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Key words: Coexistence, Co-localization, G-protein-coupled receptor, Large granular vesicle, Neuropeptides, Synaptic vesicles.

Abstract

Neuropeptides are small protein molecules produced and released by discrete cell populations of the central and peripheral nervous systems through the regulated secretory pathway and acting on neural substrates. Inside the nerve cells, neuropeptides are selectively stored within large granular vesicles (LGVs), and commonly coexist in neurons with low-molecular-weight neurotransmitters (acetylcholine, amino acids, and catecholamines). Storage in LGVs is responsible for a relatively slow response to secretion that requires enhanced or repeated stimulation. Coexistence (i.e. the concurrent presence of a neuropeptide with other messenger molecules in individual neurons), and co-storage (i.e. the localization of two or more neuropeptides within individual LGVs in neurons) give rise to a complicated series of pre- and post-synaptic functional interactions with low-molecular-weight neurotransmitters. The typically slow response and action of neuropeptides as compared to fast-neurotransmitters such as excitatory/inhibitory amino acids and catecholamines is also due to the type of receptors that trigger neuropeptide actions onto target cells. Almost all neuropeptides act on G-protein coupled receptors that, upon ligand binding, activate an intracellular cascade of molecular enzymatic events, eventually leading to cellular responses. The latter occur in a time span (seconds or more) considerably longer (milliseconds) than that of low-molecular-weight fast-neurotransmitters, directly operating through ion channel receptors. As reviewed here, combined immunocytochemical visualization of neuropeptides and their receptors at the ultrastructural level and electrophysiological studies, have been fundamental to better unravel the role of neuropeptides in neuron-to-neuron communication.

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Introduction

David de Wied (1925–2004) coined the term neuropeptides in the seventies, after his pioneering work on the activity of peptide hormones, leading to discover that the adrenocorticotropic hormone (ACTH), the melanocyte-stimulating hormone (MSH) and vasopressin acted on the brain and affected learning and memory processes (De Wied, 1971). After about 50 years of neuropeptide research, we still face some problems in finding an appropriate definition for these molecules that represent, by far, the largest and most diverse class of signaling molecules in the brain and spinal cord. Usually neuropeptide(s) are defined as small proteins produced and released by discrete neuronal populations of the central (CNS) and peripheral nervous systems through the regulated secretory pathway, and acting on neural substrates. However, recent work, particularly on glial-derived neurotrophic factor (GDNF), indicates that in the nervous system certain peptide molecules are also produced by cells other than neurons. Not only growth factors but also some cytokines synthesized by the neurons (Summy-Long et al., 2008) and glial cells can meet the require- ments for neuropeptide inclusion. As regard to this issue, emerging data from astrocytes and glial cell lines indeed show that they can also have a regulated secretory pathway, a fundamental criterion for a molecule to be considered as a neuropeptide (see below). Therefore, putative neuropeptides may be also recognized in peptide families expressed by glia (see Burbach, 2010 for a recent review of neuropeptide criteria and families).

Neuropeptides exert several different biological effects, such the regulation of gene transcription (Landgraf and Neumann, 2004), local blood flow (Cauli et al., 2004), synaptogenesis, and glial cell architecture (Theodosis et al., 1986). Notably, most of them also influence membrane excitability, and, although this is not perhaps their main biological action, it has made neuropeptides very attractive to the neurobiologists. When thinking to neuropeptides as being involved in cell-to- cell communication, the first consideration to be made is that they are about 50 times larger than low-molecular-weight ‘‘classical’’ neurotransmitters (see below). Studies on the pharmacology of cloned receptors have demonstrated that neuropeptides have a higher receptor binding affinity (about 1000x; with values in nmol/l versus mmol/l) and selectivity than ‘‘classical’’ neurotransmitters. For these reasons, neuropeptides can elicit their biological effects even when released at lower quantities. A wide array of electrophysiological, pharmacological and behavioral findings have substantiated these statements, for example in the case of somatostatin (SST—Selmer et al., 2000), cholecystokinin (CCK— Wank, 1995), the opioids (Pasternak and Wood, 1986) and many other peptides. Another remarkable

feature is that, although large neuropeptide molecules can diffuse and bind more slowly to receptors than the classical neurotransmitters, their half-lives in the brain extracellular space are remarkably long than those of low molecular weight transmitters. For example, the half-lives of oxytocin and vasopressin that are significantly more stable in proteolytic environments than many other neuropeptides are in the order of about 20 min (Mens et al., 1983), whereas that of the calcitonin gene-related peptide (CGRP) has been calculated to be comprised between 3 and 14 min (Braslis et al., 1988). These pharmacokinetic values are intermediate between that of a neurotransmitter and a hormone and are therefore consistent for a peptide with both circulatory and neurotransmitter modes of action. Altogether, the characteristics resumed above make neuropeptides ideal candidates for acting on long-lasting neuron-to-neuron communication.

This review will focus on the function of neuropeptides in neuron-to-neuron communication in CNS. The subject is part of a wider conceptual frame related to the definition of what is a neurotransmitter, how many ‘‘types’’ of neurotransmitter and neurotransmitter release do exist, and, most specifically, if neuropeptides can be considered ‘‘true’’ neurotransmitters (see Sü dhof, 2008). To put things in the right perspective it seems useful to briefly resume here some basic concepts that will be at least in part expanded in the following sections. However, one must be aware that most if not all the categorizations that we are still using in describing the molecular players in neuron-to-neuron (and neuron-to-glia) communication will very likely turn out to be merely didactic simplifications, since the borders between categories appear to be more and more labile as research proceeds further.

Communication in CNS occurs via two principal mechanisms: the release and reception of chemical messengers called neuro- transmitters and the direct transfer of intracellular signal across gap junctions (electrical synapses—see Merighi, 2002). Communi- cation via neurotransmitters takes several forms from ‘‘classical’’ synaptic transmission at specialized membrane sites (synapses) to diffuse secretion of neuromodulators (volume transmission—see Section 2.5).

It is possible to distinguish several ‘‘types’’ of neurotransmitter release (Sü dhof, 2008): (i) Synaptic neurotransmitters are released at synapses with the secretion of ‘‘classical’’ neurotransmitters such as glutamate, GABA, glycine, acetylcholine and ATP; (ii) Monoamine neurotransmitters are released by exocytosis of small dense-core vesicles (SDCVs), most often in the absence of synaptic specializations; (iii) Neuropeptides are secreted by exocytosis of large granular vesicles (LGVs), also referred to as large dense-core vesicles (LDCVs); (iv) Small permeable mediators, such as nitric oxide and endocannabinoids, are liberated by diffusion.

Only the first type of transmitter release mediates the fast point-to-point synaptic transmission, whereas all the others are collectively indicated as neuromodulators. Thus, strictly speaking, neuropeptides can be safely considered as being one ‘‘type’’ of neuromodulators.

There is a considerable overlap between classical synaptic transmission and volume neurotransmission (Sü dhof, 2008): all classical transmitters act at synapses via ionotropic receptor but also as ‘‘volume transmitters’’ via G-protein coupled receptors (GPCRs); neuromodulators, in turn, feed back onto classical synaptic transmission. Despite this, for simplicity, we will use here the term neuromodulation to indicate the neuropeptide function(s) in neuron-to-neuron communication. When possible, this review will emphasize data directly correlating histological and functional findings, primarily the subcellular localization of neuropeptides and their cognate receptors in different types of neuronal processes by multiple immunocytochemical labeling. Examples will be given for different CNS areas and different peptides, with particular attention to sensory neuropeptides in spinal cord since this is our primary field of research.

Cellular and subcellular storage of neuropeptides and their receptors

1.1. Localization to LGVs

Identification of the subcellular site of storage of neuropeptides remains crucial to the understanding of their mechanism(s) of action as synaptic (or non-synaptic) modulators and interaction with other molecules involved in chemical neurotransmission. The first demonstration that neuropeptides were packaged in granules, anterogradely transported along neuronal axons to be stored and released at synapses was obtained in the hypothalamo–neurohy- pophysial system (Brownstein et al., 1980). However, the neurohypophysial peptides oxytocin and vasopressin are some- how remarkable, since, as discussed below, it appeared that magnocellular hypothalamic neurons are purely peptidergic. Conversely, a fast growing body of evidence led to the demonstration that neuropeptides are commonly present together with low- molecular-weight classical neurotransmitters in individual nerve cells (coexistence).
Nonetheless, already from initial observations in nerve cells other than the peptidergic magnocellular neurons, the two classes of transmitter molecules appeared to be stored in different subcellular compartments: LGVs for the neuropeptides and small clear synaptic vesicles (SSVs) for the classical transmitters (Fried, 1982; Fried et al., 1985; Thureson-Klein et al., 1988; Zhu et al., 1986). Nowadays, the concept that LGVs are the sole site of neuropeptide storage is widely established (Fig. 1). The histologi- cal demonstration of differential subcellular sites of storage for neuropeptides and low-molecular-weight neurotransmitters is consistent with the possibility of a selective release upon specific stimuli. On the other hand, coexisting neuropeptides are usually stored together in LGVs. Although only a few studies have addressed this issue quantitatively, it was demonstrated by immunocytochemistry with gold labeling techniques that the neuropeptides substance P (one of the first neuropeptides to be discovered) and CGRP are co-stored in a 1:1 ratio within individual LGVs of both peripheral and central neurons (Salio et al., 2007). In addition, the same

LGVs also contained (in a precise stoichiometric ratio with the two peptides) the brain-derived neurotrophic factor (BDNF), a substance originally discovered for its growth promoting action during development that is nowa- days gaining more and more relevance as a peptide neurotrans- mitter (see below). The fact that the entire cocktail of neuropeptides produced by a single neuron is packed within individual LGVs, very likely because of fusion phenomena between immature vesicles (see Merighi, 2009 for a recent review), has a series of important functional implications. Co- stored neuropeptides, in fact, cannot be differentially released, and a modulation of biological effects is most readily accom- plished by regulating their relative proportions of synthesis. Nonetheless, cargo size apparently influences the ratio between complete and incomplete LGV release events (Perrais et al., 2004). For example, compared with the fast kinetics of neuropeptide Y (NPY) release, that of BDNF is slow (de Wit et al., 2009) offering the possibility for an additional way to modulate biological effects in vivo.

1.2. Neuropeptide synthesis, storage and targeting to processes

Neuropeptides are usually produced as large inactive pre- cursors, which are then enzymatically cleaved to yield the biologically active peptide. Precursors contain several molecules of the same neuropeptide and/or more or less structurally related compounds.

As it occurs for all protein molecules, neuropeptide precursors are synthesized within the rough endoplasmic reticulum and subsequently move to the Golgi apparatus where they are packaged into LGVs. In the marine mollusk Aplysia each neuron in the bag cell clusters, the structures that controls egg laying, synthesizes several peptides and packages them into separate vesicles. These vesicles are then differentially localized in specific neuronal processes, thus segregating peptides destined for autocrine and hormonal release sites (Jung and Scheller, 1991). In mammalian neurons, the issue of neuropeptide targeting to processes is still incompletely resolved: it remains unclear whether or not neuropeptides can be specifically targeted to different types of processes (i.e. the axon and the dendrites) and/or the different branches of the same process.

