

- as normal faults in Fig. 3 are actually complex zones with multiple offsets. Some of the faults can be interpreted as high-angle reverse faults (line S-6).
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Brain Peptides as Neurotransmitters

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It is generally agreed that information processing in the brain largely involves communication among neurons through release of neurotransmitters at synapses. In theory, the brain might make do with one excitatory and one inhibitory transmitter. Until the 1960's the amines acetylcholine, norepinephrine, and sero-

represent only 10 percent or less of the total, whose number then may exceed 200.

There has been much debate as to criteria that should be fulfilled before it can be designated a "neurotransmitter." In this article, I regard as transmitters those peptides localized in specific neuronal

addition, analgesia that follows electrical stimulation of the brainstem of rats could be partially reversed by the opiate antagonist naloxone, an indication that a morphine-like substance was being released (2).

To identify the postulated morphine-like factor two approaches were taken. Hughes (3) showed that brain extracts can mimic morphine's effects on electrically induced contractions of smooth muscle in a fashion that is blocked by naloxone. Terenius and Wahlstrom (4) and Pasternak *et al.* (5) identified in brain extracts a substance that competes for opiate receptor binding. The specificity of this effect was established by showing that the marked regional variations in opiate receptor density are paralleled by similar variations in concentrations of the morphine-like substance (1, 3, 5). Naloxone blockade of the morphine-like actions on smooth muscle and a regional distribution closely mimicking that of the opiate receptor ensured that the substance under study was biologically relevant to opiate receptors. Such guarantees of biological relevance are important, as attempts to isolate a substance solely based on its ability to inhibit the binding of a radioactive drug to membranes run the risk that the substance being isolated may not be physiologically meaningful.

Hughes *et al.* (6) isolated the morphine-like substance from pig brain and showed that it consists of two pentapeptides, methionine enkephalin (met-enkephalin) and leucine enkephalin (leu-enkephalin) which differ only in having methionine or leucine at the carboxyl terminal. Using the assay based on competition for receptor binding, Simantov and Snyder (7) isolated the same two peptides from calf brain, confirming the findings of Hughes *et al.* (6). Even before the amino acid sequence of enkephalin was established, we showed by radio-receptor assay that enkephalin was localized in nerve endings, which is consistent with a neurotransmitter role, that its detailed regional distribution in monkey brain closely paralleled that of opiate receptors, and that its phylogenetic distribution was the same as that of opiate re-

Summary. Numerous peptides appear to be neurotransmitter candidates in the brain. Some, such as the opioid peptide enkephalins, neurotensin, and substance P, were first isolated from the brain. Peptides, such as cholecystokinin and vasoactive intestinal polypeptide, were known as intestinal hormones and later recognized as brain constituents. Certain hypothalamic-releasing hormones, pituitary peptides, and blood-derived peptides like angiotensin II and bradykinin, may also be central neurotransmitters. The diversity of localization of these peptides throughout the brain implies a multiplicity of potential roles.

tonin were the only well-recognized transmitters. Then came an appreciation that amino acids such as γ -aminobutyric acid (GABA), glutamic acid, aspartic acid, and glycine, might serve as transmitters. A dramatic explosion in the number of possible neurotransmitters came with increasing recognition in the past decade that various peptides may be neurotransmitters. At present there seem to be about two dozen peptide neurotransmitter candidates, and the number is increasing rapidly (Table 1). Most of the brain peptide transmitters have been discovered serendipitously with no systematic search. It would not be surprising if the known peptide transmitters

systems and released on depolarization, which produce changes in neuronal activity, even though for the most recently identified peptides some of these criteria have not yet been examined.

Opiate Receptor and Enkephalins

In most instances, neurotransmitters are identified as endogenous substances and on this basis their receptor effects are characterized. In the case of the enkephalins, the receptors, which were discovered first, provided a means to identify and then isolate these opiate-like peptides. Dramatic properties of the opiate receptor, such as its discriminating agonists and antagonists and the intimate relation between receptor localization and central sites of pain perception (1) suggested that it might interact with a normally occurring opiate-like substance. In

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ceptors (8). Enkephalin is released in a calcium-dependent fashion with brain depolarization, further supporting a neurotransmitter role (9).

Once the amino acid sequence of enkephalin was known it became evident that the five amino acids (residues) constituting met-enkephalin are contained within the 91 amino acids of the peptide β -lipotropin isolated 10 years earlier from the pituitary by Li (10). Several groups of investigators showed that a variety of lipotropin fragments, all incorporating the met-enkephalin sequence, possess opiate activity (11). Within the pituitary most opiate-like activity can be accounted for by β -endorphin, the 31 amino acid peptide at the carboxyl terminal portion of β -lipotropin, although another recently identified pituitary peptide, dynorphin, also possesses high opiate receptor activity (12). β -Lipotropin itself derives from a 31,000-dalton precursor peptide which also incorporates the sequence of adrenocorticotrophic hormone (ACTH), and hence is referred to as 31K ACTH (13). β -Endorphin occurs in the brain at levels about 10 percent those of enkephalin in specific neuronal systems (14) other than enkephalin neurons and has been reviewed recently along with other pituitary hormones in the brain (15).

