

Neuropeptides in Psychiatric Diseases: An Overview with a Particular Focus on Depression and Anxiety Disorders

C. Belzung^{*,1}, I. Yalcin¹, G. Griebel², A. Surget¹ and S. Leman¹

¹EA3248, Psychobiologie des émotions, UFR Sciences et techniques, Parc Grandmont, 37200 Tours, France

²Sanofi-Aventis, CNS Research Department, 31 avenue Paul Vaillant-Couturier, 92220 Bagneux, France

Abstract: This paper aimed at reviewing the involvement of neuropeptides in various psychiatric diseases, particularly in depression, and anxiety disorders. General features of neuropeptides are first described, including the history of their discovery, their definition, classification, biosynthesis, transport, release, inactivation, as well as their interaction with specific neuronal receptors. The differences with classical neurotransmitters are mentioned, as well as the different patterns of co-transmission. Finally, different mechanisms, both at the cellular and at the systemic level, are proposed that may explain the involvement of these molecules in various psychiatric diseases. Indeed, at the cellular level, a neuropeptide can be involved in a psychiatric disease, either because it is co-localized with a classical neurotransmitter involved in a disease, or because the neuropeptide-containing neuron projects on a target neuron involved in the disease. At the systemic level, a neuropeptide can play a direct role in the expression of a symptom of the disease. This is illustrated by different examples.

Keywords: Neuropeptide, psychiatric diseases, depression, anxiety.

INTRODUCTION

In the past decades, there has been increasing interest and, consequently, active and dynamic research on neuropeptides. This has been facilitated by the development of biochemical, immunohistochemical and *in situ* hybridization techniques. Neuropeptides regulate physiological processes throughout all phases of development. They act as neurohormones, neurotransmitters, and/or neuromodulators, maintain homeostasis and influence cognitive, emotional and behavioural functions, including hunger, thirst, sex drive, pleasure and pain. As they are synthesized and bind to specific targets within brain areas involved in psychiatric conditions and are sometimes colocalized in neurons synthesizing classical neurotransmitters that are related to some of these diseases, compounds interfering with neuropeptide functions have been developed as pharmacological tools intending to treat a variety of mental diseases, including schizophrenia, major depression, anorexia nervosa or addiction. This paper is divided into two parts: a first one describing some very general features of these molecules (history, definition, biosynthesis, transport, biodistribution, differences and interactions with classical neurotransmitters), and a second part, based on various examples, describing putative mechanisms of action of neuropeptides in particular psychiatric diseases such as anxiety disorders and depression. The first part is not aimed at providing novel data on neuropeptide biosynthesis or interactions with classical transmitters, but presents general features of these molecules, with the hope that they may help a non specialized reader within this field.

*Address correspondence to this author at the EA3248, Psychobiologie des émotions, UFR Sciences et techniques, Parc Grandmont, 37200 Tours, France; E-mail: catherine.belzung@univ-tours.fr

I- GENERAL FEATURES

History

The term neuropeptide was initially coined by de Wied (1925-2004) at the end of the sixties. He formulated the hypothesis that peptides localized within neurons may directly influence brain function and consequently human and animal behaviour [1,2]. In fact, de Wied began research in this field at the end of the fifties when he was studying the behavioural effects of pituitary hormones on rodents confronted with an avoidance learning paradigm in a shuttle box. At this time, he showed that stress hormones such as arginine vasopressin (AVP) and adrenocorticotrophic hormone (ACTH) could act directly on the brain, an effect that was independent of their endocrine function. This led to the "neuropeptide concept", a concept that encompasses the physiological functions of neuropeptide at the cellular level (its effects on neural communication) and at the integrative level (its effects on the modulation of brain activity, cognitive functions and behaviour).