In hypothalamic magnocellular neurons galanin and vasopres- sin are only partly co-packaged and undergo a preferential targeting toward dendrites or neurohypophysis, suggesting different functions, autocrine/paracrine and endocrine, respec- tively (Landry et al., 2003).

A rather different picture is presented by the primary sensory neurons, a unique class of pseudounipolar neurons that are grouped outside the CNS in the sensory ganglia associated with certain cranial nerves and in dorsal root ganglia (DRG), and display extremely abundant and variegated neuropeptide content. Imme- diately after emerging from the cell body the single process of these neurons divides into a central and a peripheral branch that, at the same time, function as an axon and a dendrite. Due to their relatively simple histology, these neurons have represented a useful model to address the

question of process targeting of neuropeptides. Pioneering work, carried out with multiple immunogold labeling methods more than 20 years ago, led to the selective localization of different tachykinins (a group of sensory neuropeptides forming a family to which also belong the substance P and other structurally related molecules such as neurokinin A and B) and CGRP to LGVs of DRG neurons (Merighi et al., 1988). Virtually all LGVs are multiple/dually labeled in both the central and peripheral branch of the process that stems from the cell body, where, on the other hand, double (multiple)-labeled LGVs are extremely rare. These observations show that when a single neuron produces multiple peptides, they are not selectively packaged into different LGV subpopulations, but rather a cocktail of neuropeptides is consistently found within individual LGVs (Merighi, 2002, 2009). In cell bodies, LGVs containing just one component of the cocktail can be regarded as immature, as they will probably incorporate the other peptide(s) before being transported to terminals. Studies on regulated secretion of non- neuronal cells suggest that peptides are packed into immature LGVs budding from the trans-Golgi network. Fusion between immature LGVs has been demonstrated, and it is thought that after vesicle–vesicle fusion, soluble contents condense, and excess membrane are removed by vesicle budding, ultimately giving rise to the mature LGVs.
Given that LGVs (and therefore the neuropeptides packed therein) are detected at both central and peripheral branches of DRG neurons, it seems reasonable to hold that no selective transport to different branches of the same process occurs.

1.3. Localization to axon terminals and dendrites

In CNS, neuropeptide containing LGVs are most commonly targeted to axon terminals, although dendritic localization is also seen (see below).
At terminals, the most abundant SSVs (that as already mentioned above are the storage site for fast-acting low- molecular-weight neurotransmitters) are tightly tethered at synaptic specializations to the plasma membrane. The less frequently observed LGVs that store the neuropeptides are usually located away from the active zone. More precisely, the distribution of SSVs and LGVs in typical axo-dendritic central synapses follows a rather stereotyped pattern. SSVs occupy a variable, but usually large, area of the axon terminal; some are docked at the pre- synaptic grid being ready to be released at the active synaptic zone (Peters, 1976). These vesicles form the so-called readily releasable neurotransmitter pool. LGVs, on the other hand, are localized away from the pre-synaptic membrane, singularly or in clusters. Release of LGVs occurs anywhere at terminal membrane, including (less frequently) the active zones (Buma, 1988; De Camilli and Jahn, 1990; Karhunen et al., 2001; Zhu et al., 1986). It has been recently calculated that a surprisingly large proportion of release events, about 50%, indeed occurs at extra-synaptic locations (de Wit et al., 2009).
In the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the

hypothalamus, the neuropeptides oxytocin and vasopressin, besides to terminals, have also been localized to the dendrites of the magnocellular neurons (Ludwig and Leng, 2006; Pow and Morris, 1989). Similarly, dynorphin is present in the axons and dendrites of the dentate gyrus granule cells that make synapses onto the CA3 pyramidal neurons (Drake et al., 1994).

1.4. Coexistence and co-storage of neuropeptides

Tomas Hö kfelt and his collaborators have produced an impressive amount of seminal works ultimately leading to demonstrate the existence of multiple transmitters in peptidergic and monoaminergic neurons (see Hö kfelt, 2010 for a very recent historical overview).

Coexistence, the concurrent presence of two or more trans- mitters in a single neuron, is now recognized as a common feature of central (and peripheral) neurons (Salio et al., 2006). Neuropeptides coexist with other neuropeptides, low-molecular weight fast- acting neurotransmitters, certain growth factors such as BDNF and GDNF (that perhaps should be better considered as transmitters themselves—see below), and the gaseous transmitter nitric oxide (Dun et al., 1994; Yang et al., 2000). When multiple neuropeptides are present in neurons, coexistence equals to co-storage, since, as discussed above, coexisting peptides are co-stored in LGVs.

As a rule, neurons produce a combination of one (or more) low- molecular-weight transmitter(s) and one (or more) high-molecu- lar-weight neuropeptide(s). The oxytocin/vasopressin magnocel- lular neurons that contain a complex cocktail of peptides, but apparently no low-molecular-weight transmitters represent one remarkable exception to this rule. However, immunocytochemical and in situ hybridization evidence was provided that these neurons express type-2 vesicular glutamate transporter, a marker for their glutamatergic neuronal phenotype (Hrabovszky et al., 2006). Therefore, although a transmitter role for glutamate remains doubtful, these observations lead to predict that oxytocin/ vasopressin magnocellular neurons will also turn out not to be purely peptidergic.

When a neuropeptide coexists with a classical transmitter, the latter is generally the principal messenger, whereas the neuropeptide modulates neuronal response by acting on pre- and/or post- synaptic GPCRs (see Section 3.2).

Several areas of the CNS (and most ganglia in the peripheral nervous system) contain various neuropeptides often in combina- tions with each other's. In most circumstances, it is common that individual neurons in these areas produce and store more than a single peptide, and thus peptide co-storage appears to be a very widespread phenomenon. The coexistence of neuropeptides and fast-acting neurotransmitters is even more widespread. Several different neuropeptides have been demonstrated to coexist with acetylcholine, transmitter amino acids, biogenic amines, nitric oxide, and growth factors in a large number of CNS areas (for recent reviews, see Merighi, 2009; Salio et al., 2006).

1.5. Receptor localization

Neuropeptide receptors' localization also follows a well-defined pattern in terminals. Whereas receptors for low-molecular-weight fast-acting transmitters are specifically clustered at the post- synaptic membrane immediately facing the synaptic cleft, not only neuropeptide receptors are generally localized away from the synaptic differentiation, but also their distribution does not overlap with localization of ligand peptides, often leading to a so-called peptide/receptor mismatch.

At least in certain cases, receptor expression may be functionally regulated, and there is evidence for an activity- dependent insertion into terminal membranes of neuropeptide receptors translocated from the LGV membranes. Translocation of neuropeptide receptors from LGVs' to terminals' membranes has been demonstrated for kappa opioid receptors in hypothalamus (Shuster et al., 1999), and delta opioid receptors in the mesencephalic periacqueductal gray (PAG) (Commons, 2003), and spinal cord (Cahill et al., 2001).

Translocation of neuropeptide receptors is of relevance in the pre-synaptic regulation of transmitter release. The example of kappa opioid receptors is quite peculiar since it demonstrates the possibility of a regulation of neuropeptide release by another neuropeptide. Vasopressin-containing neurosecretory neurons are known to produce and co-release other neuropeptides, including dynorphin, an endogenous opioid that binds with the highest affinity to kappa opioid receptor 1 (KOR1). Stimulus-dependent translocation of KOR1 to the plasma membrane may result in an increased probability of dynorphin binding to KOR1. Increased dynorphin binding would result in a reduction of subsequent neuropeptide release (Shuster et al., 1999). In immunocytochemical studies where neuropeptide receptors have been labeled together with their peptide ligands, it clearly appears that point-to-point communication at synapses is not a major mode of signal transduction for neuropeptides.

Classic synaptic transmission at point-to-point synaptic con- tacts is designed to maintain the independence of signals. However, it is now clear that this type of neuron-to-neuron communication does not represent the sole way by which information is transferred from one neuron to another, not only when neuropeptides are acting as messengers, but also in the case of fast-acting low-molecular-weight transmitters. Interneuronal communication that takes place outside synapses has been indicated by different terms such as non-synaptic transmission (see Merighi, 2002 for review) or, more recently, parasynaptic transmission (Szapiro and Barbour, 2009). Irrespectively of this, the crucial issue is here the distance between the site(s) of neurotransmitter release and neurotransmitter receptors, an issue that is strictly related to the concept of receptor mismatch referred above. Non-synaptic/parasynaptic transmission takes several different forms that will be briefly mentioned in the following. In perisynaptic transmission there is normal vesicular transmitter

liberation at synaptic active zone(s), but receptors are activated just in proximity of the synaptic cleft (within 100–200 nm) (Fig. 1D, insert at center). Because they are exposed to lower transmitter concentrations, their activation likely requires high- frequency activity at the synapse. In ectopic release, vesicles fuse with the plasma membrane outside the active zones but still liberate their cargo directly opposite to an area of the target neuron where receptor density is sufficient to elicit detectable miniature post-synaptic currents (see Section 3.3). Synaptic spillover involves the recruitment of receptors at significant distance (at least 0.5 μm) from the site(s) of release. In intersynaptic spillover, there is significant transmitter diffusion outside synapses leading to activation of receptors at neighboring contacts of the same type and of extrasynaptic receptors on the same cells. Receptor activation is, in this case, often cooperative or nonlinear. In heterosynaptic spillover, target receptors are found on cells that do not participate in the synapses releasing the transmitter, such as other neurons or glial cells. Finally, volume transmission (Zoli et al., 1999) consists of signaling in the absence of synapses, and involves transmitter diffusion over relatively long distances (1 μm or more).

