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Abstract

The present article provides a brief overview of various aspects on neuropeptides, emphasizing their multitude and their wide distribution in both the peripheral and central nervous system. Interestingly, neuropeptides are also expressed in various types of glial cells under normal and experimental conditions. The recent identification of, often multiple, receptor subtypes for each peptide, as well as the development of peptide antagonists, have provided an experimental framework to explore functional roles of neuropeptides. A characteristic of neuropeptides is the plasticity in their expression, reflecting the fact that release has to be compensated by de novo synthesis at the cell body level. In several systems peptides can be expressed at very low levels normally but are upregulated in response to, for example, nerve injury. The fact that neuropeptides virtually always coexist with one or more classic transmitters suggests that they are involved in modulatory processes and probably in many other types of functions, for example exerting trophic effects. Recent studies employing transgene technology have provided some information on their functional role, although compensatory mechanisms in all probability could disguise even a well defined action. It has been recognized that both 'old' and newly discovered peptides may be involved in the regulation of food intake. Recently the first disease-related mutation in a peptidergic system has been identified, and clinical efficacy of a substance P antagonist for treatment of depression has been reported. Taken together it seems that peptides may play a role particularly when the nervous system is stressed, challenged or afflicted by disease, and that peptidergic systems may, therefore, be targets for novel therapeutic strategies.

Keywords: Coexistence; Feeding; Non-peptide antagonists; Non-synaptic signalling; Trophic actions

Contents

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Research on neuropeptides has been carried out in a serious and focused way for about 30 years. A major impetus was the demonstration by Guillemin, Vale and collaborators as well as by Schally, Arimura and colleagues that most hypothalamic releasing and inhibiting hormones chemically could be identified as small peptides. Moreover, a surprising early observation made in radioimmunoassay and immunohistochemical studies was that several of these peptides were not confined to the median eminence/infundibulum in the basal hypothalamus, that is the expected storage and release sites for these factors/hormones, and the brain area from which they had been isolated. In fact, thyrotropin releasing hormone (TRH), somatostatin and corticotropin releasing hormone (CRH), we now know, have a very widespread distribution in the brain with roles beyond pituitary control. The approximately concomitant work on peptides such as substance P (discovered by von Euler and Gaddum in 1931) by Leeman and collaborators, and on opioid peptides by Hughes, Kosterlitz and associates, as well as the demonstration by Vanderhaegen and collaborators that the well established gut hormone cholecystokinin (CCK), sequenced by Mutt and Jorpes, was present in the brain, sparked further interest in neuropeptides, as did the multiple peptides isolated from frog skin by Erspamer and collaborators. The recognition that peptides could be found in the nervous systems of the hydra, insects and worms suggest that the peptides in fact have played a role in nervous system function from 'the very beginning'.

The present article provides only a brief summary on some aspects of neuropeptides, and references had to be limited. For further and more detailed information see, for example, some reviews published during the 1990s by Hökfelt (1991), Kupfermann (1991), Otsuka and Yoshioka (1993), Herbert (1994), Lundberg (1996) and Darlison and Richter (1999) and books by Burbach and de Wied (1993) and Strand (1999). There are many symposium books dealing with individual peptides or families of peptides, but they are too numerous to be listed here.

1. Neuropeptides and their families

Peptides have been isolated from various organs; in particular, the porcine intestine and basal hypothalamus (see above) and, surprisingly, frog skin have been rich sources leading to the plethora of peptides now known. Ever since the mid 1970s a steady stream of peptides have been discovered and investigated, and in Table 1 mammalian peptides have been summarized. In fact, as indicated under the heading 'Novel peptides' in Table 1, the discovery period does not yet seem to be over. This is partially thanks to the use of new approaches mainly based on molecular biological techniques. Also, as described in the following article by Civelli and coworkers, a reversed approach has been taken, where the start is an orphan receptor and where the endogenous ligand is searched for. In this way some such orphan ligands could be identified as peptides.

Several new neuropeptides have been discovered during the 1990s (Table 1). Secretoneurin (Kirchmair et al., 1993), a cleavage product from secretogranin II (or chromogranin C), comprised of 33 amino acids, has interesting distribution patterns both in rat (Marksteiner et al., 1993a) and human (Marksteiner et al., 1993b) brain. This is another example that large polypeptide precursor molecules may hide interesting biological activities. Orphanin FQ or nociceptin (Meunier et al., 1995; Reinscheid et al., 1995) and prolactin releasing peptide (Hinuma et al., 1998) have both been discovered via

Table 1
Some mammalian neuropeptides and neuropeptide families[a]

Hypothalamic hormones
Oxytocin (9 amino acid residues, a.a.r.)
Vasopressin (9 a.a.r.)
Hypothalamic releasing and inhibiting hormones
Corticotropin releasing hormone (CRH) (41 a.a.r.)
Growth hormone releasing hormone (GHRH) (44 a.a.r.)
Luteinizing hormone releasing hormone (LHRH) (10 a.a.r.)
Somatostatin (SOM), growth hormone release inhibiting hormone (14 a.a.r. plus several forms)
Thyrotropin releasing hormone (TRH) (3 a.a.r.)
Tachykinins
Neurokinin α (NKA) (substance K) (10 a.a.r.)
Neurokinin β (10 a.a.r.)
Neuropeptide K (36 a.a.r.)
Substance P (SP) (11 a.a.r.)
Opioid peptides
β-endorphin (30 a.a.r.)
Dynorphin (17 a.a.r. and other forms)
Met- and leu-enkephalin (5 a.a.r.)
NPY and related peptides
Neuropeptide tyrosine (NPY) (36 a.a.r.)
Pancreatic polypeptide (36 a.a.r)
Peptide tyrosine-tyrosine (PYY) (36 a.a.r.)
VIP-glucagon family
Glucogen-like peptide-1 (GLP-1) (29 a.a.r.)
Peptide histidine isoleucine (PHI) (27 a.a.r.)
Pituitary adenylate cyclase activating peptide (PACAP) (27 or 38 a.a.r.)
Vasoactive intestinal polypeptide (VIP) (28 a.a.r.)
Other neuropeptides
Brain natriuretic peptide (32 a.a.r.)
Calcitonin gene-related peptide (CGRP) (α- and β-form) (37 a.a.r.)
Cholecystokinin (CCK) (8 a.a.r. and other forms)
Glucagon-like peptide 1 (30 a.a.r.)
Galanin (GAL) (29 or 30 a.a.r.)
Islet amyloid polypeptide (IAPP) or amylin (37 a.a.r)
Melanin concentrating hormone (MCH) (19 a.a.r.)
Melanocortins (ACTH, α-MSH and others)
Neuropeptide FF (F8Fa) (8a.a.r)
Neurotensin (13 a.a.r.)
Parathyroid hormone related protein (34 or 37 a.a.r.)
'Novel' neuropeptides
Agouti gene-related protein (AGRP) (131 a.a.r.)
Cocaine and amphetamine regulated transcript (CART)/peptide
Corticostatin (14 a.a.r., 29 a.a.r.) (in corticostatin-14, 11 a.a.r. are shared with somastotatin)
Endomorphin-I and -2 (both 4 a.a.r.)
5-HT-moduline (4 a.a.r.)
Hypocretins/orexins (29 or 39 a.a.r.)
Nociceptin/orphanin FQ (17 a.a.r.)
Nocistatin (17 a.a.r.)
Prolactin releasing peptide (20 or 31 a.a.r.)
Secretoneurin (33 a.a.r.)
Urocortin (40 a.a.r.; 45% sequence identity with CRH)

[a] No references are given in this table. However, references for 'Novel neuropeptides' can be found in the text.

