Kirk-Othmer Encyclopedia of Chemical Technology.

HORMONES, BRAIN OLIGOPEPTIDES

Neuropeptides represent the most numerous of the classes of neurotransmitters and neuromodulators (see Neuroregulators). There are at least 50 oligopeptides known to exist in the brain (1, 2), and the total list of oligopeptide neurotransmitter candidates may eventually be significantly greater. The clinical impact of these peptides on brain function, however, has been limited because most peptides are not metabolically stable, may not cross the blood–brain barrier, or there may be no specific antagonists available to test the normal functioning of a peptide system. The development of novel analogues which satisfy many of these concerns represents a significant potential. Central nervous system (CNS) disorders involving Alzheimer's disease, appetite control, depression, and analgesia are all potentially affected by drugs acting at specific neuropeptide systems. The cloning and sequencing of the receptors for these peptides and modeling of their binding sites should be important components in the design of such drugs.

Despite their relatively large number, brain oligopeptides represent the latest class of substances to be identified as neurotransmitter candidates. Although historically there has been great interest in the identification and purification of pituitary hormone-releasing factors from hypothalamic extracts (3–6), most of that interest has been focused not on their possible roles as neurotransmitters but instead on their endocrine functions. The first genuine neuropeptide to be isolated from the brain was the 11-amino acid peptide substance P, first identified in 1931 in equine intestine and brain extracts (7) and much later identified in the brain (8). The subsequent discovery that substance P [33507-63-0] plays a crucial role in the processing of pain information (9) clearly establishes its role in brain function. The field of neuropeptides exploded upon identification from porcine brain of the two pentapeptides methionine enkephalin [58569-55-4] and leucine enkephalin [58822-25-6] as endogenous opioid peptides (see Opioids, endogenous) (10). Not only did this discovery provide the key finding that neuropeptides may have significant pharmacological actions, but the existence of specific opioid antagonists, such as naloxone [465-65-6], meant that the normal function of such neuropeptide systems in the brain could be determined by their pharmacological blockade. The synthesis of peptide antagonists has greatly aided in the development of knowledge of other neuropeptide systems as well.

Neuropeptides represent potentially the most diverse group of neurotransmitters, and their potential roles in normal and diseased brain function are wide-ranging. Many oligopeptides identified in the central nervous system exert both gastrointestinal and behavioral effects and also modify pituitary function (11–16). Within the brain, almost every possible behavioral action has been associated with some type of neuropeptide. Moreover, many neuropeptides have been associated with several CNS disease states. Opioid peptides and substance P have been associated with pain states, neurotensin [39379-15-2] has been associated with schizophrenia and the actions of psychotropic drugs, and corticotropin-releasing factor [9015-75-8] (CRF) has been associated with clinical depression. All neuropeptides are distributed in discrete areas of the brain and many are strategically located to mediate many of these effects. One important aspect of neuropeptide localization is colocalization, ie, the finding that many neuropeptides are stored and released along with other peptide and nonpeptide neurotransmitters (17–20). For this reason, neuropeptides have often been termed neuromodulators because they may act to modulate the effects of other neurotransmitters.

Many neurotransmitters are remarkably potent, both *in vivo* as well as in binding to their respective receptors. Many peptides exhibit K_D, dissociation constant, values at their receptors at less than 1 n*M,* which is an affinity several orders of magnitude higher than that observed for many classical nonpeptide neurotransmitters. Most neuropeptide receptors belong to the superfamily of G-protein-coupled receptors. Thus the immediate biological response in the cell to the binding of the peptide to its receptor is the production of an intracellular second messenger system, usually either cyclic adenosine monophosphate (cAMP) or phosphoinositide turnover.

1. Analysis

Neuropeptides are assayed either by bioassays, including receptor binding assays, which measure the biologically active material as opposed to inactive precursors, or by immunological assays, eg, radioimmunoassays or immunocytochemical assays, which utilize specific antibodies generated against native peptides or against fragments of peptides (see Immunoassay). Although the latter assays have predominated since the early 1970s because of their relative simplicity and specificity, these are limited by the specificity of the antibodies used, and also may provide false-positive values based on cross-reactivity of antibodies against inactive precursors. For localization of neuropeptides, the technique of immunocytochemical localization (21) has been most useful, although this methodology may suffer from a precise identification of the peptides being measured. Another important technique is *in situ* localization of messenger ribonucleic acid (mRNA) products. The biosynthesis of neuropeptides generally occurs through breakdown from high molecular protein precursors, and these precursors can be identified by specific probes directed against their mRNA sequences. Such probes can be extremely specific for their respective mRNA sequences and this method provides an extremely specific view of a particular peptidergic system. A disadvantage is that the *in situ* technique detects only mRNA levels of inactive precursors, and the resulting localization may not match that of the final active peptide fragments.