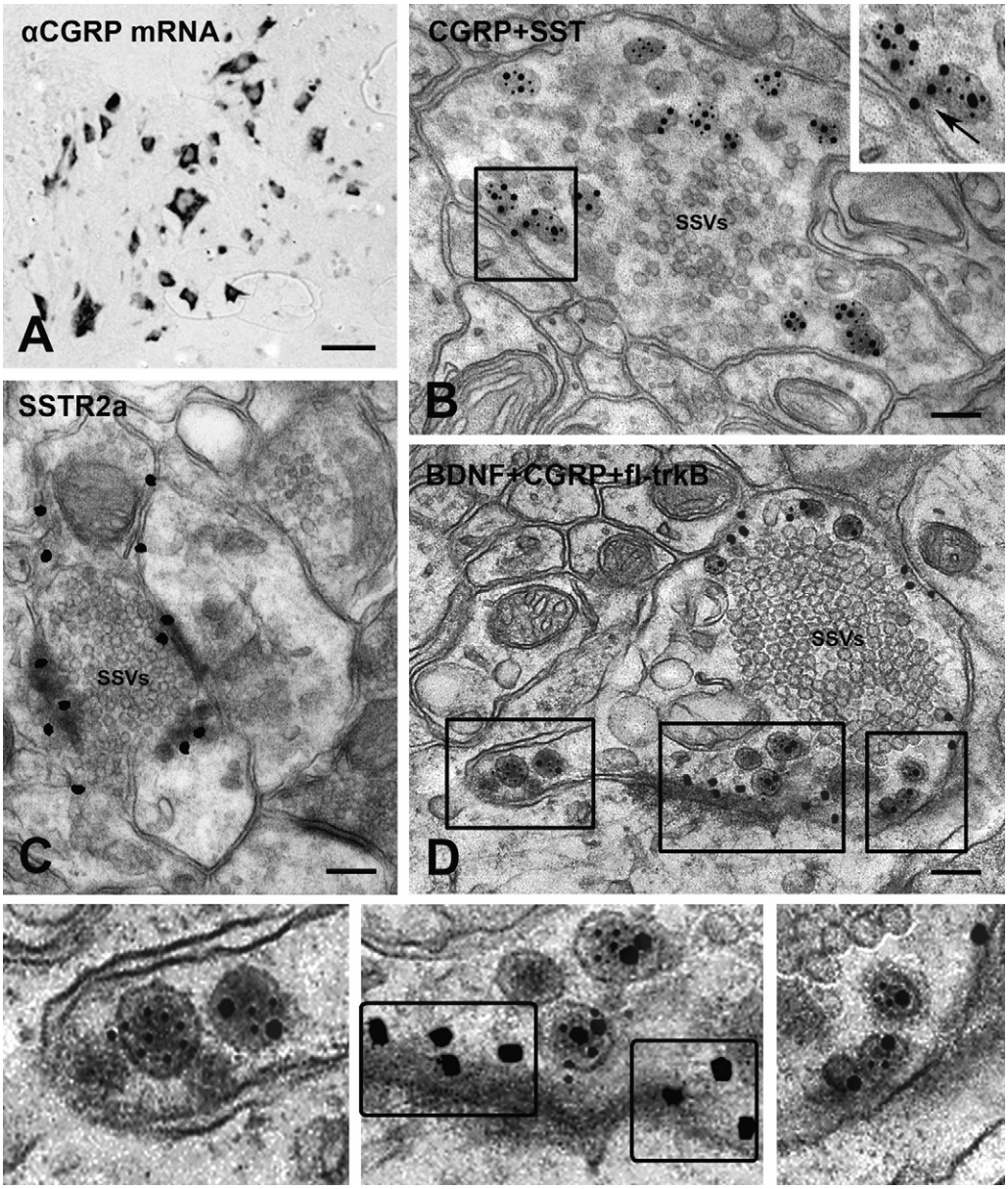

Fig. 1. Localization of neuropeptides and neuropeptide receptors. (A) Expression of the aCGRP mRNA in the mouse spinal cord motorneurons after in situ PCR. (B) Localization of CGRP (10 nm gold) and SST (20 nm gold) in an axonal varicosity of the mouse substantia gelatinosa of spinal cord. Note that the two peptides are selectively co-stored in LGVs, whereas SSVs are unlabelled. The area in the rectangle is shown at higher magnification in the insert: the two LGVs are emptying their cargo. Note that the LGV membrane is interrupted and the matrix together with SST is being released (arrow) in the absence of synaptic specializations. (C) Localization of SSTR2a in a synaptic glomerulus of mouse substantia gelatinosa after gold-intensified gold labeling with ultra small gold probes (Nanogold^{TM}). Note that receptors are clustered at both synaptic and non-synaptic sites. (D) Simultaneous localization of BDNF (10 nm gold), CGRP (20 nm gold) and fl-trkB (gold-intensified Nanogold^{TM}) in an axon terminal of mouse substantia gelatinosa. Note that also in this case SSVs are unalbeled. Fl-trkB receptors display a pre-synaptic localization as demonstrated by the location of the gold label at the inner surface of the axolemma (see Salio et al., 2005). The areas in the rectangles are shown at higher magnification in the inserts. Inserts: (left, right) exocytosis of LGVs; (center) clustering of fl-trkB receptors (rectangles with rounded corners) at synaptic and perysinaptic locations. Abbreviations: BDNF = brain-derived neurotrophic factor; CGRP = calcitonin gene-related

peptide; fl-trkB = full-length tropomyosine kinase receptor B; LGVs = large granular vesicles; SST = somatostatin; SSTR2a = somatostatin receptor isotype 2a; SSV = small synaptic vesicles. Bars: (A) 100 mm; (B and D) 150 nm.

Transmitter release is often, but not necessarily, exclusive- ly, vesicular, and can arise from any part (cell body, neurites) of the neurons or from glial cells (see Fellin, 2009 for a recent review on astrocyte modulation of synaptic activity). The development of more and more sophisticated ultrastruc- tural immunocytochemical techniques for concurrent analysis of neuropeptides (and more generally speaking neurotransmitters) and their receptors (see Salio et al., in press for a technical update) allowed for a more in depth characterization of the spatial relationship involved in ligand-receptor binding (Fig. 1C and D). These studies represent, in fact, a fundamental support to functional analysis by electrophysiology (see Section 3.3).

1.6. Neurochemical plasticity of peptidergic neurons

A remarkable feature of peptidergic neurons, which has been extensively investigated particularly in the hypothalamo–neuro- hypophyseal system and in spinal cord, is neurochemical plastici- ty. Although this area of investigation is mainly related to the response of peptidergic neurons to pathological conditions it is worth mentioning here some fundamental findings that have been of particular importance to unravel the functional role of neuropeptides also under normal conditions.

In the hypothalamo–neurohypophyseal system expression of oxytocin/vasopressin is subjected to a striking degree of plasticity in response to osmotic stimuli and stress-related mechanisms, among which lactation (Gainer et al., 2002). Studies in normal animals and the homozygous Brattleboro rat (van Leeuwen et al., 1998), which lacks vasopressin by a germ- line mutation, demon- strated a high degree of complexity both in term of specificity and quantitative nature in oxytocin/vasopressin neurons. Although initial studies suggested that the expression of the oxytocin and vasopressin genes in magnocellular neurons was mutually exclusive, it was subsequently demonstrated that all vasopressin cells contained some oxytocin mRNA, and all oxytocin cells some vasopressin mRNA, but at about two orders of magnitude lower levels than the principal peptide mRNA in the cell. The ratio of expression of the two peptide mRNAs changed under osmotic stressful conditions. In addition, a third phenotype of magnocel- lular neuron containing equivalent levels of oxytocin and vasopressin mRNAs has been identified in the SON of normal rats, and this population drastically increases at the onset of lactation (Gainer et al., 2001).

In somatosensory system, the neurochemical phenotype of peptidergic DRG neurons is profoundly influenced by injury of peripheral nerves. The neuropeptide galanin is present in a small population of DRG neurons under normal conditions but is strongly up-regulated after nerve lesion. Up-regulated galanin promotes neurite outgrowth and influences pain processing.

Both pro- and anti-nociceptive effects have been reported, probably related to activation of different receptors. It has been proposed that pre-synaptic GalR2 receptors are pro-nociceptive by enhanc- ing release of excitatory transmitters in the dorsal horn, and anti- nociceptive via an action on glutamatergic GalR1-positive inter- neurons (Xu et al., 2008). NPY is also up-regulated in parallel with galanin (Landry et al., 2005).

2. Neuromodulatory function of neuropeptides

It is far easier to demonstrate the presence of multiple transmitter molecules in neurons than to establish their physio logical role, or even to show that they have any kind of biological activity at all. Moreover, although an organic framework to describe the function(s) of individual neuropeptides at synapses and/or non-synaptic sites is now available, relatively little is known about the functional interactions and the control of release of co-stored neuropeptides at central synapses. The existence of synapses can be unequivocally demonstrated only by transmission electron microscopy, but, at the same time, ultrastructural studies have helped to show the existence of several forms of non-synaptic transmission. The identification of gases as interneuronal signals (Baranano et al., 2001), or the modulation of neuronal function by lipophilic substances (Baulieu et al., 2001) has left no doubts regarding the existence of non- synaptic information transfer in CNS. Paradoxically, it was more difficult to accept that vesicle-stored neurotransmitters were released following quantal mechanisms, and could operate at sites distant from synapses and/or devoid of any synaptic specialization, than to recognize a neurotransmitter role to gases or lipophilic substances that can diffuse across the cell membrane and do not need membrane receptor binding.

2.1. Neuropeptide release

The major functional implication of the segregation of neuropeptides and low-molecular-weight transmitters into differ- ent cellular compartments (the LGVs and SSVs, respectively), is that each may be selectively released, upon activation of different cellular pathways. Early evidence was obtained that the release of coexisting peptides and classical neurotransmitters could be differential and dependent on the frequency and pattern of firing (Hö kfelt, 1991; Hö kfelt et al., 2000; Martinez-Rodriguez and Martinez-Murillo, 1994). Again, the Hö kfelt ‘s group pioneered the work in the field, particularly in the study of the functional implications of the coexistence of serotonin, substance P and tireotrophin releasing hormone (TRH) in medullary nuclei neurons known to project to the spinal cord (Arvidsson et al., 1994). In general, neuropeptide release is triggered by a comparatively small increase in the overall intracellular Ca^{2+} concentration in neuron (estimated to be less than 1 mM), whereas release of transmitter amino-acids from SSVs requires a significant local rise of intracellular Ca^{2+} concentration (10–100 mM) in the proximity of the Ca^{2+} channels at synapses (Ghijsen and Leenders, 2005). This

apparent paradox is indeed a consequence of the very precise localization of SSVs at synapses. In terminals with both LGVs and SSVs, a focal increase in Ca^{2+} at the synaptic membrane microdomains leads to a preferential discharge from SSVs, whereas a more general elevation of Ca^{2+} inside the terminal favors the release of LGVs (Verhage et al., 1991). The remarkably short delay in response to fast-acting transmitters (about 1–3 ms) is a close reflection of this mode of discharge from SSVs. In fact, synaptotagmin, which is included in the SSV membrane, rapidly senses the rise of internal Ca^{2+} and leads to rapid fusion with the pre-synaptic membrane and release of neurotransmitter into the synaptic cleft. On the other hand, the spatial independence from Ca^{2+} channels clustered at synaptic specializations explains why neuropeptide release can occur independently from synapses (Martin, 2003). Intracellular Ca^{2+} must raise to high enough levels to permit diffusion to sites far from the active zone. This implies that neuropeptides are not released from LGVs after a single action potential. Rather, sustained action potential trains are required for Ca^{2+} inside terminals to reach overall levels sufficient to trigger release. Among the consequences of the existence of selective mechanisms of release for coexisting peptides and classical transmitters is the possibility that long-lasting intracellular Ca^{2+} elevation may cause the release of neuropeptides to outlast the duration of electrical activity, thus uncoupling release from spiking (Kits et al., 1997).