orphan receptors (see Civelli et al., 2000, this issue). Urotensin II was originally isolated from fish, and its human homologue was recently identified (Coulouarn et al., 1998) and found to be the ligand for the orphan receptor GPR14 (Ames et al., 1999). Using cocaine and amphetamine stimulation a highly abundant transcript encoding several putative bioactive peptides was discovered and named CART (cocaine- and amphetamine-regulated transcript) (Douglass et al., 1995). A new member of the CRH family has been isolated, urocortin, which has 63% sequence identity with urotensin and 45% with CRF (Vaughan et al., 1995). In a major effort, Gautvik et al. (1996) applied directional tag PCR subtractive hybridization to construct a rat hypothalamic cDNA library from which cerebellar and hippocampal sequences were depleted. They found 43 enriched clones, of which around 50% were novel and 23 highly enriched. One of the novel species 'was restricted to cells in a small bilaterally symmetric area of the paraventricular hypothalamus' (Gautvik et al., 1996) and has been named hypocretins (de Lecea et al., 1998) alternatively orexins (Sakurai et al., 1998) [Fig. 1(a)]. These and several other peptides have been shown to be involved in the regulation of feeding behaviours. Other examples are melanin-concentrating hormone (MCH) [Fig. 1(b)] (Vaughan et al., 1989) and the endogenous melanocortin antagonist agouti gene-related protein (AGRP) (Ollmann et al., 1997; Shutter et al., 1997). Some further peptides involved in pain sensation have been discovered, that is two potent and selective endogenous agonists for the μ-opiate receptor called endomorphin-1 and -2 (Zadina et al., 1997), and nocistatin which is part of the same precursor as orphanin FQ/nociceptin and which blocks the effect of orphanin FQ/nociceptin (Okuda-Ashitaka et al., 1998). Cortistatin is expressed in a distinct subset of cortical neurons, shares eleven of its 14 amino acids with somatostatin, and has depressant and sleep-modulating properties (de Lecea et al. 1996, 1997). Massot et al. (1996) have described a small peptide, 5-hydroxytryptamine (5-HT)-moduline, which influences serotonergic transmission via interaction with $5\text{-HT}_{1B/1D}$ receptors.

2. Evolutionary aspects

Peptides occur in the whole animal kingdom (as well as in plants), including the hydra, a member of the coelenterates with a very simple nervous system. In this species numerous peptides, mainly belonging to the FMRF amide family, have been identified (see Grimmelikhuijzen et al., 1996). FMRF amide-like peptides are also expressed in ~10% of the neurons in *Caenorhabditis elegans*, a nematode, and are encoded by at least 14 genes which are transcribed (Nelson et al., 1998). There are multiple peptides (see Schoofs et al., 1997)

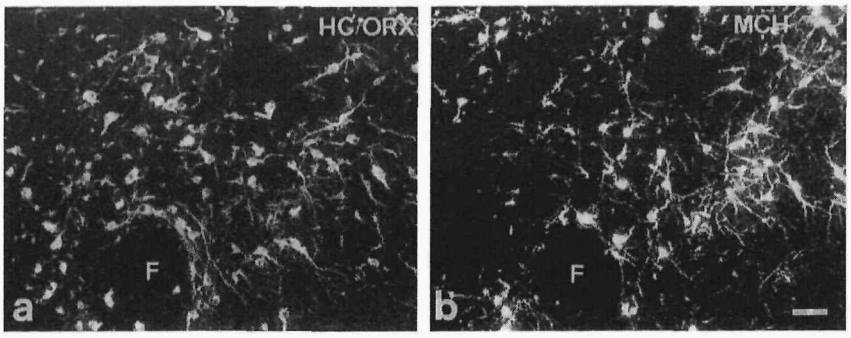

Fig. 1. Immunofluorescence micrographs show hypocretin/orexin (HC/ORX) and melanin concentrating hormone (MCH) neurons in the rat lateral hypothalamus. Double staining reveals that these peptides are present in two distinctly different neuron populations without any coexistence. F=fornix. Bar indicates 50 μm for (a) and (b).

and peptide receptors (see Vanden Broeck et al., 1998) in insects.

The peptide families have their origin in many million year-old primordial genes that through various processes such as gene duplication and point mutations have developed into the presently known ensemble (see Hoyle, 1998). The most thoroughly examined family consists of the two hypothalamic magnocellular neurosecretory peptides oxytocin and vasopressin, in fact the first discovered and chemically identified neuropeptides (Du Vigneaud et al., 1953). They have high structural homology, but are produced in different neurons, have different physiological activities with two molecular lineages: the isotocyin–mesotocyn–oxytocin line associated with reproductive functions and the vasotosin–vasopressin line primarily concerned with water and electrolyte balance (see Acher et al., 1995; Hoyle, 1998).

3. Peptide biosynthesis

The synthesis of neuropeptides is a complex process, distinctly different from that of classic transmitters (see Eipper and Mains, 1999). Virtually all bioactive neuropeptides are part of a larger, inactive molecule precursor which, after synthesis in the endoplasmic reticulum, is transferred to the Golgi apparatus for packaging, followed by centrifugal transport and exocytotic release. The precursor proteins are stored in the so called large dense core vesicles or secretory granules together with processing enzymes, also called convertases, which cut out the bioactive peptides. Several such enzymes have now been identified (see Seidah and Chretien, 1997; Steiner, 1998). The majority of endoproteolytic cleavages occur in the secretory granules, mainly at pairs of basic amino acid residues. In addition, important post-translational enzymatic modifications of the final peptide may occur, such as glycosylation, C-terminal amidation, acetylation, phosphorylation and sulfation.

The precursor molecules may contain several copies of one and the same peptide but also different types of peptides. For example, the precursor for TRH contains five copies of the tripeptide TRH, and the proopiomelanocortin (POMC) precursor gives rise to adrenocorticotropin, (ACTH; corticotropin), melanotropins and the opioid peptide β-endorphin, all of which have distinct biological effects (de Wied, 1999). The neuropeptides are in principle degraded in the extracellular space by peptidases, of which several have been identified (see Roques and Noble, 1995).

4. Peptide expression patterns

Peptides are expressed in neurons in at least three types of mode (Table 2) (see also Tohyama, 1992). Thus, some peptides are present at high levels under normal circumstances, which indicates that they are functionally available at any time. A second type are peptides normally expressed at low or undetectable levels. They are then upregulated under certain conditions, for example after nerve injury (see below). Thus a specific stimulus is required for such a peptide to become functionally relevant. The third type of peptide is expressed early during development, often only prenatally, and they are then downregulated postnatally. There seems to be a connection between the second and third type in that many of the peptides expressed transiently during ontogenesis can be 'reactivated' in adulthood, for example by nerve injury. It is important to note that one and the same peptide can belong to each of these three groups, and that the classification in Table 2 is dependent on the type of neuron, in which the peptide is expressed and how a neuron 'uses' a particular peptide.

5. Distribution and coexistence of messengers

Peptides are present in all parts of the nervous system — the brain, spinal cord, gastrointestinal tract, autonomic and sensory ganglia. However, each peptide has its unique distribution pattern as exemplified in Fig. 2,

Table 2
Neurons handle peptides in different ways[a]

Mode 1:	Substantial levels synthesized and stored under normal conditions Examples: SP and CGRP in primary sensory neurons, GAL in hypothalamic neurons, VIP and NPY in cortical neurons
Mode 2:	Very low levels under normal conditions Stimulus for upregulation of synthesis required Examples: VIP, GAL and NPY in sensory neurons
Mode 3:	Transient expression during development Examples: SOM in many central systems, CGRP in chicken motor neurons, SP in spinal guiding neurons, GAL in primary sensory neurons

[a] For abbreviations, see Table 1.

showing four peptides in the mouse dorsal hippocampus, galanin [Fig. 2(a)], NPY [Fig. 2(b)], CCK [Fig. 2(c)] and enkephalin [Fig. 2(d)]. Initially, it was assumed that these 'peptidergic' systems were different from and complementary to previously transmitter-characterized neurons, for example the catecholamine and serotonin systems (Carlsson et al., 1962; Dahlström and Fuxe 1964, 1965). Therefore, an important perspective on neuropeptides, and on chemical transmission in general, was added with the recognition that peptides almost always coexist with one or more classic messengers (see Hökfelt, 1991; Lundberg, 1996). Thus, peptides are either complementary to classic transmitters, for example by modulating their actions, or may play a completely different role in nervous system function, such as exerting trophic actions (Table 3), as has been shown for example for vasoactive intestinal polypeptide (VIP) (see Gozes and Brenneman, 1990) (see below). Peptide coexistence with classic transmitters should also be seen in the perspective of multiple messengers in general. Thus, more than one of the classic transmitters such as glutamate, γ-amino butyric acid (GABA), glycine and ATP may occur together in one neuron (see Hökfelt et al., 2000). For example, glycine and GABA have been shown to coexist in cerebellar Golgi terminals and cells (Ottersen et al., 1988) and in spinal cord boutons (Todd and Sullivan, 1990), and a recent study reports that they can in fact be co-released in the spinal cord (Jonas et al., 1998). Here we will focus on coexistence situations where peptides are involved.

