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NONOPIOID NEUROPEPTIDES IN MAMMALIAN CNS

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INTRODUCTION

During the past decade there has been a remarkable change in our understanding of the process of chemical signalling within the mammalian CNS. Ten years ago some eight or nine monoamine and amino acid neurotransmitter candidates were known, but to these must now be added thirty or more small peptides, each with a potential chemical messenger function. The purpose of the present review is to give a progress report on some recent developments with nonopioid neuropeptides. As there are some two dozen such peptides (Table 1), it is impossible to describe each in detail; instead this review focuses on a few of the most intensively studied examples, in the hope of revealing some general principles, concepts, and problems in this area. For each of the peptides described here there is evidence that a calcium-dependent release mechanism exists in the CNS (2), suggesting that they are probably released and serve some chemical messenger function. Several recent reviews provide more detailed information (1–6).

Our difficulties in understanding the functions of the bewildering variety of neuropeptides are related to our generally poor knowledge of chemical neurotransmission in CNS. Although the presence of monoamines in CNS has been recognized for more than thirty years, we still lack a clear picture of their functions in particular CNS pathways (7). Are they to be viewed as "neuromodulators" that set the level of excitability of groups of neurons in CNS, or do they function as more conventional "neurotransmitters"? It is possible to take the iconoclastic view that fast point-to-point neural circuits in CNS operate only with amino acid transmitters (GABA, glycine, glutamate, aspartate), and that the other ingredients of the chemical soup (monoamines and neuropeptides) act in modulatory roles. The distinction

Table 1 Nonopioid neuropeptides, July 1982[a]

ACTH	α-MSH
Angiotensin II	Motilin
Avian pancreatic polypeptide	Neuropeptide Y
Boinbesin	Neurotensin
Bradykinin	Oxytocin
Calcitonin	Pancreatic polypeptide
Carnosine	Proctolin
Cholecystokinin	Secretin
Gastrin	Somatostatin
Glucagon	Substance P
Growth hormone	TRH
Insulin	Vasopressin
β-Lipotropin	Vasoactive intestinal polypeptide (VIP)
LHRH	

[a] These peptides have been described in neurons and nerve terminals within mammalian CNS, other than those related to endocrine or neuroendocrine functions.

between neurotransmitters and neuromodulators is not an easy one to make (8), but it will be important to try to develop some conceptual framework to guide research on the neuropeptides.

In thinking of a future "neuropeptide pharmacology" there are also few precedents to guide us. How can drugs be designed to manipulate CNS peptide functions? Will peptides themselves ever prove useful as orally administered agents? How can nonpeptide drugs be discovered that will act on CNS peptide mechanisms?

SUBSTANCE P

Introduction

The undecapeptide substance P, (Table 2) discovered more than fifty years ago in extracts of horse intestine, is probably the most studied to date of all the nonopioid neuropeptides. Recent reviews have summarized the evidence for the view that SP may function as a neurotransmitter in CNS (9–11) and accounts of its detailed distribution in CNS are available (12).

SP in Sensory Nerves

It is in its location in primary afferent nerves that the evidence for a neurotransmitter role of SP is strongest. The peptide is present in a subpopulation of small sensory neurons, probably belonging to the C-fiber category. Estimates of the size of this subpopulation of sensory cells vary according to species and the particular sensory ganglion examined. In visceral afferents as many as 50% of the unmyelinated fibres may contain

Table 2 Structures of substance P and some synthetic analogs

	1 2 3 4 5 6↓7↓8↓9 10 11 Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Substance P	[Arrows indicate sites of attack by substance P degrading enzyme (28)]
	pGlu-Gln-Phe-MePhe-Sar-Leu-Met-NH_2
DiMe-C7	[Metabolically stable substance P agonist in CNS (29)]
	Arg-D-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Met-NH_2
D-Pro^2, D-$Trp^{7,9}$-Substance P	[Substance P antagonist (36, 37)]

SP (13), but in spinal ganglia not more than 10–20% of the sensory neurons are SP-positive (14). SP can be released on stimulation from the central terminals of such neurons in the substantia gelatinosa of spinal cord, or in cranial nerve nuclei, and it acts as a powerful excitant of potential target cells in the dorsal horn (9–11). It thus fulfils the criteria expected of a sensory transmitter, and is widely believed to act in this way, although dissent has also been expressed (15). The corollary, that SP may be particularly involved in the transmission of nociceptive inputs into CNS and, thus, function as a "pain transmitter," is also widely believed, although it is much less firmly based. The association of SP with pain mechanisms rests entirely on circumstantial evidence: its location in C-fibers, its reported ability to excite selectively nociceptive units in dorsal horn, and its ability to cause transient changes in pain sensitivity when administered intrathecally (9–11). The finding that the neurotoxin capsaicin (8-methyl-N-vanillyl-6-noneamide) (pungent factor of red peppers) could damage or destroy SP-containing sensory fibers, and that it caused a decreased sensitivity to pain, appeared at first to support the hypothesis of an involvement of SP in pain mechanisms (16). However, subsequent work with capsaicin (usually administered as a single systemic dose to infant animals) has shown that it causes indiscriminate damage to all unmyelinated sensory fibers and may also damage small diameter myelinated fibers of the Aδ category if administered in high doses (> 50 mg/kg) (16). Thus, although capsaicin has an interesting pattern of specificity (it does not damage small diameter unmyelinated fibers in the autonomic nervous system, for example) it cannot be regarded as a selective neurotoxin for SP-containing neurons. The case for SP as a "pain transmitter," thus, remains to be established. It would be prudent to remember that several other neuropeptides, present in other

subpopulations of C-fibers, are also destroyed by capsaicin treatment. A priori these peptides (somatostatin, vasoactive intestinal polypeptide, cholecystokinin) should all be considered putative candidates for a "pain transmitter" role.