Definition

According to Holmgren and Jensen [3], "A neuropeptide is a peptide that can be released from a neurone as a signalling molecule. This signalling molecule will have an effect as a transmitter or a modulator on other excitable cells (and sometimes on its own cell, actually)." It is important here to note that a neuropeptide is not only a peptide being present in neurons; it should also be involved in cellular communication, that is, elicits effects on another cell *via* high affinity binding to specific targets (receptors). These molecules are expressed in the central nervous system (CNS) as well as in the peripheral nerves (for example sensory and motor nerves); however, in this review of involvement of neuropeptides in psychiatric conditions, we will focus on neuropeptides expressed in the CNS. Indeed, when a given peptide is involved in a psychiatric disease, this generally

refers to its central action. This has for example been suggested for corticotropin releasing factor (CRF), a peptide present both in the endocrine and nervous systems, which is involved in the modulation of stress-related behaviours. In fact, using conditional knock-out mice in which CRF₁-receptors (CRF₁-Rs) are inactivated postnatally in limbic brain structures (hippocampus, amygdala, striatum) but not in the pituitary, Müller and coll. [4] showed that the anxiolytic-like action of the mutation may involve an extrahypothalamic CRF/CRF₁-R system that is independent from the hypothalamic/pituitary CRF/CRF₁-R system.

Classification

There are over 100 neuropeptides that have been identified and are currently being scrutinized. Many different classifications of the various neuropeptides have been proposed. For example, they can be classified into the six following categories: opioid neuropeptides (met-enkephalin, leu-enkephalin, α -endorphin, dynorphin), gut-brain peptides (substance P, cholecystokinin, galanin, bombesin, neurotensin, neuropeptide Y), hypothalamic releasing hormones (corticotropin-releasing factor, hypocretin, melanin-concentrating hormone (MCH)), pituitary hormones (AVP, ACTH), miscellaneous peptides (bradykinin). Other classifications exist, which are based for example upon the structure or the function of these various peptides (stress-related peptides, sleep-related peptides). However, as the same peptide is sometimes involved in different functions, we will use the first classification in the present paper.

Biosynthesis, Transport, Release, Inactivation

Biosynthesis of neuropeptides occurs according to the following steps, which are the classical steps described for protein synthesis:

- 1) The gene of a specific molecule (the pre-propeptide) within the neuron nucleus is first transcribed in the corresponding messenger RNA (mRNA); this mRNA leaves the nucleus, and is translated on the ribosomes of the rough endoplasmatic reticulum into the pre-propeptide that is a precursor of the active peptide. A prepropeptide consists of a signal peptide, one or several copies of a neuropeptide, and spacer parts. In the nucleus, alternative splicing can occur. Splicing occurs when immature mRNA is processed into mature mRNA: introns are cut out, and in some cases parts of exons. Therefore, the same gene can give rise to different neuropeptides. For example, opioid peptides derive from three different precursor proteins: proopiomelanocortin (POMC), proenkephalin and prodynorphin. POMC is the precursor of endorphins (β -endorphin, α -endorphin) and a family of non-opioid peptides, including melanocortins and ACTH. Proenkephalin is the source of both enkephalins and several longer opioid peptides, such as Met-enkephalin-Arg-Phe. Moreover, dynorphin A, dynorphin B, α - and β -neoendorphin and several larger molecules can be generated from prodynorphin [5-7]. Another interesting example can be seen with MCH as it illustrates the possible occurrence of alternative splicing, thus generating different kinds of molecules. MCH is derived from the precursor prepro-MCH,

which generates MCH and two other peptides: the 13 amino acids neuropeptide-Glu-Iso (NEI) and the 19 amino acids neuropeptide-Gly-Glu (NGE) [8]. This prepro-MCH may also potentially give rise to an alternative splice variant named MCH-gene-overprinted-polypeptide (MGOP, [9]) as well as encode a portion of the antisense-RNA-overlapping-MCH (AROM, [10]).

- 2) On the rough endoplasmatic reticulum, a part of the pre-propeptide is cleaved off by the activation of specific molecules termed as signal peptide, to form the propeptide.
- 3) A part of the propeptide is then split off by converting enzymes (specific endo- and exopeptidases) to form the active neuropeptide; this occurs in the Golgi apparatus.

After synthesis, neuropeptides are packed in large and dense vesicles budding off from the Golgi apparatus and shipped by axonal transport to the nerve terminal. No formation or packaging of neuropeptides can occur at the nerve terminal, because of the absence of mRNA and necessary cellular organelles in this part of the neuron. Therefore, new neuropeptides can be available only by synthesizing more propeptide in the soma and after axonal transport to the nerve terminals.