Coexistence seems to be such a general phenomenon that the question may be posed whether or not there are any peptide neurons lacking a classic transmitter. One possible example is the hypothalamic magnocellular neurons, which contain a host of peptides with oxytocin and vasopressin as their principal messengers (see Brownstein and Mezey, 1986), and where no classic transmitter has so far been identified with certainty. Also in the parvocellular hypothalamic neurons involved in anterior pituitary control peptides are presumably the most important messengers, except for some tuberoinfundibular neurons which seem to have dopamine, the prolactin inhibitory factor, as the main messenger (see Hökfelt and Fuxe, 1972). Some CRH neurons in the paraventricular nucleus express glutamic acid decarboxylase (GAD) and synthesize GABA (Meister and Hökfelt, 1988). Again CRH is still likely the main messenger and GABA a modulator of, for example, CRH release. In virtually all other neurons, however, non-peptide messengers are the principal transmitters.

Most attention has been focused on colocalization of peptides with biogenic amines and acetylcholine. A general rule seems to be that there is no general rule in terms of combinations. For example, in the rat the most caudal medullary 5-HT neurons projecting to the spinal ventral horn express substance P and TRH, whereas the rostral medullary neurons innervating the dorsal horn seem to lack these peptides (see Hökfelt et al., 2000). The pontine raphe nuclei of the rat projecting rostrally to the forebrain also seem to lack these two peptides. However, in man these 5-HT dorsal raphe neurons express substance P (Baker et al., 1991; Sergeyev et al., 1999), pointing to distinct species differences. Furthermore, many 5-HT neurons in the raphe nuclei and noradrenaline neurons in the rat locus coeruleus synthesize galanin, and galanin can be visualized in most noradrenergic nerve terminals in cortex and hippocampus [Fig. 2(a)], and exerts actions both at the cell body and nerve terminal level to inhibit firing and classic transmitter release (see Hökfelt et al., 1998; Xu et al., 1998a,b), possibly involving dendritic/somatic release of the peptide (Fig. 3). In fact, there is evidence for the latter type of peptide release both from magnocellular (Morris et al., 1993) and dorsal root ganglion (Huang and Neher, 1996) neurons. There is immunohistochemical evidence that these 5-HT neurons *in addition* may express an amino acid transmitter, such as GABA (Belin et al., 1983) or glutamate (Kaneko et al., 1990; Nicholas et al., 1990). Thus, using triple-staining methodology, evidence for coexpression of glutamate-, 5-HT- and substance P-like immunoreactivities in bulbo-spinal neurons has been presented (Nicholas et al., 1992). In an elegant in vitro study, Johnson (1994) has shown co-release of serotonin and glutamate from rat mesopontine neurons. In the peripheral nervous system certain neurons may release ATP, noradrenaline and neuropeptide tyrosine (NPY)

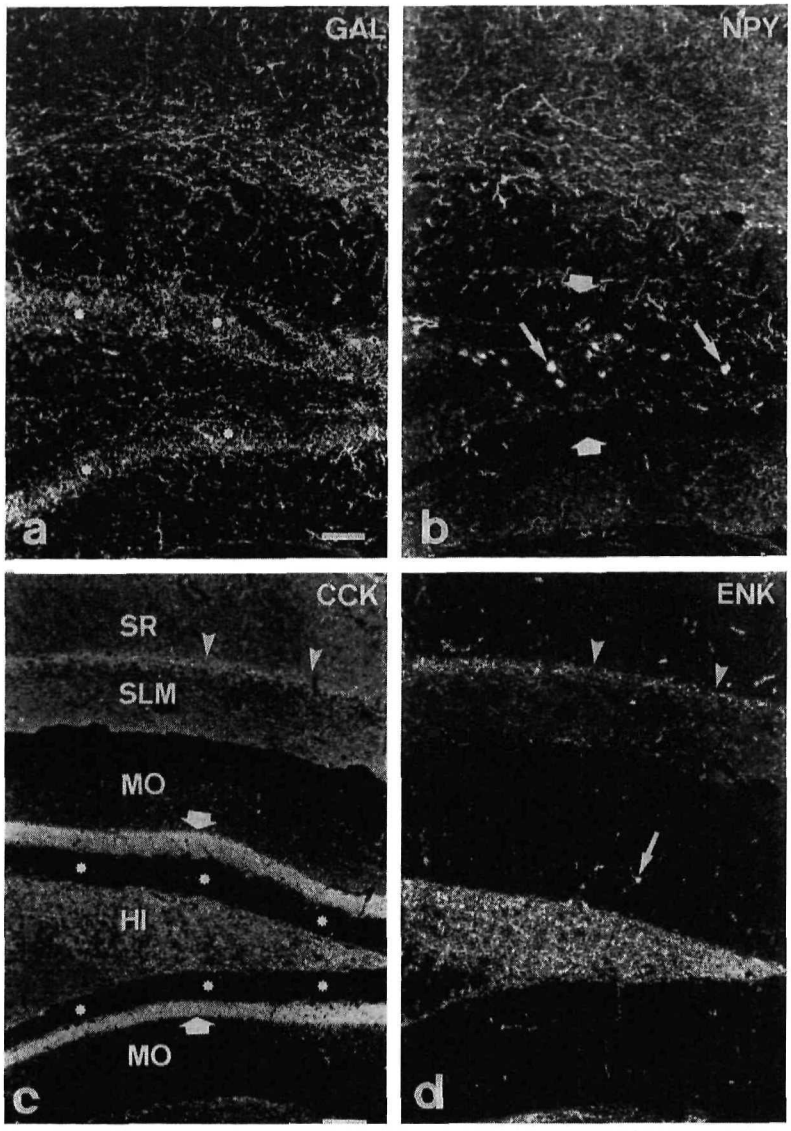

Fig. 2. Immunofluorescence micrographs of semi-adjacent sections showing four peptides, galanin (GAL) (a), neuropeptide Y (NPY) (b), cholecystokinin (CCK) (c) and enkephalin (ENK) (d) in the mouse dorsal hippocampus. Note the distinctly different distribution patterns. Galanin is present in fibers, with a fairly even distribution, probably representing noradrenergic terminals. (b) NPY is mainly present in interneurons (arrows) giving rise to a fiber plexus especially in stratum radiatum (SR) and stratum lacunosum moleculare (SLM). CCK is present in the supragranular layer (thick arrows), whereas the granule cell layer (*) lacks immunoreactivity. Note dense fiber plexus (mossy fibers) in the hilus (HI) of the dentate gyrus and thin fiber layer between SR and SLM. (d) Enkephalin has a more restricted distribution, mainly being present in the hilus of the dentate gyrus (mossy fibers). Note single enkephalin cell body (arrow) as well as the thin fiber plexus (arrowheads) between SR and SLM. MO=molecular layer of the dentate gyrus. Bar indicates 50 μm for (a–d).