SP is present in high concentrations not only in the central but also in the peripheral terminals of primary sensory neurons. It is possible that SP may be released from sensory terminals in response to tissue damage, and that its powerful local effects on blood flow, increased capillary permeability, and ability to release histamine from mast cells contribute importantly to the vasodilatation and plasma extravasation components of the inflammatory response [see Lembeck in (11)]. SP-containing sensory nerve endings are widely distributed in the vascular system (17,18), where fine networks of peptide-containing fibers are present in the walls of blood vessels in all vascular beds including the cerebral vasculature, and in the heart. The possibility may have to be considered that the C-fibers that contain SP have dual functions—as carriers of sensory information and as local regulators of tissue blood flow (17, 18).

Another unexpected location for SP-containing sensory nerve terminals is in autonomic ganglia. Some sympathetic ganglia contain a dense network of SP fibers, which appear to represent the collateral branches of primary sensory neurons (1). Guinea pig inferior mesenteric ganglia receive a particularly rich SP innervation of this type from visceral afferents and have proved valuable models for studying the electrophysiological actions of SP on a defined neuronal population. Konishi et al (19) and Dun & Jiang (20) found that the principal ganglionic neurons exhibit a slow depolarizing response to exogenously applied SP that mimics precisely a slow EPSP component elicited by low frequency stimulation of a ganglionic input. In this tissue SP appears to act as an excitatory modulator; not able itself to fire the ganglionic cells, but making them more easily excited by the conventional preganglionic cholinergic input.

SP in Brain and Retina

In addition to its presence in sensory neurons, SP is also present in many intrinsic neuronal pathways within the CNS. More than thirty different groups of SP-containing neurons have been described in rat brain (12), and new systems are still being discovered. Karten & Brecha (21), for example, found SP in a morphologically distinct subpopulation of amacrine cells in the pigeon retina. The same authors were able to identify some half dozen other neuropeptides in other types of amacrine cell (22), neatly illustrating our newfound ability to place chemical labels onto neuron subtypes hitherto

identified only by their distinctive morphological appearance in Golgi-stained material.

Within the brain we still know little of the possible functions of SP pathways. The finding that SP terminals are particularly concentrated in regions containing the cell bodies of noradrenergic (locus coeruleus) and dopaminergic (substantia nigra and ventral tegmentum) neurons suggests a possible function of SP in controlling the activity of cerebral adrenergic systems. This idea has received support from neurophysiological studies showing that microiontophoretically administered SP exerts a direct excitatory effect on the firing of locus coeruleus neurones (23). Behavioral studies, furthermore, have shown that microinfusions of SP in the region of the dopaminergic neurons in the ventral tegmentum of rat brain elicit an amphetamine-like hyperactivity response, which appears to be due to activation of forebrain dopamine pathways (24). As is the case for other chemical mediators, SP seems likely to play a number of different functional roles in various parts of the nervous system.

Metabolism

It is assumed that the synthesis of neuropeptides takes place by cleavage of larger precursor polypeptides, synthesized on ribosomes in the neuronal cell bodies. The hypothetical SP precursor, however, has so far not been identified. Harmar et al (25) have shown a cycloheximide-sensitive incorporation of ^{35}S-methionine and ^{3}H-proline into SP when rat sensory ganglia were incubated in vitro. The incorporation occurred with a delay of 1–2 h, suggesting the existence of a precursor stage. ^{35}S-methionine was also incorporated into a second immunoreactive peak, which did not appear to represent a C-terminal fragment of SP or a larger precursor molecule. The chemical identity of this SP-like "peak X," however, remains unknown.

There is considerable interest in the possibility that specific peptidases may exist in CNS as mechanisms for inactivating neuropeptides after their release from neurons. In the case of SP a number of peptidases have been described that are capable of degrading the molecule, but of these only two appear to be serious contenders for an inactivation role [see Lee in (11)]. One, known as the postproline cleaving enzyme, is an endopeptidase purified from bovine brain that cleaves on the C-terminal side of Pro residues and attacks SP and several other proline-containing peptides (TRH, LHRH, neurotensin) (26). It cleaves SP to yield an N-terminal tetrapeptide (Arg-Pro-Lys-Pro) and a C-terminal heptapeptide. However, since the C-terminal fragment retains full biological activity, this enzyme by itself

would not constitute an inactivation step, but would have to be followed by further cleavage of the product by aminopeptidases, or as suggested by Nakata et al (27), an active uptake of the C-terminal heptapeptide into brain tissue. An alternative inactivation mechanism for SP is represented by a novel metallo-endopeptidase, SP-degrading enzyme, recently purified from human brain [(28); see also Lee in (11)]. This is a membrane-bound peptidase that exhibits a remarkably high substrate specificity for SP. It requires a substantial portion of the SP sequence for full recognition, and acts on SP with a Km of about 25 μM. Cleavages occur at three alternative points in the region of the phenylalanine residues at positions 7 and 8 (Table 2), any of which would cause complete loss of biological activity. Whether this enzyme, which also exists in animal brain, plays a role in SP inactivation remains to be established. A synthetic analogue of SP (DiMe-C7), which contains N-methyl amino acid residues in positions 8 and 9 (Table 2), was protected against degradation by the purified SP-degrading enzyme, and was, furthermore, resistant to metabolic degradation in rat brain in vivo and exerted prolonged behavioral arousal effects (29). DiMe-C7 seems likely to prove a useful new research tool as a stable CNS agonist.

Pharmacology of SP Receptors

SP exerts a wide variety of effects on biological systems, including contraction of smooth muscle in various regions of the gastrointestinal and urinogenital systems, secretagogue activity in salivary glands and pancreas, and direct excitant actions on neurons in the peripheral and central nervous systems (30,31). In all of its actions certain fundamental structure-activity relations apply: full activity resides in the C-terminal hexapeptide sequence, and the actions of SP are mimicked by other naturally occurring peptides, the tachykinins, which share the common C-terminal sequence: -Phe-X-Gly-Leu-Met-NH_2.