After storage in the large vesicles, neuropeptides can be released through exocytosis. This occurs when an action potential arrives down the axon in the nerve terminal, causing a calcium influx through calcium channels. The increased calcium concentration causes a cascade of molecular events leading to exocytosis. Once in the synaptic cleft, the neuropeptide is catabolized to metabolites by catabolic peptidases: the neuropeptides are broken down through hydrolyzation into inactive fragments. There are two broad categories of peptidases: exopeptidase and endopeptidase. The first acts by cleaving off one amino acid at the very end of the peptide, while the second acts by hydrolyzing peptide bonds within the interior of the molecule.

An important point to remember is that each cell of a given organism (endocrine cells, epithelial cells, neurons, etc.) is able to express all genes of a genome: therefore, every cell could potentially synthesize all neuropeptides. However, this is not the case because the gene regulating systems in this cell may decide which gene will be transcribed. This depends upon pre- as well as post-transcriptional regulation. For example, cholecystokinin (CCK) distribution differences are due to tissue-specific post-translational processing events of the same precursor. Indeed, the brain contains CCK4, CCK8 and several other CCK-desoctapeptides; whereas the gut contains intact CCK33, CCK39 and CCK58 as well as CCK-octapeptide and CCK-desoctapeptides [11].

Interactions with Receptors And Second Messenger Systems

Once released, neuropeptides bind to specific post-synaptic high-affinity binding sites. Even if some exceptions have been described (for example the Phe-Met-Arg-Phe-amide (FMRF amide), which is the most studied invertebrate

neuropeptide, directly activates a ionotropic receptor, the amiloride-sensitive sodium channel), most neuropeptide receptors belong to the G protein-coupled receptor superfamily, which possesses seven putative transmembrane domains (TM1 to TM7). This is for example the case for the three main opioid receptors that all belong to the family of the G protein-coupled receptors composed of a single polypeptide chain that forms seven hydrophobic transmembrane regions [12]. This association with G-coupled proteins is also found for oxytocin (OT) receptors, AVP V_{1a} and V_{1b} receptors, CCK_B receptors and galanin receptors.

G protein-coupled receptors correspond to a superfamily of proteins that can bind ligands and initiate signal transduction through the activation of large G proteins. Many neurotransmitter receptors (e.g., dopamine, serotonin or glutamate) or odorant receptors belong to this superfamily. When a neuropeptide binds to its receptor, it triggers off the activation or the inhibition of a variety of second messenger systems, resulting in cell firing, modulation of membrane threshold, phosphorylation and dephosphorylation events, and alteration of gene expression. They can thus activate various intracellular pathways using phospholipase C, adenylate cyclase *via* Gs or protein kinase A. For example, the two subtype CCK receptors are coupled with G-proteins, using phospholipase C as second messenger [11,13] while the two CRF receptors are G protein-coupled and trigger diverse intracellular signalling pathways essentially through an activation of adenylate cyclase *via* Gs and protein kinase A [14-16].

At least one receptor has been cloned for almost all of the neuropeptides discovered so far; however, in many cases, several receptors have been described for a given neuropeptide. For example, five different somatostatin receptors (sst1 to sst5) [17,18], five neuropeptide Y receptors (Y1, Y2, Y4, Y5 and Y6) [19], three AVP receptors (V_{1a} , V_{1b} and V_2) [20], three galanin receptors (GAL-R1, GAL-R2 and GAL-R3) [21] and three tachykinin receptors (NK_1 , NK_2 and NK_3) [22] have been discovered.

A typical characteristic of G protein-coupled receptors is that they are subject to fast desensitization (mainly through receptor phosphorylation) and to subsequent internalization (reduction in the number of receptors at the cell surface) upon prolonged exposure to agonists. This has for example been reported for opioid receptors.

Differences With Classical Neurotransmitters

Like the "classical" small molecule neurotransmitters, neuropeptides function as chemical mediators enabling neuronal communication. However, contrary to such classical neurotransmitters, neuropeptides often act as local neuronal modulators as well as endocrine hormones, thus mediating complex integrated behaviours. Further, a paracrine mechanism of action of some specific neuropeptides with particular physiological actions has been described. This is for example the case of neurotensin, gastrin, CCK and AVP [23].