Table 3
Trophic effects of neuropeptides[a]

Peptide	Effect	Reference
Opioid peptides	Affect DNA synthesis in brain	Vertes et al. (1982)
Substance P, NKA	Stimulate growth of fibroblasts and smooth muscle	Nilsson et al. (1985)
VIP	Alters bone mineralization	Hohmann et al. (1986)
CGRP	Stimulates acetylcholine receptor synthesis in myoblasts	Fontaine et al. (1986), New and Mudge (1986)

[a] This table only shows some early examples. For abbreviations, see Table 1.

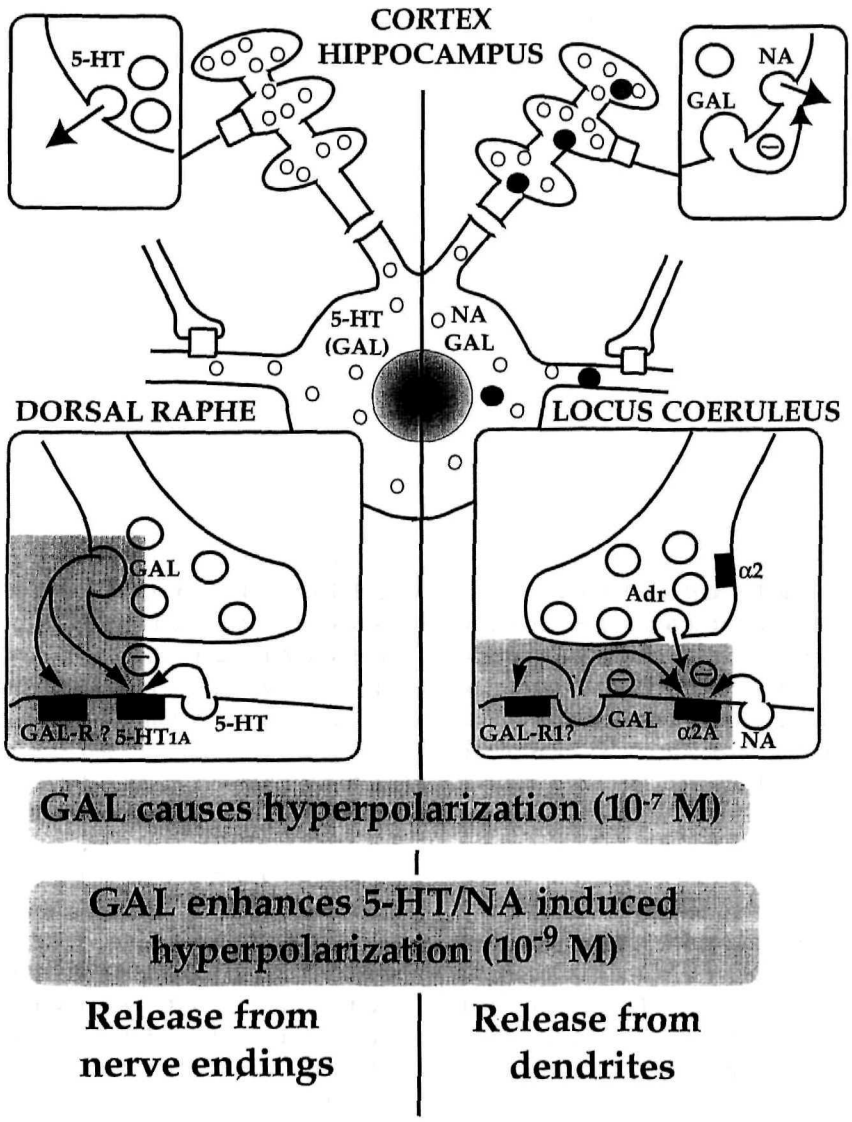

Fig. 3. Schematic illustration of effects of galanin (GAL) on a serotoninergic dorsal raphe neuron (left panel) and a noradrenergic locus coeruleus neuron (right panel). Under normal circumstances the 5-HT neuron expresses low levels of GAL and the peptide cannot be detected in cell bodies or forebrain nerve terminals. In contrast, the noradrenergic neuron has a robust GAL synthesis, and GAL can be detected in cell bodies as well as cortical and hippocampal nerve terminals. The 5-HT neurons are innervated by non-serotonergic, GAL-containing boutons of unknown origin. There are comparatively few GAL nerve endings surrounding and synapsing on locus coeruleus neurons. Therefore, under normal circumstances GAL acting on 5-HT neurons may mainly originate from synaptic nerve endings in the dorsal raphe, but from dendrites and soma in the locus coeruleus. Especially under conditions when GAL synthesis is upregulated in 5-HT neurons, GAL may also in this region be released from dendrites and soma. The effect of GAL on 5-HT and noradrenaline neurons is similar, causing hyperpolarization via a direct action on a GAL receptor of not yet defined type. Somewhat higher concentrations are needed in the dorsal raphe as compared to locus coeruleus. In addition, GAL at low concentrations (10^{-9} M) enhances the inhibitory effect of 5-HT on the 5-HT_{1A} receptor on the 5-HT neurons and of the α_{2A} receptor on the noradrenaline neurons. These effects, obtained at low peptide concentrations, may in fact be in the physiological range. From (Hökfelt et al., 1998).

(Kasakov et al., 1988; Sneddon and Westfall, 1984). In summary, neurons may release a 'cocktail' of messenger molecules providing a spectrum of biological actions, including different temporal information (fast, intermediate and slow signalling). The functional implications of coexistence have been discussed in several reviews (see, for example, Kupfermann, 1991; Lundberg, 1996).

A major difference between principal transmitters and peptides is the mode of synthesis and replacement after release. Thus, classic transmitters often have a membrane reuptake mechanism through specific transporter molecules, which allow reutilization of the transmitter. Classic transmitters can also be synthesized locally in the nerve endings. In contrast, it has been assumed that peptides can only be produced ribosomally, de novo in the cell bodies (see above), and that replacement after release has to occur via axonal transport from the cell

bodies to the nerve endings. However, occurrence of a high affinity uptake for CCK has been reported (Migaud et al., 1995).

An important issue is to understand the mechanisms underlying the release of these different classes of messenger molecules. There was early evidence that the release of classic transmitter and peptide can be differential and dependent on frequency and patterns of firing, presumably due to different subcellular storage sites (see Lundberg, 1996; Lundberg and Hökfelt, 1983). Verhage et al. (1991) have provided evidence that neuropeptide release is triggered by small elevations in the Ca^{2+} concentration in the bulk cytoplasm, whereas secretion of amino acids requires higher elevations, as produced in the vicinity of Ca^{2+} channels', i.e. near the active zone at synapses. In agreement, morphological evidence indicates that peptides stored in so called large dense core vesicles, are released extrasynaptically (Thureson-Klein and Klein, 1990). Huang and Neher (1996) have shown that Ca^{2+}-dependent exocytosis of substance P can occur from dorsal root ganglion neuron somata, at Ca^{2+} levels at least ten times lower than those required for transmitter release from nerve terminals. A number of proteins have been isolated related to exocytosis and transmitter release (see Südhof, 1995). However, there are distinct physiological differences between release of classic transmitters from small synaptic vesicles and of peptides from large dense core vesicles (see DeCamilli, 1991), which should have molecular correlates. It has recently been reported that CAPS (calcium-dependent activator protein for secretion; mammalian UNC-31) is localized to neuronal large dense core vesicles but not to small synaptic vesicles, suggesting that this protein is selectively associated with the large dense core vesicle exocytotic pathway (Berwin et al., 1998).

Interestingly, there are indications that neuropeptide-encoding mRNAs such as oxytocin and vasopressin mRNAs can be transported centrifugally into the axons (Jirikowski et al., 1990; Lehman et al., 1990; Levy et al., 1990; Mohr et al., 1990; Murphy et al., 1989). The functional significance of mRNA within the axonal compartment still remains to be elucidated.

