from other research on human problem-solving and that are usually described as processes for selective heuristic search through spaces of possible solutions. The less blind the search—that is, the more existing theoretical knowledge is available to guide it and turn it from unprofitable directions—the more readily and directly are the regularities hidden in empirical data discovered.

References and Notes

1. H. Poincaré, in *The Foundations of Science* (Science Press, New York, 1913), p. 387; in *The World of Mathematics*, J. R. Newman, Ed. (Simon & Schuster, New York, 1956), vol. 4; J. S. Hadamard, *Essays on the Psychology of Invention in the Mathematical Field* (Princeton Univ. Press, Princeton, N.J., 1945).
2. A. Newell, J. C. Shaw, H. A. Simon, in *Models of Thought*, H. A. Simon, Ed. (Yale Univ. Press, New Haven, Conn., 1979), pp. 144–174; H. A. Simon, in *Mind and Cosmos*, R. Colodny, Ed. (Univ. of Pittsburgh Press, Pittsburgh, 1966), pp. 22–40.
3. Many of the source documents relating to this and most of the other scientific discoveries discussed in this paper can be found in H. M. Leicester and H. S. Klockstein [*A Source Book in Chemistry* (McGraw-Hill, New York, 1952)] and W. F. Magie (*7*); Black's law is described by Magie (*7*), pp. 134–139.
4. Black saw that it did not matter which of the two measures of amount was used.
5. A description of the basic BACON program will be found in H. A. Simon, P. W. Langley, G. L. Bradshaw [*Synthese* **47**, 1 (1981)]. BACON.4's heuristics were devised before our work on Black's law; BACON.5 incorporates modifications partly motivated by our experience with that law. There are a number of programs antecedent to BACON that are capable of inducing laws from data. Early examples include a program for extrapolating letter series [H. A. Simon and K. Kotovsky, *Psychol. Rev.* **70**, 534 (1963)] and a program for fitting functions to data [D. G. Gerwin, *Behav. Sci.* **19**, 314 (1974)]. The METADENDRAL program [B. G. Buchanan, G. Sutherland, E. B. Feigenbaum, in *Machine Intelligence 4*, B. Meltzer and D. Michie, Eds. (Elsevier, New York, 1969)] constructs rules for explaining mass spectrogram data. The AM and EURISKO programs [D. B. Lenat, *Proc. 5th Int. Jt. Conf. Artif. Intell.* (1977), p. 833; *Artif. Intell.* **21**, 31 (1983)] discover new concepts and make conjectures. This is by no means an exhaustive list of programs with such inductive capabilities.
6. We have described the last stages of the inductive process only in the roughest terms. Parenthetically, it should be mentioned that the derivation of Black's law by BACON.4 [G. L. Bradshaw, P. W. Langley, H. A. Simon, *Proc. Natl. Conf. Can. Soc. Comput. Stud. Intell.* (1980), p. 19] is incomplete, but the derivation by BACON.5 (P. W. Langley, G. L. Bradshaw, H. A. Simon, *Proc. 7th Int. Jt. Conf. Artif. Intell.* (1981), p. 121] is correct.
7. W. F. Magie, *A Source Book in Physics* (McGraw-Hill, New York, 5/1935).
8. This is not always true; whether it is in any given case is a matter of physics and chemistry. But early in life we encounter many situations where such conservation holds, and we build up a representation for thinking about that broad class of situations.
9. A first crude version of a computer program capable of carrying out this kind of reasoning has been constructed, and our experiences with it will be reported in future publications.

10. Supported by NIMH grant MH-07722 and contract N00014-82-K-0168 from the Office of Naval Research.

Brain Peptides: What, Where, and Why?

Dorothy T. Krieger

Summary. Within the past decade, a large number of peptides have been described within the vertebrate central nervous system. Some of these peptides were previously known to be present in nonneural vertebrate tissues, as well as in lower species, in which they may serve as primitive elements of intercellular communication prior to the development of neuronal or endocrine systems. In vertebrates, these peptides are thought to have neurotransmitter or neuromodulatory roles and appear to be involved in the regulation of a number of homeostatic systems, although the mechanisms of their actions are still unclear.

In recent years, a large number of peptides, many of which were originally characterized in nonneural tissues, have been reported to be present in the central nervous system. Table 1 indicates the rapidity of recent progress in this field. It can be seen that, in addition to those peptides that were originally termed "hypothalamic-releasing hormones," all of the other peptides identified within the central nervous system and which were previously known to occur within the gastrointestinal tract—that is, vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), insulin, and glucagon—and within the pituitary—that is, adrenocorticotropic hormone (ACTH) and other peptides derived from its precursor, proopiomelanocortin (POMC), and possibly prolactin, thyrotropin-stimulating hormone (TSH), and a luteinizing hormone (LH)—have been described essentially within the last 10 years.

The detection of these peptides within the central nervous system has raised many questions regarding their source, mechanism of action, and function. Many of the initial studies in this field focused on the delineation of the sites of peptide distribution and the demonstration of peptide synthesis within the central nervous system. Distribution studies were of value in delineating localization of peptide-containing cell bodies in which synthesis could potentially occur, as well as delineating fiber pathways for investigation of possible physiological function (or functions). Demonstration of synthesis was deemed important since conceptually the mechanism of action and the function of a peptide detected in the central nervous system would differ if the peptide were locally synthesized or were transported thereto from another source. Regulation of a neuronally synthesized peptide is governed by both "neuronal" and "metabolic" inputs impinging on the neuron; the synthesized material would be acting as a neurotransmitter—that is, being liberated at presynaptic terminals with subsequent postsynaptic membrane effects. In the case of a peripherally synthesized peptide having access to the central nervous system, its synthesis is regulated by the multiplicity of factors known to affect its tissue of origin, and its central nervous system actions would presumably be mediated via peptidergic receptors present on neurons or their terminals.

Until recently, the monoamines (norepinephrine, epinephrine, dopamine, and serotonin), acetylcholine, and the amino acids (such as glycine, glutamine, and γ-aminobutyric acid) were thought to be the only neurotransmitters that classically mediated synaptic communication (*1*). Prior to the last decade, the only peptide chemically characterized within the brain was substance P (*2*), previously identified therein and in the gastrointestinal tract in terms of its biological activity. Table 1 indicates the time frame in the past decade during which some of the other peptides present

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in the central nervous system have been characterized.

Also indicated in Table 1 are the tentative estimates (*3*) of the number of synapses occupied by each of the types of "classical" neurotransmitters present in the central nervous system. All of these "classical" neurotransmitters may therefore account for only approximately 40 percent of known synapses present in the central nervous system. It is most likely, therefore, that delineation of the neurophysiological functions and interactions of the peptide neurotransmitters will account for the occupancy of the majority of thus far unclassified synaptic sites. In view of the considerable body of information that has accumulated concerning central nervous system function, as mediated by the "classical" neurotransmitters, it would appear that major additional functional insights will be obtained by elucidating the role of the brain peptides.