Localization of Enkephalin

Neurons and Opiate Receptors

Considerable insight into the functions of neurotransmitters has been attained by light microscopic examination of neuronal systems containing them. Histochemical techniques mapping norepinephrine, dopamine, and serotonin pathways were crucial for our present appreciation of their role in brain function. For peptide neurotransmitters, antibodies have been raised against the peptides themselves usually linked to a carrier protein. Opiate receptors were visualized microscopically prior to the isolation of enkephalin by means of the autoradiographic techniques developed by Kuhar and co-workers (16). Maps localizing opiate receptors and enkephalin neurons coincide fairly closely and involve brain structures whose functions are linked to opiate actions.

Localizations of opiate receptors and enkephalins can explain many of the pharmacological actions of opiates (16, 17). For instance, it appears that small enkephalin-containing interneurons in the dorsal spinal cord synapse on opiate receptors localized to nerve endings of sensory neurons, inhibiting their release

Table 1. Peptide neurotransmitter candidates.

Gut-brain peptides	
Vasoactive intestinal polypeptide (VIP)	
Cholecystokinin octapeptide (CCK-8)	
Substance P	
Neurotensin	
Methionine enkephalin	
Leucine enkephalin	
Insulin	
Glucagon	
Hypothalamic-releasing hormones	
Thyrotropin-releasing hormone (TRH)	
Luteinizing hormone-releasing hormone (LHRH)	
Somatostatin (growth hormone release-inhibiting factor, SRIF)	
Pituitary peptides	
Adrenocorticotropin (ACTH)	
β -Endorphin	
α -Melanocyte-stimulating hormone (α -MSH)	
Others	
Angiotensin II	
Bradykinin	
Vasopressin	
Oxytocin	
Carnosine	
Bombesin	

of sensory "pain" neurotransmitters such as substance P. Enkephalin tracts and opiate receptors in the limbic system, which regulates emotional behavior, may explain euphoric effects of opiates. Respiratory depression, which accounts for lethal effects of opiates, may involve receptors in the solitary nucleus of the brainstem, which regulates visceral reflexes including respiration.

The existence of two distinct enkephalin molecules differing only at the carboxyl terminal raises questions about their disposition. Does the same neuron synthesize both met- and leu-enkephalin or are they contained in distinct neuronal populations? Most antisera to the individual enkephalins fail to discriminate them histochemically. A recently developed antiserum to met-enkephalin adsorbed with leu-enkephalin shows absolute specificity for met-enkephalin, while oxidative destruction of met-enkephalin with permanganate permits selective staining of leu-enkephalin (18). Using these procedures, we showed that met-enkephalin and leu-enkephalin occur in completely separate neurons in brain and intestine (18). The neuronal patterns of met- and leu-enkephalin differ considerably. For instance, in the globus pallidus, leu-enkephalin nerve fibers are disposed as narrow bands surrounding axon bundles while the met-enkephalin fibers are in dense clusters between the leu-enkephalin bands.

Pharmacological and biochemical evidence suggests the existence of multiple populations of opiate receptors (19). In binding studies two distinct receptors

have been identified, mu receptors, with distinct preference for morphine, and delta receptors with selectivity for certain enkephalin derivatives, such as [D-Ala²-D-Leu⁵]enkephalin (Ala, alanine). Differential autoradiographic localizations suggest that the two types of receptors mediate different functions (20). Thus, the mu receptors are preferentially localized to layers 1 and 4 of the cerebral cortex, which (especially layer 4) are involved preferentially in integrating sensory perception. The nucleus accumbens and olfactory tubercle, parts of the emotion-regulating limbic system, are selectively enriched in delta receptors. Enkephalin derivatives with preferential actions at mu receptors are more potent analgesics, suggesting that mu receptors mediate analgesic actions. Perhaps delta receptors preferentially regulate emotional behavior.

My co-workers and I have proposed that the two types of opiate receptors interact, respectively, with met- and leu-enkephalins (20). Accordingly, the two types of enkephalin neurons may be involved, in part, in different brain functions. In support of this notion is the finding that brain regions with more mu than delta receptors, such as the hippocampus and thalamus, also have more met-enkephalin neurons than leu-enkephalin neurons (20, 21). Conversely, the central amygdala, which has more delta than mu receptors, has more leu-enkephalin than met-enkephalin neurons (20, 21). The substantia gelatinosa of spinal cord and the caudate, which have similar levels of mu and delta receptors, also have similar numbers of met-enkephalin and leu-enkephalin neurons (20, 21).

Enkephalin and Opiate Receptor

Interactions

The central question in synaptic transmission is how recognition of a neurotransmitter is translated into an alteration in cell functioning, be it a change in ion permeability or cyclic nucleotide formation. The first generation of receptor studies dealt with the binding or recognition site. Subsequent work has shed light on how recognition of a transmitter alters cellular function and how receptors differentiate agonists and antagonists. Very low concentrations of sodium ion enhance the binding of opiate antagonists and reduce the binding of opiate agonists, thus providing a way to predict whether a test drug is a pure agonist, or has both agonist and antagonist functions (22).