6. Plasticity in peptide expression

There is evidence that peptide levels may vary considerably during different conditions, including endogenous diurnal variations and after experimental manipulations. Since replacement after release almost exclusively seems to occur via new synthesis in cell bodies, neuronal activation and peptide release from nerve endings are followed by a rapid upregulation of mRNA levels (see, for example, Schalling et al., 1987). Thus, there is a considerable delay until peptide levels in the nerve endings are restored. In contrast, classic transmitters, such as catecholamines can be locally synthezised in nerve endings and replaced by efficient reuptake mechanisms, and their rate of synthesis is regulated by phosphorylation of the synthetic enzymes. Thus, classic transmitter levels can be kept constant under various conditions. The quite dramatic regulation of peptide synthesis has been particularly evident when using the in situ hybridization technique (see Young, 1990) for analysis of peptide mRNA levels in neuronal somata under various experimental conditions. Some examples are primary sensory neurons and neurons in the rat striatum, but similar regulations occur in many other systems (Table 4).

6.1. Peptides in primary sensory neurons

Primary sensory neurons contain substantial amounts of peptides and provide a good model for analysis of peptidergic mechanisms. Some of these peptides, e.g. substance P and calcitonin gene-related peptide (CGRP) are present in many DRG neurons under normal circumstances. They facilitate transmission in the dorsal horn (in addition to having effects in the periphery, for example vasodilation), and are down-regulated after axotomy (Nielsch et al., 1987; Noguchi et al., 1990) (Table 4). Other peptides such as VIP/peptide histidine isoleucine (PHI), galanin and NPY are normally expressed at low levels, but are dramatically increased after experimental manipulation, especially axotomy (Hökfelt et al., 1987; Shehab and Atkinson, 1986; Wakisaka et al., 1991) (Table 4). In addition, peptide receptors are regulated in DRG neurons after axotomy, including CCK_B (Zhang et al., 1993) and NPY Y1 (Zhang et al., 1994a) and Y2 (Zhang et al., 1997) receptors. Such findings suggest that changes in sensitivity to peptides also help primary sensory neurons to adapt to trauma. Taken together, primary sensory neurons change their phenotype both with regard to messengers, receptors and function after peripheral nerve injury, the implications being that DRG neurons adapt to the new situation by suppressing excitatory transmitters, enhancing inhibition and promoting survival and regenerative mechanisms. This is in agreement with the general view of the reaction of neurons in response to injury, mainly based on studies of motoneurons (see Kreutzberg, 1982), emphasizing that the synthetic machinery of the neuron is reprogrammed from transmitter synthesis to production of molecules of importance for survival and recovery. There is evidence that similar changes occur when neurons in the brain are injured (Table 4) and eventually degenerate. In fact, recent studies on an Alzheimer mouse model suggest that galanin and other peptides are upregulated in a system-specific manner in hippocampus and ventral cortices in old transgenic mice with high plaque density (Diez et al., 1999). Another dramatic example of disease-related changes in peptide expression

Table 4
Effect of axotomy on messengers and receptors in DRG, motor and magnocellular neurons

Neuropeptide/receptor	L5 DRG[a]	L5 MN[a]	SON/PVN[b]
Substance P	⇊(↑)[c]	↓	
CGRP	⇊(↑)	⇈	
Somatostatin	⇊	↑	
VIP/PHI	⇈	↑	
Galanin	⇈	⇈	⇈
Galanin R1-R	↓		⇊
CCK	(↑)		⇈
CCK_B-R	⇈		⇈

[a] Complete sciatic nerve transection; DRG, dorsal root ganglion; L, lumbar; MN, motoneuron.
[b] Hypophysectomy. SON, nucleus supraopticus; PVN, nucleus paraventricularis magnocellularis.
[c] Bracket indicates weak regulation. In the case of substance P and CGRP, a subpopulation of neurons upregulates these peptides, that is opposite to the large number of neurons in which the peptides are downregulated.

in the brain has been provided by studies on various epileptic animal models, where NPY and several other peptides are strongly upregulated, especially in the hippocampal formation (see Gall et al., 1990; Schwarzer et al., 1996). For further information, (see Hökfelt et al. 1994, 1999; Wiesenfeld-Hallin et al., 1992; Zigmond et al., 1996).

6.2. *Peptides in striatum*

The basal ganglia and related structures are associated with several serious central nervous system disorders such as Parkinson's disease and Huntington's chorea. Much interest has been focused on dopamine and its functional role, but neuropeptides may also participate (see Gerfen and Wilson, 1996; Graybiel, 1990). Thus, many dopamine neurons contain peptides, in particular CCK, and the spiny projection neurons in the striatum express peptides at high or low levels. Of the latter, one population contains substance P and dynorphin projects to the substantia nigra zona reticulata, whereas the other one expresses enkephalin and mainly seems to project to the globus pallidus (Gerfen and Wilson, 1996; Graybiel, 1990). Double staining techniques have demonstrated that many of these peptide neurons utilize GABA as their principal transmitter (Penny et al., 1986).

The three peptides mentioned above, enkephalin, substance P and dynorphin, are regulated by the dopaminergic input from the substantia nigra, as evidenced by various manipulations. Thus, attenuation of dopamine transmission in the striatum, for example by lesioning the nigrostriatal pathway with 6-hydroxydopamine or treatment with dopamine receptor antagonists, results in increased expression of enkephalin and a decrease in dynorphin and substance P, whereas dopamine agonists increase levels of dynorphin and substance P but not enkephalin (see Gerfen and Wilson, 1996).

Dopamine also regulates neurotensin in the striatum, a peptide having interesting clinical and behavioural interactions with dopamine (see Kitabgi, 1989; Nemeroff, 1986). Thus, drugs that *attenuate* dopamine transmission, such as haloperidol, 6-hydroxydopamine and reserpine upregulate neurotensin in many cell bodies in the rat striatum via D2 receptors (see Deutch and Zahm, 1992). Moreover, dopamine receptor *stimulation*, but in this case of D1 receptors (with methamphetamine), upregulates neurotensin (Letter et al., 1987; Wachi et al., 1987). D1-regulated neurotensin is present in striato-nigral neurons (Castel et al., 1993), whereas the D2-regulated neurotensin occurs in neurons projecting to the globus pallidus. This is in agreement with the general view that striato-nigral neurons are mainly under the control of D1 receptors and that the striato-pallidal neurons contain D2 receptors (see Gerfen and Wilson, 1996).

7. Neuropeptides and drug development

There are at least three types of 'peptide' drugs, antagonists, agonists and peptidase inhibitors, the latter preventing peptide breakdown and thus strengthening the peptidergic transmission (agonist effect). The early antagonists were of peptidergic nature, mostly D-substituted analogs, one of the first being [D-Pro^2, D-Phe^7, D-Trp^9] substance P (Folkers et al., 1982), a substance P antagonist. A non-peptide substance P antagonist was then developed ten years later (Snider et al., 1991). Another important finding was reported by Peikin and collaborators, showing that buturyl derivatives of cGMP antagonize the action of CCK, and this was followed by development of more potent CCK antagonists at the Rolla Laboratories (Italy), Merck Sharp and Dohme (USA and UK) and Parke-Davis (UK) (Table 5). To achieve this, various types of approaches were taken, mainly extensive screening efforts, resulting in lead compounds which were modified into powerful and specific, non-peptide antagonists. In addition, rational drug design starting out from the peptide molecule itself has been employed (see Hughes et al., 1990).

Table 5
The first non-peptide antagonists[a]

Receptor	Code name	Drug company	Reference
Cholecystokinin A	L-364,718	MSD	Evans et al. (1986)
	LY 219057	Eli Lilly	Howbert et al. (1992)
Cholecystokinin B	L-356,260	MSD	Bock et al. (1989)
	CI 988	Parke-Davis	Hughes et al. (1990)
	LY 262691	Eli Lilly	Barrett et al. (1991)
Neurokinin 1	CP 96345	Pfizer	Snider et al. (1991)
	RP 67580	Rhône-Poulenc	Garret et al. (1991)
	WIN 51708	Sterling Winthrop	Appell et al. (1992)
Neurokinin 2	SR 48968	Sanofi	Advenier et al. (1992)
Neurotensin	SR 48692	Sanofi	Gully et al. (1993)

[a] For further information, see Betancur et al. (1997).