There has been considerable discussion as to whether brain peptides should be classified as neurotransmitters or "neuromodulators." A neurotransmitter has previously been considered to be a substance liberated at presynaptic terminals which, after diffusing across the narrow synaptic gap, acted on the postsynaptic membrane. Its action is highly localized to the synaptic region, with a duration of milliseconds. Termination of its action is accomplished by removal of the transmitter, either by enzymatic degradation or via a reuptake mechanism into the presynaptic terminal. It is becoming increasingly clear, however, that putative transmitters are capable of a wider range of action, both with regard to duration of action and distance between release sites and targets (*4*). Perhaps a broader definition of a neurotransmitter is that of a substance liberated at presynaptic terminals which can produce physiological changes in the postsynaptic cell identical with those produced by stimulating the presynaptic cell. In addition, antagonists of such a "neurotransmitter" substance should block this nerve-evoked response. The term "neuromodulator" has been used to refer to substances with nonclassical transmitter actions. However, there is no consensus on such a definition, and the term has been used to define peptide actions when they (i) modify the known actions of the so-called "classical" neurotransmitters, (ii) act to block the release of a given neurotransmitter via their release at presynaptic endings on the terminals releasing that transmitter, or (iii) alter the turnover of other neurotransmitters. It may well be that such classification is a semantic one. The best evidence of peptides existing as conventional transmitters has been adduced for the "hypothalamic-releasing hormone"—luteinizing hormone–releasing hormone (LHRH), present in sympathetic ganglia. It has been demonstrated that an LHRH-like peptide can be released from such ganglia by nerve stimulation by way of a calcium-dependent process, and that this peptide can act directly on sympathetic neurons to produce a depolarizing response lasting for minutes. It has been suggested that LHRH functions as the transmitter for the previously observed long-lasting late slow excitatory postsynaptic potential (EPSP), which is known to occur after nerve stimulation. The responses to LHRH and the late slow EPSP are associated with similar changes in membrane conductance and in the excitability of the neuron; both are blocked by LHRH analogs that inhibit LHRH-induced release of LH from pituitary gonadotrophs (*5*).

Table 1. Chronology of the description of the presence of neurotransmitters and neuropeptides in the central nervous system. Indicated in column 3 are the estimated percentages of brain synapses occupied by the monoamine, amino acid, and acetylcholine neurotransmitters as estimated in (*3*).

Year	Transmitter	Percent of brain synapses
1920	Acetylcholine	5 to 10
	Epinephrine	
1930		
1940		
	Norepinephrine	0.5
1950		
	Amino acids (GABA, glutamic acid, aspartic acid)	25 to 40
	Dopamine	0.5
1960		
	Substance P	
1970	Serotonin	0.5
1971		
1972		
1973		
1974	"Hypothalamic-releasing hormones" (TRH, LHRH, SRIF)	
1975	Enkephalin	
1976	VIP, CCK	
1977	ACTH	
1978	Other pituitary hormones	
	Insulin	
	Vasopressin, oxytocin	
	Angiotensin	
1979	Glucagon	
1980		
1981	Corticotropin-releasing hormone	
1982	Growth hormone–releasing hormone	
1983		

Categories of Brain Peptides

Table 2 lists the major categories of brain peptides described to date. The initial identification of all these peptides in the vertebrate brain has been greatly facilitated by application of the techniques of radioimmunoassay and immunohistochemistry, which have been used to characterize within the central nervous system peptides previously detected elsewhere in vertebrate tissues. With the advent of new insights, these techniques have been used to demonstrate within the vertebrate central nervous system peptides originally thought to be present only in invertebrates. Indeed, the converse has also been shown. An innovative strategy has been described by Mutt and his co-workers for detection of "new peptides," based on the hypothesis that molecules with a COOH-terminal amide are biologically important (*6*). Using this approach, these investigators have isolated hitherto uncharacterized peptides from both intestine and brain, such as neuropeptide Yy. Additional peptides have been characterized as a result of studies of precursor messenger RNA's (mRNA) or the genes that encode them (for example, CGRP, calcitonin gene-related product) (*7*).

The classification indicated is a somewhat arbitrary one, based on previous attributions of localization of a given peptide. In Table 2 the hypothalamic-releasing hormones are cited within quotation marks to indicate that, although the original impetus for the isolation of these hormones was to confirm physiological observations suggesting hypothalamic control of anterior pituitary function, it is now known that these peptides have a widespread extrahypothalmic distribution, which presumably relates to other functions. The neurohormones vasopressin and oxytocin are included in this list of "brain" peptides, since although, as noted (*2*), their presence within the magnocellular neurons of the hypothalamus has long been known, it has only recently been recognized that projections from these neurons occur to regions elsewhere in the central nervous system other than to the posterior pituitary (*8*). A number of peptides are listed—that is VIP, CCK, insulin, glucagon, and gastrin—which were originally demonstrated to occur in secretory elements of the gastrointestinal tract. Others of these so-called "gastrointestinal" peptides, such as substance P, neurotensin, the enkephalins, and somatostatin, were initially described in neural tissues prior to their description within the gastrointestinal tract. In addition, in the gastrointestinal tract some of the peptides, such

as gastrin, somatostatin, CCK, and VIP, are present within both nerves and endocrine cells (similar to the occurrence of the same peptide in different tissues, that is, gastrointestinal tract and brain). In the gastrointestinal tract thus far, secretin, motilin, insulin, and glucagon have been described only in secretory cells, not in nerves, while enkephalin, bombesin, substance P, and VIP have been localized only to nerves and not in endocrine cells. With regard to the pituitary-like hormones in brain, there is evidence that peptides derived from POMC—such as ACTH, β-endorphin, and α-melanocyte-stimulating hormones (α-MSH)—are locally synthesized therein (*9*). Prolactin mRNA and POMC mRNA have been detected within the central nervous system (*10*). Direct evidence of synthesis of the other pituitary hormones has not yet been presented, although there is considerable indirect evidence for this (*11*). These considerations of the demonstration of synthesis are particularly stressed with regard to pituitary hormones, in view of the close physical proximity of the pituitary to the brain, which has led to suggestions that the presence of such pituitary-like peptides in brain was secondary to diffusion or transport from pituitary sites (*12*).

Concentrations of Peptides in the Central Nervous System

Concentrations of brain peptides are several orders of magnitude less than those of the previously defined neurotransmitters (acetylcholine, monoamines, and amino acids), which are present at concentrations of 10^{-9} to 10^{-10} moles per milligram of protein (acetylcholine and monoamines) and 10^{-6} to 10^{-8} moles per milligram of protein (amino acids). The concentrations of the "hypothalamic-releasing hormones," gastrointestinal hormones, and pituitary-like hormones for the most part vary between 10^{-12} to 10^{-15} moles per milligram of protein. In a comparison of concentrations of a given peptide in brain and in its sites of localization outside the central nervous system, of those peptides initially described as occurring in the gastrointestinal tract, only CCK appears to have a central nervous system concentration that is greater than its concentration in gastrointestinal tract. Concentrations of pituitary-like peptides in brain are several orders of magnitude less than in pituitary. These relatively low peptide concentrations in the central nervous system by no means minimize their importance. For example, the high concentrations of peptides in the pituitary or the gastrointestinal tract, for the most part, reflect the levels necessary for peptides with hormonal roles. Such hormones are exported by way of the circulation and hence their concentrations are diluted before reaching their target organs. In the central nervous system, these peptides act over short distances where dilution of such magnitude would not occur. It should also be realized that determination of peptide concentrations gives no information with regard to their turnover. Finally, specific regional concentrations of peptides (many of which still remain to be determined) may be greater than those thus far described for the concentrations of these peptides in gross areas of the central nervous system.

Much of the recent interest in brain peptides has been focused on several major areas which are considered in the remainder of this article. These are (i) evolutionary considerations which have been raised by the demonstration of brain peptides, (ii) evidence for synthesis and processing of brain peptides, (iii) localization of brain peptides, (iv) function of brain peptides, and (v) possible clinical applications of studies of brain peptides.

Table 2. Categories of mammalian brain peptides.