Other differences include biosynthesis, storage, axonal transport, release and inactivation. These differences are summarized in Table 1. First, while classical neurotransmitters are synthesized from dietary precursors by

specific enzymes in nerve terminals and in some cases in perikarya, neuropeptides are synthesized in the cell body only from gene transcription and translation. Classical neurotransmitters are then stored in small vesicles, while neuropeptides are stored in large vesicles. The synthesizing enzymes as well as the storage vesicles of the classical neurotransmitters are then transported down the axon while, in the case of neuropeptides, only the storage vesicles are transported. The release dynamics also differ. For example, in neurons with slow firing, release is limited to small neurotransmitter vesicles. When rapid burst firing and prolonged depolarization occurs, the calcium concentration in the presynaptic terminal is elevated, leading to the release of neuropeptide vesicles in addition to neurotransmitter.

Interaction with Classical Neurotransmitters

It was once thought that each neuron synthesizes and releases only a single transmitter. This belief was often erroneously referred as the "Dale's principle", after Sir Henry Dale, a British physiologist. In fact, the famous Dale's principle was first proposed by Eccles in 1954, based on lectures given by Dale in the 1930s. However, neither Dale nor Eccles stated literally the one neuron-one transmitter concept. It is now commonly admitted that, in a given neuron, neuropeptides may coexist with another transmitter, this one being either a classical transmitter or another neuropeptide. This corresponds to a phenomenon termed as co-transmission. Notable examples are the co-transmission of dopamine and cholecystokinin in the mesolimbic and mesocortical pathways [24], of galanin and acetylcholine in the basal forebrain [25], of GABA and opioids in the striatum [26] or of hypocretin and dynorphin, galanin or glutamate in the lateral hypothalamus [27-29].

Different patterns of co-transmission involving neuropeptides have been described:

- a) one neuropeptide and one or several classical neurotransmitters.
- b) several neuropeptides, all derived from the same pre-peptide.
- c) several neuropeptides, derived from different pre-peptide.

For simplification purpose, we will only consider the case of co-transmission between two, and not more, different molecules. Different kinds of interactions have been described:

- a) Both molecules act on the same postsynaptic neuron. For example, if the presynaptic neuron contains a classical neurotransmitter and a neuropeptide, both being released at different firing patterns, the same postsynaptic neuron will exhibit different responses to different presynaptic activity. So, at a low frequency, only the classical neurotransmitter is released, causing a fast onset, short-duration postsynaptic response. If this neurotransmitter is an excitatory one, this will result in an excitatory postsynaptic potential (EPSP). If the firing of the presynaptic neuron is increased, it elicits co-release of the two transmitters and may modify the postsynaptic response. For example, if the neuropeptide is inhibitory, and if this inhibitory action dominates the excitatory action of

Table 1. Comparison of the Cellular Processes (Synthesis, Storage, Transport, Release and Inactivation) Between Classical Neurotransmitters and Neuropeptides

	Classical neurotransmitters	Neuropeptides
Type of molecule	Small water-soluble molecules containing amine (amino acid transmitters) and carboxyl groups	Large molecules
Biosynthesis	Synthesized from dietary precursors by specific enzymes, mainly in nerve terminals, in some cases in perikarya	Transcribed and translated from prepropeptide genes. Then, after activation by peptidases, transformed in propeptide and finally, after activation of converting enzymes, neuropeptide is obtained. This occurs in the cell body
Storage	In small vesicles	In large vesicles
Transport	Synthesizing enzymes as well as storage vesicles transported down the axon	Only storage vesicles transported down the axon
Release	Occurs through exocytosis at specialized regions (active zones) from the nerve terminal	Occurs through exocytosis, but no specific active zones
Possibility of paracrine action	No	Yes
Inactivation	Metabolism in synaptic cleft or reuptake by transporter and recycling	Catabolism by specific peptidase, no reuptake, no recycling

the classical transmitter, the observed result will be an inhibition of the postsynaptic neuron. So, in the postsynaptic cell, one may observe an initial fast EPSP, eliciting an action potential, followed by a hyperpolarization resulting from the inhibitory action of the co-released neuropeptide. Alternatively, when two neuropeptides are present at the nerve terminal, there might be a constant ratio of the neuropeptides released as a function of presynaptic firing rate [30].