2. Synthesis and Biosynthesis

2.0.1. Synthesis

In contrast to pituitary hormones, which usually can be obtained in pure form only after extraction from animal tissues, brain oligopeptides are readily available because of their small size. The synthetic replica represents the most economical and readily accessible source for the oligopeptides. Two techniques are available for laboratory synthesis of oligopeptides, ie, solution chemistry and solid-phase peptide synthesis (SPPS). These methodologies have been reviewed (22). In both methods, synthesis involves assembly of protected peptide chains, deprotection, purification, and characterization. However, the solid-phase method, pioneered by Merrifield, dominates the field of peptide chemistry (23). In SPPS, the C-terminal amino acid of the desired peptide is attached to a polymeric solid support. The addition of amino acids (qv) requires a number of relatively simple steps that are easily automated. Therefore, SPPS contains a number of advantages compared to the solution approach, including fewer solubility problems, use of less specialized chemistry, potential for automation, and requirement of relatively less skilled operators (22). Additionally, intermediates are not isolated and purified, and therefore the steps can be carried out more rapidly. Moreover, the SPPS method has been shown to proceed without racemization, whereas in fragment synthesis there is always a potential for racemization. Solution synthesis provides peptides of relatively higher purity; however, the addition of hplc methodologies allows for pure peptide products from SPPS as well.

Several improvements have been made in the original SPPS methodology which have extended the general usefulness of this technique and enabled routine synthesis of peptides 50 amino acids in length (22). Longer

peptides also have been reported using this technique. Although the original SPPS technique allowed for synthesis of only one peptide at a time, more recent developments have allowed multiple concurrent syntheses. For example, a method synthesizing peptides permanently bound to solid supports has been reported (24). Such peptides then undergo enzyme immunosuppressant assays (elisa). Another variation of SPPS has been described in which the solid support is contained within solvent-permeable packets. This is often referred to as the T-bag method (25) and it allows concurrent synthesis of extremely large numbers of different peptide sequences (26). It has become useful (ca 1993) in construction of extremely large peptide libraries.

The use of SPPS allows synthesis of both native oligopeptides and important synthetic analogues. Many of these fragments or substituted analogues have been found to be equal to, or more potent than, the parent molecule and to exhibit long-acting and antagonistic activities.

2.0.2. Biosynthesis

The biosynthesis of neuropeptides is much more complex and involves the multistep process of transcription of specific mRNA from specific genes, formation of a high molecular weight protein product by translation, post-translational processing of the protein precursor to allow for proper packaging within the cell, and final enzymatic cleavage to produce the active peptide product (2). The latter processing step is summarized by the schemes in Figure 1, which illustrates the precursors of several oligopeptides, including CRF, β-endorphin/ACTH/MSH, tachykinins, enkephalin, CGRP, and dynorphin. An example of this process is the ACTH/MSH/endorphin precursor proopiomelanocortin [78321-08-1] (POMC). Synthesized in the pituitary, this 31,000 mol wt protein is heavily glycosylated in several species before it is cleaved into at least three different classes of active peptide, ie, ACTH, β-endorphin, and three different variants of MSH (see Fig. 1). Like most precursors, the active peptides from POMC are hydrolyzed from the precursor by trypsin-like enzymes which cleave between double-basic amino acid residues, including Lys—Lys, Arg—Arg, Lys—Arg, and Arg—Lys. These cleavages result in at least four active oligopeptide products from POMC, including γ-MSH, ACTH, β-MSH, and β-endorphin (Fig. 1).

Peptide biosynthesis is affected by regulation of mRNA synthesis (27). For example, levels of some neuropeptides are increased by cyclic AMP, which stimulates gene expression through a specific promoter element known as the cyclic AMP responsive element (CRE). A CRE was first identified with the nucleotide sequence TGACGTCA in the somatostatin gene (28). Other neuropeptide genes contain homologous CRE sites. In addition, the AP-1 site on many peptide genes is activated by the product of the proto-oncogene *c-jun* and probably mediates protein kinase C-activated synthesis (27).

3. Individual Brain Oligopeptides

Table 1 lists neuropeptides, along with their common acronyms, CAS Registry Numbers, and molecular weights. The amino acid sequences of these neuropeptides are known. Although many peptides contain free amino- and carboxy-terminals, some peptides contain altered amino acids and termini. Particularly common is the presence of an amidated carboxy-terminal. In every case, this is caused by enzymatic decarboxylation of a carboxy-terminal glycine residue by glycine-converting enzyme. Other amino acids are also altered enzymatically, eg, in cholecystokinin, Tyr^{27} is sulfated. In thyrotropin-releasing factor (TRF), the amino-terminal Gln residue has been converted into a pyro-Glu. Many synthetic analogues also contain altered amino acids, including D-amino acids. Such alterations may increase potencies, change pharmacological properties, eg, antagonists vs agonists, or increase stability to enzymatic breakdown.