"Hypothalamic-releasing hormones"
Thyrotropin-releasing hormone
Gonadotropin-releasing hormone
Somatostatin
Corticotropin-releaseing hormone
Growth hormone–releasing homone
Neurohypophyseal hormones
Vasopressin
Oxytocin
Neurophysin(s)
Pituitary peptides
Adrenocorticotropic hormone
β-Endorphin
α-Melanocyte-stimulating hormone
Prolactin
Luteinizing hormone
Growth hormone
Thyrotropin
Invertebrate peptides
FMRF amide*
Hydra head activator
Gastrointestinal peptides
Vasoactive intestinal polypeptide
Cholecystokinin
Gastrin
Substance P
Neurotensin
Methionine-enkephalin
Leucine-enkephalin
Insulin
Glucagon
Bombesin
Secretin
Somatostatin
TRH
Motilin
Others
Angiotensin II
Bradykinin
Carnosine
Sleep peptide(s)
Calcitonin
CGRP
Neuropeptide Yy

*Phenylalanylmethionylargininylphenylalanylamide.

Brain Peptides in Evolution

The evolutionary origins of brain peptides and the larger issue of the evolutionary origins of the polypeptide hormones per se are interrelated questions and have aroused great interest. Such interest has arisen from several types of observations. These include the identification in vertebrate nervous tissue of peptides that had been considered to be of glandular origin in these species; the identification in invertebrate neurons and in unicellular organisms of peptides previously thought to occur only in vertebrate glandular tissue; the presence of peptide receptors in unicellular organisms; the identification in vertebrate tissue of peptides previously described only in invertebrates; and the presence of similar sequences in diverse peptides both within and between species.

The accumulated data indicate that substances similar to known hormonal peptides and chemical neurotransmitter molecules are present in plants and unicellular organisms (*13*) (Fig. 1). It has been proposed that these peptides serve as primitive elements of intercellular communication in these organisms. Evidence of a specific neuronal system is not present until the appearance of the sponges, at which time some of these primitive messenger molecules became localized within this system to function as neurotransmitters. The use of peptides as both neurotransmitters and neurohormones is apparently present in phyla which have no endocrine tissue per se (for example, coelenterates and annelids). The anatomical elements of classic glandular endocrine tissues first appear in vertebrates. Commonality of brain-gut peptides is present as early in evolution as the lamprey (the living representative of the earliest vertebrate group, the Agnatha)—that is, somatostatin and the terminal octapeptide (CCK-8) of the 33- or 39-amino acid peptide CCK have been detected in both of these tissues in this organism. It would therefore appear that what are considered to be products of the nervous system and of the endocrine system were present before these systems evolved. As nervous and glandular tissue appeared, these tissues expressed peptides previously present in a diffuse cell system in lower organisms.

Given the commonality of many invertebrate and vertebrate peptides, it is of interest to see in which tissues they are expressed. Peptides in invertebrates may be present in both secretory (gastrointestinal) and neuronal cells, and in other instances only in neurons. One previously characterized neuronal peptide, hydra head activating factor, is present in both vertebrate gastrointestinal tract and in neurons, while another—molluscan cardioexcitatory peptide—has thus far been demonstrated only in vertebrate neuronal tissue. Some peptides (such as ACTH, β-endorphin, and prolactin), which are present in both endocrine and neural tissue of vertebrate species, have thus far been detected only in invertebrate neuronal tissue. Other peptides, such as insulin and pancreatic polypeptide, which occur only in vertebrate endocrine cells, are present in both gastrointestinal secretory cells and neurons of invertebrates. These findings are in keeping with the concepts set forth in Fig. 2 regarding the evolutionary precedence of the neuronal system over the endocrine system.

In addition to evidence of peptide evolution, there is also evidence for the evolution of peptide receptors, as in the case of those for secretin and vasoactive intestinal polypeptide in mammalian and avian species (*14*). The target cell for a given peptide may also undergo evolutionary change. For example, prolactin in teleosts is concerned with the regulation of fluid balance, whereas in mammals it is concerned with the regulation of secretory epithelium of the mammary gland and has lost, for the most part, its effects on fluid homeostasis.

Studies of peptide structure in multiple species have indicated that in a given peptide some portions of its sequence have been conserved across evolution. Such conserved sequences are believed to comprise functionally active portions of the molecule. The observation that there are common sequences in different peptides in a given species (for example, the "family"—CCK, gastrin, glucagon, secretin, and VIP) and the presence of different peptides with similar functions in different species (such as the above-noted effects of secretin and VIP), may be explained by gene duplication followed by point mutation and amino acid substitution. (Other types of mutation may also occur, such as frame shifts, deletions, or insertions in a structured gene.) It is of interest that when such peptide "families" have been described, the peptide of the group that has been identified as occurring only in neurons (such as VIP for the secretin "family") is presumed to be the ancestral molecule (*15*).

In unicellular organisms, ACTH-like β-endorphin-like, insulin-like, somatostatin-like, vasotocin-like, and relaxin-like peptides have been reported in the protozoan *Tetrahymena pyriformis* (*16–19*). Insulin-like material has also been reported in *Escherichia coli* (*20*), and bacterial production of a choriogonadotropin-like factor has also been reported (*21*). Although in some of these instances the extent of the physicochemical identity of the peptide-like material to its vertebrate form has not been firmly established, in other instances characterization has been extensive (*22*). Alpha factor, a mating pheromone of yeast (*Saccharomyces cerevisiae*), not only has extensive sequence homology with the hypothalamic decapeptide, LHRH, but synthetic and natural preparations of alpha mating factor bind specifically to rat pituitary LHRH receptors and can stimulate the release of LH from cultured gonadotrophs (*23*). The studies published to date are not consistent with the proposal that the detected presence of peptides in these unicellular organisms is the result of assay artifacts or inadvertent exogenous contamination of the extracts used, but rather suggests that these peptides originated in the unicellular organisms and have been maintained in higher organisms during the course of evolution. An alternative hypothesis, that the genes for these peptides arose in vertebrates and were introduced by plasmids into the unicellular organisms, cannot be excluded until the genes are demonstrated and analyzed.

When considering the observation that similar peptides occur in different tissues and organisms, it is important to realize that the same peptide can act via different modes of intercellular communication, depending on the tissue in which it is present. Figure 1 illustrates how a given peptide can, in some instances, act as a local factor (that is, autocrine or paracrine secretion), a neurotransmitter, a neurohormone, or a hormone. In the course of evolution, therefore, the same peptide may have functioned in one and then in another of these modes of communication. Even in a single vertebrate species, products of a precursor molecule, as in the case of POMC (the ACTH precursor molecule), can act by four of these mechanisms, that is, as *neurotransmitters* in brain, as *hormones* when secreted from pituitary, possibly as *neurohormones*, as adduced from their presence in portal blood and their reported