Table 2. Sites for enzymatic cleavage of enkephalins. All the peptide bonds of the enkephalins can be cleaved by one or another known peptidase. Aminopeptidases and carboxypeptidases can remove the NH₂-terminal and COOH-terminal amino acids, respectively, but they have no known selectivity for enkephalins. Enkephalinase A₁, enkephalinase A₂, and angiotensin-converting enzyme display substantial regional variations in mammalian brain which parallel to some extent regional variations in opiate receptor and enkephalin distribution. Enkephalinase B activity does not vary much regionally. The regional distribution of cathepsin C is not well characterized.

Met-enkephalin:	Tyrosine	↑	Glycine	↑	Glycine	↑	Phenylalanine	↑	Methionine
Leu-enkephalin:	Tyrosine	↑	Glycine	↑	Glycine	↑	Phenylalanine	↑	Leucine
	Aminopeptidases		Enkephalinase B		Cathepsin C		Enkephalinase A ₁		
					Enkephalinase A ₂		Angiotensin-converting enzyme		
							Carboxypeptidases		

The actions of sodium at the opiate receptor suggested that sodium permeability is altered in synaptic actions of opiates and enkephalins. Indeed, Zieglansberger and his co-workers showed that opiates block the excitatory effects of glutamic acid and acetylcholine on cerebral neurons by altering sodium channel functioning (23). Peripheral pharmacologic actions of morphine are also regulated by sodium and other ions in parallel with their effects on opiate receptor binding (24).

Receptor interactions of opiate agonists and antagonists are also distinguishable by their reactivity with guanosine triphosphate (GTP). Like sodium, GTP decreases receptor affinity of opiate agonists but not antagonists (25). The GTP effects on numerous hormonal neurotransmitter receptors are associated with linkage of the receptor to adenylate cyclase. Opiates and enkephalins diminish the adenylate cyclase activity and reduce cyclic adenosine monophosphate (cyclic AMP) levels in neuroblastoma clones, which possess specific opiate receptors (26). Thus, cyclic AMP also seems to be a second messenger for enkephalin. Are the sodium and cyclic AMP effects related or are they independent second messengers communicating different kinds of information to the cell? It has been shown that cyclic AMP reduction elicited by opiates is crucially dependent on both extracellular sodium and guanine nucleotides (27).

Sodium also regulates receptors for other neurotransmitters such as alpha-adrenergic, histamine H₁, and muscarinic cholinergic receptors (28). In addition, opiate receptors as well as alpha-adrenergic, dopamine, and histamine H₁-receptors are also regulated by divalent cations with manganese generally being the most effective and calcium relatively inactive (28, 29). Receptor regulation by divalent cations appears associated with regulation by GTP and therefore is prob-

ably also linked to the adenylate cyclase alterations.

The chain of cellular events following opiate receptor stimulation has recently been traced yet another step. Opiate receptor stimulation in neuroblastoma clones reduces synthesis of gangliosides (lipid related molecules on membrane surfaces), while phospholipid, protein, nucleic acid, and other metabolic constituents are not affected (30). Another lipid, cerebroside sulfate, may play a role in opiate recognition at receptors (31).

Enkephalin Metabolism

Most biological peptides are formed by cleavage from larger precursor peptides, just as β -endorphin is formed by cleavage from β -lipotropin. Although met-enkephalin is contained in the amino acid sequence of β -endorphin, it appears unlikely that β -endorphin could be the biological precursor of met-enkephalin, since they are localized in different brain regions. Several attempts have been made to identify large peptides in the brain which generate enkephalin. Labeled amino acids are incorporated into enkephalin in brain slices (32). Large peptides in the brain can be converted into small peptides with enkephalin-like immunoreactivity and opiate-like activity in systems such as the guinea pig intestine (33). Peptides of 6, 7, and 15 amino acids containing enkephalin sequences have been identified in brain and adrenal tissue (34). For example, Kimura *et al.* (35) identified peptides incorporating both met-enkephalin and leu-enkephalin sequences in the adrenal gland of the cow, which contains as much enkephalin as brain. If these putative enkephalin precursors occur also in the brains of rodents, then our finding of distinct neurons that contain met- and leu-enkephalin (18) implies that the enzymes

cleaving enkephalin from its precursor differ in the two types of neurons.