2.1.1. **LGV secretion**

Even upon prolonged stimulation, not all vesicles at synapses unload their transmitter content. A variable fraction of SSVs (Harata et al., 2001) and LGVs (Kits and Mansvelder, 2000) is readily releasable, but the remaining ones, forming the reserve pool, need further steps to become competent (see inserts in Fig. 1B and D). Two different mechanisms of transmitter emptying occur in SSVs (Harata et al., 2001) and LGVs (Artalejo et al., 1998). They are the slower classical exocytosis, with complete fusion of the vesicle to the plasma membrane, or a faster mechanism whereby vesicles come in close proximity to the membrane and, with the formation of a transient pore, release part of their transmitter content by kiss and run (Artalejo et al., 1998; Tsuboi and Rutter, 2003). The transient pore mechanism would allow the quick simultaneous passage of amine transmitters and, perhaps, other small molecules that may be present in LGVs together with neuropeptides, from LGVs into the extracellular fluid (Artalejo et al., 1998; Elhamdani et al., 2001). On the other hand, neuropeptides contained within LGVs remain trapped inside the retrievable vesicle, because of their higher molecular weight. Therefore, release of most neuropeptides from the LGVs is unlikely to occur through kiss and run for several reasons. These include the larger size of the peptides relative to the transient pore (Barg et al., 2002), and the slow emptying of peptide content from LGVs upon exocytosis (Balkowiec and Katz, 2000; Brigadski et al., 2005; Lessmann et al., 2003). In support,

simultaneous capacitance measurements and confocal imaging have shown that peptide release by kiss and run is negligible (Barg et al., 2002). On the other hand complete vesicle fusion is usually required for LGVs to release their cargo, through a mechanism involving a priming step followed by retrieval of the vesicle as a coated vesicle (Artalejo et al., 1998; Elhamdani et al., 2001). A divergence in this respect between peptide-containing LGVs and amine-containing SDCVs may merely be additional to the several differences between these two classes of neurotransmitters. Indeed, neuropeptides do not have a known re-uptake mechanism, as opposed to biogenic amines, so that there is no way locally to refill the peptide containing LGVs after empting. Moreover, neuropeptides are synthesized at the RER in the neuronal cell body, but not in axon terminals (which are devoid of RER), whereas amines can also be synthesized inside SDCVs.

2.1.2. **Release of coexisting/co-stored neuropeptides**

As it appears to be the rule, peptidergic neurons produce more than a single peptide. Heterogeneity in neuropeptide content in neurons, in other words neuropeptide coexistence, derives from precursor processing, expression of different neuropeptide encod- ing genes, or both. As to precursor processing, it appears that the biologically active form of the peptide is stored into LGVs together with other peptide products that are devoid of biological activity, such is, for example, the case of TRH in PVN neurons (see Nillni, 2010 for a recent review), although some isolated exceptions can be found to this rule in the case of opioid peptides (Hirsch and Millington, 1991) or melanocortins (Pritchard et al., 2002). It is obvious that release of non-biologically active peptide fragments is of limited interest in functional terms. On the other hands when LGVs are loaded with cocktails of bioactive peptides derived from different genes, the mode of peptide release assumes a clear functional relevance. It is possible that all co-stored neuropeptides can be released all at once at all processes (Harling et al., 1991; Holst et al., 1987). Alternatively or in addition, individual neuropeptides can be liberated singularly or in different combinations at different processes. For example, the co-release of CGRP and SP (and other tachykinins) has been demonstrated to occur at both central and peripheral endings of the DRG neurons (Arvieu et al., 1996; Collin et al., 1993, 1994; Garry et al., 1994; Vanner, 1994). Moreover, if the co-release of co-stored neuropep- tides is indeed the rule, then it should occur from any neuronal process containing LGVs, although this latter issue needs also further clarification. The major functional implication is that co- released biologically active peptides probably act together in determining the response of target cells (Bean et al., 1994). A differential release of co-stored peptides (if indeed this occurs in vivo) would more likely rely on mechanisms different from those that apply to co-stored biogenic amines and/or co-existing low-molecular weight neurotransmitters. From this perspective, the relative rate of peptide dissolution from the LGV core might be of primary relevance, since this appears to be the critical determinant of the speed of peptide secretion in vitro (Brigadski et al., 2005). In addition, interaction with the LGV matrix is also

relevant in the local retention of secretory vesicle cargo (de Wit et al., 2009). Finally, a further issue of complication derives from the fact that certain peptides, such as the opioids, the tachykinins and CGRP, in addition to enzymatic processing and degradation by tissue peptidases, have been shown to undergo enzymatic conversion to fragments with retained or modified biological activity. Sometimes the released fragment shares the activity of the parent compound. However, in many cases the conversion reaction is linked to a change in the receptor activation profile, i.e. the generated fragment acts on and stimulates a receptor not recognized by the parent peptide (see Hallberg and Nyberg, 2003).

3.2. Receptor binding

Most neuropeptides (opioids, tachykinins, neurotensin—NTS, SST, CCK, numerous gut-brain peptides and most endocrine- releasing factors) use cell-surface receptors members of the large superfamily of GPCRs. These receptors share similar three- dimensional structure (a common seven-transmembrane-span- ning domain architecture), and the ability to modulate intracellu- lar metabolism through the activation of heterotrimeric GTP- binding proteins (G proteins) (Hamm and Gilchrist, 1996; Watson and Arkinstall, 1994). Each receptor subtype can couple to and activate only certain G protein types, each leading to distinct downstream signals. G proteins are classified based on their a-subunits. There exist at least 20 different Ga subunits, which are separated into four main families: Gi (inhibitory), Gs (stimulatory), Gq and G12/13. In the absence of the appropriate activating ligand or agonist, both GPCRs and their cognate G proteins are generally inactive. In the CNS, GPCRs function primarily, but not exclusively, as mediators of slow neuromodulators rather than fast neurotransmitters, and their role is critical to normal brain function.

Modulation of fast neurotransmission

When neuropeptides are co-released with other neurotrans- mitters, the wealth of responses of target neurons increases dramatically (Kupfermann, 1991). The neurotransmitters pro- duced and released by a single neuron are often defined as co- transmitters. However, it is probably unsafe to consider that co- transmitters must display some kind of interaction simply because they are co-released, even though such a co-release occurs under physiological conditions. The common existence of a combination of neuropeptides and classical neurotransmitters in neurons enables fast (2–5 ms) and slow (100–500 ms) synaptic communi- cation to take place (see Merighi, 2009 for a recent review). Fast and slow-acting co-transmitters, at least theoretically, can act on completely independent targets and, therefore, do not interact at all (Yang et al., 1996). However, there is a general consensus that, when multiple neurotransmitters are released within the extra- cellular space, they usually display at least some

type of interactive actions, irrespective of the finding that such a release occurs from the same neuron, i.e. they are true cotransmitters, or from separate sources. The simplest mode of the interaction of two (or more) neurotransmitters occurs when two (or more) distinct receptor complexes are present in the post-synaptic membrane of target cells. When neuropeptides coexist with low-molecular-weight neurotransmitters, the neuropeptide(s) usually act(s) on GPCRs, whereas the low-molecular-weight transmitter generally opens a ligand-gated ion channel. The low-molecular-weight transmitter is generally the principal messenger, and the neuropeptide interacts with it by altering the ion channel gating properties or its response to further signals, this being often referred to as a modulation of signal transduction. The modulatory action of neuropeptides may occur by direct operation on the receptor complex or by the activation of second messenger systems that, in turn, act on the receptor complex. Hence, one neurotransmitter may, for example, alter the number of receptors or the affinity of the receptor to the other(s) simultaneously released. Interestingly, receptor recruit- ment from the interior of the cell to the plasma membrane or vice versa, may be an additional mechanism of modulation of signal transduction, respectively leading to a reinforcement or depression of neurotransmission. Receptor recruitment seems to be a common feature of opioid receptors. It was already mentioned that kappa receptors are translocated to the plasma membrane of magnocellular hypothalamic neurons after a physiological stimulus (salt-loading) that elicits vasopressin release (Shuster et al., 1999), this representing a mode of pre-synaptic regulation of neuropeptide release. Similarly, delta opioid receptor-mediated analgesia is enhanced in the complete Freund's adjuvant model of inflammation because of the translocation of receptors in the plasma membrane of DRG and spinal cord neurons (Gendron et al., 2006). Interestingly, in both circumstances LGVs storing a different neuropeptide (vasopressin or substance P) appear to act as cargoes for these receptors (Shuster et al., 1999). On the other hand, neuropeptide receptor internalization can also occur, at least in certain circumstances, following ligand binding, and eventually leading to receptor de-sensitization, as for example for the preferred substance P receptor NK_1 (Mantyh et al., 1995a,b). The interaction of co-transmitters also occurs through pre-synaptic regulation. This implies the existence of pre-synaptic receptors for one or more messengers. In this case, one of the neurotransmitters feeds back on the pre-synaptic receptors and thus affects its own release (Malcangio and Bowery, 1999) or the release of the co-transmitter(s) (Glowinski et al., 1993; Marco et al., 1998). Since neuropeptides are able to target receptors at distant sites from release, an additional element of complexity is added by the possibility that neuropeptide receptors not only are localized at the same synapse where release of the neuropeptide(s) occurs, but also at different synaptic sites. This leads to the existence of homosynaptic and heterosynaptic effects, respectively (Weisskopf et al., 1993). Broadly speaking, neuropeptide modulation of fast

neurotrans- mission occurs by both pre- and post-synaptic mechanisms. Both can affect excitatory or inhibitory neurotransmission. It is not easy to dissect out with certainty the intracellular pathway(s) involved in the modulatory effects of neuropeptides, mainly because there are only few examples where anatomical and functional analysis have been carried out co-jointly. Electrophysi- ological experiments have helped to elucidate the dependence of release from Ca^{2+} by analysis of evoked or spontaneous (action potential-dependent) post-synaptic currents (ePSCs or sPSCs) which rely on Ca^{2+}-dependent release from one side, and miniature PSCs (mPSCs) where transmitter release is independent from Ca^{2+}. However, results on sPSCs must be interpreted with caution since it is difficult to exclude polysynaptic effects or actions on the soma of the pre-synaptic neuron.

3.3.1. Glutamatergic neurotransmission

Modulation of excitatory glutamatergic neurotransmission by neuropeptides occurs with several different mechanisms and homosynaptic or heterosynaptic effects. Both direct and indirect underlying mechanisms have been described, and often shown to occur in combination with each other. A few examples are given to highlight the broad spectrum of possibilities of neuropeptide action.

Enhancement of glutamatergic neurotransmission has been reported for orexin B/hypocretin 2 in several brain areas including the median preoptic nucleus, the ventral tegmental area (VTA) of mesencephalon, and the nucleus tractus solitarius. Pre- (Borgland et al., 2008; Smith et al., 2002) and/or post-synaptic (Borgland et al., 2008; Kolaj et al., 2008) mechanisms were involved. The latter consisted of a potentiation of NMDA receptors mediated by activation of orexin/hypocretin 2 receptors coupled with Gq proteins and protein kinase C (PKC) (Borgland et al., 2008). An interaction with the melanin concentrating hormone (MCH) system in lateral hypothalamus has also been reported (Rao et al., 2008). Similarly, pituitary adenylate cyclase-activating polypeptide (PACAP) was shown to enhance glutamatergic neurotransmission by both pre- and post-synaptic mechanisms in hippocampus (Costa et al., 2009; Macdonald et al., 2005) and hypothalamus (Michel et al., 2006). Ligands (among which aMSH) of pre-synaptic melanocortin-4 receptors on vagal afferent fibers modulate the excitability of neurons in the nucleus tractus solitarius (Wan et al., 2008). Nociceptin/orphanin FQ (N/OFQ) and its receptor (NOP) facilitate glutamate release in the mesencephalic substantia nigra, a brain area containing dopamine neurons that degenerate in Parkinson's disease (Marti et al., 2005a,b). In the amygdala, the modulation of excitatory neuro- transmission by corticotropin releasing factor (CRF) appears to be quite complex, since positive, negative or null effects have been described in specific synapses (Gallagher et al., 2008). The mechanism, by which CRF enhances glutamatergic neurotrans- mission in the bed nucleus of the stria terminalis (BNST), a CRF-rich component of the extended amygdala, is even more complex than those exemplified above. In this case an additional player, dopamine, directly and rapidly interacts with CRF systems and appears to be

responsible for the regulation of excitatory glutamatergic transmission (Kash et al., 2008). Many neuropeptides have been found to inhibit glutamate release from pre-synaptic terminals, likely by indirect interaction of bg G-protein subunits with pre-synaptic proteins (see Tallent, 2008 for a recent review). Coupling more often occurs with Gi/Go G-proteins, but also with Gq-coupled receptors. At present, the most common mechanism described for Gi/Go-coupled neuro- peptide receptors appears to be independent from Ca^{2+} entry in terminals and to act downstream of axonal excitability. Nonethe- less both Ca^{2+}-dependent and -independent mechanisms have been demonstrated. Another common mechanism for Gi/Go- coupled neuropeptide receptors is the facilitation of K^{+} channels. Examples for both types of mechanisms can be found for different neuropeptides, including NPY, opioids, SST, N/OFQ, and galanin in several areas of the brain, such as the olfactory bulb (Blakemore et al., 2006), hippocampus (Tallent et al., 2001), striatum (Barral et al., 2003), amygdala (Meis and Pape, 2001; Zhu and Pan, 2005), thalamus (Meis et al., 2002), hypothalamus (Iremonger and Bains, 2009), PAG (Vaughan and Christie, 1997; Vaughan et al., 1997), and spinal cord (Bencivinni et al., 2010).