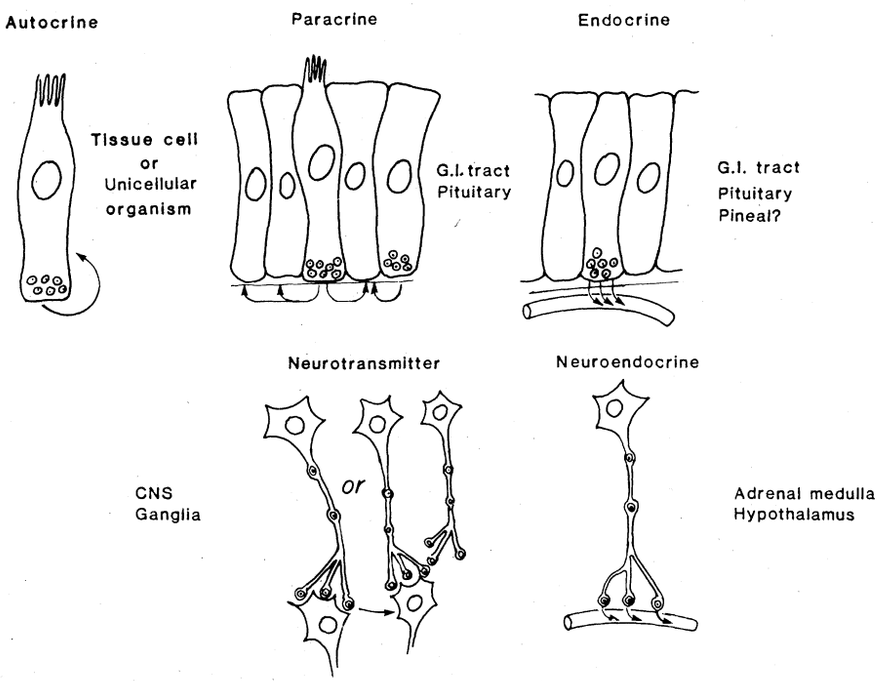

Fig. 1. Examples of possible modes of peptide secretion. In autocrine secretion, the secreted product acts locally on the cell of origin. Paracrine secretion refers to the local action of a secretory product on neighboring cells, either by extracellular fluid transport systems or via intracellular gap junctions. In endocrine secretion, a product is secreted into the bloodstream to affect distant targets. In neuronal cells (lower portion of figure), cell-to-cell communication occurring via axodendritic or presynaptic axo-axonic synapses may be looked on as a form of paracrine communication (neurotransmitter). Neurohumoral secretion (neuroendocrine) refers to release of a neuronal product into the bloodstream to act on other tissues. Representative tissues utilizing such forms of communication are cited. [Courtesy of *Clinical Research* (*39*)]

actions on pituitary gonadotroph function (*24*), and as *paracrine* factors (in the reproductive and gastrointestinal tracts) (*25, 26*). In vertebrate neurons and in *Tetrahymena*, POMC-derived peptides may function via neurotransmitter and autocrine or paracrine mechanisms, respectively. In some instances, evolution has actually changed the morphological configuration of a peptide-secreting cell. The pinealocyte, whose peptide product or products are still incompletely defined, and which in lower vertebrates is a photoreceptor and has afferent nerve connections, has in mammals lost both of these attributes, and the cell has taken on the attributes of a typical glandular hormonal secretory cell.

Changes in tissue peptide expression are also seen during the course of ontogeny. There is preliminary evidence that some of the embryonal pancreatic cells, which transiently express a dopaminergic phenotype, also express insulin or glucagon, indicating transformation of catecholaminergic precursor-type cells into peptidergic cells (*27*). Such studies can be considered as complementary to experiments in rats of different embryonic ages, which indicate that catecholaminergic expression is detected earlier in both brain and peripheral nerves (E 10.5) than peptidergic expression (E 16) (*28*). These findings, however, have been based only on immunohistochemical and radioimmunoassay techniques. It is still possible that nonimmunoreactive precursor molecules are present for such peptides earlier in gestation.

Characterization, Synthesis, and Processing of Brain Peptides

While the foregoing has provided evolutionary perspectives that a given peptide can arise independently in different tissues, it is important to demonstrate both that the peptide is structurally similar and that it is synthesized in the various tissues and species in which it is described as being present. Available methodologies for characterization of the nature of a peptide in a given tissue include immunoassay and immunocytochemistry (*29*), bioassay, physicochemical characterization, and sequence determination.

Synthesis of peptides in brain appears to be governed by the same general mechanisms as have been described for all polypeptide hormones, these being derived from post-translational proteolytic cleavage of larger precursor molecules, which in themselves have little, if any, inherent biological activity. Synthesis of neuropeptide precursors, as is true for those of other peptides, occurs in ribosomes within the perikarya, at a considerable distance from the secretory site of the axon terminal. This is in contrast to the local synthesis, uptake, and recycling mechanisms at the axon terminal, which occur in the case of the enzymatically synthesized neurotransmitters, such as acetylcholine and the biogenic amines.

Many approaches have been utilized to demonstrate peptide synthesis in brain. One indirect approach, in the case of peptides that were initially localized to other tissues, has been the demonstration of the peptide in brain after removal of the tissue presumed to be the major site of its production. More direct approaches include demonstration of a net gain in peptide concentration during culture of central nervous system tissue, incubation of central nervous system tissue with labeled amino acids and demonstration of incorporation of label into the peptide in question, demonstration by either solution hybridization or in situ hybridization of the presence in central nervous system of mRNA coding for the peptide, or extraction of mRNA from the central nervous system and its translation in a cell-free system to yield the peptide of interest (*30*).

The term "processing" has been used to describe the liberation of active peptide substances from their precursors by proteolytic enzymes. It is thought that most such cleavages occur within the Golgi apparatus in the developing secretory granules. Cleavage sites are usually at points in the peptide backbone at which pairs of basic amino acids occur. Various types of post-translational enzymatic modifications also exist, such as amidation, acetylation, sulfation, glycosylation, and phosphorylation. Some of these modifications appear to occur within the secretory granule; the site of others is still uncharacterized.

POMC as a prototype precursor molecule. The most extensive studies of multitissue and multispecies localization, synthesis, and processing of a peptide have been performed with regard to POMC. These are described in detail below.

Synthesis of POMC in brain. These approaches with regard to characterization and demonstration of synthesis are considered, with POMC as a prototype molecule (*31*). Figure 3 is a schematic diagram of the POMC molecule, indicating the potential peptides that can be derived therefrom by proteolytic processing. Prior to the initiation of studies attempting to demonstrate synthesis of this previously characterized pituitary peptide in the central nervous system, it was important to ascertain the location of the cell bodies in the central nervous

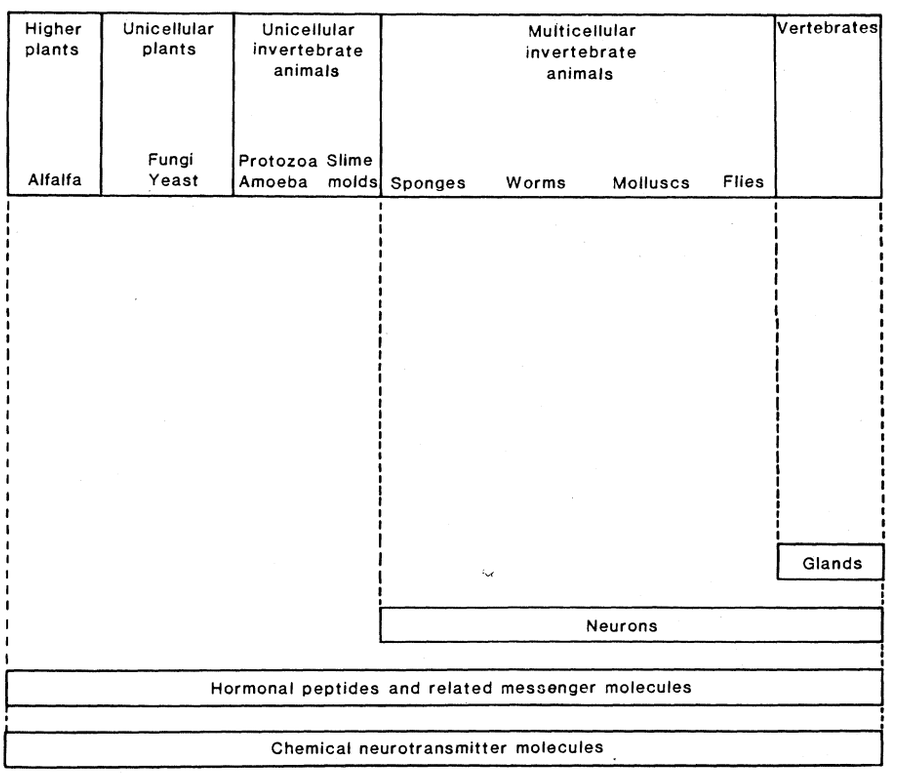

Fig. 2. Evolutionary origins of the biochemical elements of the endocrine system and the nervous system. [Courtesy of *Peptides* (*13*)]