Identification of a specific inactivating system for a neurotransmitter facilitates an understanding of its function. For instance, behavioral effects of drugs that block the inactivating enzyme provide clues to transmitter actions and may afford new therapeutic drugs. Could enkephalin be inactivated by a specific peptidase analogous to the enzymatic hydrolysis of acetylcholinesterase? Like all peptides, enkephalin can be acted on by numerous peptidases (Table 2). Recently, an enzyme activity that cleaves enkephalin between glycine and phenylalanine has been described (36, 37) generating the Tyr-Gly-Gly fragment (Tyr, tyrosine; Gly, glycine), referred to here as enkephalinase A. This membrane-associated enzyme was first reported to have affinity for enkephalin detectable in the nanomolar range, to display regional variations like those of opiate receptors, and to double in activity in morphine-addicted mice (37). Our subsequent studies (38) and those of others (39) showed that the affinity of the enzyme for enkephalin is only 1 percent of that originally reported, and its activity increases only 20 percent in addicted mice. We separated two enkephalinase A activities (A₁ and A₂) in solubilized brain membranes and purified both to homogeneity. We also detected a second peptidase, designated enkephalinase B, which cleaves enkephalin between the two glycines and then gives rise to the Tyr-Gly fragment (38). This enzyme has a similar affinity as enkephalinase A for enkephalin. In addition, we characterized a membrane-associated aminopeptidase that cleaves tyrosine from enkephalin.

Whether any of these enzymes represents a specific synaptic inactivating mechanism for enkephalin is unclear. Enkephalinase A and B have similar affinities for enkephalin, whereas aminopeptidase has somewhat lesser affinity. Although there are regional variations in activity of all three enzymes, the most marked variations occur for enkephalinase A₁ and A₂ and angiotensin-converting enzyme. Initially, it had been suggested that enkephalinase A is identical with the angiotensin-converting enzyme that generates the physiologically active angiotensin II from its inactive precursor angiotensin I (40). Although both enzymes can cleave enkephalin between glycine and phenylalanine, we separated by physical means the two enzymes, indicating that they are distinct entities (38) with different sensitivities to metals, reagents, and enzyme inhibitors (38, 39).

Neurotensin

Neurotensin was identified as a by-product of substance P isolation (41). Besides lowering blood pressure by dilating blood vessels, neurotensin alters pituitary hormone release (41), and, when injected in the brain, lowers body temperature (42). Highest concentrations of neurotensin occur in the hypothalamus and the basal ganglia of the brain; high concentrations are also found in nerve ending fractions of brain homogenates (41, 43). Neurotensin is released in a calcium-dependent fashion when brain slices are depolarized (44). It produces selective inhibition of neuronal firing in areas such as the locus coeruleus, which has a high density of neurotensin neurons (45). Abundant neurotensin receptor binding sites have been demonstrated throughout the brain (46). All of these findings are consistent with a neurotransmitter role.

Histochemical mapping of neurotensin reveals dramatic similarities to the disposition of enkephalin. Indeed, neurotensin localizations bear a closer resemblance to those of enkephalin than other peptides do (47). For instance, as with enkephalin, neurotensin neurons are highly localized to the dorsal gray, substantia gelatinosa region of the spinal cord, implying a role in pain perception; this implication has been confirmed by direct studies revealing neurotensin's potent analgesic effects (48). The neurotensin-elicited analgesia is unrelated to the opiate system, since it is not blocked by opiate antagonists such as naloxone. Drugs that mimic neurotensin might have analgesic properties but lack typical opiate side effects.

The most dense collection of neurotensin cells is in the central nucleus of the amygdala, a distribution similar to that of enkephalin. Another similarity to enkephalin is that a neurotensin neuronal pathway has cell bodies in the central nucleus of the amygdala with axons proceeding through the stria terminalis to terminate in part in its bed nucleus. But although neurotensin and enkephalin have closely similar localizations, they are stored in distinct neurons (47).

Substance P

Next to enkephalin, substance P is the most studied brain peptide. Its localization throughout the brain resembles neurotensin and enkephalin, although the similarities are not as great as between neurotensin and enkephalin. Substance P's role as a sensory transmitter

of pain has been extensively supported. Substance P occurs in 20 percent of dorsal root ganglia cells with some processes extending to the skin and others entering the spinal cord and giving rise to terminals in the substantia gelatinosa (49, 50). Whereas cutting the dorsal root has no influence on spinal cord enkephalin, a similar lesion causes degeneration of substance P terminals in the spinal cord. Removal of the tooth pulp, which contains only pain-sensitive sensory fibers, causes a loss of nerve endings that contain substance P in the trigeminal nucleus of the brainstem, where sensory fibers terminate (51). Since the tooth pulp contains only pain-sensitive fibers, the substance P sensory neurons presumably mediate pain perception. Moreover, spinal cord neurons excited by substance P are the same as those that respond selectively to painful stimuli (52). Substance P sensory fibers may also regulate axon reflexes in the skin. Axon reflexes are responsible for the local vasodilation mediated by sensory nerves that occurs around an injured area. Sir Henry Dale (53) suggested that the vasodilation was elicited by release from sensory nerves of the same transmitter that the primary afferent terminals release in the spinal cord, a proposal consistent with the potent vasodilatory action of substance P.