A summary of the properties of some of these neuropeptides is available herein; other neuropeptides have been reviewed (1, 2, 19, 29–32).

Table 1. Names and Properties of Selected Brain Oligopeptides

Peptide[a]	Acronym[b]	CAS Registry Number	Species	Molecular weight
adrenocorticotrophic hormone	ACTH	[9002-60-2]	human	4538
angiotensin				
angiotensin I		[9041-90-1]	human	1296
angiotensin II		[11128-99-7]	human	1046
bombesin		[31362-50-2]		1619
bradykinin		[58-82-2]		1060
calcitonin gene-related peptide	CGRP	[83652-28-2]	human	3787
cholecystokinin	CCK			
CCK-33		[93443-27-7]	human	3950
CCK-8				1149
dynorphins				
dynorphin-A	DYN A	[88161-22-2]	porcine	2146
dynorphin-B	DYN B	[85006-82-2]	porcine	1570
α-neo-endorphin	α-NEO END	[69671-17-6]	porcine	1228
endorphins				
β-endorphin	β-END	[59887-17-1]	human	3463
α-endorphin		[59004-96-5]		1745
enkephalins				
Leu-enkephalin	L	[58822-25-6]		555
Met-enkephalin	M	[58569-55-4]		573
galanin		[88813-36-9]	human	3156
gastrin		[9002-76-0]	human	2097
hypothalamic-releasing factors				
corticotropin-releasing factor	CRF[c]	[9015-71-8]	human	4754
growth hormone-releasing factor	GRF[d]	[83930-13-6]	human	5037
luteinizing hormone- releasing hormone	LHRH[e]	[9034-40-6]		1182
somatostatin	SRIF[f]	[51110-01-1]		1637
thyrotropin-releasing factor	TRF[g]	[24305-27-9]		362
melanocyte-stimulating hormone	MSH			
αMSH		[37213-49-3]		1664
β-MSH		[9034-42-8]	human	2659
γ-MSH		[72711-43-4]		1570
neurotensin		[39379-15-2]		1672
neuropeptide Y	NPY	[82785-45-3]	human	4269
peptide YY		[106388-42-5]	human	4307
secretin		[1393-25-5]	human	3038
tachykinins				
substance K	SK	[102577-24-2]		1133
neurokinin B[h]		[102577-19-5]		1210
substance P	SP	[33507-63-0]		1347
vasoactive intestinal peptide	VIP	[40077-57-4]	human	3324
vasopressin	ADH	[11000-17-2]		1083

[a] Alternative nomenclature for hypothalamic releasing factors are indicated.
[b] See Figure 1.
[c] Corticotropin-releasing factor (CRF) = corticoliberin.
[d] Growth hormone-releasing factor (GRF) = growth hormone-releasing hormone (GHRH) = somatoliberin.
[e] Luteinizing hormone-releasing hormone (LHRH) = gonadotropin − releasing hormone (GnRH) = gonadoliberin − luteinizing hormone-releasing factor (LRF) = luliberin.
[f] Somatostatin (SS) = somatotropin − releasing inhibiting factor (SRIF).
[g] Thyrotropin-releasing factor (TRF) = thyrotropin − releasing hormone (TRH) = thyroliberin.
[h] Neurokinin B = neuromedin K.