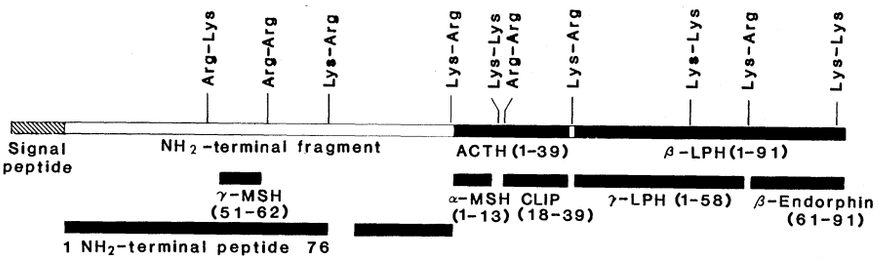

Fig. 3. Schematic representation of the bovine pro-opiomelanocortin precursor molecule (*100*). The indicated points at which dibasic amino acids occur represent potential cleavage sites by trypsin-like enzymes. The peptides that can potentially be generated by such trypsin-like cleavages are indicated below the pro-opiomelanocortin structure depicted in the upper horizontal bar. *LPH*, lipotropin; *CLIP*, corticotropin-like intermediate lobe peptide. [Courtesy of *Clinical Research* (*39*)]

system in which it was present. Such localization was predominantly in cells in the arcuate nucleus of the hypothalamus. Although hypothalamic concentrations of both ACTH and β-endorphin are not decreased after hypophysectomy, central nervous system concentrations, but not pituitary concentrations, are markedly diminished after arcuate nuclear lesions produced by neonatal administration of monosodium glutamate. Subsequently, dispersed hypothalamic cell preparations were shown to secrete ACTH- and β-endorphin-like materials into medium in a nearly linear fashion without any notable change in cellular peptide content. Arcuate area hypothalamic cells incubated in the presence of labeled amino acids were shown to incorporate radioactivity into high molecular weight material containing both ACTH and β-endorphin antigenic determinants. Such high molecular weight material was similar to pituitary POMC with regard to size, apparent isoelectric point, and carbohydrate unit content. Tryptic digestion of the hypothalamic high molecular weight material yielded peptides similar to those derived from tryptic digestion of anterior pituitary POMC. In the labeling studies, the major products were physicochemically similar to β-endorphin and α-MSH. Recently, utilizing a labeled complementary DNA (cDNA) POMC probe, this has been shown to hybridize with mRNA derived from hypothalamus and, to a lesser extent, from amygdala, similar to hybridization with pituitary mRNA (*10*). Similar hybridization has been detected with in situ techniques, demonstrating localization of POMC mRNA within the same arcuate nuclear cells shown immunocytochemically to contain ACTH (*32*).

POMC synthesis in other nonpituitary tissues. The brain is not the only nonpituitary tissue in which POMC-derived peptides have been identified. Other tissues include placenta (*33*), various tissues of the male and female reproductive tract (*25, 34*), gastrointestinal tract (*35*), lymphocytes (*36*), and lung (*37*). Of these, synthesis has been demonstrated only in placenta (*38*) and gastrointestinal tract (*26, 35*), while physicochemical characterization of the component peptides has been reported in testis (*25, 34*). In other instances, identification has been only by immunoassay and immunocytochemistry.

Processing of POMC in nonpituitary tissues. Studies in these different tissues have also yielded information that POMC is differentially processed in the tissues in which it is present (*39*). In anterior pituitary lobe, the precursor molecule is processed to yield ACTH and β-LPH as predominant products, whereas in intermediate lobe these peptides are further processed so that α-MSH-like and β-endorphin-like material predominate. In all extrapituitary tissues studied to date (brain, reproductive tract, placenta), the major proteolytic cleavages are similar to those in intermediate lobe (*31, 33*). In the intermediate lobe, β-endorphin and α-MSH are present predominantly in their acetylated forms (*40*). However, in hypothalamus and reproductive tract, there is little or no acetylation of these forms (*34, 41*). Such differential processing of the same precursor molecule in different tissues enables expression of different activities derived from the same precursor. Acetylated α-MSH is a much more potent melanotropic hormone than is des-acetylated α-MSH, while acetylated β-endorphin has 1/1000 of the opiate binding activity of the unacetylated form (*42*).

Presence of POMC in nonvertebrate species. In keeping with what has already been referred to in the section on "brain peptides in evolution," immunoreactive POMC-related peptides have also been detected in the annelid—earthworm (β- and α-endorphin); in the molluscan—pond snail (ACTH) and octopus (α-MSH); in insecta—the fruit fly (ACTH), locust, and silkworm (α-endorphin) and hover fly (β-endorphin); and the protochordate—sea squirt (β-endorphin). In all of these, detection has been confined to neural elements (*43*), save in the fruit fly, in which reproductive localization was also noted (*44*). ACTH and β-endorphin have also been detected in the protozoan *Tetrahymena pyriformis*. In this instance, these peptides have been further characterized by bioassay and physicochemically. High molecular weight material containing the antigenic determinants for both of these peptides was also identified (*16*).

Other peptides. The originally described tetradecapeptide, somatostatin, is now recognized to be part of a molecule expanded at the NH_2-terminal and consisting of 28 amino acids (SS-28) (*45*). The SS-28 peptide itself is derived from a precursor molecule which also contains a pro-region of 64 amino acids upstream from the SS-28 sequence (*46*). Radiolabeling experiments have demonstrated transfer of radioactivity from high molecular weight material to material corresponding to the SS-14 and SS-28 forms (*47, 48*).

Vasopressin and oxytocin precursors are also present in brain. Characterization of their primary structure by cDNA sequencing has indicated that these contain the peptide neurophysin and a previously uncharacterized glycopeptide (*49*). Radiolabeling studies have indicated that these precursors (present in the magnocellular nuclei) undergo modification during axonal transport to the posterior pituitary to yield the respective peptide and neurophysin (*50*). Evidence for large precursor forms for CCK, thyrotropin-releasing hormone (TRH), and gonadotropin-releasing hormone in brain has also been reported (*51*).