Since substance P is a "pain" transmitter, the demonstrated blockade of its release in spinal cord by opiates (54) may account in part for opiate analgesia. Interactions between enkephalin and substance P may occur in other parts of the central nervous system, since neurons containing the two peptides are juxtaposed in such areas as the raphe nuclei, the ventral tegmental area, the septum, and the amygdala (49, 50). In the substantia nigra of the brainstem, substance P release is regulated by another transmitter, GABA. Substance P is contained in a long pathway with cell bodies in the corpus striatum and fibers that descend to terminate in the substantia nigra, which contains the highest concentration of substance P in the brain (55). GABA, which occurs in a parallel striatonigral pathway, inhibits the release of substance P that follows the depolarization of substantia nigra slices (55).

Substance P also seems to be closely associated with serotonin, perhaps in regulating pain perception. Areas that are enriched in serotonin as well as substance P and enkephalin include the amygdala, periaqueductal gray, raphe nuclei, and substantia nigra. Stimulation of the nucleus raphe magnus of the brainstem, many of whose neurons contain serotonin, activates a descending sero-

tonin pathway to the spinal cord which in turn elicits analgesia (56). Certain raphe nuclei contain both substance P and serotonin (57), indicating that a single neuron can contain more than one neurotransmitter.

More neuronal pathways have been demonstrated for substance P than for any of the other peptide transmitters. The pathway of sensory neurons that contain substance P and the striatonigral pathway have already been discussed. A substance P-containing pathway with cells in the caudate and terminals in the globus pallidus is analogous to the apparent enkephalin-containing pathway that connects these structures. Cells in the medial habenular nucleus that contain substance P give rise to axons that terminate in the interpeduncular nucleus, paralleling a similar acetylcholine-containing pathway. Within the amygdala, substance P-containing cells in the medial nucleus give rise to terminals in the central nucleus, differing from the amygdaloid disposition of neurotensin and enkephalin, whose cells are in the central nucleus. Substance P cells in the bed nucleus of the stria terminalis project to the medial preoptic area. These pathways within the limbic system may play a role in emotional behavior.

Cholecystokinin and Gastrin

Cholecystokinin (CCK) was originally isolated from the duodenum as a substance that contracted the gall bladder, hence its name. Independently, a duodenal substance that stimulated pancreatic secretion was designated pancreozymin (58). Chemical isolation, sequencing, and synthesis of CCK in classical studies by Mutt and colleagues (59) revealed that it is a 33 amino acid peptide and is identical to pancreozymin. In radioimmunoassay of brain extracts CCK was first mistaken for gastrin (60). Dockray (61) then showed that the immunoreactivity was due to CCK and not gastrin, the cross-reactivity deriving from the fact that gastrin and CCK share the same COOH-pentapeptide sequence at the COOH-terminal. Whereas intestinal CCK largely consists of the 33 amino acid residue peptide, the major CCK entity in the brain is the COOH-terminal octapeptide (CCK-8) with a lesser amount of the COOH-terminal tetrapeptide and very little CCK-33 (62).

True gastrin is contained in the large cell system of the hypothalamus, which projects to the posterior pituitary gland (61). Conceivably, gastrin occurs in a hypothalamic-pituitary pathway sim-

ilar to the one containing enkephalin.

Initial histochemical staining for CCK in rabbit cerebral cortex suggested labeling of the majority of cells as well as much white matter (63). Quite different results were obtained in the rat and guinea pig (64). Both rat and guinea pig cerebral cortex contain many CCK cells and fibers, coinciding with the high levels of CCK in the cerebral cortex. CCK and vasoactive intestinal peptide (VIP) are the only brain peptides with cells in the cerebral cortex. Because of the considerable mass of the cerebral cortex, the total brain content of CCK, about 1 to 2 milligrams in the human, is far greater than that of any other peptide. CCK is a rapid and powerful excitant of cerebral cortical cell firing (65).

The most prominent group of CCK cells in the brain occurs in the periaqueductal gray area of the brainstem (64), where CCK might be involved in the pain-integrating functions of this region. Like substance P, CCK occurs in sensory fibers with cell bodies in dorsal root ganglia and terminals in the dorsal gray matter of the spinal cord (64). As with substance P, neurotensin, and enkephalin, CCK cells are abundant in the hypothalamus, whereas the central nucleus of the amygdala has a dense collection of CCK fibers but no cells (64).

Just as substance P coexists with serotonin in some neurons, CCK appears to coexist with dopamine not in the substantia nigra neurons, which project to the caudate nucleus, but in brainstem dopamine neurons, which project to the limbic systems (66). This neuronal system is implicated in the actions of antischizophrenic neuroleptic drugs, suggesting a role for CCK in schizophrenia.

Recently CCK receptor binding has been identified in the pancreas (67, 68) and brain (68). In the pancreas the peptide specificity of binding sites corresponds to relative CCK-like biological potencies in the pancreas, with pentagastrin, the COOH-terminal pentapeptide of CCK, having no activity. By contrast, at the brain receptors, pentagastrin and CCK-4 are highly potent (68). The characteristic CCK receptor binding sites in the brain may therefore not represent receptors for the CCK-8 or CCK-33, the major forms in brain, but for CCK-4 (the COOH-terminal tetrapeptide of CCK), which has recently been described in specific brain areas and is quite distinct from the localization of CCK-8 (69).