The C-terminal amide moiety also plays a key role in the tachykinin sequence, the free carboxylic acid analogues being approximately 1000 times less potent. Erspamer and colleagues (32) were among the first to note that although the different tachykinins possessed broadly similar pharmacological properties, there are marked differences in relative potencies among the different peptides when tested in parallel in a variety of peripheral tissue bioassays. We confirmed their findings (33) and have described two distinct patterns of tissue response: the "SP-P" type, in which SP, physalaemin, kassinin, and eledoisin are approximately equipotent (e.g. guinea pig ileum) and the "SP-E" type, in which eledoisin and kassinin are up to several hundred-fold more potent than SP and physalaemin, which act only at micromolar concentrations (e.g. mouse urinary bladder, rat vas deferens)

[see Iversen et al in (11)]. Which of these possible receptor subtypes is present in CNS remains unclear, although the specificity of sites revealed by the high affinity binding of ^{3}H-SP to rat brain membranes in vitro (34) appears to reflect that of a SP-P type. Another approach to biochemical studies of SP receptors is offered by the coupling of such receptors in some target tissues to phosphatidylinositol turnover, which can be measured as a secondary metabolic response to receptor occupation (35).

Considerable interest has been raised by the recent description of novel synthetic analogues of SP that possess antagonist properties (36, 37) (Table 2). The most potent compunds so far described are analogues of SP with D-Trp in positions 7 and 9. In some tissues these are simple competitive antagonists, but in other bioassays they retain agonist or partial agonist properties, making them less than ideal as research tools. However, the presently available antagonists will no doubt be improved, and this lead represents an important advance.

CHOLECYSTOKININ-LIKE PEPTIDES

Introduction

Cholecystokinin-like peptides are present in CNS at concentrations that exceed those of most other neuropeptides. Although it was at first thought that the closely related peptide gastrin was present in brain, it is now clear that the major immunoreactive species in CNS are related to cholecystokinin (CCK), although they may cross-react with gastrin antisera. The CCK-related peptides represent an interesting group, illustrating the principle of molecular heterogeneity among a family of peptides all derived from the same precursor sequence, and having some intriguing effects on behaviour. For recent reviews see (38, 39).

Molecular Species and Distribution

Whereas native CCK from gut is a 33 amino acid peptide, it is now generally recognized that the principal form present in CNS is the C-terminal octapeptide fragment, CCK-8 (38, 39). Rehfeld and colleagues (40) have put forward the interesting suggestion that an even smaller C-terminal tetrapeptide fragment, CCK-4, may be the principal component present in nerve terminals in the endocrine pancreas, where it may play a key role in the neural regulation of hormone secretion. They have also suggested that CCK-4 may be an important species in neurons in CNS, although this conclusion is disputed (38, 39). The precise molecular nature of CCK in CNS remains an open question. There are some puzzling discrepancies between results obtained by radioimmunoassay, usually designed to measure CCK-8, and those obtained by immunohistochemical procedures.

Thus, two groups have independently reported that immunostaining shows CCK-like material to be present in high concentration in substantia gelatinosa of spinal cord, and that much of this material disappears after dorsal rhizotomy or after capsaicin treatment, suggesting a location of CCK-like peptides in the terminals of primary afferent fibres. Radioimmunoassay, however, has failed to detect any significant loss of CCK-immunoreactivity from spinal cord dorsal horn after rhizotomy or capsaicin (41, 42).

Radioimmunoassay indicates that CCK-like peptides are most concentrated in cerebral cortex, hippocampus, amygdala, and hypothalamus. In the cortex the CCK concentration greatly exceeds that of other neuropeptides, and immunostaining shows CCK to be present in numerous local interneurons, with largely radially oriented processes (38).

Central Actions of CCK

Receptor binding studies in brain membranes have been performed with ^{125}I-labelled CCK-33 by three different groups (see 38). The CCK-binding sites are of high affinity (Kd approx. 0.3-0.5 nM) and their regional distribution in brain is similar to that of endogenous CCK-peptides. Innis & Snyder (43) reported some differences in the specificity of brain binding sites when compared with those present in pancreas. The sulphated form of CCK-8 was more potent than the non-sulphated form in both brain and pancreas, but the difference between sulphated and nonsulphated forms was much less marked in brain, where the C-terminal tetrapeptide CCK-4 was also potent. The results suggest that the receptors in brain may represent a distinct subclass, differing from those in peripheral tissues in recognising CCK-4 as well as the larger forms of CCK.

A number of studies have demonstrated excitatory effects of microapplied CCK-8 on neuronal firing in cerebral cortex, hippocampus and spinal cord dorsal horn (see 38). Dodd & Kelly (44), for example, applied CCK-8 by microiontophoresis and by micropressure to hippocampal pyramidal cells in slices of rat hippocampus maintained in vitro. CCK-8 elicited a potent excitatory response, whose onset and offset was as rapid as that produced by L-glutamate, thus dispelling the myth that neuropeptide actions are invariably slow in onset and prolonged. Other studies indicate that locally applied CCK-like peptides can excite dopaminergic neurons in ventral tegmentum (45).

Surprisingly, intravenously administered CCK was also reported to increase the firing of the A10 dopaminergic neurons (45), and Kovacs et al (46) have also observed increases in dopamine turnover in rat brain after intraperitoneally administered CCK-8 (200 μg/kg). These phenomena are difficult to understand, as it seems unlikely that systemically administered

CCK-8 would gain access to CNS. Indeed, no penetration of CCK into csf could be demonstrated after intravenous administration of the radiolabelled peptide (47).

CCK and Postprandial Satiety

The similar effects of systemically and centrally administered CCK on dopaminergic systems is paralleled by the satiety effects of both peripherally and centrally administered peptide. CCK has long been considered the most likely candidate as the major peripheral satiety signal (48). A rise in circulating CCK, presumably of gastrointestinal origin, towards the end of a meal is thought to trigger satiety mechanisms that lead the animal to terminate the meal. This mechanism appears to depend entirely on peripheral effects of CCK rather than on any direct action of circulating peptide on CNS, since the anorexic effects of peripherally administered CCK are absent after vagotomy (49). Thus, circulating CCK presumably affects the CNS only indirectly by activating visceral afferent fibres, probably as a result of an action on gastric or intestinal smooth muscle (49). However, numerous studies have shown that CCK and the structurally related peptide caerulein can also inhibit feeding behaviour in hungry animals when adminstered directly into CNS (see 38, 48, 50). The central effects are elicited by doses smaller than those needed to elicit satiety effects systemically, and are thus unlikely to be due to leakage of intracerebrally administered peptide into circulation, although some such transfer does occur (47).