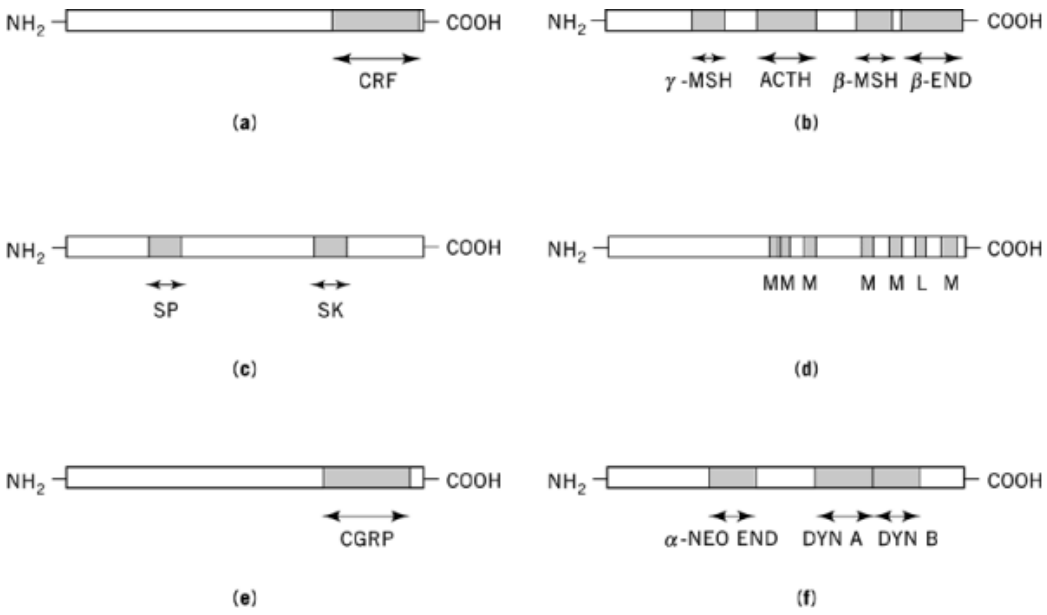

Fig. 1. Schematic drawing of precursors for selected brain oligopeptides. Shaded areas represent the location of sequences of active peptide products which are normally cleaved by trypsin-like enzymes acting on double-basic amino acid residues. Precursors are not necessarily drawn to scale. (**a**) CRF precursor; (**b**) proopiomelanocortin (POMC); (**c**) β-protachykinin; (**d**) proenkephalin A; (**e**) CGRP precursor; (**f**) preprodynorphin, ie, preproenkephalin B. Terms are defined in Table 1.

4. Tachykinins

The group of peptides known as tachykinins include substance P, substance K or neurokinin A, and neuromedin K, ie, neurokinin B, as well as a number of nonmammalian peptides. All members of this family contain the conserved carboxy-terminal sequence Phe-X-Gly-Leu-Met-NH_2, where X is an aromatic, ie, Phe or Tyr, or branched aliphatic, eg, Val or Ile, amino acid. In general, this C-terminal sequence is crucial for tachykinin activity (33); in fact, both the methionineamide and the C-terminal amide are crucial for activity. The nature of the X residue in this sequence determines pharmacological identity (34, 35); thus the substance P group contains an aromatic residue in this position, while the substance K group contains an aliphatic residue (33).

Substance P was the first neuropeptide to be discovered. It was originally detected in the acid—alcohol extracts of equine brain and intestine (7). Its role in the brain was confirmed by isolation and sequence of substance P from bovine hypothalamus (8, 36). Although other members of the tachykinin family have been identified in nonmammalian species (37), it was assumed for years that substance P was the only tachykinin in the brain. That assumption was invalidated in the early 1980s when substance K and neuromedian K were isolated from brain extracts (38–41). The identification of these other tachykinins in the brain not only broadened the potential role of this family as neurotransmitters but also confounded many previous localization studies, because it is likely that some of the antisera used in earlier studies may have cross-reacted with these other tachykinins (33). Nevertheless, the highest density of substance P in the brain is found in the *substantia nigra* (43, 43), with relatively large levels also observed in hypothalamus. In several areas, substance K and substance P are colocalized (44), which would be predicted based on their common precursors.

4.0.2.1. Biosynthesis. Two closely related genes encode the three mammalian tachykinins. The preprotachykinin A gene encodes both substance P and substance K, while the preprotachykinin B gene encodes neuromedin K (45–47). The active sequences are flanked by the usual double-basic amino acid residues, and

the carboxy-terminal amino acid is a glycine residue which is decarboxylated to an amide. As with most neuropeptide precursors, intermediates in peptide processing can be detected, but their biological activities are not clear (ca 1994).

4.0.2.2. Biological Activities. Substance P was first isolated for its hypotensive action and stimulation of rabbit jejunum contraction and later for following its ability to produce salivation in rats. It also stimulates glucagon secretion and produces hyperglycemia in rats, stimulates smooth muscle contraction in the guinea pig vas deferens and ileum, and elevates growth hormone and prolactin secretion in humans (48). In the brain, one of the most commonly discussed roles of substance P is the mediation of pain information, where this peptide acts as one of the primary neurotransmitters in the sensory afferent fibers which conduct pain information (49, 50). Thus compounds which increase substance P release, eg, capsaisin, tend to increase pain, while potential substance P antagonists are sought-after analogues for analgesic purposes.

4.0.2.3. Analogues. The smallest sequence possessing most of the substance P spectrum of activity and high potency is the hexapeptide C-terminus. There are a number of analogues of substance P which are potent antagonists at substance P receptors; these include [D-Arg^1, D-Phe^5, D-Trp^{7,9}, Leu^{11}]-substance P (51), [Arg^4, Gly^5, D-Trp^7, Asp(OC$_4$H$_9$^{t})^{10}$_2$]-substance P (52), and [Arg^6, D-Trp^{7,9}, CH$_3$-Phe^8]-substance P 6–11 (53).