Cholecystokinin is a good example of an intestinal hormone subsequently shown to occur in the brain. A possible link between the intestinal and central

activities of CCK is suggested by findings that very low doses of CCK-8 injected intraperitoneally cause satiety in previously hungry rats (70). The CCK-8 satiety is elicited by lower doses when injected peripherally than when administered directly in the brain (70). Where might the peripheral "satiety receptor" for CCK be located? The vagal nerves of the liver or stomach are reasonable candidates. Evidence for a central influence of CCK in regulating fluid intake derives from experiments showing that as little as 0.01 picomole of CCK-8 per minute infused into the lateral ventricles of sheep suppress feeding behavior (71).

Vasoactive Intestinal Polypeptide

Vasoactive intestinal polypeptide was identified in extracts of the gut as a substance that causes vasodilation. It was isolated as a 28 amino acid peptide with many similarities in amino acid sequence and biological activity to the intestinal peptides secretin and glucagon (72). Thus, besides causing vasodilation, VIP stimulates the conversion of glycogen to glucose, enhances lipolysis and insulin secretion, inhibits the production of gastric acid, and stimulates secretion by the pancreas and small intestine (72).

Seven years after its isolation from the intestine, VIP was demonstrated in the brain (73). Like CCK, highest levels of VIP occur in the cerebral cortex, although cortical VIP levels are only one-tenth those of CCK. VIP appears to be contained in small neurons within the cerebral cortex since severing connections between the cerebral cortex and the rest of the brain does not lower cortical levels of VIP. The VIP nerve terminals are also localized in the central amygdaloid nucleus and in the medial preoptic and anterior hypothalamic nuclei (74).

In the cerebral cortex, VIP and CCK neurons are bipolar and oriented perpendicular to the surface. This pattern makes these peptidergic neurons ideally suited to activate and synchronize neuronal activity within the vertical columns of cerebral cortical cells. Like CCK, VIP is a potent and rapid neuronal excitant in the CCK-enriched hippocampus, is stored in vesicles, and is released with neuronal depolarization.

Bradykinin

Enkephalin, neurotensin, substance P, CCK, and VIP all fit in the group of brain-gut hormones with distributions

essentially restricted to the intestine, its associated organs, and the central nervous system. Bradykinin presents a different pattern. It was discovered 30 years ago as a biologically active factor released from α_2 -globulin fractions of blood by incubation with trypsin or snake venom, and called bradykinin because of the slow "brady" contractions it elicited from the guinea pig ileum (75). Being generated in the circulation, bradykinin acts (as would be expected) in many parts of the body and has been implicated in the genesis of inflammation, cardiovascular shock, hypertension, pain, and even rheumatoid arthritis. Bradykinin is probably the most potent pain-producing substance known. Its release by tissue damage and action at specific receptors on sensory neurons has been suggested as a first step in pain perception. Interestingly, when injected near the periaqueductal gray of the brainstem, bradykinin has potent analgesic effects; in the lateral septal area, bradykinin injections increase blood pressure (76).

Immunohistochemical studies have revealed the location of bradykinin neurons (77). The principal group of bradykinin cells occurs in the hypothalamus with fibers throughout the hypothalamus as well as in the lateral septal area and periaqueductal gray. The septal bradykinin neurons might participate in the regulation of blood pressure, which would be consistent with the hypertensive effects of injections there, whereas bradykinin neurons in the periaqueductal gray might have a role in pain perception.

Angiotensin

The angiotensin story is probably older than that of almost any biologically active peptide. In 1898, the kidney was shown to contain a proteolytic activity, renin, which converts a large peptide in blood plasma, angiotensinogen, to the decapeptide angiotensin I (78). Angiotensin I is almost devoid of biological activity in the periphery and the brain. It is "activated" by angiotensin-converting enzyme which removes a COOH-terminal histidyl-leucine (His-Leu) to form angiotensin II. Most of the peripheral actions of angiotensin II center around the regulation of the circulatory system. Angiotensin II is a potent vasoconstrictor and causes renal sodium retention by stimulating aldosterone secretion from the adrenal cortex.

Interestingly, the effects of centrally administered angiotensin II are complementary to its peripheral actions.

Central injections of angiotensin II potently stimulate drinking behavior and raise blood pressure (79). Regional microinjections have localized the dipsogenic effects of angiotensin II to the subfornical organ where application of angiotensin II activates neuronal firing and enhances drinking behavior (80).

The striking central actions of angiotensin suggest that endogenous angiotensin should exist in the brain. The brain does contain renin-like activity as well as considerable angiotensin-converting enzyme activity. Attempts to isolate endogenous angiotensin itself have indicated extremely low levels and these amounts may not in fact represent authentic angiotensin (81). In contrast to the very low levels of apparent endogenous angiotensin, the brain possesses abundant angiotensin receptor binding higher than in any other tissue of the body (82).