The mechanisms by which Gq-coupled receptors inhibit glutamate release are still far from being fully understood. Gq stimulates phospholipase C (PLC), which, in turn, increases intracellular Ca^{2+}. Therefore, it is paradoxical that Gq-coupled receptors reduce neurotransmitter release. Numerous studies indicate that the inhibition of glutamate release by this type of receptors is indeed indirect. Examples can be found for substance P, orexin B/hypocretin 2, and CCK in several areas of the brain including PAG (Sekizawa et al., 2003), parabrachial nucleus (PBN) (Saleh, 1997), dorsal raphe nucleus (DRN) (Haj-Dahmane and Shen, 2005), and nucleus accumbens (Kombian et al., 2004).

3.3.2. **GABAergic/glycinergic neurotransmission**

Neuropeptides have also been demonstrated to enhance GABA and/or glycine release from terminals by pre-synaptic and/or post- synaptic mechanisms.

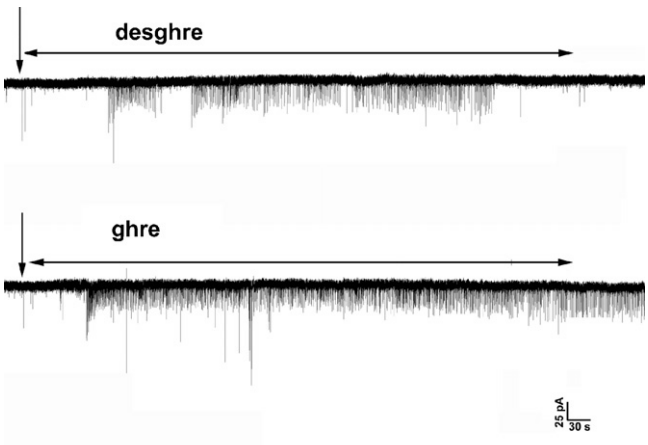

Fig. 2. Spontaneous inhibitory post-synaptic currents (sIPSCs) in neurons of lamina V of the mouse spinal cord dorsal horn after challenge (100 nM) with des-acyl ghrelin

and ghrelin in the presence of glutamatergic neurotransmission block. The vertical arrow indicates the start of drug pulse, the horizontal arrow the duration of pulse. In both cases there is a strong increase in the frequency of sIPSCs, related to a neuropeptide-dependent release of GABA/glycine (see Vergnano et al., 2008). Abbreviations: desghre = des-acyl ghrelin; ghre = ghrelin.

These latter include inhibition of N-P/Q-type Ca^{2+} channels, reduction in the pre-synaptic baseline Ca^{2+} concentration, and inhibition of the vesicle-release machinery. Examples include: CRF in DRN neurons (Kirby et al., 2008), ghrelin in spinal cord dorsal horn (Fig. 2) (Vergnano et al., 2008), N/ OFQ in hippocampus (Bongsebandhu-phubhakdi and Manabe, 2007) and central amygdala (Roberto and Siggins, 2006), orexins in vagal motor neurons (Davis et al., 2003), SST in neostriatum (Lopez-Huerta et al., 2008), substance P in spinal cord dorsal horn (Ferrini et al., 2007, 2010; Vergnano et al., 2004), and VIP in hippocampal CA1 pyramidal cells (Cunha-Reis et al., 2004). Neuropeptides have also been found to inhibit GABAergic synapses. In the hypothalamic arcuate nucleus, ghrelin receptors are expressed on axon terminals of NPY/AgRP inhibitory neurons. Receptor activation by ghrelin induces the release of NPY that, in turn, inhibits anorexygenic pro-opiomelanocortin (POMC) expres- sing neurons by acting on post-synaptic receptors, and disinhibits orexygenic CRF neurons by binding on pre-synaptic receptors, therefore reducing GABA release (see Ferrini et al., 2009 for a recent review). In the mesencephalic PAG, NTS produces a direct neuronal depolarization via NTS1 receptors, and inhibits GABAer- gic synaptic transmission (Mitchell et al., 2009).

3.3.3. Dopaminergic and serotoninergic neurotransmission

Among the neuropeptides enhancing dopamine release are NTS (Fawaz et al., 2009) and NPY (Quarta et al., 2011; Silva et al., 2005) in nucleus accumbens, CRF (Wanat et al., 2008) and galanin (Weiss et al., 2005) in VTA. NTS also induces a release of serotonin in the rostral ventromedial medulla (Buhler et al., 2005).

3.3.4. Growth factors as modulatory neuropeptides

Growth factors are among potential candidate neuropeptides (see Burbach, 2010 for a recent review). For many of them expression in the mature nervous system and their participation in neural communication is not known. One remarkable exception is BDNF (see Merighi, 2002 for a review of earlier literature) that since more than 15 years has been recognized to promote long- term potentiation (LTP) in hippocampus, visual system and spinal cord, and, more recently, in dorsal striatum (Jia et al., 2010). BDNF is secreted in precursor form (pro-BDNF) having a signal peptide and cleavage sites for typical prohormone convertases. In addition, mature BDNF is present in LGVs, and subject to stimulated release (Salio et al., 2007; Yang et al., 2009). BDNF and pro-BDNF have different

functional roles, since BDNF acts on the full length tropomyosine receptor kinase B isoform (trkB) that serves as its high affinity receptor, whereas pro-BDNF acts on a different receptor, the low affinity common neurotrophin receptor $p75^{NTR}$ (Teng et al., 2005; Woo et al., 2005). BDNF modulates fast excitatory and inhibitory neurotransmis- sion in several areas of the brain including the neocortex (Lemtiri- Chlieh and Levine, 2010), hippocampus (Holm et al., 2009; Paredes et al., 2007), hypothalamic SON (Ohbuchi et al., 2009), and spinal cord (see Merighi et al., 2008b for a recent review). The negative modulation of GABAergic neurotransmission in the neocortex is particularly complex with the intervention of endocannabinoid retrograde signaling (Lemtiri-Chlieh and Levine, 2010). The modulation of fast neurotransmission in spinal cord affects both the glutamatergic and GABAergic systems trough pre- and post- synaptic mechanisms (see Bardoni and Merighi, 2009 for a recent review). The lamina II of the spinal cord dorsal horn appears to be the main site where these interactions occur. Combined structural and functional analysis of lamina II synapses has demonstrated the existence of two separate populations of terminals each endowed with a different combination of slow-acting transmitters in LGVs in association to fast-acting glutamate. In the first, BDNF is stored in LGVs together with substance P and CGRP (Salio et al., 2007), whereas in the second LGVs contain a cocktail of GDNF, SST and CGRP (Merighi and Salio, 2010). Therefore, by concurrent receptor localization (Salio et al., 2005; Merighi and Salio, 2010) and functional observations (Bardoni et al., 2007; Merighi et al., 2008a) one can start to envisage a more precise definition of the neurotransmitter role of neuropeptides and growth factors in this area of CNS (Fig. 1D).

3. Conclusion

Studies on the endocrine system in the sixties–seventies of the last century led to the definition of neuropeptides as a new and special group of molecules involved in neuron-to-neuron communication. Nowadays over 70 genes have been recognized in the mammalian genome that encode for neuropeptide precursors, and this number will surely increase as far as a broader view for neuropeptide inclusion is emerging from combined structural and functional studies. When studying the neuromodulatory function of neuropeptides that, as mentioned, is only one of the several roles served by this class of molecules in central and peripheral neurons, the combination of different approaches is mandatory, since simple neurochemical localiza- tion can only be supportive to demonstrate an effect of biological relevance. The refinement of ultrastructural immunocytochemi- cal techniques in parallel with the availability of more and more reliable antibodies against not only the neuropeptides themselves but also their receptors represents a real milestone in the field. It offers a unique until recently unforeseen

possibility to dissect the role of neuropeptides in chemical transmission and their relationship with low-molecular-weight neurotransmitters, the earlier recognized actors in the exchange of information between the nerve cells. Most likely, some of the barriers that we are still facing to our comprehension of the function of neuropeptides in cell-to-cell communication will be removed as we start thinking to this class of molecules in a more integrated fashion. The lesson that we should have learnt after more than half century of neuropeptide research is that the same molecule can exert different functions in relation to the type of cell that produces it, the time and site of production, the coexistence/co-storage with other transmitters, and the functional status.

Acknowledgements
The experimental work described in this paper has been funded by grants of the Italian MIUR (PRIN 2008), and Compagnia di San Paolo, Torino.

References

Arvidsson, U., Cullheim, S., Ulfhake, B., Luppi, P.H., Kitahama, K., Jouvet, M., Hö kfelt, T., 1994. Quantitative and qualitative aspects on the distribution of 5-HT and its coexistence with substance P and TRH in cat ventral medullary neurons. J. Chem. Neuroanat. 7, 3–12.

Artalejo, C.R., Elhamdani, A., Palfrey, H.C., 1998. Secretion: dense-core vesicles can kiss-and-run too. Curr. Biol. 8, R62–R65.

Arvieu, L., Mauborgne, A., Bourgoin, S., Oliver, C., Feltz, P., Hamon, M., Cesselin, F., 1996. Sumatriptan inhibits the release of CGRP and substance P from the rat spinal cord. Neuroreport 7, 1973–1976.

Balkowiec, A., Katz, D.M., 2000. Activity-dependent release of endogenous brain- derived neurotrophic factor from primary sensory neurons detected by ELISA in situ. J. Neurosci. 20, 7417–7423.

Baranano, D.E., Ferris, C.D., Snyder, S.H., 2001. Atypical neural messengers. Trends Neurosci. 24, 99–106.

Bardoni, R., Merighi, A., 2009. BDNF and trkB mediated mechanisms in the spinal cord. In: Malcangio, M. (Ed.), Synaptic Plasticity in Pain. Springer, New York.

Bardoni, R., Ghirri, A., Salio, C., Prandini, M., Merighi, A., 2007. BDNF-mediated modulation of GABA and glycine release in dorsal horn lamina II from postnatal rats. Dev. Neurobiol. 67, 960–975.