4.0.3. Endorphins, Enkephalins, and Dynorphins

The discovery of Met- and Leu-enkephalin as the first opioid peptides in 1975 (10) was not only a breakthrough in the understanding of endogenous opioids, but also represented the beginning of the explosion of neuropeptide discoveries that would highlight the following 10 years (54). In 1994 it is clear that three separate opioid peptide families exist, each representing different gene products, ie, enkephalins, β-endorphin, and dynorphin. All of these peptides share the common sequence Tyr-Gly-Gly-Phe-X, where X is either Leu or Met. The shortest members of this family, the enkephalins, are pentapeptides. β-Endorphin contains 31 amino acids, and also the sequence of Met-enkephalin. Dynorphin A contains 17 amino acids, and the sequence of Leu-enkephalin. Both of the longer peptides, especially dynorphin, are relatively basic in nature, with a number of Lys and Arg residues. The enkephalin sequence is crucial for the biological activity of the longer peptides; in all members of this family, the N-terminal Tyr is also crucial for activity.

These three peptide groups exhibit different localization patterns in the brain. β-Endorphin cell bodies are mostly localized to the arcuate nucleus of the hypothalamus (55). Fibers from this area project to many different areas of the brain. However, pituitary is the principal organ of POMC synthesis, where it exists in anterior and intermediate lobes (56). In the anterior lobe, β-endorphin exists in corticotrophic cells, along with ACTH, whereas in the intermediate lobe it exists in melanotropic cells together with MSH. Thus the processing of the POMC precursor varies in different cells. In contrast to β-endorphin, enkephalins are found in cell bodies in many different brain areas (57). In some of these areas, enkephalins form local circuits and are located in interneurons, while in other areas-enkephalin cell bodies form long projections. Moreover, enkephalins are colocalized with a large number of other peptide and nonpeptide neurotransmitters, including acetylcholine [51-84-3], epinephrine [51-43-4], norepinephrine [51-41-2], serotonin [50-67-9], γ-aminobutyric acid [56-12-2] (GABA), and substance P (57). Like enkephalin, dynorphin peptides also are widely distributed throughout the brain, although their presence in the supraoptic and paraventricular nuclei of the hypothalamus are particularly well known (57).

Several peptides are related in different ways to these classical opioid peptides. FMRFamide (Phe-Met-Arg-Phe-NH$_2$) contains the first four amino acids of enkephalin and is active in various invertebrates (58); FMRFamide-related peptides also have been located in the mammalian brain. Although these peptides share structural similarities with enkephalins, they do not have appreciable affinities for opioid receptors. Casomorpins, originally isolated from milk, do not contain the enkephalin sequence, but do bind to opioid receptors (59). Another group of nonenkephalin-like opioid peptides are derived from dermophin [77614-16-5], an

unusual D-amino acid–containing peptide from frog skin (60). One derivative of dermophin, termed deltorphin, is extremely specific for delta-opioid receptors (61).

4.0.3.1. Biosynthesis. Three separate genes encode the opioid peptides (see Fig. 1). Enkephalin is derived from preproenkephalin A, which contains six copies of Met-enkephalin and extended peptides, and one copy of Leu-enkephalin (62–66). β-Endorphin is one of the many products of POMC, and represents the N-terminal 31 amino acids of β-lipotropin (67, 68). Three different dynorphin peptides are derived from the third opioid gene, preproenkephalin B, or preprodynorphin (69). The dynorphin peptides include dynorphin A, dynorphin B, and α-neo-endorphin.

4.0.3.2. Biological Activities. All opioid peptides elicit a number of morphinomimetic activities following intracerebroventricular injection, eg, analgesia and catatonia, and exert numerous behavioral effects (54). *In vitro,* they decrease the amplitude of muscle contractions induced electrically in the guinea pig ileum and in the mouse vas deferens. β-Endorphin also stimulates prolactin and growth hormone secretion *in vivo* (70). All of these are reversed by the opiate antagonist, naloxone [465-65-6]. Although they are not as selective as some of the synthetic analogues, the natural peptides preferentially bind to different types of opioid receptor. Both Met- and Leu-enkephalin preferentially bind to delta-opioid receptors, whereas dynorphin peptides bind to kappa-receptors. On the other hand, β-endorphin is relatively nonselective between mu-, delta-, and kappa-opioid receptors (54).

