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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
Bombesin	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