Barg, S., Olofsson, C.S., Schriever-Abeln, J., Wendt, A., Gebre-Medhin, S., Renstrom, E., Rorsman, P., 2002. Delay between fusion pore opening and peptide release from large dense-core vesicles in neuroendocrine cells. Neuron 33, 287–299.

Barral, J., Mendoza, E., Galarraga, E., Bargas, J., 2003. The presynaptic modulation of corticostriatal afferents by mu-opioids is mediated by K+ conductances. Eur. J. Pharmacol. 462, 91–98.

Baulieu, E.E., Robel, P., Schumacher, M., 2001. Neurosteroids: beginning

of the story. Int. Rev. Neurobiol. 46, 1–32.
Bean, A.J., Zhang, X., Hö kfelt, T., 1994. Peptide secretion: what do we know. FASEB J. 8, 630–638.
Bencivinni, I., Ferrini, F., Salio, C., Beltramo, M., Merighi, A., 2010. The somatostatin analogue octreotide inhibits capsaicin-mediated activation of nociceptive pri- mary afferent fibres in spinal cord lamina II (substantia gelatinosa). Eur. J. Pain, doi:10.1016/j.ejpain.2010.11.001.
Blakemore, L.J., Levenson, C.W., Trombley, P.Q., 2006. Neuropeptide Y modulates excitatory synaptic transmission in the olfactory bulb. Neuroscience 138, 663– 674.
Bongsebandhu-phubhakdi, S., Manabe, T., 2007. The neuropeptide nociceptin is a synaptically released endogenous inhibitor of hippocampal long-term potenti- ation. J. Neurosci. 27, 4850–4858.
Borgland, S.L., Storm, E., Bonci, A., 2008. Orexin B/hypocretin 2 increases glutama- tergic transmission to ventral tegmental area neurons. Eur. J. Neurosci. 28, 1545–1556.
Braslis, K.G., Shulkes, A., Fletcher, D.R., Hardy, K.J., 1988. Pharmacokinetics and organ-specific metabolism of calcitonin gene-related peptide in sheep. J. Endo- crinol. 118, 25–31.
Brigadski, T., Hartmann, M., Lessmann, V., 2005. Differential vesicular targeting and time course of synaptic secretion of the mammalian neurotrophins. J. Neurosci. 25, 7601–7614.
Brownstein, M.J., Russell, J.T., Gainer, H., 1980. Synthesis, transport, and release of posterior pituitary hormones. Science 207, 373–378.
Buhler, A.V., Choi, J., Proudfit, H.K., Gebhart, G.F., 2005. Neurotensin activation of the NTR1 on spinally-projecting serotonergic neurons in the rostral ventromedial medulla is antinociceptive. Pain 114, 285–294.
Buma, P., 1988. Synaptic and nonsynaptic release of neuromediators in the central nervous system. Acta Morphol. Neerl. Scand. 26, 81–113.
Burbach, J.P., 2010. Neuropeptides from concept to online database. Eur. J. Phar- macol. 626, 27–48., www.neuropeptides.nl.
Cahill, C.M., Morinville, A., Lee, M.C., Vincent, J.P., Collier, B., Beaudet, A., 2001.
Prolonged morphine treatment targets delta opioid receptors to neuronal plasma membranes and enhances delta-mediated antinociception. J. Neurosci. 21, 7598–7607.
Cauli, B., Tong, X.K., Rancillac, A., Serluca, N., Lambolez, B., Rossier, J., Hamel, E., 2004. Cortical GABA interneurons in neurovascular coupling: relays for subcortical vasoactive pathways. J. Neurosci. 24, 8940–8949.
Collin, E., Mantelet, S., Frechilla, D., Pohl, M., Bourgoin, S., Hamon, M., Cesselin, F., 1993. Increased in vivo release of calcitonin gene-related peptide-like material from the spinal cord in arthritic rats. Pain 54, 203–211.

Collin, E., Frechilla, D., Pohl, M., Bourgoin, S., Mauborgne, A., Hamon, M., Cesselin, F., 1994. Differential effects of the novel analgesic, S 12813-4, on the spinal release of substance P- and calcitonin gene-related peptide-like materials in the rat. Naunyn Schmiedebergs Arch. Pharmacol. 349, 387–393.

Commons, K.G., 2003. Translocation of presynaptic delta opioid receptors in the ventrolateral periaqueductal gray after swim stress. J. Comp. Neurol. 464, 197– 207.

Costa, L., Santangelo, F., Li, V.G., Ciranna, L., 2009. Modulation of AMPA receptor- mediated ion current by pituitary adenylate cyclase-activating polypeptide (PACAP) in CA1 pyramidal neurons from rat hippocampus. Hippocampus 19, 99–109.

Cunha-Reis, D., Sebastiao, A.M., Wirkner, K., Illes, P., Ribeiro, J.A., 2004. VIP enhances both pre- and postsynaptic GABAergic transmission to hippocampal interneurones leading to increased excitatory synaptic transmission to CA1 pyramidal cells. Br. J. Pharmacol. 143, 733–744.

Davis, S.F., Williams, K.W., Xu, W., Glatzer, N.R., Smith, B.N., 2003. Selective enhancement of synaptic inhibition by hypocretin (orexin) in rat vagal motor neurons: implications for autonomic regulation. J. Neurosci. 23, 3844–3854.

De Camilli, P., Jahn, R., 1990. Pathways to regulated exocytosis in neurons. Annu. Rev. Physiol. 52, 625–645.

De Wied, D., 1971. Long term effect of vasopressin on the maintenance of a conditioned avoidance response in rats. Nature 232, 58–60.

de Wit, J., Toonen, R., Verhage, M., 2009. Matrix-dependent local retention of secretory vesicle cargo in cortical neurons. J. Neurosci. 29, 23–37.

Drake, C.T., Terman, G.W., Simmons, M.L., Milner, T.A., Kunkel, D.D., Schwartzkroin, P.A., Chavkin, C., 1994. Dynorphin opioids present in dentate granule cells may function as retrograde inhibitory neurotransmitters. J. Neurosci. 14, 3736– 3750.

Dun, N.J., Dun, S.L., Wong, R.K., Forstermann, U., 1994. Colocalization of nitric oxide synthase and somatostatin immunoreactivity in rat dentate hilar neurons. Proc. Natl. Acad. Sci. U.S.A. 91, 2955–2959.

Elhamdani, A., Palfrey, H.C., Artalejo, C.R., 2001. Quantal size is dependent on stimulation frequency and calcium entry in calf chromaffin cells. Neuron 31, 819–830.

Fawaz, C.S., Martel, P., Leo, D., Trudeau, L.E., 2009. Presynaptic action of neurotensin on dopamine release through inhibition of D(2) receptor function. BMC Neu- rosci. 10, 96.

Fellin, T., 2009. Communication between neurons and astrocytes: relevance to the modulation of synaptic and network activity. J. Neurochem. 108, 533–544.

Ferrini, F., Salio, C., Vergnano, A.M., Merighi, A., 2007. Vanilloid receptor-1 (TRPV1)- dependent activation of inhibitory neurotransmission in spinal substantia gelatinosa neurons of mouse. Pain 129, 195–209.

Ferrini, F., Salio, C., Lossi, L., Merighi, A., 2009. Ghrelin in central neurons. Curr.
Neuropharmacol. 7, 37–49.

Ferrini, F., Salio, C., Lossi, L., Gambino, G., Merighi, A., 2010. Modulation of inhibitory neurotransmission by the vanilloid receptor type 1 (TRPV1) in organotypically cultured mouse substantia gelatinosa neurons. Pain 150, 128–140.

Fried, G., 1982. Neuropeptide storage in vesicles. In: Klein, R.L., Lagercrantz, H., Zimmermann, H. (Eds.), Neurotransmitter Vesicles. Acedemic Press, London/New York, pp. 361–374.

Fried, G., Terenius, L., Hö kfelt, T., Goldstein, M., 1985. Evidence for differential localization of noradrenaline and neuropeptide Y (NPY) in neuronal storage vesicles isolated from rat vas deferens. J. Neurosci. 5, 450–458.

Gainer, H., Fields, R.L., House, S.B., 2001. Vasopressin gene expression: experimental models and strategies. Exp. Neurol. 171, 190–199.

Gainer, H., Yamashita, M., Fields, R.L., House, S.B., Rusnak, M., 2002. The magno- cellular neuronal phenotype: cell-specific gene expression in the hypothalamo– neurohypophysial system. Prog. Brain Res. 139, 1–14.

Gallagher, J.P., Orozco-Cabal, L.F., Liu, J., Shinnick-Gallagher, P., 2008. Synaptic physiology of central CRH system. Eur. J. Pharmacol. 583, 215–225.

Garry, M.G., Richardson, J.D., Hargreaves, K.M., 1994. Sodium nitroprusside evokes the release of immunoreactive calcitonin gene-related peptide and substance P from dorsal horn slices via nitric oxide-dependent and nitric oxide-independent mechanisms. J. Neurosci. 14, 4329–4337.

Gendron, L., Lucido, A.L., Mennicken, F., O'Donnell, D., Vincent, J.P., Stroh, T., Beaudet, A., 2006. Morphine and pain-related stimuli enhance cell surface availability of somatic delta-opioid receptors in rat dorsal root ganglia. J. Neurosci. 26, 953–962.

Ghijsen, W.E., Leenders, A.G., 2005. Differential signaling in presynaptic neuro- transmitter release. Cell Mol. Life Sci. 62, 937–954.

Glowinski, J., Kemel, M.L., Desban, M., Gauchy, C., Lavielle, S., Chassaing, G., Beaujouan, J.C., Tremblay, L., 1993. Distinct presynaptic control of dopamine release in striosomal- and matrix-enriched areas of the rat striatum by selective agonists of NK1, NK2 and NK3 tachykinin receptors. Regul. Pept. 46, 124–128.

Haj-Dahmane, S., Shen, R.Y., 2005. The wake-promoting peptide orexin-B inhibits glutamatergic transmission to dorsal raphe nucleus

serotonin neurons through retrograde endocannabinoid signaling. J. Neurosci. 25, 896–905.

Hallberg, M., Nyberg, F., 2003. Neuropeptide conversion to bioactive fragments—an important pathway in neuromodulation. Curr. Protein Pept. Sci. 4, 31–44. Hamm, H.E., Gilchrist, A., 1996. Heterotrimeric G proteins. Curr. Opin. Cell Biol. 8, 189–196.

Harata, N., Pyle, J.L., Aravanis, A.M., Mozhayeva, M., Kavalali, E.T., Tsien, R.W., 2001. Limited numbers of recycling vesicles in small CNS nerve terminals: implica- tions for neural signaling and vesicular cycling. Trends Neurosci. 24, 637–643.

Harling, H., Messell, T., Poulsen, S.S., Rasmussen, T.N., Holst, J.J., 1991. Galanin and vasoactive intestinal polypeptide: coexistence and corelease from the vascularly perfused pig ileum during distension and chemical stimulation of the mucosa. Digestion 50, 61–71.

Hirsch, M.D., Millington, W.R., 1991. Endoproteolytic conversion of beta endorphin-1-31 to beta-endorphin-1-27 potentiates its central cardioregulatory activity. Brain Res. 550, 61–68.