4.0.3.3. Analogues. Hundreds of enkephalin analogues have been synthesized in an effort to find a nonaddictive opioid. Some analogues have been synthesized as metabolically stable agonists for longer durations of action; these include [D-Ala^2, Met^5]-enkephalinamide (71) and [D-Ala^2-*N*-CH_3-Phe^4, Met(O)^5-ol]-enkephalin (72). The latter peptide is about 30,000 and 1,000 times more potent when injected intracerebroventricularly than Met-enkephalin and morphine [57-27-2], respectively, and 23 times as active as β-endorphin. Other analogues have high selectivity for different receptor types. DAMGO [78123-71-4] (sometimes abbreviated DAGO, [D-Ala^2-*N*-CH_3-PH^4, Gly^5-ol]-enkephalin), is selective for mu-receptors (73), whereas DSLET ([D-Ser^2, Leu^5, Thr^6]-enkephalin) and DPDPE ([D-Pen^{2,5}]-enkephalin) are selective for delta-receptors (74, 75).

The opioid peptides are unique from a pharmacological point of view because there historically exists a number of specific nonpeptide antagonists, typified by naloxone. Nevertheless, there are several peptide antagonists available for these receptors, particularly for delta-opioid receptors. In 1994 the most selective peptide antagonist for delta-receptors is Tyr-Tic-Phe-Phe-OH (TIPP), where Tic is tetrahydroisoquinoline-3-carboxylic acid (76). Finally, several somatostatin analogues exhibit reasonable affinities at various opioid receptors. One example is CTOP (Cys^2-Tyr^3-Orn^8-Pen^7 amide) somatostatin analogue[103429-31-8], which is a potent and highly selective mu receptor antagonist (77). This finding is particularly interesting because the cloned delta-opioid receptor has significant sequence homology with the cloned somatostatin receptor (78).

4.0.4. Corticotropin-Releasing Factor

Corticotropin-releasing factor (CRF) stimulates the release of ACTH. However, because ACTH, MSH, and β-endorphin all arise from the same precursor, POMC, CRF regulates the release of these peptides as well. Therefore, CRF plays a significant regulatory role in brain and hormonal function. CRF activity was first identified in the hypothalamus in the 1950s (3, 4), but difficulties in the bioassay for this material precluded isolation and sequencing of this peptide until 1981 when the sequence of the 41-amino acid peptide CRF was reported (79, 80). As shown in Table 1, CRF is one of the largest of the brain oligopeptides and activity appears to reside in several areas of its sequence, although much of its potency lies in the C-terminal 27 amino acids (81).

CRF is localized in several brain areas. The best studied structure is the parvocellular region of the periventricular nucleus of the hypothalamus, where fibers project to the median eminence. Other areas containing CRF include amygdala, lateral hypothalamus, central gray area, dorsal tegmentum, *locus coereleus,*

parabrachial nucleus, dorsal vagal complex, and inferior olive (31). CRF is colocalized with a number of other peptides, including enkephalin and vasopressin (82, 83).

4.0.4.1. Biosynthesis. CRF is derived from a precursor of 196 amino acids (84, 85). This gene contains one copy of CRF, which is flanked by double basic amino acids. The amino acid sequence of the CRF precursor suggests that it may arise from proteins related to POMC and neurophysins (31). The CRF precursor contains a cAMP responsive element which allows stimulation of mRNA synthesis when intracellular levels of cAMP are increased (86).

4.0.4.2. Biological Activity. The principal function of CRF is to regulate the release of POMC-derived peptides, especially ACTH. Because the role of ACTH is to release glucocorticoids, CRF is a significant regulator of pituitary–adrenal function. CRF also regulates several aspects of the immune system (31). Perhaps most interestingly, from the point of view of brain function, is the suggestion that CRF plays a principal role in the etiology of CNS depression. In this view, CRF causes many of the known signs of clinical depression, including decreased appetite, decreased libido, and insomnia (32). Such findings suggest that specific CRF antagonists may be valuable tools in the treatment of depression.

4.0.4.3. Analogues. There is a definite lack of practical synthetic CRF analogues (ca 1994). The only CRF antagonist available is α-helical CRF 9-41 (87). Although this compound acts as an antagonist by binding to the CRF receptor and not producing a biological response, it is relatively weak and its role as a clinical tool is limited by the fact that it does not cross the blood–brain barrier (32).

4.0.5. Somatostatin

Somatostatin is somatotropin-releasing inhibiting factor (SRIF or SS). Somatostatin was isolated from ovine hypothalamic extracts because of its characteristic inhibition of the spontaneous release of growth hormone by cultured pituitary cells (88). It also inhibits a large number of other neurotransmitters and hormones, including thyroid-stimulating hormone [9002-71-5] (TSH), prolactin [9002-62-4], insulin [9004-10-8], glucagon [9007-92-5], acetylcholine, gastrin [9002-76-0], gastric acid, and other digestive enzymes.