Immunohistochemical studies have revealed neuronal systems that stain with antiserum to angiotensin (83). Whether this material represents authentic chemical angiotensin is unclear. Some cells stain in the paraventricular and perifornical area of the hypothalamus, while dense terminal patterns are observed in the substantia gelatinosa of the spinal cord, the spinal cord, the spinal nucleus of the trigeminal nerve, the central amygdala, locus coeruleus, and the periventricular gray. This pattern resembles that of substance P, neurotensin, and enkephalin. Dorsal root lesions reduce the angiotensin staining in the dorsal spinal cord, suggesting that the angiotensin-like material may be contained in sensory neurons.

Hypothalamic-Releasing Factors

The hypothalamic-releasing factors were identified as agents that are contained within the median eminence of the hypothalamus and pass through the portal capillaries to the pituitary gland where they regulate the synthesis and release of pituitary hormones. Immunohistochemical procedures have shown that most of these factors are fairly widely distributed outside of the hypothalamus.

Thyrotropin-releasing hormone (TRH) was the first of these factors to be isolated and has now been identified as the tripeptide pyroglutamylhistidylprolinamide (84). In the rat brain, 80 percent of TRH occurs outside the hypothalamus (85). Immunohistochemical studies reveal TRH-containing fibers in many areas of the brain, including motor nuclei

of the trigeminal, facial, and hypoglossal nerves, the ventral spinal cord, the nucleus accumbens, the lateral septal nuclei, and the bed nucleus of the stria terminalis (86). We identified specific TRH receptor binding in numerous brain regions with receptor specificity essentially the same as for pituitary receptors (87). TRH elicits behavioral excitation and anorexia in animals and may cause mood enhancement in humans (88).

Somatostatin is a hypothalamic, cyclic peptide consisting of 14 amino acids; it inhibits the release of growth hormone from the pituitary gland (89). In addition, it blocks the release of pituitary thyrotropin (TSH) and prolactin. Apart from its role as a hypothalamic release inhibiting factor, somatostatin appears to be a typical "gut-brain" peptide, being localized both to neurons throughout the brain and the stomach, intestine, and pancreas (50, 90); it inhibits the secretion of glucagon, insulin, and gastrin (91). Somatostatin-containing cells occur in the amygdala, parts of the hypothalamus, the hippocampus, and the cerebral cortex with terminals located in all of these areas as well as the caudate nucleus, nucleus accumbens, and the olfactory tubercle (50, 90). Somatostatin also occurs in about 20 percent of primary sensory neurons as well as dorsal root ganglion cells. Like substance P, somatostatin in these unmyelinated sensory neurons may be a major transmitter of pain sensation.

Luteinizing hormone-releasing hormone (LHRH), a hypothalamic decapeptide, stimulates the secretion of both luteinizing and follicle-stimulating hormones from the anterior pituitary (92). There is less evidence for a widespread distribution of LHRH than for TRH or somatostatin outside the hypothalamus or brain. LHRH is contained within the pre- and suprachiasmatic and arcuate nuclei of the hypothalamus (93). It is not clear which of these LHRH systems specifically regulates pituitary secretion. Most LHRH nerve fibers outside the hypothalamus probably originate from hypothalamic cell bodies, although studies of lesions separating the hypothalamus from the rest of the brain have been ambiguous (94). Some LHRH fibers project to the organum vasculosum of the lamina terminalis, a portion of the central nervous system outside of the blood brain barrier, the suprachiasmatic nucleus, the mammillary bodies, and the ventral tegmental area. Some LHRH neurons in the medial septal area, diagonal band, and olfactory tubercle seem to contact blood vessels rather than other neurons. Conceivably, these neurons regulate blood supply.

Insulin and Glucagon

While all the peptide transmitters discussed above are relatively small molecules, generally with fewer than 20 amino acids, insulin is substantially larger, a protein comprising 86 amino acids. Insulin secreted from the pancreas does not penetrate into the brain. Recent evidence reveals that the brain possesses its own insulin (95). Radioimmunoassay reveals that insulin concentrations in the brain are 10 to 100 times higher than in plasma. The immunoreactive insulin is biologically active at receptor binding sites and in stimulating glucose oxidation in fat cells. Highest brain insulin concentration (80 nanograms per gram) occurs in the hypothalamus with similar levels in the olfactory bulb.

What might be the role of insulin in the brain? Could such a large molecule function as a neurotransmitter? The possibility that insulin has some neurotransmitter or neuromodulator function is suggested by the demonstration in brain of specific insulin receptor binding sites (96). Properties of insulin receptors in the brain are essentially the same as those of peripheral insulin receptors.