Hö kfelt, T., 1991. Neuropeptides in perspective: the last ten years. Neuron 7, 867– 879.

Hökfelt, T., 2010. Looking at neurotransmitters in the microscope. Prog. Neurobiol. 90, 101–118.

Hökfelt, T., Broberger, C., Xu, Z.Q., Sergeyev, V., Ubink, R., Diez, M., 2000. Neuropep- tides—an overview. Neuropharmacology 39, 1337–1356.

Holm, M.M., Nieto-Gonzalez, J.L., Vardya, I., Vaegter, C.B., Nykjaer, A., Jensen, K., 2009. Mature BDNF, but not proBDNF, reduces excitability of fast-spiking interneurons in mouse dentate gyrus. J. Neurosci. 29, 12412–12418.

Holst, J.J., Fahrenkrug, J., Knuhtsen, S., Jensen, S.L., Nielsen, O.V., Lundberg, J.M., Hö kfelt, T., 1987. VIP and PHI in the pig pancreas: coexistence, corelease, and cooperative effects. Am. J. Physiol. 252, G182–G189.

Hrabovszky, E., Csapó , A.K., Kallό , I., Wilheim, T., Tú ri, G.F., Liposits, Z., 2006. Localization and osmotic regulation of vesicular glutamate transporter-2 in magnocellular neurons of the rat hypothalamus. Neurochem. Int. 48, 753–761.

Iremonger, K.J., Bains, J.S., 2009. Retrograde opioid signaling regulates glutamatergic transmission in the hypothalamus. J. Neurosci. 29, 7349–7358.

Jia, Y., Gall, C.M., Lynch, G., 2010. Presynaptic BDNF promotes postsynaptic long- term potentiation in the dorsal striatum. J. Neurosci. 30, 14440–14445.

Jung, L.J., Scheller, R.H., 1991. Peptide processing and targeting in the neuronal

secretory pathway. Science 251, 1330–1335.

Karhunen, T., Vilim, F.S., Alexeeva, V., Weiss, K.R., Church, P.J., 2001.

Targeting of peptidergic vesicles in cotransmitting terminals. J. Neurosci. 21, RC127.
Kash, T.L., Nobis, W.P., Matthews, R.T., Winder, D.G., 2008. Dopamine enhances fast excitatory synaptic transmission in the extended amygdala by a CRF-R1-de- pendent process. J. Neurosci. 28, 13856–13865.
Kirby, L.G., Freeman-Daniels, E., Lemos, J.C., Nunan, J.D., Lamy, C., Akanwa, A., Beck, S.G., 2008. Corticotropin-releasing factor increases GABA synaptic activity and induces inward current in 5-hydroxytryptamine dorsal raphe neurons. J. Neu- rosci. 28, 12927–12937.
Kits, K.S., Mansvelder, H.D., 2000. Regulation of exocytosis in neuroendocrine cells: spatial organization of channels and vesicles, stimulus-secretion coupling, calcium buffers and modulation. Brain Res. Brain Res. Rev. 33, 78–94.
Kits, K.S., Dreijer, A.M., Lodder, J.C., Borgdorff, A., Wadman, W.J., 1997. High intracellular calcium levels during and after electrical discharges in molluscan peptidergic neurons. Neuroscience 79, 275–284.
Kolaj, M., Coderre, E., Renaud, L.P., 2008. Orexin peptides enhance median preoptic nucleus neuronal excitability via postsynaptic membrane depolarization and enhancement of glutamatergic afferents. Neuroscience 155, 1212–1220.
Kombian, S.B., Ananthalakshmi, K.V., Parvathy, S.S., Matowe, W.C., 2004. Cholecystokinin activates CCKB receptors to excite cells and depress EPSCs in the rat rostral nucleus accumbens in vitro. J. Physiol. 555, 71–84.
Kupfermann, I., 1991. Functional studies of cotransmission. Physiol. Rev. 71, 683– 732.
Landgraf, R., Neumann, I.D., 2004. Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. Front. Neuroendocrinol. 25, 150–176.
Landry, M., Vila-Porcile, E., Hö kfelt, T., Calas, A., 2003. Differential routing of coexisting neuropeptides in vasopressin neurons. Eur. J. Neurosci. 17, 579–589. Landry, M., Liu, H.-X., Shi, T.-J., Brumovsky, P., Nagy, F., Hö kfelt, T., 2005. Galani- nergic mechanisms at the spinal level: focus on histochemical phenotyping. Neuropeptides 39, 223–231.
Lemtiri-Chlieh, F., Levine, E.S., 2010. BDNF evokes release of endogenous cannabinoids at layer 2/3 inhibitory synapses in the neocortex. J. Neurophysiol. 104, 1923–1932.
Lessmann, V., Gottmann, K., Malcangio, M., 2003. Neurotrophin secretion: current facts and future prospects. Prog. Neurobiol. 69,

341–374.
Lopez-Huerta, V.G., Tecuapetla, F., Guzman, J.N., Bargas, J., Galarraga, E., 2008. Presynaptic modulation by somatostatin in the neostriatum. Neurochem. Res. 33, 1452–1458.
Ludwig, M., Leng, G., 2006. Dendritic peptide release and peptide-dependent behaviours. Nat. Rev. Neurosci. 7, 126–136.
Macdonald, D.S., Weerapura, M., Beazely, M.A., Martin, L., Czerwinski, W., Roder, J.C., Orser, B.A., MacDonald, J.F., 2005. Modulation of NMDA receptors by pituitary adenylate cyclase activating peptide in CA1 neurons requires G alpha q, protein kinase C, and activation of Src. J. Neurosci. 25, 11374–11384.
Malcangio, M., Bowery, N.G., 1999. Peptide autoreceptors: does an autoreceptor for substance P exist? Trends Pharmacol. Sci. 20, 405–407.
Mantyh, P.W., Allen, C.J., Ghilardi, J.R., Rogers, S.D., Mantyh, C.R., Liu, H., Basbaum, A.I., Vigna, S.R., Maggio, J.E., 1995a. Rapid endocytosis of a G protein-coupled receptor: substance P-evoked internalization of its receptor in the rat striatum in vivo. Proc. Natl. Acad. Sci. U.S.A. 92, 2622–2626.
Mantyh, P.W., DeMaster, E., Malhotra, A., Ghilardi, J.R., Rogers, S.D., Mantyh, C.R., Liu, H., Basbaum, A.I., Vigna, S.R., Maggio, J.E., Simone, D.A., 1995b. Receptor endocytosis and dendrite reshaping in spinal neurons after somatosensory stimulation. Science 268, 1629–1632.
Marco, N., Thirion, A., Mons, G., Bougault, I., Le, F.G., Soubrie, P., Steinberg, R., 1998. Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK3 receptor stimulation: an in vivo microdialysis approach in guinea pig. Neuropeptides 32, 481–488.
Marti, M., Manzalini, M., Fantin, M., Bianchi, C., Della, C.L., Morari, M., 2005a. Striatal
glutamate release evoked in vivo by NMDA is dependent upon ongoing neuronal activity in the substantia nigra, endogenous striatal substance P and dopamine. J. Neurochem. 93, 195–205.
Marti, M., Mela, F., Fantin, M., Zucchini, S., Brown, J.M., Witta, J., Di, B.M., Buzas, B., Reinscheid, R.K., Salvadori, S., Guerrini, R., Romualdi, P., Candeletti, S., Simonato, M., Cox, B.M., Morari, M., 2005b. Blockade of nociceptin/orphanin FQ transmission attenuates symptoms and neurodegeneration associated with Parkinson's disease. J. Neurosci. 25, 9591–9601.
Martin, T.F., 2003. Tuning exocytosis for speed: fast and slow modes. Biochim. Biophys. Acta 1641, 157–165.
Martinez-Rodriguez, R., Martinez-Murillo, R., 1994. Molecular and cellular aspects of neurotransmission and neuromodulation. Int. Rev. Cytol. 149, 217–292.
Meis, S., Pape, H.C., 2001. Control of glutamate and GABA release by nociceptin/orphanin FQ in the rat lateral amygdala. J. Physiol. 532,

701–712.
Meis, S., Munsch, T., Pape, H.C., 2002. Antioscillatory effects of nociceptin/orphanin FQ in synaptic networks of the rat thalamus. J. Neurosci. 22, 718–727.
Mens, W.B., Witter, A., Wimersma Greidanus, T.B., 1983. Penetration of neurohy- pophyseal hormones from plasma into cerebrospinal fluid (CSF): half-times of disappearance of these neuropeptides from CSF. Brain Res. 262, 143–149.
Merighi, A., 2002. Costorage and coexistence of neuropeptides in the mammalian CNS. Progr. Neurobiol. 66, 161–190.
Merighi, A., 2009. Neuropeptides and coexistence. In: Squire, L.R. (Ed.), Encyclo- pedia of Neuroscience. Academic Press, Oxford, pp. 843–849.
Merighi, A., Salio, C., 2010. Use of gold-labeled antibodies for ultrastructural localization of neurotrophic factors and their receptors in somatosensory path- ways. In: 2nd International Congress on Antibodies, BIT Life Sciences, Bejing. Merighi, A., Polak, J.M., Gibson, S.J., Gulbenkian, S., Valentino, K.L., Peirone, S.M., 1988. Ultrastructural studies on calcitonin gene-related peptide-, tachykinins- and somatostatin-immunoreactive neurones in rat dorsal root ganglia: evidence for the colocalisation of different peptides in single secretory granules. Cell Tissue Res. 254, 101–109.
Merighi, A., Bardoni, R., Salio, C., Lossi, L., Ferrini, F., Prandini, M., Zonta, M., Gustincich, S., Carmignoto, G., 2008a. Presynaptic functional trkB receptors mediate the release of excitatory neurotransmitters from primary afferent terminals in lamina II (substantia gelatinosa) of postnatal rat spinal cord. Dev. Neurobiol. 68, 457–475.
Merighi, A., Salio, C., Ghirri, A., Lossi, L., Ferrini, F., Betelli, C., Bardoni, R., 2008b. BDNF as a pain modulator. Prog. Neurobiol. 85, 297–317.
Michel, S., Itri, J., Han, J.H., Gniotczynski, K., Colwell, C.S., 2006. Regulation of glutamatergic signalling by PACAP in the mammalian suprachiasmatic nucleus. BMC Neurosci. 7, 15.
Mitchell, V.A., Kawahara, H., Vaughan, C.W., 2009. Neurotensin inhibition of GABAergic transmission via mGluR-induced endocannabinoid signalling in rat periaqueductal grey. J. Physiol. 587, 2511–2520.
Nillni, E.A., 2010. Regulation of the hypothalamic thyrotropin releasing hormone (TRH) neuron by neuronal and peripheral inputs. Front. Neuroendocrinol. 31, 134–156.
Ohbuchi, T., Yokoyama, T., Saito, T., Hashimoto, H., Suzuki, H., Otsubo, H., Fujihara, H., Suzuki, H., Ueta, Y., 2009. Brain-derived neurotrophic factor inhibits spon- taneous inhibitory postsynaptic currents in the rat supraoptic nucleus. Brain Res. 1258, 34–42.