Somatostatin is widely distributed throughout the brain and the gastrointestinal tract; it also is found in mammalian plasma (89), in the rat retina (90), and in the human adrenal medulla (91). In the brain, the highest levels are found in the amygdala and hypothalamus, followed by several areas of frontal cortex (29). Significant loss of somatostatin occurs in the frontal cortex of Alzheimer's patients (92, 93), and behavioral studies in rats suggest that this peptide may play a role in mediating recognition and memory (94).

4.0.5.1. Biosynthesis. Somatostatin exists in longer forms in several biological tissues (95, 96). One of the longer forms, which has been isolated from porcine intestine, has been characterized as a 28-amino acid peptide (97). Somatostatin is derived from a precursor containing 116 amino acids (98, 99). The precursor contains one copy of the somatostatin tetradecapeptide, which is contained within the sequence of the 28-amino acid peptide at the carboxy-terminal end of the precursor. The 28-amino acid somatostatin is preceded by a single Arg residue, while somatostatin 1-14 is preceded by a pair of basic residues.

4.0.5.2. Biological Activities and Analogues. Somatostatin exerts some neurotropic actions, eg, as a tranquilizer and as a spontaneous motor activity depressor. It also lengthens barbiturate anesthesia time and induces sedation and hypothermia. These actions are consistent with the strong association between somatostatin and GABA; in the primate cerebral cortex, 90–95% of somatostatin-positive cells also contain GABA (100).

Substitution of Trp^8 by D-Trp^8 increased the potency of somatostatin (101). Most other substitutions, however, are deleterious to biological activity. Cyclic octapeptide analogues of somatostatin retain high potency; one of them, CTOP, is a potent mu-opioid antagonist.

4.0.6. Hypothalamic-Releasing Peptides

4.0.6.1. Gonadoliberin. Gonadoliberin (LRF) has been isolated from porcine (102) and ovine (103) hypothalamic extracts. Its highest concentration is found in the hypothalamus; however, it also has been reported to be present in extrahypothalamic central nervous regions, blood, urine, and placenta (104–108). LRF acutely stimulates lutenizing hormone (LH) and follicle-stimulating hormone (FSH) secretion (see Hormones, anterior pituitary hormones). Additionally, there is evidence that LRF can act on the central nervous system to modulate sexual behavior (109). Paradoxically, however, its long-term administration is associated with antigonadal effects which include termination of pregnancy, decreased gonadal weights, and lowered steroid secretion, possibly through desensitization at pituitary and gonadal levels and through alterations in steroidogenesis (110).

Potent and long-acting analogues of LRF have been designed. These peptides generally have a D-amino acid at the 6-position and a modified C-terminus. Specific LRF antagonists also have been designed. Although the first antagonist to LRF, ie, [des-His^2]-LRF, was very weak, more recent analogues are considerably more potent; these include [D-Phe^2, Pro^3, D-Phe^6]-LRF and [D-$pGlu^1$, D-Phe^2, D-$Trp^{3,6}$]-LRF (111).

4.0.6.2. Thyroliberin. Thyroliberin (TRF) originally was purified from alcohol-acid extracts of ovine (112) and porcine (113) hypothalamic extracts. Its greatest hypothalamic concentration is found in the mammalian median eminence; immunoreactive TRF has been found throughout the central nervous system as well as in the blood, urine, cerebrospinal fluid, and endocrine pancreas (114). The principal function of TRF is to stimulate thyroid-stimulating hormone (TSH) release, but it also releases prolactin and growth hormone under specific conditions (see Hormones, human growth hormone). TRF has been reported to alleviate depressive symptoms, to reverse the duration of anesthesia and hypothermia induced by a number of substances, and to increase spontaneous motor activity. It is derived from a precursor that contains five separate copies of this decapeptide, flanked as usual by double basic residues (115). After cleavage from the precursor, the N-terminal Gln is converted to Glu to produce the active peptide product.

Several hundred analogues to TRF have been synthesized. Some of them, like 1-CH_3-S-Dio-His-Pro-NH_2 are more potent than TRF (116); others are conformationally restricted and equally as potent as TRF (117).

4.0.7. Neurotensin

This hormone has been isolated and characterized from acid–acetone extracts of bovine hypothalamus (118) on the basis of its hypotensive activity. Immunoreactive neurotensin is present in mammalian gut and is distributed throughout the central nervous system; its highest concentration is in the hypothalamus and in the substantia gelatinosa of the spinal cord (119). Its overall brain distribution is not unlike that of enkephalin (1).