Localization of Brain Peptides

Although it is not possible to present detailed descriptions of the distribution of the numerous neuropeptides described in the central nervous system, several generalizations can be offered (*52*) (see Fig. 4). Some peptides, such as CCK and VIP, have their highest concentrations within cortical areas. Others are present with highest concentrations in the hypothalamus. These include the "hypothalamic-releasing hormones," the POMC family, bombesin, neurotensin, and angiotensin. There are also differences in the distribution of peptide-containing cell bodies. Some peptides are present in cell bodies restricted to one central nervous system area. Exam-

ples of these are arginine vasopressin, oxytocin, and angiotensin, which are localized to the hypothalamic supraoptic and paraventricular nuclei; LHRH, which is found, depending on the species, in the mediobasal hypothalamus or the preoptic area (or both); and the peptides in the POMC family, which are essentially localized to the arcuate nucleus. These peptides have long projection systems throughout the remainder of the central nervous system, such projections accounting for the concentrations of these peptides reported in these other central nervous system areas. In contrast, there are peptides that appear to have cell bodies in multiple areas, with short projection systems, such as substance P, VIP, enkephalin, TRH, neurotensin, CCK, and somatostatin. It is also evident that several peptides can occur in the same nuclear area. For example, vasopressin, oxytocin, angiotensin, CCK, and enkephalin have been described in the supraoptic nucleus; neurotensin, LHRH, and the POMC family have been described within the arcuate nucleus. Multiple peptides are also present in peripheral nerves; that is, substance P, VIP, enkephalin, CCK, and somatostatin are present in vagus, splanchnic, and ischial nerves.

Hökfelt *et al.* (*53*) have demonstrated the coexistence of neuropeptides and neurotransmitters within the same neuron. For example, serotonin has been found together with substance P and TRH in neurons of the medulla oblongata; CCK and dopamine coexist in mesencephalic cells, VIP and acetylcholine within autonomic ganglia, and, very recently, corticotropin-releasing factor (CRF) and vasopressin have been colocalized in cells in the paraventricular nucleus. Recently, Pelletier *et al.* (*54*) have provided evidence for the coexistence of serotonin and substance P within the same dense-core secretory granule. This latter observation raises a major question with regard to the mechanisms of such storage, since neuropeptides and neurotransmitters arrive within secretory granules by different routes; neurotransmitters arrive by an active reuptake process at the synaptic cleft, whereas peptides are synthesized within the cell body and transported to terminals, and packaged in the secretory granules. The physiological significance of the coexistence of neuropeptides and neurotransmitters remains to be explored. Studies with vasoactive intestinal polypeptide and acetylcholine suggest functional interactions (*55*). A marked increase in the affinity of acetylcholine for muscarinic receptors in the cat submandibular gland is produced by physiologically relevant concentrations of VIP (*55*). A VIP antiserum partially blocks the atropine-resistant vasodilation produced by parasympathetic nerve stimulation (*55*).

Functions of Brain Peptides

The functions of peptides in the central nervous system have been assessed by several types of studies. First, the effect of the peptide in question has been observed on a variety of behaviors. The peptide is usually administered intraventricularly or intracerebrally. Since some of the observed events may represent pharmacological rather than physiological effects, studies have been performed observing the behavioral effects of deletion of the action of an endogenous peptide by administration of antagonists or antiserum to the peptide in question. A second type of study has been that of determination of peptide concentrations in central nervous system diseases. This has been done either on postmortem samples of cerebral tissues or in cerebrospinal fluid. Cerebrospinal fluid (CSF), because of its accessibility, might appear to offer a more suitable approach, in view of the problems presented in evaluating peptide concentrations in postmortem specimens obtained when available after death of the patient rather than at a fixed time as in animals. Most such studies, however, have been performed on lumbar fluid; in view of the distribution of peptides throughout the neuraxis, measurement of concentrations in lumbar CSF may reflect release of spinal cord peptides rather than of brain peptides. Furthermore, in the case of those peptides with diffuse central nervous system distribution, there could be alter-

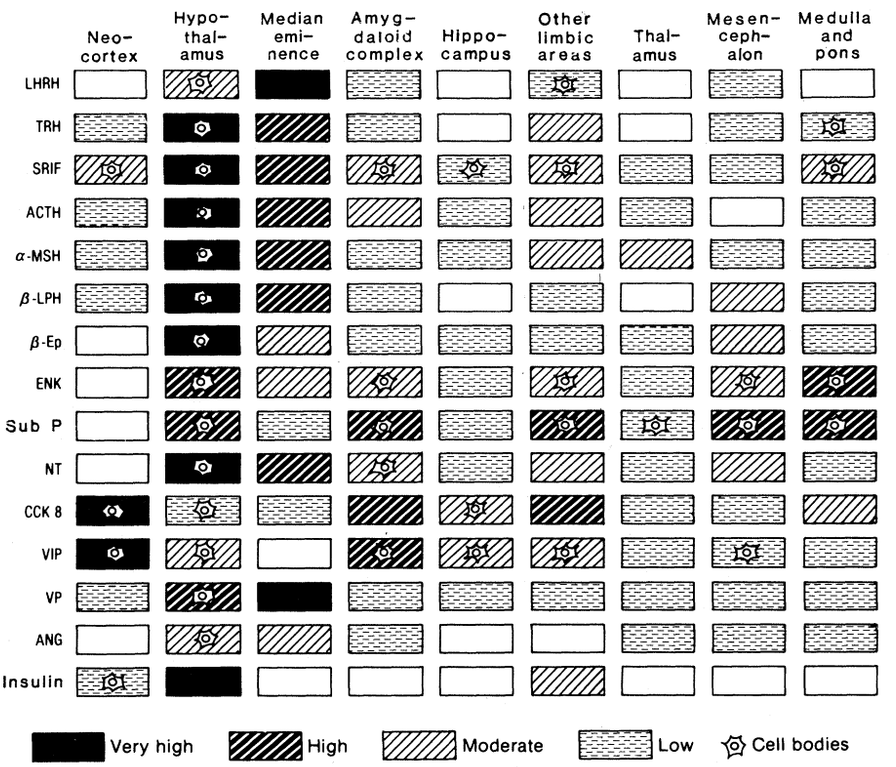

Fig. 4. Relative concentrations of selected neuropeptides in selected central nervous system areas. The amygdaloid complex and hippocampus are considered part of the limbic system. The median eminence refers to a specialized area of the hypothalamus located in the inferior portion of the third ventricle, containing endings of the hypophysiotropic and other neurons in the capillaries of the hypophyseal portal circulation. Also depicted are those areas in which cell bodies containing the designated peptides have been demonstrated immunocytochemically. Data are those of demonstration by various types of criteria. In the instances of the known neurotransmitters, releasing hormones, substance P, and enkephalin, their chemical identity has been established. The presence of the ACTH precursor molecule and other "pituitary" hormones contained therein has been established by immunocytochemical, immunoassay, biosynthetic, and cDNA hybridization techniques. The other peptides cited have thus far been characterized by immunoassay or immunocytochemistry. The data cited for vasopressin, long known to occur in the magnocellular nuclei and the tuberohypophyseal tract, represents that of the demonstration of its more widespread central nervous system distribution. Abbreviations used: *SRIF*, somatostatin; β-*Ep*, β-endorphin; *ENK*, enkephalin; *NT*, neurotensin; *VIP*, vasoactive intestinal polypeptide; *VP*, vasopressin; *ANG*, angiotensin; *ACTH*, adrenocorticotropic hormone; α-*MSH*, α-melanocyte-stimulating hormone; *Sub P*, substance P.

ations in concentration only in a localized area, which would be insufficient to be reflected in the levels determined in CSF.

With these caveats, selected aspects of the effects of brain peptides on several major homeostatic systems are considered below. It will become apparent that each of these systems is affected by multiple neuropeptides, as well as by conventional neurotransmitters. Defining the interactions of these substances, their individual roles in the response of a given system to different types of inputs, as well as elucidating which may be the transmitter employed in the "final common pathway" in a given system, remain challenging questions.