The anorexic effects of CCK have aroused considerable interest, although it is not the only neuropeptide to possess such actions. Similar satiety effects have been observed after both central and peripheral administrations of TRH and bombesin (51). The anorexic actions of CCK may possibly be related to its other reported behavioral actions as an anticonvulsant and an antinociceptive agent when administered systemically (52). It is possible that at least some of the actions of CCK in CNS are related to its function as a postprandial satiety signal. On the other hand it is impossible to believe that this is the only function of CCK in CNS. Its widespread localization in interneurons in all regions of neocortex, for example, hardly seems compatible with such a restricted function.

VASOACTIVE INTESTINAL POLYPEPTIDE (VIP)

Introduction

The presence of VIP in brain was detected almost as soon as radioimmunoassay methods for the peptide became available (see 38, 53, 54). The immunoreactive material in CNS appears to correspond to the 28 amino

acid residue peptide originally characterized in gut extracts, and there is no evidence for the molecular heterogeneity found in the gastrin/cholecystokinin family of peptides. In addition to its presence within neurons in CNS, VIP-containing nerve terminals are also abundant in many regions of the peripheral autonomic system, especially in the gastrointestinal, urinogenital, and respiratory tracts (53). Its possible function as a neurotransmitter involved in various peripheral inhibitory nonadrenergic, noncholinergic reflexes has attracted considerable attention (53), with rather less interest so far in its possible neurotransmitter role in CNS (38, 54).

Distribution in CNS

As with cholecystokinin, the distribution of VIP in mammalian brain is unusual in that the highest concentrations are found in neocortex. VIP-containing interneurons are found in all regions of cerebral cortex and in hippocampus. The cortical VIP cells are located in layers II–IV with radially oriented long processes spanning the entire depth of the cortical gray matter. These cells appear to correspond to "nonspiny bipolar" neurons previously described in Golgi stained preparations (54). VIP-containing neurons and terminals are also concentrated in hypothalamus (the lateral preoptic area and the supraoptic and suprachiasmatic nuclei), amygdala (central nucleus), bed nucleus of the stria terminalis, and the periaqueductal grey. In several of these areas the VIP innervation may arise from long-fibered tracts, some of which run in the stria terminalis and in the medial forebrain bundle, although the exact topography of these pathways remains unclear (38, 54).

VIP is also present in some primary afferent neurons, with cell bodies in sensory ganglia. Except in visceral afferents, however, the proportion of VIP-containing sensory fibers appears relatively low.

VIP terminals in CNS are often found in close association with cerebral blood vessels, suggesting a possible function in regulating local blood flow. However, VIP fibers are also found in the walls of many blood vessels in the periphery, and it is not clear whether the fibers seen in cerebral vessels originate from VIP neurons in brain, or whether they arise extrinsically, for example, as the terminals of VIP-containing sensory neurons.

Central Actions of VIP

VIP can be released in a calcium-dependent manner from brain slice or synaptosome preparations in vitro in response to depolarizing stimuli (2, 38, 55). There have been relatively few direct neurophysiological studies of VIP action in CNS at a single cell level, but when applied locally it has

been found to excite cell firing in cerebral cortex, hippocampus, and spinal cord (38).

VIP receptors in brain can be detected biochemically by radioligand binding assays, using ^{125}I-VIP (38, 56, 57, 59), or by measuring the ability of VIP to stimulate cyclic AMP formation in brain slice, synaptosome, or membrane preparations (38, 58, 59). The regional distribution of high affinity VIP binding sites parallels the distribution of VIP-containing neurons (57), and the specificity of both the binding sites and the VIP-stimulated adenylate cyclase are compatible with those expected for VIP receptors. The only related peptides that are recognised are shorter fragments of VIP, or the closely related peptide secretin and its analogues. As in peripheral tissues, the N-terminal sequence His-Ser-Asp-Gly appears essential for activity, with potency increasing as the chain length increases. In the secretin analogue [Gln^9, Asn^{15}]-secretin [5-27] the neutralization of two negative charges present in secretin makes this fragment more VIP-like. This analogue acts as a competitive antagonist of VIP-stimulated adenylate cyclase in brain, as it does in peripheral tissues (59).

Although there are some differences in the specificity of brain VIP receptors by comparison with those in peripheral tissues (38), these do not seem major. The receptors in both CNS and in peripheral target cells are coupled to adenylate cyclase, and exhibit some degree of overlapping specificity with secretin and related peptides.

The exact cellular location of VIP receptors in CNS remains unknown. The VIP-stimulated cyclic AMP response in rat striatal slices was reduced considerably after kainic acid lesions, suggesting a predominantly neuronal location (60). However, the ability of VIP to stimulate glycogenolysis in cerebral cortical slices (61) might suggest a glial location for some VIP actions, as astrocytes are thought to represent the main glycogen-containing elements in CNS. In addition to its possible function as a chemical messenger within brain and spinal cord, VIP may also play a neuroendocrine regulatory role. It is present in high concentrations in anterior hypothalamus and median eminence, and relatively high concentrations are present in hypophyseal blood, suggesting a possible role as a hypothalamic releasing factor (62). In anterior pituitary it acts on mammatrophs to stimulate prolactin secretion.

Within the brain, however, we have no clear picture of the possible functions of VIP. There is no obvious involvement in any particular behavioral response, and the notion of an involvement of VIP in the regulation of local cerebral blood flow is based only on circumstantial evidence. The concentration of this peptide, with cholecystokinin, in neocortex, and the presence of both peptides in cortical interneurons with radially oriented processes has suggested that VIP and cholecystokinin may be involved in

local vertical modulatory control systems, perhaps controlling activity within cortical columns, in contrast to the diffuse horizontally organised monoamine modulatory inputs to cerebral cortex (38, 61).

NEUROTENSIN

Introduction

This tridecapeptide is an example of a gut-brain peptide, first discovered in brain extracts, and later found to be present in gut (see 63, 64). In the gastrointestinal system it seems increasingly likely that neurotensin represents "enterogastrone", a hormone released especially in response to fat-containing food, which inhibits gastric secretions and motility (64). Neurotensin also has powerful hypotensive actions when administered systemically (64) and it may have a neuroendocrine role as a hypothalamic releasing factor (63). A comprehensive preview of the distribution and possible functions of neurotensin in CNS is available (63).