Many of these compounds pass the blood–brain-barrier and can thus be used to probe for central peptide functions under normal circumstances and after various types of manipulations, and potentially in disease states. In a recent review article by Betancur et al. (1997) well over 100 such compounds were listed for a number of peptide receptors, with, for example, around 20 compounds for both the CCK_B and NK-1 (substance P) receptors, and almost 30 for the angiotensin AT1 receptor. The binding characteristics of such antagonists are now being investigated (see Schwartz et al., 1995).

It has turned out to be more difficult to develop potent and specific agonists. Successful examples are a potent CCK_B agonist that passes the blood brain barrier (Durieux et al., 1992), and a potent analgesic opioid extracted from a synthetic combinatorial library containing 52.128.400 D-amino acid hexapeptides (Dooley et al., 1994). An alternative to peptide agonists are peptidase inhibitors. For example, inhibition of enkephalin catabolism represents a potential drug treatment for pain and opioid addiction. In fact, drugs have been developed which inhibit two enzymes, neutral endopeptidase 24.11 and aminopeptidase N, involved in enkephalin degradation (see Roques and Noble, 1995).

Why could peptide antagonists be suitable as drugs, and even advantageous over antagonists to classic transmitters? In general terms, the effects of peptides seem to be 'milder' than those of monoamines and especially than those of amino acid transmitters. The peptides are thus of modulatory nature, and blockade of their receptors should result in less dramatic effects than for example interfering with glutamate or GABA transmission. Moreover, and perhaps more important, peptides may be preferentially released, at least in some systems, when neurons are strongly activated and/or under pathological conditions (see below). Thus, antagonists have normally no effect and will only act on deranged systems with increased peptide release. These characteristics together should lead to less pronounced side effects. The recent demonstration of clinical efficacy of an NK-1 antagonist in depression (Kramer et al., 1998) may represent an example of this, since in this study only few side effects were recorded. In contrast, the new generation of antidepressants based on selective blockade of uptake of serotonin, such as Prozac, show considerable side effects, especially related to sexual dysfunction (see Kramer et al., 1998). This is not surprising, since the uptake blocker will act on *all* 5-HT neurons, not only those associated with mood control. Finally, the fact that peptides often have more than one receptor (see Branchek et al., 2000, this issue) provides possibilities for the design of antagonists for receptor subtypes which may be involved in specific functions. This principle has been important in the monoamine field, where already many subtype specific agonists and antagonists have been developed.

8. Neuropeptide function

8.1. General aspects

In spite of studies demonstrating interesting actions of peptides when exogenously applied, and in spite of the wealth of peptides and all efforts invested in peptide research, including studies with antagonists, it has been difficult to define an exact role for many of these molecules in nervous system function. There are of course, several peptides for which an unequivocal physiological function has been defined, such as the posterior pituitary hormones (oxytocin, vasopressin) originating in the magnocellular hypothalamic nuclei, or the above mentioned hypothalamic releasing and inhibiting factors controlling the anterior pituitary hormone secretion. However, these messengers are not typical neuropeptides, since they are released into the blood and thus act like hormones. No, it is the peptides that are released, presumably extrasynaptically, from nerve endings to act within the nervous system or on peripheral tissues that

represent a problem in terms of establishing their function.

An array of methods has been employed to study the functional significance of neuropeptides. Early on, radioimmunoassay, immunhistochemistry, behavioural methods and electrophysiology played important roles to establish basic knowledge of neuropeptides. More recently modern molecular biology has had a strong impact. The histochemical approach has benefitted in particular from various in situ hybridization procedures (see Young, 1990), made possible by cloning of peptides and peptide receptors. The discovery of numerous receptors has per se been crucial for assigining functional significance to neuropeptides, as was the discovery of peptide antagonists which pass the blood brain barrier (see above). An alternative approach is the use of antisense probes, which are complementary to a sequence of a certain peptide mRNA and can be used to block or attenuate translation of mRNA into protein (see Wahlestedt, 1994). Finally, an increasing number of transgenic mice contribute to understanding of peptide functions (see Bloom et al., 1998; Palmiter et al., 1998). In the following we will mention some interesting examples, where specific functions could be associated with release of endogenous neuropeptides. Experiments with transgenic animals will also be mentioned.

8.2. *Some interesting examples*

One of the first pieces of evidence for a physiologic involvement of a peptide in CNS functions has been obtained for dynorphin in the hippocampus. This opioid peptide is expressed in the granule cells and their mossy fibers (Fallon and Ciofi, 1990; McGinty et al., 1983; Watson et al., 1982), and it was shown that endogenous dynorphin can block long term potentiation (LTP) via kappa opioid receptors (Wagner et al., 1993; Weisskopf et al., 1993). Another case evidencing endogenous release and function of a peptide involves studies on long-term depression in the cerebellum by Miyata et al. (1999). It is known from histochemical studies that olivo-cerebellar climbing fiber afferents express CRH (Palkovits et al., 1987; Sakanaka et al., 1987), and that CRH receptor mRNA, mainly for type I receptors, is present in Purkinje cells as well as in granule cells (Chang et al., 1993; Potter et al., 1994). CRH has been shown to directly activate Purkinje cells and to enhance the sensitivity of these neurons to glutamate and aspartate and to reduce their sensitivity to GABA (Bishop, 1990). It is well known that climbing fibers are important in triggering long-term depression (LTD) of parallel fiber synapses (see Ito, 1989). It has now recently been shown that LTD induction is blocked by a specific CRH receptor antagonist (Miyata et al., 1999). Moreover, after destruction of the climbing fiber system by 3-acetylpyridine, stimulation of parallel fibers combined with Purkinje cell depolarization failed to induce LTD in a slice preparation, but LTD could be restored after addition of CRH (0.1 μm) to the bath. Taken together these comprehensive studies suggest an involvement of a neuropeptide in the LTD phenomenon in cerebellum.

Of the many central peptides that recently have received increased attention, we would also like to mention some of those involved in the neural control of feeding and body weight. These peptides include CCK, NPY and galanin as well as the orexins/hypocretins [Fig. 1(a)], melanocortins, MCH [Fig. 1(b)], AGRP and CART peptides, which either increase or attenuate food intake (Kalra, 1997; see also Kristensen et al., 1998; Lambert et al., 1998; Leibowitz, 1995; Woods et al., 1998). The MCH neurons [Fig. 1(a)] (Bittencourt et al., 1992; Skofitsch et al., 1985) intermingle with, but are distinct from the orexin/hypocretin neurons [Fig. 1(b)] (Broberger et al., 1998; Elias et al., 1998). The central circuitries involved in regulation of feeding behavior are now being investigated (see Broberger, 1999; Elmquist et al., 1999; Sawchenko, 1998). This field has also been stimulated by the discovery of several receptor subtypes, especially for NPY (see Balasubramaniam, 1997; Larhammar et al., 1998), which could provide selectivity for functions such as food intake regulation, as well as by the discovery of leptin, an adipose tissue-derived signaling factor encoded by the obesity (OB) gene (Zhang et al., 1994b). The hypothalamic neuropeptide systems have recently been reviewed (Broberger, 1999; Elmquist et al., 1999; Sawchenko, 1998).

de Bono and Bargmann (1998) have described distinct peptide-related effects in *C. elegans*. This worm exhibits either solitary or social feeding behaviour, the latter characterized by aggregation. In this worm the *npr-1* gene encodes a member of the NPY receptor family. In a series of elegant experiments de Bono and Bargmann show that a loss of function mutation of this gene converts the solitary strain to a social behaviour, and that two isoforms of the receptor, differing only at a single residue and both occuring in the wild, are associated with solitary and social feeding behaviour, respectively, and that gene manipulation induces transformation from the social to the solitary behaviour. These elegant *elegans* studies strongly suggest that a peptide and a peptide receptor play an important role in the social behaviour in this worm.