4.0.7.1. Biological Activities and Analogues. The many pharmacological actions of neurotensin include hypotension, increased vascular permeability, hyperglycemia, increased intestinal motility, and inhibition of gastric acid secretion (120). In the brain, it produces analgesia at remarkably low doses (121).

The smallest sequence possessing most of the neurotensin spectrum of activities and its high potency is the hexapeptide C-terminus (1). [D-Trp^{11}]-Neurotensin acts like a neurotensin antagonist in perfused heart preparations, but acts like a full agonist in guinea pig atria and rat stomach strips (122).

4.0.8. Calcitonin Gene-Related Peptide

Most peptides are discovered in a classical way, ie, they are first identified by their biological or receptor-binding activity, then they are isolated and purified from extracts and finally sequenced. The advent of molecular cloning, however, has revolutionized this approach. Several neuropeptides have been isolated by the fact they are derived from precursors closely related to known peptide precursors. An example of this approach is calcitonin gene-related peptide (CGRP), the existence of which was not even suspected before an analysis of the calcitonin precursor gene revealed that alternative splicing of mRNA would give rise to a structurally

related sequence (123). By generating antibodies to this sequence, it has been possible to isolate CGRP from the brain and determine its localization (123). Unlike calcitonin itself, which is present primarily in the thyroid gland, CGRP is present throughout the nervous system. It contains 36 amino acids having a C-terminal amide.

CGRP has a wide distribution in the nervous system (19) and was the first peptide to be localized to motoneurons (124). It is also found in primary sensory neurons where it is colocalized with substance P (125). CGRP is derived from a precursor structurally related to the calcitonin precursor. The latter precursor produces two products, calcitonin itself and katacalcin, while the CGRP precursor produces one copy of CGRP (123). Like other peptides, CGRP is cleaved from its precursor by tryptic breakdown between double basic amino acid residues.

4.0.8.1. Biological Activity and Analogues. The colocalization of CGRP with substance P in primary sensory afferents indicates an important relationship between these two peptides. CGRP promotes release (126) and inhibits degradation of substance P (127), thus increasing its effects. Like substance P, CGRP is a vasodilator (124). Finally, CGRP may have trophic actions on motoneurons.

Relatively few CGRP analogues are available (ca 1994). One interesting analogue is CGRP 8–37, which appears to be an antagonist at CGRP receptors and an agonist at calcitonin receptors (128, 129).

4.0.9. Cholecystokinin

Cholecystokinin (CCK) was first isolated from duodenal mucosa as a substance that produced contractions of the gallbladder (130). It is a 3-amino acid peptide that shares the same carboxy-terminal pentapeptide sequence with gastrin. In fact, its existence in the brain was first determined with gastrin antibodies (131) and this cross-reactivity has produced confusion regarding the relative localization of these two peptides in the brain. Authentic gastrin is known to be primarily localized to the magnocellular nuclei of the hypothalamus; all other gastrin-like immunoreactivity in the brain is actually CCK (132). There are relatively high quantities of CCK in the brain, eg, it has been calculated that the human brain contains 1–2 mg CCK, compared to microgram quantities of many other neuropeptides (1). One unusual aspect of CCK is the presence of a sulfated Tyr residue (see Table 1) which is added to the peptide during post-translational processing.

CCK has been detected in two principal forms, ie, the traditional 33-amino acid peptide, and an octapeptide CCK-8. The intestine produces mainly CCK-33 (133) and the brain produces mainly CCK-8 (132). The CCK precursor contains one copy of CCK-33 (133, 134); this peptide is flanked on both ends with double basic residues, whereas CCK-8 is formed from CCK-33 by cleavage of a single basic residue.

4.0.9.1. Biological Activity and Analogues. In addition to its actions on the gastrointestinal tract, CCK in the brain has been associated with control of eating behavior (1). Like somatostatin, CCK has a strong relationship with GABA, ie, 90–95% of CCK immunoreactive cells colocalize with GABA cells (100). However, CCK and somatostatin do not coexist in the same GABA-containing cells (100). There is also colocalization of CCK with dopamine (135), which correlates with biochemical studies suggesting that CCK is a neuromodulator of dopamine function (29).

The CCK system shares one property with the opioid system, ie, the existence of selective nonpeptide antagonists. These include asperlicine, a natural benzodiazepine (136), and Devazepide (L-364,718; MK-329) (137). Selective, potent peptide antagonists for CCK, eg, Cl-988 and PD 134308, have been developed that may be useful as anxiolytics and as drugs which increase the analgesic effect of morphine but at the same time prevent morphine tolerance (138) (see Hypnotics, sedatives, anticonvulsants, and anxiolytics).

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