Distribution in CNS

Neurotensin is present in locally high concentrations in various regions of CNS. Unlike the previously described peptides, it is not present in primary afferent fibers, although like them it is found in high concentrations in the substantia gelatinosa of spinal cord dorsal horn, and in cranial nerve sensory nuclei in brain stem. In these regions, however, neurotensin is present in local short axoned interneurons.

In contrast to cholecystokinin and VIP, very little neurotensin is present in cerebral cortex or hippocampus. Instead the highest densities of neurotensin-containing nerve terminals are found in various regions of the limbic system: especially in hypothalamus (preoptic area and median eminence), central amygdaloid nucleus, bed nucleus of stria terminalis, and the nucleus accumbens. Locally high densities of neurotensin fibers also occur in locus coeruleus, dorsal raphe nuclei, periaqueductal grey, substantia nigra, and ventral tegmentum (63, 65, 66).

Central Actions of Neurotensin

Neurotensin receptors can be identified in brain membranes by radioligand binding (67), and by using ^{3}H-neurotensin as the ligand the distribution of the high affinity binding sites has been mapped autoradiographically in tissue sections (68). In general the distribution of presumptive neurotensin receptors corresponds to the distribution of neurotensin-containing nerve terminals, with particularly high receptor densities in hypothalamus, amygdala, substantia gelatinosa, substantia nigra, and nucleus accumbens (68).

Neurotensin elicits a number of easily measured responses when administered intracerebrally. It causes a fall in rectal temperature in rodents maintained at an ambient temperature of 23°C or below. In animals maintained at 4°C core temperature may drop by as much as 10°C after intracisternal injection of 1 μg of the peptide (69). The intracisternal route is the most effective, although larger doses of peptide will also elicit hypothermic responses when administered intraventricularly or intrathecally (70).

Intracerebrally administered neurotensin also elicits an antinociceptive response, increasing latency in the mouse hot plate test (70, 71). This response is not blocked by naloxone and thus appears to involve a nonopioid analgesic mechanism. The antinociceptive response is not simply due to the fall in body temperature caused by the peptide, since falls of core temperature of similar magnitude elicited by other drugs or peptides do not result in changes in pain thresholds. Furthermore, intrathecally administered neurotensin elicits a fall in body temperature without any significant change in hot plate latency (70). This suggests that the analgesic mechanism activated by neurotensin is located supraspinally, although from the anatomical localization of neurotensin in local interneurons in substantia gelatinosa one might have suspected a possible controlling influence on nociceptive inputs at this level. A detailed study of the localization of neurotensin in dorsal horn of rat spinal cord, however, failed to reveal any connection between neurotensin terminals and primary afferent fibers, nor was there any evidence for a localization of neurotensin receptors on primary afferent fibers, although such fibers appear to possess significant numbers of opiate receptors (72).

Considerable interest is currently focussed on the possible interaction between neurotensin and catecholamine-containing neurons in brain. Neurotensin and neurotensin receptors are strategically located in high densities in the nuclei of origin of catecholamine pathways: locus coeruleus, ventral tegmentum, and substantia nigra. Furthermore, the loss of substantial numbers of neurotensin binding sites from rat substantia nigra after 6-hydroxydopamine-induced lesions of the dopaminergic neurons suggests that neurotensin receptors may be located in part on the catecholamine neurons (73). This fits well with other evidence first proposed by Nemeroff and colleagues suggesting an interaction between neurotensin and central dopamine systems (see 63, 74). Thus, intracerebrally administered neurotensin is able to block the stereotyped behavior and hyperactivity responses normally elicited by *d*-amphetamine and possesses some other similarities to the dopamine-blocking neuroleptic drugs (74). In contrast, if the peptide is administered locally into the region of the A10 dopamine cell bodies in ventral tegmentum, it appears to *activate* this dopamine pathway, causing an increase in exploratory behavior (75). Similarly, when tested by local

application from microelectrodes in rat cerebellum, neurotensin causes an inhibition of Purkinje cell firing, apparently due to local release of noradrenaline from locus-coeruleus-derived fibers (76). The interactions of neurotensin with central adrenergic mechanisms are clearly complex, but this remains a promising area for further research.

HYPOTHALAMIC RELEASING HORMONES

Introduction

Just as the individual gut-brain peptides are seen to be quite inappropriately named, so the hypothalamic releasing hormones are inaccurately so described, as it is clear that they are involved in a variety of other functions outside the hypothalamus. These peptides are widely distributed in CNS and in peripheral tissues, and the particular specialized neuroendocrine functions for which they were initially discovered probably represent their cooption from more general biological functions. Only a very brief summary of their extrahypothalamic functions can be given here.

TRH

The tripeptide TRH is widely distributed in brain and spinal cord, with particularly high concentrations in spinal cord ventral horn (where TRH-positive terminals surround the large motor neurons), in amygdala, cortex, and retina (77). TRH receptors in brain have been studied by radioligand binding techniques using ^{3}H-TRH as radioligand (78, 79). They are particularly abundant in amygdala, septum, interpeduncular nucleus, periaqueductal grey, hippocampus, and hypothalamus. Intracerebrally administered TRH elicits a variety of behavioral responses, apparently reflecting increases in arousal. Thus, TRH increases wakefulness, grooming, and rearing; it potentiates the hyperactivity responses elicited by L-DOPA and 5-HTP, and it antagonizes the sedative effects of barbiturates and ethanol. There has been considerable interest in this profile, because of early reports that TRH might possess antidepressant effects in man, although these claims have not been substantiated by later studies (77, 80, 81). TRH may be inactivated by deamidation, catalyzed by the postproline cleavage enzyme, mentioned previously as a possible candidate for substance P inactivation (82). Metabolically protected analogs have been described that are active after systemic administration in various standard animal tests of antidepressant potential, and cause prolonged EEG arousal (81, 83). These analogs have an interesting neuropharmacological profile, although it is unclear whether compounds of this type are likely to have any therapeutic applications.