8.3. *Neuropeptide knock-out mice*

One approach to understanding the functional role of proteins in general is the creation of knock-out mice, that is deletion of a specific gene, using modern molecular biological tools. This has been practiced only to a limited extent in the neuropeptide field, but more recently several such animals have been described including knockouts, where the gene for NPY and some of its receptors

receptors have been deleted (Baraban et al., 1997; Erickson et al., 1996; Marsh et al., 1998; Naeilhan et al., 1999; Palmiter et al., 1998; Pedrazzini et al., 1998). There are also knock-out mice where substance P (Cao et al., 1998; Zimmer et al., 1998) or its NK-1 receptor (De Felipe et al., 1998) have been targeted. The phenotypic changes observed are not as dramatic as might have been expected (hoped for?) on the basis of pharmacological and morphological studies, possibly due to compensatory mechanisms, but are still very interesting. Thus, the substance P/NK1 receptor knockouts show altered nociception (Cao et al., 1998; De Felipe et al., 1998; Zimmer et al., 1998). Perhaps surprisingly, little effect on weight was observed in the NPY knock-out mouse (Erickson et al., 1996). However, leptin-deficient ob/ob mice rendered NPY-deficient by crossing with NPY-knock-out mice have a much attenuated obese phenotype as compared to their NPY-expressing controls (see Palmiter et al., 1998). This experiment suggests that combinatorial mutants may provide the greatest use in studies of neuropeptide function. In the Y1R knock-out mouse daily food intake as well as NPY-stimulated feeding were only slightly diminished, whereas fast-induced refeeding was markedly reduced (Pedrazzini et al., 1998). The Y1-R knock-out mouse had increased body fat with no change in protein content. The Y5-R knock-out mouse showed a late-onset obesity characterized by increased body weight, food intake and adiposity (Marsh et al., 1998). Whereas Y1 knock-out mice had a lower metabolic rate resulting in increased fat deposit, the Y5 knock-outs were rather hyperphagic. The Y2-R knock-out mice developed increased body weight, food intake and fat deposition. The reaction of NPY-deficient mice in an epileptic seizure model was interesting, in that kainic acid induced essentially qualitatively similar seizures in both knock-out and wild-type mice, but that seizures did not terminate in the knock-out mice, and virtually all of them died while wild type mice survived (Baraban et al., 1997). This suggests an important role of NPY in the control of electrical activity in brain (Baraban et al., 1997). The regulation of NPY and other peptides in epilepsy models has been reviewed by Schwarzer et al. (1996). Finally, NPY-deficient mice are more prone to ethanol consumption (Thiele et al., 1998).

Very recently a surprising association between a peptidergic system and sleep disturbance has been revealed. Thus, the hypocretins/orexins, previously thought mainly to be involved in feeding as mentioned above, now are linked to narcolepsy. The hypocretins/orexins are present in large neurons in the lateral hypothalamus [Fig. 1(a)] projecting to widespread areas of the brain (de Lecea et al., 1998; Peyron et al., 1998; Sakurai et al., 1998). Lin et al. (1999) have found a deletion in the transcripts of the *hypocretin receptor 2* gene both in the narcoleptic Doberman pinscher and the narcoleptic Labrador retriever, although at different locations. Moreover, Chemelli et al. (1999) have described sleep abnormalities resembling narcolepsy in mice with deleted *hypocretin/orexin* gene. This may be the first example of a genetic linkage of a neuropeptide and its receptors to an important human (and animal) disease, narcolepsy, characterized by sleepiness and sudden loss of muscle tone (see Siegel, 1999).

An alternative route to knock-out by homologous recombinantion has been taken by Piccioli et al. (1995) who generated transgenic mice expressing antibodies to substance P and showed a marked inhibition of neurogenic inflammation and motor deficits.

9. Neuropeptides in glia

There is evidence that neuropeptides, in spite of their 'name' (!), are also expressed in glial cells. Most studies focus on expression in cell cultures, including demonstration of pre-proenkephalin mRNA, preprosomatostatin mRNA and enkephalin peptides in astrocytes (Klein and Fricker, 1992; Melner et al., 1990; Schwartz and Simantov, 1988; Shinoda et al. 1992, 1989; Spruce et al., 1990; Steine-Martin et al., 1991; Vilijn et al., 1988). However, atrial natriuretic peptide has been found in canine brain astrocytes in situ (McKenzie, 1992). Also galanin, a 29-amino acid peptide, is present in glial cells in vivo in the rat brain [Fig. 4(a,b)], but only after treatment with mitosis inhibitors such as colchicine (Zhang et al., 1992). The identity of these cells has not been established, but they may represent microglia. In a recent study it could be demonstrated that the colchicine-induced upregulation of galanin in such glial cells is almost completely abolished in hypothyroid rats, clearly indicating a hormone dependency of this effect (Calzà et al., 1998). Another example of in vivo expression is the presence of NPY in ensheating cells surrounding the olfactory nerves in the olfactory bulb (Ubink et al., 1994) [Fig. 4(c,d)]. Here, robust mRNA and peptide levels can be seen without any preceding experimental manipulations. The role of neuropeptides in glial cells is so far only subject to speculation. Regarding the presence of NPY in ensheating cells, this may indicate a possible role for this peptide in guiding of axons, since this has been the assigned role for the ensheating cells in the olfactory bulb (Doucette, 1990). The olfactory sensory neuron is the best known example of a mammalian neuron population that is continuously renewed during adult life (Graziadei and Monti Graziadei, 1979). Interestingly NPY has previously been shown to indirectly, possibly together with neurotrophin 3, increase neurite outgrowth of dissociated dorsal root ganglion cells (White, 1998; White and Mansfield, 1996).