Paredes, D., Granholm, A.C., Bickford, P.C., 2007. Effects of NGF and BDNF on baseline glutamate and dopamine release in the hippocampal formation of the adult rat. Brain Res. 1141, 56–64.

Pasternak, G.W., Wood, P.J., 1986. Multiple mu opiate receptors. Life Sci. 38, 1889– 1898.

Perrais, D., Kleppe, I.C., Taraska, J.W., Almers, W., 2004. Recapture after exocytosis causes differential retention of protein in granules of bovine chromaffin cells. J. Physiol. 560, 413–428.

Peters, W., 1976. In: Peters, A., Palay, S.L., Webster, H.d. (Eds.), The Fine Structure of the Nervous System. WB Saunders Company, Philadelphia.

Pow, D.V., Morris, J.F., 1989. Dendrites of hypothalamic magnocellular neurons release neurohypophysial peptides by exocytosis. Neuroscience 32, 435–439. Pritchard, L.E., Turnbull, A.V., White, A., 2002. Pro-opiomelanocortin processing in the hypothalamus: impact on melanocortin signalling and obesity. J. Endocrinol. 172, 411–421.

Quarta, D., Leslie, C.P., Carletti, R., Valerio, E., Caberlotto, L., 2011. Central administration of NPY or an NPY-Y5 selective agonist increase in vivo extracellular monoamine levels in mesocorticolimbic projecting areas. Neuropharmacology 60, 328–335.

Rao, Y., Lu, M., Ge, F., Marsh, D.J., Qian, S., Wang, A.H., Picciotto, M.R., Gao, X.B., 2008. Regulation of synaptic efficacy in hypocretin/orexin-containing neurons by melanin concentrating hormone in the lateral hypothalamus. J. Neurosci. 28, 9101–9110.

Roberto, M., Siggins, G.R., 2006. Nociceptin/orphanin FQ presynaptically decreases GABAergic transmission and blocks the ethanol-induced increase of GABA release in central amygdala. Proc. Natl. Acad. Sci. U.S.A. 103, 9715–9720.

Saleh, T.M., 1997. Visceral afferent stimulation-evoked changes in the release of peptides into the parabrachial nucleus in vivo. Brain Res. 778, 56–63.

Salio, C., Lossi, L., Ferrini, F., Merighi, A., 2005. Ultrastructural evidence for a pre- and postsynaptic localization of full-length trkB receptors in substantia gelatinosa (lamina II) of rat and mouse spinal cord. Eur. J. Neurosci. 22, 1951–1966.

Salio, C., Lossi, L., Ferrini, F., Merighi, A., 2006. Neuropeptides as synaptic trans- mitters. Cell Tissue Res. 326, 583–598.

Salio, C., Averill, S., Priestley, J.V., Merighi, A., 2007. Costorage of BDNF and neuropeptides within individual dense-core vesicles in central and peripheral neurons. Dev. Neurobiol. 67, 326–338.

Salio, C., Lossi, L., Merighi, A. Combined light and electron microscopic localization of neuropeptides and their receptors in central neurons. In: Merighi, A. (Ed.), Neuropeptides Methods and Protocols. Methods in Molecular Biology. Humana Press, New York, in press.

Sekizawa, S., Joad, J.P., Bonham, A.C., 2003. Substance P presynaptically depresses the transmission of sensory input to bronchopulmonary neurons in the guinea pig nucleus tractus solitarii. J. Physiol. 552, 547–559.

Selmer, I., Schindler, M., Allen, J.P., Humphrey, P.P., Emson, P.C., 2000. Advances in understanding neuronal somatostatin receptors. Regul. Pept. 90, 1–18.

Shuster, S.J., Riedl, M., Li, X., Vulchanova, L., Elde, R., 1999. Stimulus-dependent translocation of kappa opioid receptors to the plasma membrane. J. Neurosci. 19, 2658–2664.

Silva, A.P., Xapelli, S., Pinheiro, P.S., Ferreira, R., Lourenco, J., Cristovao, A., Grouz- mann, E., Cavadas, C., Oliveira, C.R., Malva, J.O., 2005. Up-regulation of neuro- peptide Y levels and modulation of glutamate release through neuropeptide Y receptors in the hippocampus of kainate-induced epileptic rats. J. Neurochem. 93, 163–170.

Smith, B.N., Davis, S.F., van den Pol, A.N., Xu, W., 2002. Selective enhancement of excitatory synaptic activity in the rat nucleus tractus solitarius by hypocretin 2. Neuroscience 115, 707–714.

Sü dhof, T.C., 2008. Neurotransmitter release. Handb. Exp. Pharmacol. 184, 1–21.

Summy-Long, J.Y., Hu, S., Long, A., Phillips, T.M., 2008. Interleukin-1beta release in the supraoptic nucleus area during osmotic stimulation requires neural func- tion. J. Neuroendocrinol. 20, 1224–1232.

Szapiro, G., Barbour, B., 2009. Parasynaptic signalling by fast neurotransmitters: the cerebellar cortex. Neuroscience 162, 644–655.

Tallent, M.K., 2008. Presynaptic inhibition of glutamate release by neuropeptides: use-dependent synaptic modification. Results Probl. Cell Differ. 44, 177–200.

Tallent, M.K., Madamba, S.G., Siggins, G.R., 2001. Nociceptin reduces epileptiform events in CA3 hippocampus via presynaptic and postsynaptic mechanisms. J. Neurosci. 21, 6940–6948.

Teng, H.K., Teng, K.K., Lee, R., Wright, S., Tevar, S., Almeida, R.D., Kermani, P., Torkin, R., Chen, Z.Y., Lee, F.S., Kraemer, R.T., Nykjaer, A., Hempstead, B.L., 2005. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. J. Neurosci. 25, 5455–5463.

Theodosis, D.T., Chapman, D.B., Montagnese, C., Poulain, D.A., Morris, J.F., 1986. Structural plasticity in the hypothalamic supraoptic nucleus at lactation affects oxitocin-but not vasopressin-secreting neurones. Neuroscience 17, 661–678.

Thureson-Klein, Å .K., Klein, R.L., Zhu, P.C., Kong, J.-Y., 1988. Differential release of transmitters and neuropeptides co-stored in central and peripheral neurons. In: Zimmermann, H. (Ed.), Cellular and Molecular

Basis of Synaptic Transmission. Springer-Verlag, Berlin/New York, pp. 171–183.
Tsuboi, T., Rutter, G.A., 2003. Insulin secretion by 'kiss-and-run' exocytosis in clonal pancreatic islet beta-cells. Biochem. Soc. Trans. 31, 833–836.
van Leeuwen, F.W., Verwer, R.W., Spence, H., Evans, D.A., Burbach, J.P., 1998. The magnocellular neurons of the hypothalamo-neurohypophyseal system display remarkable neuropeptidergic phenotypes leading to novel insights in neuronal cell biology. Prog. Brain Res. 119, 115–126.
Vanner, S., 1994. Corelease of neuropeptides from capsaicin-sensitive afferents dilates submucosal arterioles in guinea pig ileum. Am. J. Physiol. 267, G650–G655.
Vaughan, C.W., Christie, M.J., 1997. Presynaptic inhibitory action of opioids on synaptic transmission in the rat periaqueductal grey in vitro. J. Physiol. 498, 463–472.
Vaughan, C.W., Ingram, S.L., Connor, M.A., Christie, M.J., 1997. How opioids inhibit GABA-mediated neurotransmission. Nature 390, 611–614.
Vergnano, A.M., Salio, C., Merighi, A., 2004. NK1 receptor activation leads to enhancement of inhibitory neurotransmission in spinal substantia gelatinosa neurons of mouse. Pain 112, 37–47.
Vergnano, A.M., Ferrini, F., Salio, C., Lossi, L., Baratta, M., Merighi, A., 2008. The gastrointestinal hormone ghrelin modulates inhibitory neurotransmission in deep laminae of mouse spinal cord dorsal horn. Endocrinology 149, 2306–2312. Verhage, M., McMahon, H.T., Ghijsen, W.E., Boomsma, F., Scholten, G., Wiegant, V.M., Nicholls, D.G., 1991. Differential release of amino acids, neuropeptides, and catecholamines from isolated nerve terminals. Neuron 6, 517–524.
Wan, S., Browning, K.N., Coleman, F.H., Sutton, G., Zheng, H., Butler, A., Berthoud, H.R., Travagli, R.A., 2008. Presynaptic melanocortin-4 receptors on vagal affer- ent fibers modulate the excitability of rat nucleus tractus solitarius neurons. J. Neurosci. 28, 4957–4966.
Wanat, M.J., Hopf, F.W., Stuber, G.D., Phillips, P.E., Bonci, A., 2008. Corticotropin- releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of Ih. J. Physiol. 586, 2157–2170.
Wank, S.A., 1995. Cholecystokinin receptors. Am. J. Physiol. 269, G628–G646. Watson, S., Arkinstall, S., 1994. The G-protein Linked Receptor—Facts Book. San Diego Academic, San Diego.
Weiss, J.M., Boss-Williams, K.A., Moore, J.P., Demetrikopoulos, M.K., Ritchie, J.C., West, C.H., 2005. Testing the hypothesis that locus coeruleus hyperactivity produces depression-related changes via galanin. Neuropeptides 39, 281–287.

Weisskopf, M.G., Zalutsky, R.A., Nicoll, R.A., 1993. The opioid peptide dynorphin mediates heterosynaptic depression of hippocampal mossy fibre synapses and modulates long-term potentiation. Nature 362, 423–427.

Woo, N.H., Teng, H.K., Siao, C.J., Chiaruttini, C., Pang, P.T., Milner, T.A., Hempstead, B.L., Lu, B., 2005. Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. Nat. Neurosci. 8, 1069–1077.

Xu, X.J., Hö kfelt, T., Wiesenfeld-Hallin, Z., 2008. Galanin and spinal pain mechan- isms: where do we stand in 2008? Cell. Mol. Life Sci. 65, 1813–1819.

Yang, J., Siao, C.J., Nagappan, G., Marinic, T., Jing, D., McGrath, K., Chen, Z.Y., Mark, W., Tessarollo, L., Lee, F.S., Lu, B., Hempstead, B.L., 2009. Neuronal release of proBDNF. Nat. Neurosci. 12, 113–115.

Yang, L., Thomas, N.D., Helke, C.J., 1996. Characterization of substance P release from the intermediate area of rat thoracic spinal cord. Synapse 23, 265–273.

Yang, Y., Ozawa, H., Yuri, K., Kawata, M., 2000. Postnatal development of NADPH-diaphorase activity in the rat: the role of nitric oxide in the ontogeny of arginine vasopression and oxytocin. Endocr. J. 47, 601–613.

Zhu, P.C., Thureson-Klein, Å .K., Klein, R.L., 1986. Exocytosis from large dense cored vesicles outside the active synaptic zones of terminals within the trigeminal subnucleus caudalis:a possible mechanism for neuropeptide release. Neuroscience 19, 43–54.

Zhu, W., Pan, Z.Z., 2005. Mu-opioid-mediated inhibition of glutamate synaptic transmission in rat central amygdala neurons. Neuroscience 133, 97–103.

Zoli, M., Agnati, L.F., Jansson, A., Fuxe, K., Syková , E., 1999. Volume transmission in the CNS and its relevance for neurophyschopharmacology. Trends Physiol. Sci. 20, 142–150.
20, 142–150.