Effects of Peptides on Behavioral Systems

Pain. Peripheral sensory information that is perceived as pain reaches the central nervous system by way of nerves that terminate in the dorsal horn of the spinal cord. Neurons within the dorsal horn send afferent processes to the substantia gelatinosa of the central nervous system and also receive inputs from other neighboring neurons, as well as descending inputs from brainstem centers. Although most reports have stressed an interaction between substance P (which has been shown to be a sensory transmitter released by certain classes of afferent neurons) and enkephalin and related opioid peptides, whose inhibitory effects on dorsal horn neurons may explain their analgesic action, it should be stressed that a number of peptides are present at all the different levels of the pathways subserving pain (*56*). In addition to substance P, somatostatin, VIP, CCK-8, and angiotensin have been demonstrated in primary sensory neurons. Within the dorsal horn, neurons containing enkephalin, substance P, CCK-8, somatostatin, neurotensin, bombesin, and pancreatic polypeptide have been described. Substance P, TRH, enkephalin, oxytocin, vasopressin, and angiotensin are present in the descending supraspinal pathway to the dorsal horn. Interactions of these peptides in pain perception appear to be extremely complex.

Memory, learning, and behavior. Available data suggest that fragments of ACTH and α-MSH (the ACTH sequences 4 to 7 and 4 to 10), which are devoid of hormonal effects, as well as β-endorphin and the enkephalins, facilitate acquisition of avoidance behavior, an effect interpreted as memory formation. In addition, these peptides also can inhibit the extinction of avoidance behavior on stimulus withdrawal, an effect interpreted as persistence of a learned response (*57, 58*). Products of β-endorphin, such as α- and γ-endorphin [β-endorphin (1 to 16) and (1 to 17), respectively], have opposite behavioral effects, α-endorphin being excitatory whereas γ-endorphin is neuroleptic. In some instances, the ACTH(4 to 7)-like peptides and β-endorphin have opposite effects on various types of behavior. Since all of these peptides are derived by processing from the POMC precursor molecule, this raises the question as to whether different types of processing occur in different terminal areas, and whether the different peptides may interact with each other as a local feedback modulatory system.

Vasopressin has also been reported to have similar effects on acquisition and extinction (*59*), although it has been proposed that this peptide is involved in long-term memory, in contrast to the effect of the ACTH fragments, which is postulated to be on short-term memory processes. Oxytocin is reported to have behavioral effects opposite to those of vasopressin (*60*). It has been suggested that the effects of vasopressin are mediated by its interaction with central catecholaminergic systems. Others have interpreted the behavioral effects of vasopressin as secondary to its action on blood pressure regulation, with the central catecholaminergic effects occurring in response to such changes rather than their representing direct central nervous system effects of vasopressin (*57*).

There are preliminary data that the recently discovered CRF may produce central behavioral activation (*61*). The relation of such effects to alterations in brain concentrations of POMC-related peptides and those of vasopressin, presumably by stimulating adrenal medullary catecholamine secretion, remains to be investigated.

Although the preceding studies suggest a role for peptides in cognitive function, the specificity of these responses, the question of their central as compared to their peripheral actions, and their biochemical and physiological bases require further investigation. In clinical studies, there are both positive and negative reports that systemic administration of ACTH(4 to 10) can increase attention and visual discrimination (*58, 62*). Most such studies have been performed in young, healthy volunteers; it may well be that studies in elderly persons whose cognitive functions are disturbed may provide more significant information. Administration of both lysine vasopressin to elderly subjects and a synthetic analog of vasopressin (1-des-amino-D-arginine-8 vasopressin) to young control subjects (*63, 64*) was associated with improved performance in tests measuring long-term memory. Vasopressin has also been reported to improve memory in patients suffering from retrograde amnesia (*65*). Still unexplained is the long duration of the effect of a single dose of vasopressin on the behaviors tested.

Psychiatric disease. Although early studies had suggested that TRH administration might be effective for treating depressed patients, such reports have not been substantiated (*66*). There has been considerable recent interest in the role of endogenous opioids in psychiatric disease. This has been occasioned by the discovery of the endogenous opioid ligands, detection of opiate receptors in the limbic system (an area implicated in emotional behavior), the demonstration of interactions between opioid systems and the monoamine systems which had previously been implicated in psychiatric disease, and reports of behavioral effects of central administration of opioids.

Studies performed in schizophrenic subjects has suggested the presence of both increased and decreased opiate activity. Terenius (*67*) has reported the presence of fractions containing increased opioid-like activity in CSF obtained from unmedicated schizophrenic subjects, such activity being in fractions other than those that would contain β-endorphin and the enkephalins. Such increased levels decreased in some patients who manifested clinical improvement on therapy. There have also been reports in which no alterations in CSF opioid levels were detected in schizophrenic patients. Many reported studies have not adequately characterized the opioid being measured, nor has there been adequate diagnostic definition of the psychiatric subgroup studied.

On the basis of the suggestion that increased opioid activity is present in schizophrenia, naloxone (an opioid antagonist) has been administered therapeutically, with reports of decreased hallucinations following high-dose naloxone administration (*68*). In studies based on the hypothesis that an opioid deficiency exists in schizophrenic subjects, the therapeutic response to intravenous administration of β-endorphin has been studied, with inconclusive results (*69*). De Wied *et al.* (*70*) have suggested that schizophrenics cannot convert β-endorphin to des-tyrosine γ-endorphin, postulated by them to normally function as a neuroleptic agent. Although this group has reported encouraging clinical responses utilizing des-tyrosine γ-endorphin for treatment of schizophrenia, negative results have been reported by others (*71*).

Feeding. Previous studies of central nervous system regulation of ingestive behavior had indicated the presence of a medial hypothalamic satiety center receiving stimulatory serotonergic and inhibitory adrenergic inputs, and a lateral hypothalamic "feeding center" receiving a prominent dopaminergic input. Reciprocal interactions appear to occur between these two areas, characterized by mutual inhibition; that is, activation of one area results in inhibition of the other, whereas suppression of one results in activation of the other. More recently, peptide involvement in the regulation of feeding behavior has been described. For example, various studies suggest that opioids participate in regulation of events that result in ingestive behavior; for example, (i) administration of opioid peptides into discrete central nervous system regions will stimulate feeding (*72*); (ii) higher levels of brain endorphin are present in the obese Zucker rat (*73*); and (iii) fasting is associated with decreased hypothalamic β-endorphin concentrations (*74*). Since β-endorphin can modulate the turnover of central monoamines, which, as noted above, affect feeding behavior, this action may represent the basis for the opiate effect.

A large number of other peptides appear to suppress feeding behavior, perhaps by inhibiting such opioid stimulation. CCK-8, the predominant form of CCK present in brain, and which is also present in intestinal tract, appears to decrease feeding behavior after either its parenteral or central administration (*75*). Other peptides, such as insulin (when administered centrally), bombesin, calcitonin, CRF, and TRH, also depress feeding behavior. Their site of action has been presumed to be within the central nervous system, with controversy over whether their effects are mediated via interactions with catecholaminergic systems. In addition, a number of these peptides have also been described as having effects on glucoregulation; hypoglycemia is known to be a potent stimulus to feeding behavior, and hyperglycemia may be associated with satiety.

The only clinical correlate of these studies has been with regard to CCK-8, for which it has been reported that its peripheral administration decreases food intake in obese males (*76*).