Somatostatin

Somatostatin is present in various extrahypothalamic regions of CNS, both as the originally described 14 amino acid residue peptide, and in higher molecular weight forms which include somatostatin-28 and somatostatin-25 (= somatostatin 4–28) (84, 85). Somatostatin-containing nerve terminals are found in particularly high densities in amygdala, bed nucleus of stria terminalis, and in some areas of cerebral cortex. In addition somatostatin is found in a subpopulation of small sensory neurons, distinct from those containing substance P (14). Somatostatin receptors have been identified by radioligand binding, using ^{125}I-labelled somatostatin or somatostatin-28 (86, 87). When applied by microiontophoresis, somatostatin appears to excite a large proportion of neurons in cortex, hippocampus, and corpus striatum (88).

In its location in primary afferent fibers somatostatin is a priori another of the putative peptide sensory transmitter candidates. Its possible role elsewhere in the CNS, however, remains quite unknown. Little is known of the mechanisms involved in the inactivation of somatostatin in CNS. A potent metabolically stable cyclic hexapeptide analog has a prolonged duration of action when administered systemically (89), and this and related synthetic analogs may prove valuable tools in probing the central actions of this peptide.

Other Hypophysiotrophic Peptides

Although LHRH-containing neurons appear to be largely confined to the hypothalamus, its actions apparently are not concerned only with neuroendocrine regulation. LHRH-containing nerve terminals are not confined to the median eminence, but are found in various regions of the hypothalamus (90, 91). The local administration of minute quantities of LHRH to the preoptic area of hypothalamus in estrogen-primed ovariectomized female rats elicits elements of female sexual behavior, notably the lordosis response (92). This is a striking example of a central action of a neuropeptide, switching on a specific item of the animal's behavioral repertoire.

Kuffler and his colleagues described a surprising action of LHRH (or some closely related peptide) in sympathetic ganglia (93, 94). They found that LHRH closely mimicked a slow excitatory synaptic potential in bullfrog sympathetic ganglia, a small amplitude depolarizing event lasting for up to 5 min after peptide application. This phenomenon is similar in many ways to the slow depolarization elicited in mammalian ganglia by substance P (19,20), and it represents another clear example of a peptide modulatory action, in this case of very long duration. The amphibian ganglia appear to receive a long fiber LHRH-containing innervation, originating from

LHRH-neurons in spinal cord (93, 94) and there is evidence that the peptide can diffuse for several μm from sites of release to its target cells (94).

The recently described corticotropin releasing factor (CRF) is a 41 amino acid residue peptide that also appears capable of exerting direct actions on CNS. Intracerebrally administered CRF has been reported to elicit a syndrome of behavioral arousal, similar to that seen after *d*-amphetamine (95). Whether CRF-containing neurons are found in extrahypothalamic areas of CNS is not yet clear.

NEUROHYPOPHYSIAL HORMONES

Distribution in CNS

Like the other neuropeptides described above, the classical neuropeptides, vasopressin and oxytocin, of the neurohypophysial system also probably perform several different functions apart from the one for which they were originally discovered. Radioimmunoassay and immunohistochemical studies have revealed vasopressin- and oxytocin-containing fibers, apparently all originating from magnocellular neurons in the hypothalamus, or from a separate group of parvocellular vasopressin neurons in the suprachiasmatic nucleus, and distributed in many areas of brain and spinal cord (96, 97). Vasopressin- and oxytocin-containing terminals are found in various regions of limbic system (especially septum and amygdala), in diencephalon, mesencephalon, brain stem, and spinal cord dorsal horn. Of particular interest is the observation of high densities of such terminals in monoaminergic nuclei, especially the locus coeruleus and substantia nigra, which in human and animal brain are the richest vasopressin-containing areas outside hypothalamus (97, 98) (Table 3).

Biosynthesis

The convenient anatomical arrangement of the magnocellular neurosecretory neurons, with perikarya clearly separated from the remote terminals in neurohypophysis, has made them the subjects of choice for detailed studies of neuropeptide biosynthesis. The hypothesis that vasopressin and neurophysin originate as cleavage products from a single polypeptide precursor, first put forward by Sachs some twenty years ago (99), can now be seen to be correct (see 100). Gainer and his colleagues (101) have shown that ^{35}S-cysteine injected in the region of the supraoptic nucleus of the rat hypothalamus is incorporated into two macromolecules, each about 20,000 daltons molecular weight, which represent the precursors for vasopressin and oxytocin and their respective neurophysins. Maturation of these large precursors to the smaller peptides and neurophysins can be followed as the

newly synthesized materials are transported along the axons of the neurosecretory neurons to the pituitary terminals (101).

Similar mechanisms are believed to operate in all other neuropeptide neurons, but nowhere else is the experimental description of the biosynthetic process as complete. The requirement for ribosomal synthesis in the neuronal perikaryon, followed by axonal transport and processing of the precursor form, must impose limitations on the rate at which nerve terminal stores of neuropeptides can be replenished. The peptide neuron faces an entirely different set of logistic problems from cells using conventional monoamine or amino acid transmitters, in which a rapid local resynthesis of neurotransmitter stores can take place in the nerve terminals. In the case of the magnocellular neurosecretory neurons this particular problem appears to be solved by maintaining very large stores of neuropeptide in the nerve terminals; the amounts of vasopressin and oxytocin in the neurohypophysis are sufficient to last for up to 14 days at normal rates of secretion. Whether other neuropeptide neurons maintain a similarly slow rate of turnover of nerve terminal peptide stores is not yet known.

Central Actions of Vasopressin and Oxytocin

A great deal of interest has focused on the central actions of vasopressin (and to a lesser extent oxytocin), almost entirely because of the work of de Wied and his colleagues (for review see 102). They found that vasopressin, when administered either intracerebrally or systemically, has remarkable effects in prolonging the extinction of learned avoidance behaviour in the rat. They suggested that vasopressin has the effect of facilitating memory consolidation and retrieval. Although less intensively studied, oxytocin is reported to exert effects of opposite character to those of vasopressin (102).

These animal findings have prompted a number of trials of vasopressin (usually Arg^8-vasopressin given as a nasal spray) to assess its possible effects on human memory and cognitive performance. Although such studies have yielded variable results, there have been positive reports of the ability of vasopressin to facilitate learning-memory tasks in normals and in patients with various forms of dementia (102, 103).