- b) Both molecules may act on two different postsynaptic cells. This can for example occur in a situation wherein all presynaptic terminals release both co-transmitters, but different target neurons have receptors for only one of the co-transmitters. In this case, if only one of the co-transmitters requires high frequency stimulation for its release (the neuropeptide case), then at times the corresponding synapse might be functionally silent when the other synapse carries information. Another possibility occurs when each co-transmitter is specifically localized in different presynaptic branches of the same presynaptic neuron, thus liberating only a subset of its co-transmitters from each of its endings.
- c) One substance may influence the sensitivity of the target cell to the other substance.
- d) A neuropeptide can bind to presynaptic receptors, thus modifying release of a classical neurotransmitter. This is for example the case with 5-hydroxytryptamine-moduline (serotonine- or 5-HT-moduline), a tetrapeptide discovered by Fillion and Fillion in 1981 [31]. Indeed, this compound binds with a high affinity on 5-HT_{1B/1D} presynaptic receptors [32] on which it exerts an antagonistic action [33]. The 5-HT_{1B/1D} receptors are located presynaptically either on serotonergic neurons or on neurons releasing other neurotransmitters such as dopamine. When 5-HT-moduline is administered in the striatum, it increases dopamine release *via* blockade of 5-HT_{1B/1D} receptors located on

dopaminergic terminals [34]. Further, in acute stress conditions, 5-HT-moduline concentration increases in several brain areas, where it prevents serotonin binding to 5-HT_{1B/1D} receptors and causes their desensitization [35-37]. 5-HT-moduline may thus modulate anxiety-related behaviours. Indeed, blockade of 5-HT-moduline by icv administration of specific antibodies induces anxiolysis in mice in the elevated plus-maze and open-field tests [38]. On the opposite, HG1, a 5-HT-moduline antagonist, has been shown to produce anxiolytic-like effects in three mouse tests [39].

II. INVOLVEMENT OF NEUROPEPTIDES IN ANXIETY DISORDERS AND DEPRESSION

There is a vast body of evidence demonstrating that neuropeptides are involved in numerous mental diseases, such as anxiety disorders and depression.

Studies in animals:

- a) In rodents, administration of several neuropeptides within specific brain areas attenuates behaviours that have been suggested to relate to certain aspects of human psychiatric diseases. For example, intracerebroventricular galanin injection has anxiolytic-like effects in the Vogel punished drinking test [40], whereas intra-amygdala injection leads to anxiogenic-like effects in the Vogel conflict test, but not in the elevated plus-maze [41]. Thus, one can tentatively propose that this peptide may play a role in the modulation of emotional behaviors. In another example, Monzon and de Barioglio [42] showed that icv administration of MCH in rats induced anxiolytic-like effects in the elevated plus-maze [43]. These data are in line with the recent study of Kela and collaborators [44], reporting anxiolytic-like effects of MCH in the Vogel punished drinking test. Overall these findings suggest that the activation of several neuropeptide receptors may represent opportunities

for the pharmacological treatment of anxiety disorders.