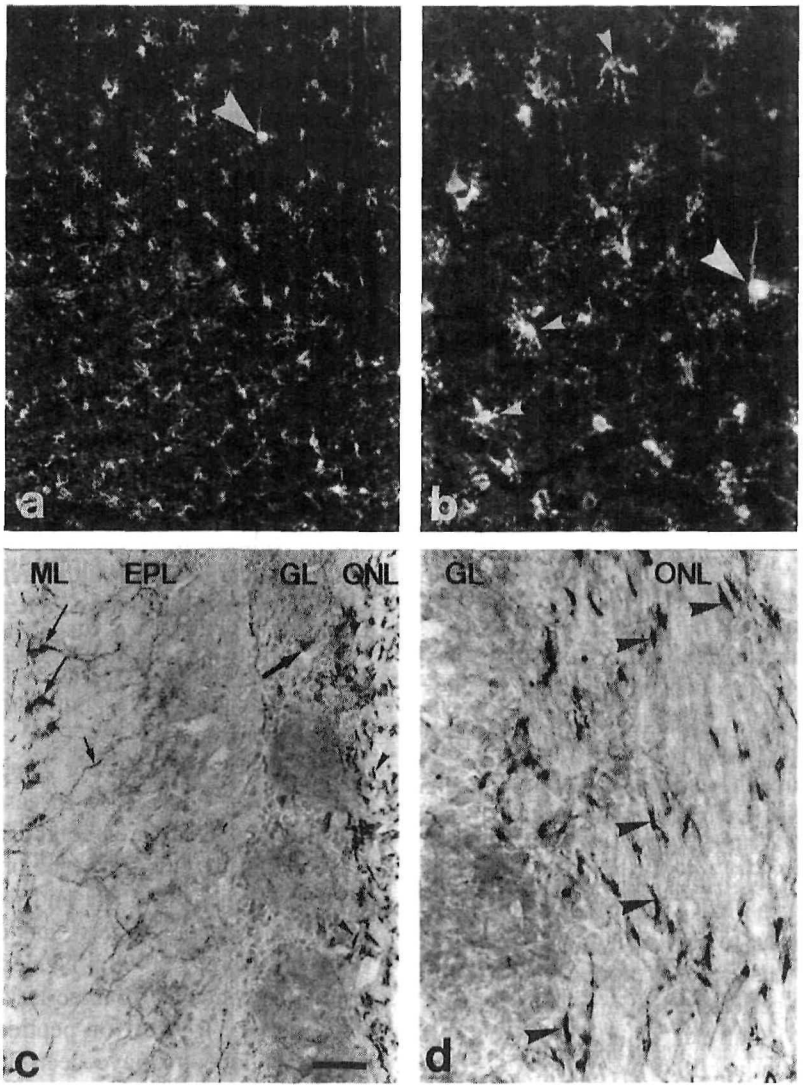

Fig. 4. (a,b) Many galanin-positive glial cells (small arrowheads) are seen in cortex after intraventricular colchicine treatment, at low (a) and high (b) magnification. Big arrowheads point to a galanin-positive neuron. (c,d) In the olfactory bulb NPY is present in neurons (long arrows) in the mitral layer (ML), in nerve fibers (short arrow) and in neurons (arrow) in the glomerular layer (GL). Many small cells (arrowheads) are seen in the olfactory nerve layer (ONL), especially distinctly at high magnification in (d). EPL=external plexiform layer. Bar indicates 50 μm for (a) and (c) and 25 μm for (b) and (d).

10. Trophic effects of peptides

Increasing evidence indicates that peptides exert trophic actions and have roles during the embryonic period (see Schwartz, 1990; Strand et al., 1991). It was initially noticed that several peptides, for example somatostatin, can only be seen during the embryonic period in certain systems and then disappear, suggesting a developmental role (see Tohyama, 1992). Some early results are listed in Table 5. To mention a few recent studies, VIP has been shown to have a dramatic effect on growth of whole fetuses in vitro (Gressens et al., 1993) which may be due to the fact that it shortens the G1 and S phases of the neural cell cycle (Gressens et al., 1998). De Felipe et al. (1995) have provided evidence for involvement of substance P and NK1 receptors during embryogenesis in the spinal cord, where transiently expressed substance P is released from pioneer neuronal pathways and, via NK1 receptors in floor plate cells, regulates the release of a chemoattractant to guide the permanent commissural axons to the midline.

11. Clinical studies

In the beginning of the peptide era it was predicted that peptides are involved in disease, and that consequently drugs interfering with peptidergic mechanisms could have therapeutic effects. For example, the realization that substance P is present in small diameter fibers of presumptive nociceptive sensory neurons, and the discovery of endogenous ligands for the opiate receptors

suggested a rapid progress in understanding and treatment of various pain states. To date this prediction has not materialized. The insight that most hypothalamic releasing and inhibitory factors are peptides clearly suggested neuroendocrine therapeutical applications. In fact, some of the hypothalamic releasing peptides such as TRH are used diagnostically to probe for pituitary function. Moreover, the demonstration that the growth hormone-inhibiting hormone, somatostatin, has a wide spread distribution and strong inhibitory effects, initiated work to produce powerful and long-lasting somatostatin analogues. In fact, such analogues are now used to treat and localize peripheral endocrine tumors (see de Herder et al., 1996).

Little information is available on the role of most neuropeptides in humans. Nevertheless, unquestionably morphine via opioid peptide receptors is the most efficient analgesic drug so far known, underlining the importance of peptide receptors as drug targets. More recently promising treatment of alcoholism with the opioid receptor antagonist naltrexone suggests that this type of receptor may be of importance for alcohol dependency (O'Malley et al., 1992; Volpicelli et al., 1992).

Much focus has been on a possible involvement of CCK and CCK receptors in anxiety, and it has been shown in man that CCK-4-induced panic attacks can be significantly attenuated by the CCK_B antagonist CI-988 (Bradwejn et al., 1995), although several not published clinical studies using such CCK_B antagonists given to patients with anxiety appear to have failed to reveal significant effects. It has also been reported that CCK antagonists have an analgesic effect in man (McCleane, 1998; Price et al., 1985), but lack of efficacy has also been reported (Lehmann et al., 1989).

NK-1 antagonists have shown efficacy in treatment of nausea induced by cytostatic drugs (Diemunsch et al., 1999; Navari et al., 1999), and, although limited, there are some clinical studies showing an effect of a substance P receptor antagonist on acute postoperative pain (Dionne et al., 1998).

An apparent breakthrough may have occurred in the field of substance P. Thus, the Merck NK-1 receptor antagonist MK869 has demonstrated antidepressant and anxiolytic activity when given alone and orally once daily (Kramer et al., 1998). In fact, the NK-1 antagonist is at least as efficient as a serotonin uptake inhibitor prototype (paroxetine), and has fewer side effects, in particular with regard to sexual dysfunctions and nausea. This appears to be the first evidence for derangement of a central neuropeptide system in a major mental disease and the possibility to orally treat this with a non-peptide peptide antagonist. Previous indirect (Baker et al., 1991) and recent direct evidence based on double-labelling in situ hybridization (Sergeyev et al., 1999) demonstrate that in human, in contrast to rat, many 5-HT dorsal raphe neurons express substance P. It may be speculated that these 5-HT neurons in depressed patients are affected in a more general way, that is involving not only low extracellular 5-HT levels but also disturbed substance P-ergic mechanisms.

If, and where, a further peptide drug(s) will show clinical efficacy in a CNS disease must be awaited, but CRH antagonists are candidates for treatment of depression.Thus, there is a major literature on the possible involvement of CRH and its receptors in affective disorders such as anxiety and depression (De Souza, 1995; Holsboer, 1999; Nemeroff, 1998), including results from studies on mice lacking the CRH1 receptor (Timpl et al., 1998). Non-peptide antagonists acting on the CRH1 receptor have been developed (McCarthy et al., 1999), opening up promising venues for clinical trials.

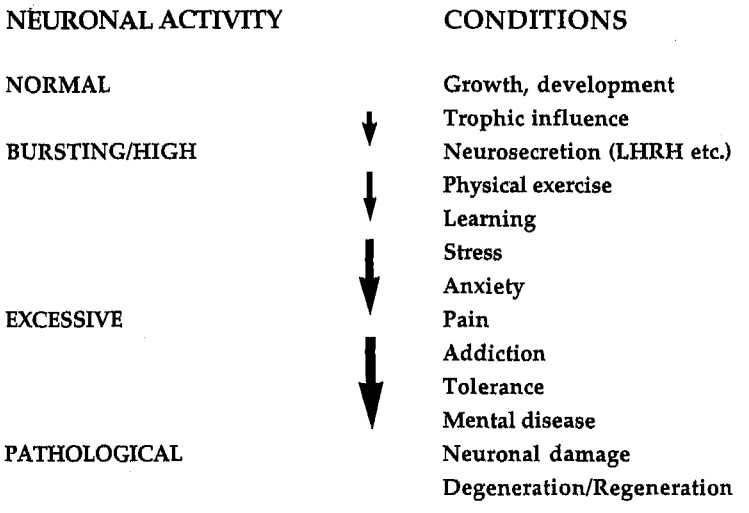

Fig. 5. Some conditions under which peptides may preferentially be released, emphasizing relation to firing activity. From Hökfelt (1991).

12. Conclusion

Major progress has been made in the field of neuropeptides. Peptide receptors have been identified, drugs have been developed and novel insights into the regulation of peptide synthesis have been obtained. Still, the physiological roles of neuropeptides often remain elusive, and both transmitter-like functions, modulation and trophic actions have to be considered. There is evidence that peptides may exert their main actions when the nervous system is 'stressed', challenged or afflicted by disease (Fig. 5). In fact, this suggests that peptides are important in signalling under these circumstances and that peptidergic communication may be a 'language' of the diseased brain which should make peptidergic mechanisms targets for drug development. Nevertheless, a number of questions have to be posed and answered to be able to carry out targeted drug development in a fruitful way. With the improved tools now available, including genetically manipulated animals, it should be possible to clarify many of these open questions.

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