An important question concerns the site at which vasopressin acts to produce its effects on memory. De Wied and colleagues have argued for a central site of action, even for systemically administered peptide. However, as the effects of systemically administered vasopressin can be blocked by systemic administration of a peptide antagonist of vasopressin which does not penetrate CNS, it seems likely that these effects are mediated peripherally (104). Indeed the behavioral effects occurred after doses of vasopressin similar to those needed to elicit pressor responses, and it was suggested that

Table 3 Regional distribution of some neuropeptides in human brain[a]

Brain region	Immunoreactive peptide—pmol/g wet weight						
	TRH[110]	Cholecystokinin[108]	VIP[112]	Somatostatin[111]	Substance P[107]	Neurotensin[109]	Vasopressin[98]
Frontal cortex	3.2 ± 1.4	419 ± 69	17.3 ± 1.4	65 ± 5	14 ± 10	1.2 ± 0.3	n.d.
Cingulate cortex	4.6 ± 0.5	864 ± 241	—	—	—	3.6 ± 2.0	n.d.
Parietal cortex	2.2 ± 0.6	305 ± 24	—	—	—	—	n.d.
Temporal cortex	3.8 ± 0.5	676 ± 52	13.8 ± 1.5	103 ± 9	—	2.9 ± 1.1	n.d.
Hippocampus	5.5 ± 1.8	516 ± 74	10.7 ± 1.0	82 ± 7	104 ± 29	11.2 ± 5.6	n.d.
Amygdala	21.2 ± 4.8	—	20.8 ± 8.9	304 ± 32	25 ± 12	8.7 ± 1.9	n.d.
N. accumbens	8.5 ± 1.7	318 ± 67	—	223 ± 34	159 ± 21	—	n.d.
Caudate n.	4.7 ± 1.3	223 ± 26	—	107 ± 15	138 ± 14	3.7 ± 1.2	n.d.
Putamen	6.1 ± 1.2	151 ± 21	2.3 ± 0.5	113 ± 10	112 ± 29	5.2 ± 1.2	n.d.
Globus pallidus (lateral)	8.7 ± 3.9	37 ± 10	2.4 ± 1.4[b]	60 ± 15	197 ± 99	12.4 ± 3.3	0.3 ± 0.1
Globus pallidus (medial)	6.0 ± 2.2	71 ± 25	—	16 ± 4	877 ± 253	15.0 ± 2.0	n.d.
Hypothalamus	109.3 ± 31.2	255 ± 34	23.1 ± 6.0	309 ± 60	112 ± 19	54.9 ± 5.6	41.8 ± 14.4
Substantia nigra (pars compacta)	9.7 ± 2.7	111 ± 21	1.9 ± 1.3[b]	58 ± 5	1264 ± 239	28.8 ± 2.9	2.4 ± 0.3
Substantia nigra (pars reticulata)	6.8 ± 1.8	—	—	62 ± 15	1535 ± 177	21.4 ± 2.9	2.1 ± 0.4
Thalamus (anteromedial n.)	8.3 ± 2.7	87 ± 23	0.3 ± 0.1	112 ± 21	n.d.	8.1 ± 2.5	n.d.
Periaqueductal grey	19.5 ± 5.0	113 ± 63	—	212 ± 30	378 ± 130	23.2 ± 3.4	1.7 ± 0.5
Cerebellum	1.5 ± 0.7	10 ± 4	0.4 ± 0.1	1 ± 0.3	n.d.	4.9 ± 1.1	n.d.
Locus coeruleus	—	—	—	—	310 ± 72	—	5.5 ± 1.1

[a] References are indicated by superscript numbers. n.d. = not detectable.
[b] Value for combined subareas of this brain region. All values are means ± SEM for 3–30 individual brains.

the reported effects of vasopressin on behavior might be due to the peripheral triggering of visceral integrative centers, via visceral afferents (104). Nevertheless, intracerebrally administered vasopressin may still act centrally—to produce similar behavioral consequences. It is difficult to see how the very small doses of peptide needed by the intracerebral route could act peripherally. The parallel behavioral actions of vasopressin when acting peripherally or centrally are reminiscent of the parallel actions of cholecystokinin and bombesin on satiety mechanisms, again capable of being elicited by the same peptides at either central or peripheral sites (50, 51). Is this how neuropeptides integrate body and brain (see 105)?

The mechanisms involved in either the peripheral or central actions of vasopressin on memory processes, however, remain unclear. Is it possible that a direct central action on catecholamine systems might converge, with the peripheral activation of sensory inputs leading to activation of the same final common pathways? As described above, vasopressin terminals in brain are known to exist in high density in the region of the catecholamine cells in locus coeruleus and substantia nigra, and Kovacs and colleagues (106) have argued on other grounds that the effects of vasopressin on memory processes may involve activation of central noradrenergic pathways.

NEUROPEPTIDES IN HUMAN BRAIN

A variety of neuropeptides can be detected in post-mortem human brain tissue. All of the peptides so far examined have proved surprisingly stable in post-mortem brain and both radioimmunoassays and immunostaining methods can be applied (98, 107–112). We have published data on the regional distribution in normal human brain of various neuropeptides; these include substance P (107); cholecystokinin (108); neurotensin (109); TRH (110); somatostatin (111); and VIP (112). Some of these results are summarized in Table 3. The general patterns of distribution observed in human brain and spinal cord are similar to those found in animal material. A series of studies are under way to compare the neuropeptide content of different regions of brain in a variety of pathological conditions. There are, for example, a number of changes associated with the loss of peptide-containing neurons from basal ganglia in Huntington's disease; these include a marked loss of substance P from globus pallidus and substantia nigra (107). Measurements of peptide receptors by radioligand binding techniques are also possible in human post-mortem brain tissue; a loss of cholecystokinin receptors from basal ganglia and cerebral cortex in Huntington's disease has been reported (113). In senile dementia of Alzheimer's type, measurements of cholecystokinin, somatostatin, and VIP have been used as indices of cortical damage, and surprisingly little change was found in the concentration of any of these in various regions

of cerebral cortex, except for a reduction in somatostatin in temporal cortex (114).