Temperature. Recent studies have implicated a number of brain peptides in the regulation of body temperature (*77*). In many instances, information is lacking as to their specific locus and mode of action (that is, whether they act by way of alteration of the central set-point at which heat loss or conservation is elicited, via activation of efferent thermoregulatory pathways involved in heat production or heat loss, or by altering behavioral mechanisms which involve a choice of alternative thermal environments). Intracranial injection of TRH is associated with hyperthermia (*78*), whereas administration of an antiserum to TRH into the lateral ventricle is associated with a decrease in core body temperature (*79*). These effects of TRH are also observed in hypophysectomized animals, implying a central rather than a peripheral effect secondary to increased thyroid hormone release. Hyperthermia induced by TRH is prevented by indomethacin, suggesting the involvement of endogenous prostaglandins, which are thermogenic. [Such an effect may be mediated via the facilitative mitochondrial calcium uptake produced by prostaglandins, since a decrease in local calcium concentrations elevates core temperature (*80*).] A hypothermic effect of β-endorphin and other opioids has also been reported. Such effects are dose-dependent, high doses producing hypothermia and low doses producing hyperthermia (*81*). Naloxone reverses some of the thermoregulatory effects of β-endorphin, but not of enkephalins. Naloxone can also produce disordered thermal regulation in animals exposed to cold or hot environments, suggesting a role of endogenous opioids in temperature adaptation (*82*). Concentrations of α-MSH in the septum rise during fever (*83*), and central or peripheral administration of α-MSH and ACTH induces hypothermia or produces antipyresis, an effect present in adrenalectomized animals (*84*). Vasopressin release in the septal area after induction of fever has also been demonstrated (*85*). There appear, however, to be species differences regarding the efficacy of vasopressin as an antipyretic agent (*86*).

Blood pressure. There is a considerable body of evidence that, in addition to adrenal, renal, and dietary factors, the central nervous system occupies an important role in blood pressure control. Since all of the components of the renin-angiotensin system appear to be present in brain, a natural area of inquiry was the role of this central system in blood pressure regulation, in comparison to that of its peripheral counterpart. Central administration of angiotensin increases blood pressure, an effect blocked by central angiotensin receptor blockade with saralasin. Intraventricular saralasin, as well as an angiotensin-converting enzyme inhibitor, will decrease blood pressure in the spontaneously hypertensive rat of a stroke-prone strain (*87*). There are no data on possible interactions between the peripheral and the central angiotensin systems. As in all of the other homeostatic systems, opioids have also been implicated in the control of blood pressure. Consideration will be given only to their possible central role, although effects of peripheral opioids, especially the adrenal medullary enkephalins, have also been described. Much of the evidence for a central role of opioids comes from studies in which the opiate antagonist naloxone has been used to reverse the manifestations of various forms of hypotensive shock, such as endotoxic shock, hemorrhagic shock, or spinal shock (*88*). Even more than naloxone, TRH can also reverse the hypotensive manifestations of various forms of shock (*89*). Clonidine, an α-adrenergic agonist which increases central β-endorphin levels (*90*), can reduce blood pressure of spontaneously hypertensive rats (*91*). These findings may have clinical applicability. There are recent reports that continuous naloxone infusion was associated with reversal of hypotension in a ventilator-dependent patient (*87*), and transient increases in blood pressure have been reported after naloxone administration to patients with septic shock (*92*).

Overview of functions of brain peptides. From the foregoing, it is apparent that given behaviors are governed by multiple peptides. The hierarchy of such involvement and the interrelations of the various components affecting a given behavior still remain to be determined. Reports that the multiple peptides involved in the expression of the behavioral patterns associated with egg-laying are all part of a single gene family (*93*) raise the possibility that a similar explanation may exist for some of the multiple peptides involved in a given homeostatic system. For many of the peptides described in brain, their major functional role is unknown, and for some no role has yet been defined. With additional knowledge of the central nervous system, active forms of these peptides (which may differ from their forms in other tissues), their physiological regulation, and the physiological effects of antagonists thereto, new insights should be forthcoming.

Alterations of Concentrations of Brain Peptides in Disease

Alterations in concentrations of brain peptides are being studied for their use as possible markers in a number of neurological diseases. This field is still in its scientific infancy. Nevertheless, interesting findings have been reported to date in two major degenerative neurological diseases of unknown etiology: Alz-

heimer's disease and Huntington's disease (an autosomal dominant hereditary disorder). In Alzheimer's disease, there appears to be a decrease in concentrations of somatostatin within the cerebral cortex in the areas affected by the neuritic plaques and neurofibrillary tangles which are characteristic of this disease, although levels of two other peptides with prominent cortical distribution (CCK-8 and VIP) appear to be normal (*94*). In Huntington's disease, which is associated with marked neuronal loss in the basal ganglia, concentrations of CCK-8, substance P, and enkephalin are reported to be decreased in this area, while somatostatin concentrations are increased (*95*), so that the peptide losses noted do not wholly appear to be a consequence of neuronal loss. CCK receptor binding is also reduced in both the basal ganglia and cortex of patients with Huntington's disease, whereas it is normal in patients with Alzheimer's disease (*91*).

There have also been two clinical reports implicating endorphin in diseases with altered central nervous system function. Naloxone administration was associated with improvement in a syndrome associated with obesity, abnormal temperature control, respiratory depression, decreased pain perception, and alterations in sleep and mood. In this instance, Met-enkephalin and immunoreactive β-endorphin in CSF were normal (*96*). In another case of subacute necrotizing encephalopathy, characterized by periods of prolonged apnea, unconsciousness, hypothermia, and restlessness, such symptoms were reversed on some occasions after naloxone administration. Analysis of CSF demonstrated an increase in uncharacterized opioid-like activity, while determination of opiate concentrations in brain (autopsy performed 26 hours after death) revealed that Met- and Leu-enkephalin concentrations were increased in the cortex but not in other central nervous system areas examined, while β-endorphin concentrations were normal (*98*).

The role of any of these described alterations in peptide concentrations as primary events in the etiologies of the diseases noted, or whether they are secondary to other neurotransmitter disturbances, still requires clarification.

From the foregoing, it would appear that peptides have the potential for therapeutic intervention in diseases characterized by malfunctioning of a number of body homeostatic systems. The observation that multiple neuroactive substances appear to be involved in the regulation of a given system and the observation that a given peptide has multiple effects pose some problems in the development of possible therapeutic agents. Development of analogs that may provide specificity for a given effect, of long-acting agonist derivatives (in view of the very short half-life of the naturally occurring substances), and of modified peptide structures which can circumvent the blood-brain barrier present for a given peptide represent some possible approaches. Development of peptide antagonists may be required in instances in which an action of a given peptide may be excessive.

Conclusion

A large number of peptides are present in the central nervous system, some of which also have been shown to be synthesized within the brain. Some of these peptides have been described previously as occurring in other tissues or in other species, raising questions as to their evolutionary origin and significance. These peptides are believed to function as neurotransmitters, although the mechanism of action for many of these peptides is still unclear, as is their functional role. Their involvement in the regulation of a number of homeostatic systems appear to occur in concert with that of the other previously described neurotransmitters (for example, catecholamines and acetylcholine). Delineation of such interactions should aid in the understanding of the intricacy of central nervous system function. Additional physiological and pharmacological studies are necessary to assess the role of these peptides, both in health and in disease, and to offer therapeutic approaches in a variety of disease states. As stated by Scharrer, "Neurons and endocrine cells specializing in peptide production are not too far apart. The search for general principles has revealed a remarkable degree of unity as well as diversity in the organization of the family of peptidergic neural mediators and their cellular sources. A solid foundation has been created on which future basic and clinical research efforts can be built" (*99*).

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