Glucagon is a pancreatic hormonal peptide of 29 amino acids. A much larger form (12,000 daltons) of glucagon has recently been identified in the intestine (97). Whether or not this is a precursor of pancreatic glucagon is unclear. Intestinal glucagon, also referred to as glicentin, has been detected in brain tissue (98). Intestinal glucagon may represent another very large peptide that serves as a neurotransmitter candidate. Support for this possibility comes from histochemical studies in our own and other (99) laboratories, demonstrating central glucagon-containing neurons. We have detected a dense plexus of glucagon-containing nerve fibers in the hypothalamus, but have not yet localized cell bodies.

Other Peptides

The posterior pituitary nonapeptides, vasopressin and oxytocin, were the first peptides isolated from the brain. Vasopressin is the pituitary's antidiuretic hormone, and oxytocin plays a role in regulating uterine contraction and milk ejection. Vasopressin- and oxytocin-containing cells, which project to the pituitary, are localized in the supraoptic and paraventricular nuclei of the hypothalamus. Besides innervating the pituitary, they may give rise to small recurrent collateral neurons that make connections with the vasopressin and oxytocin cells of the

hypothalamus (100). These peptides are stored along with carrier hormones referred to as neurophysin I, which binds oxytocin, and neurophysin II, which binds vasopressin. Neurophysin-containing pathways have been traced histochemically from the hypothalamic nuclei to various autonomic centers in the brainstem and the spinal cord (101). In the brainstem these fibers project to the locus coeruleus, parabrachial nucleus, Edinger-Westphal nucleus, various vagal nuclei, the septum, the subfornical organ, the bed nucleus of the stria terminalis, and the medial nucleus of the amygdala. Although they have similar pathways, oxytocin and vasopressin neurons are physically distinct. Oxytocin and vasopressin synthesis has been traced by injecting radioactive precursor amino acids into the supraoptic and paraventricular nuclei and then by following incorporation into the peptides at different points in the hypothalamic-pituitary pathway (102).

Behavioral studies suggest a role for vasopressin in learning and memory. DeWied and Versteeg showed that impaired learning behavior in Brattleboro rats, which lack vasopressin-synthesizing mechanisms, can be reversed by intraventricular injections of vasopressin (103). In normal rats, peripheral vasopressin injections influence learning and memory, and in clinical trials vasopressin may improve memory in brain-damaged human subjects.

The dipeptide carnosine (β -alanylhistidine) is the smallest of the neurotransmitter candidate peptides in the brain. It is highly concentrated in the primary olfactory pathway, which passes from the nasal epithelium to the olfactory bulb (104). Destroying the nasal epithelium or severing the olfactory nerve dramatically decreases carnosine levels and the activity of the carnosine-synthesizing enzyme in the olfactory bulb. Moreover, stereospecific and saturable carnosine binding has been demonstrated in the olfactory bulb. Unlike most other brain peptides, which are presumably synthesized as large precursors by ribosomal mechanisms, carnosine is formed in a single enzymatic step. The olfactory bulb appears to be the only area of the central nervous system that contains high levels of carnosine ($10^{-3}M$), which are ten to a hundred times higher than levels in any other part of the brain.

Bombesin is a 14 amino acid peptide that has been isolated from frog skin (105). In mammals, bombesin influences the gastrointestinal tract, stimulating secretion of gastrin and gastric acid, mimicking cholecystokinin effects on the gall

bladder, stimulating enzyme secretion from the exocrine pancreas, and altering intestinal motility. Radioimmunoassays with antibodies to bombesin have revealed bombesin-like material in the gastrointestinal tract, the lung, and brain (106). However, in the brain the material reacting with the bombesin antibodies is chemically distinct from authentic bombesin. High affinity binding of bombesin to brain membranes has been reported; the regional distribution of this bombesin binding differs considerably from that of immunoreactive bombesin (107). When injected intraventricularly in rats, bombesin quite potently lowers body temperature. Thus, whether bombesin or a related peptide has a biological role in the brain is as yet unclear.

In summary, rapidly increasing numbers of peptides seem likely to have neurotransmitter-related roles in the brain. Detailed investigation over many years of individual neurotransmitters, such as norepinephrine and dopamine, has revealed links to normal and abnormal behavior and facilitated development of major psychotropic drugs. The properties of any one of the peptides discussed in this article are as potentially "interesting" as those of norepinephrine or dopamine. The characterization of most of these peptides is just commencing. Development of drugs with selective effects on peptide disposition awaits clarification of the biosynthesis and inactivation of most of the peptides. The ability to identify specific receptor binding sites for several peptides provides a simple means to screen for drugs that might mimic or block the effects of peptides. Thus, in addition to enhancing our understanding of brain function, it is likely that further studies of peptide neurotransmitters may result in important therapeutic applications.

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 108. Supported by PHS grants MH-18501, DA-00266, and DA-01645 and grants of the McKnight and John A. Hartford Foundations. I thank the following individuals for advice and for providing unpublished data: C. Gorenstein, V. Mutt, G. Aghajanian, S. Watson, J. Kelly, A. Blume, T. Hökfelt, and L. L. Iversen. I particularly thank P. Emson for advice and access to the manuscript form of his review, in *Prog. Neurobiol.* **13**, 61 (1979).