary portal circulation
Cells of the hypothalamic supraoptic nucleus	Acetylcholine or norepinephrine	Vasopressin
Pituitary lactotropes?	Dopamine	Prolactin (inhibition)
Adrenal medulla	Acetylcholine (preganglionic sympathetic neurons)	Epinephrine
Pineal organ	Norepinephrine (postganglionic sympathetic neurons)	Melatonin
Juxtaglomerular cells of the kidney	Norepinephrine (postganglionic sympathetic neurons)	Renin
β-Cells of pancreatic islets	Norepinephrine (postganglionic sympathetic neurons)	Insulin
Thyroid follicular cells?	Monoamines	Thyroid hormones
β- and α_2-cells of pancreas, parafollicular cells of the thyroid, gastrointestinal gland cells?	Somatostatin	Insulin (inhibition), glucagon (inhibition), calcitonin, gastrin, pepsin (inhibition)

islets) are not. Thus, not necessarily all of them have to be classified as true neurosecretory cells. A unique feature of the neuroendocrine transducers thus far identified is the frequent involvement of catecholamines and indoleamines in their communication function. These aminergic links are widespread; the monoamines either transmit messages between cells over long distances as hormones (e.g., epinephrine release from the adrenal medulla) or act over short distances as transmitter substances (neuroendocrine transducers of the hypothalamus). In addition, MA's are normal constituents of many polypeptide hormone-producing cells (see also below), and evidence suggests that activation of the cells to induce hormone release is associated with changes in the amine levels and turnover within the cell (Owman *et al.*, 1973).

1. The Neurohypophysis

In mammals the major neuroendocrine transducers are the supraoptic and paraventricular–hypophyseal neurons which constitute the

neurohypophysis, and the neurons of the base of the hypothalamus which secrete hypophysiotropic hormones into the blood supplying the AP gland.

The neurohypophysis consists of neurons whose cell bodies are situated in the supraoptic and paraventricular nuclei of the hypothalamus. The biologically active peptides which they synthesize, vasopressin and oxytocin, are released from the endings of their axons into the posterior pituitary where they are stored. They are then released into the general circulation in response to either suckling or genital stimulation (oxytocin) or changes in extracellular fluid osmolarity and volume (vasopressin).

Two kinds of vesicles are found in the terminals of the supraoptic hypophyseal neurons: the neurosecretory vesicle (about 1500 Å in width), related to hormone synthesis and transport, and a smaller electron-lucent vesicle (about 400 Å) (Palay, 1957), which was termed "synaptic vesicle" and was by analogy with ordinary nerves believed to contain neurotransmitter substances (such as acetylcholine or norepinephrine) that are liberated during stimulation to initiate hormone release. Interestingly enough, these vesicles stain preferentially with the zinc iodide osmic acid (ZIO) procedure as do the classic synaptic vesicles of ordinary nerves (Christ and Back, 1970; Rufener and Dreifuss, 1970). In accordance with the above hypothesis, both morphologic (Shute and Lewis, 1966; Fuxe and Hökfelt, 1967) and electrophysiologic studies (see Cross, 1973) produced evidence for neurotransmitter involvement of the cholinergic and adrenergic systems in the release of neurohypophyseal hormones.

To summarize the results of many studies, some of which refer to the identification of neurosecretory cells *in vivo* by antidromic stimulation in conjunction with iontophoretic injection of drugs into hypothalamic cells (see Cross, 1973), it would appear that the cholinergic afferent connections are mainly responsible for the stimulation of hypothalamic nuclei, while afferent noradrenergic fibers exert inhibitory effects on hormone release.

Koelle (1961) proposed that ACh might be stored in, and released from, hormone- and ACh-containing nerve endings on stimulation of the secretory neurons and then act on the same nerve endings to influence the release of neurohypophyseal hormones. This would produce the unique situation of a neuron's own transmitter substance providing the stimulus for the release of its own endocrine product. Subsequent studies, however, have challenged Koelle's theory of the "double neurohumoral role of acetylcholine" in the neurohypophysis. Under *in vitro* conditions, when the release of hormones can be easily stimulated and measured (Douglas, 1963; Douglas and Poisner, 1964), ACh was shown to stimulate the release of neurohypophyseal hormones (Douglas and Poisner, 1964; Dicker,