It is possible to measure by radioimmunoassay a variety of neuropeptides in human cerebrospinal fluid (csf), and no doubt there will be many attempts to determine whether neuropeptide abnormalities exist in various human disease conditions by using this approach (see 115). Although measurements in csf so far represent the only direct approach to assessing cerebral peptide function in the living patient, much more research will be needed to determine how well measurements of peptide concentration in lumbar csf reflect the state of activity or integrity of peptide systems in brain.

NEURONAL COEXISTENCE OF PEPTIDES WITH MONOAMINES AND OTHER PEPTIDES

As first shown by T. Hökfelt and his colleagues (1), the application of multiple histochemical staining techniques to tissue sections has revealed the unexpected finding that neuropeptides are sometimes found in the same neurons that contain a monoamine neurotransmitter or another neuropeptide (1, 116). There are now many examples of such peptide/-monoamine coexistences (for review see 116), mainly in the peripheral autonomic nervous system. However, some clear examples also exist within the CNS.

Substance P, Serotonin, and TRH in Raphe Neurons

A well studied case is the coexistence of substance P and 5-hydroxytryptamine (5-HT) in some neurons in the medullary raphe nuclei (1). Using a combination of multiple immunostaining of adjacent thin serial sections and serial restaining of individual sections, Johansson et al (117) have recently shown that some neurons in these nuclei exhibit a three-way coexistence of 5-HT, substance P, and TRH. All possible combinations of one, two, or three substances in the same cell seem to exist, and there were differences in the proportions of these types between subgroups of cells in the raphe complex. Thus, cells exhibiting three-way coexistence were particularly abundant in the nucleus raphe pallidus in which, at certain levels, virtually all cells stained for all three substances.

These immunohistochemical findings are supported by measurements of substance P, TRH, and 5-HT in the projection areas of the raphe nuclei cells in spinal cord, where a depletion of all three materials occurs in parallel following destruction of the raphe neurons with 5,7-dihydroxytryptamine (118).

The significance of this remarkable three-way coexistence is quite unknown. It is not a phenomenon observed in relation to any of the three substances in other neuronal locations in CNS. As with other coexistence phenomena one can see that the ability to synthesize and presumably to secrete multiple chemical messengers increases the diversity of chemical signalling in the nervous system. Thus, the raphe neurons, by virtue of the three-way coexistence and all possible permutations of it, can exist in seven different sub-categories, each distinguished by a different "chemical code". How such a code is read, however, remains a challenge for future work.

Cholecystokinin and Dopamine in Mesolimbic Neurons

Another example of coexistence that has aroused considerable interest is the discovery that a cholecystokinin-like peptide is present in some dopaminergic neurons in the ventral tegmental area (A10 group) (119, 120). About half of the ventral tegmental dopaminergic cells appear to contain cholecystokinin, and the axons of these neurons project principally to certain limbic forebrain areas, including nucleus accumbens, olfactory tubercle, and amygdala. The local application of cholecystokinin to dopaminergic neurons in the ventral tegmentum by microiontophoresis consistently activated cell firing (45), suggesting a possible role for cholecystokinin in regulating the activity of some mesolimbic dopaminergic neurons.

Neuropeptides in Magnocellular Neurosecretory Neurons

A bewildering variety of neuropeptide staining reactions have been observed in the magnocellular neurons of the supraoptic and paraventricular nuclei in hypothalamus (for review see 116). These include glucagon-like, cholecystokinin-like, dynorphin-like, enkephalin-like, prolactin-like, and angiotensin II-like materials. Whether some of these represent staining artifacts is not clear, but there is confirmatory evidence from chemical characterization of the immunoreactive materials in neurohypophysis that dynorphin, enkephalins, and cholecystokinin (CCK-8) exist normally in the posterior lobe, although the functional significance of these additional peptides in the neurosecretory cells is unclear. The additional peptides are present in only minute amounts by comparison with the massive content of vasopressin or oxytocin in these neurons.

CONCLUSIONS

Eight years ago I wrote, "The possibility that 'peptidergic' neurons may exist, releasing peptide neurotransmitter substances, or that peptides may

exert other long-term modulatory influences on neuronal function is one to delight the neuropharmacologist and the pharmaceutical industry" (121). The early scientific promise has certainly been fulfilled, although the application of this wealth of new knowledge to the pharmaceutical industry remains a challenge. In so rapidly moving a field the time is probably not yet ripe for generalities. Some concepts do begin to emerge, however, and some directions for future work seem clear.

One of the most important general principles that has emerged is that a peptide will rarely if ever be found to serve only a single biological function. The same chemical may be used as an endocrine hormone, a paracrine factor, a neuroendocrine releasing factor, a neuromodulator, or a neurotransmitter in different parts of the body or in different species. Within the nervous system an individual neuropeptide may be involved in many different functions. Thus, substance P may be involved in sensory transmission in spinal cord and in entirely unrelated functions within the brain and in the retina. This should come as no surprise, as we have long been accustomed to thinking of acetylcholine and the other monoamines in this way, but somehow the neuropeptides were at first regarded as more specialized in their likely neural functions.

In terms of future directions for neuropharmacological research in the peptide area there are many obvious needs. It will be vital to have more information on the properties and pharmacological classification of peptide receptors in the nervous system. Are the neural receptors identical to those found on peripheral target cells for the same peptides? Do pharmacologically distinct subclasses of peptide receptor exist? What can we learn of the interaction of peptides with each other or with monoamines at the cellular or receptor level?

For the biochemically inclined, there is a large new territory to be explored in characterizing the enzymic machinery involved in the biosynthesis and degradation of peptides in the nervous system, an area that also offers the potential for developing enzyme inhibitors as drugs to manipulate neuropeptide function.

Reluctantly perhaps, we also have to contemplate the virtual certainty that many more neuropeptides remain to be discovered. When one considers the hit-or-miss basis by which we have arrived at today's list of brain peptides, there must be scope for further discovery.

In preparing this review I have been conscious of the near impossibility of the task, and apologize to the many scientists whose important publications in this area could not be cited because of the limited space available.

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