- b) Administration of ligands for the neuropeptide receptor induces a modification in the behaviour that relates to symptoms of particular mental diseases. This has been observed in bio-assays for anxiety and depression. For example, recent reports demonstrate that infusion of an antagonist of κ -opioid receptors, the primary receptor for dynorphin, produces an antidepressant-like effect in the forced-swimming test [45] and the learned helplessness paradigm [46]. Further, anxiolytic- and antidepressant-like effects of the non-peptide V_{1b} antagonist SSR149415 have been observed in various animal models of affective disorders [47,48]. These effects were maintained after administration of this compound in the lateral septum, suggesting that an extrahypothalamic V_{1b} /AVP system contributes to the antidepressant-like action of SSR149415 [49]. Finally, Borowsky and coll. [50] showed that the selective high affinity MCH1-R receptor antagonist, SNAP-7941 produced a profile which is consistent with an antidepressant- and anxiolytic-like activity in rats, an effect which is in line with a previous study of Gonzalez and coll. [51] showing anxiogenic-like effects of MCH after an infusion in the preoptic area.
- c) Factors contributing to the etiology of psychiatric disorders may modify the concentration of the neuropeptides. This is for example the case with stress, a phenomenon involved in the etiology of depression. Shirayama and coll. [52] found that exposure to immobilization stress increases levels of dynorphin A and dynorphin B in the hippocampus and the nucleus accumbens. Further, cortical extracellular CCK concentrations are increased in anxiogenic situations [53].
- d) An alternative approach for the investigation of neuropeptides or their receptor function is the characterization of mice having a null mutation of the gene of interest or mutant mice overexpressing the gene of interest. In this context, abnormal behaviors reminiscent of symptoms of human psychiatric diseases have been observed in mice lacking genes encoding for neuropeptides or for their receptors. For example, autistic-like behaviours (absence of social attachment, decrease of social investigation, impairment of social memory and increased aggression) have been observed in mice lacking the OT gene [54,55]. Mice lacking the GAL-R1 gene display impairment of cued fear conditioning [56] and anxiety-like behaviour in the elevated plus-maze [57].
- e) Antisense oligonucleotides infusion is another strategy that allows the inhibition of the expression of targeted proteins specifically in sites where the antisense RNA is administered. Thus, studies which used antisense oligonucleotides against a neuropeptide or its receptor have also allowed investigating the contribution of neuropeptides in psychiatric diseases. For instance, CRF receptor antisense studies showed a differential role of CRF-R1 and CRF-R2 in anxiety and depression tests.

While CRF-R1 antisense oligonucleotide induced anxiolytic-effects and antidepressant-like effects in the forced-swimming test, CRF-R2 antisense oligonucleotide had no effect in anxiety tests, such as the defensive-withdrawal and elevated plus-maze models, but potentiated despair in the forced-swimming test [58,59].

Studies in humans:

- a) Injection of the neuropeptide elicits symptoms of a specific psychopathology or reduces the symptoms of the disease. For example, in healthy volunteers, CCK4 has been reported to elicit panic attacks [60], whereas OT infusion was found to reduce repetitive behaviours in adults with autistic and Asperger's disorders [61].
- b) Pharmacological treatments of mental diseases elicit modifications in the activity or concentration of neuropeptides. For example, the anxiolytic benzodiazepines have been found to reduce the activity of CCK in the brain [62]. Lithium, a treatment used in bipolar disorder, was shown to decrease plasma and cerebrospinal fluid (CSF) vasoactive intestinal peptide (VIP) levels, while increasing the affinity of the VIP lymphocyte receptor, an effect that may be relevant to the action of lithium in manic-depressive illness [63].
- c) In psychiatric patients, the concentration of neuropeptides or the number of neurons expressing the neuropeptide may be altered. For example, an increase of AVP-expressing neurons in the hypothalamus has been shown in patients with depression [64]. Other studies also showed that plasmatic VIP is decreased in depressed and anxious patients [65]. In some cases, these modifications are not specifically related to a particular disease. Indeed, alterations in CRF and OT have been described in several psychiatric conditions. For example, elevated CSF CRF levels are found in anorexia nervosa [66,67] and in post-traumatic stress disorders (PTSD) patients [68,69]. Similarly, the activity of the OT system has been shown to be altered in several psychiatric conditions, such as schizophrenia [70,71], depression [64] and obsessive-compulsive disorder (OCD) [72,73]. CSF OT has also been found to be altered during the starvation phase of anorexia nervosa [74].
- d) Physiological response to neuropeptide or neuropeptide ligands is altered in patients. For example, in OCD and panic disorder patients, a blunted ACTH response to intravenously administered CRF has been reported [75-79]. Further, in a dexamethasone (DEX)-CRF challenge test, Schreiber and coll. [80] showed a substantial disturbance of the hypothalamic-pituitary-adrenal axis (HPA) system regulation in panic disorder patients.
- e) Polymorphism of genes encoding for certain neuropeptide receptors are associated with several psychiatric disease. For example, occurrence of depression is increased in subjects carrying one haplotype of the V_{1b} gene [81].

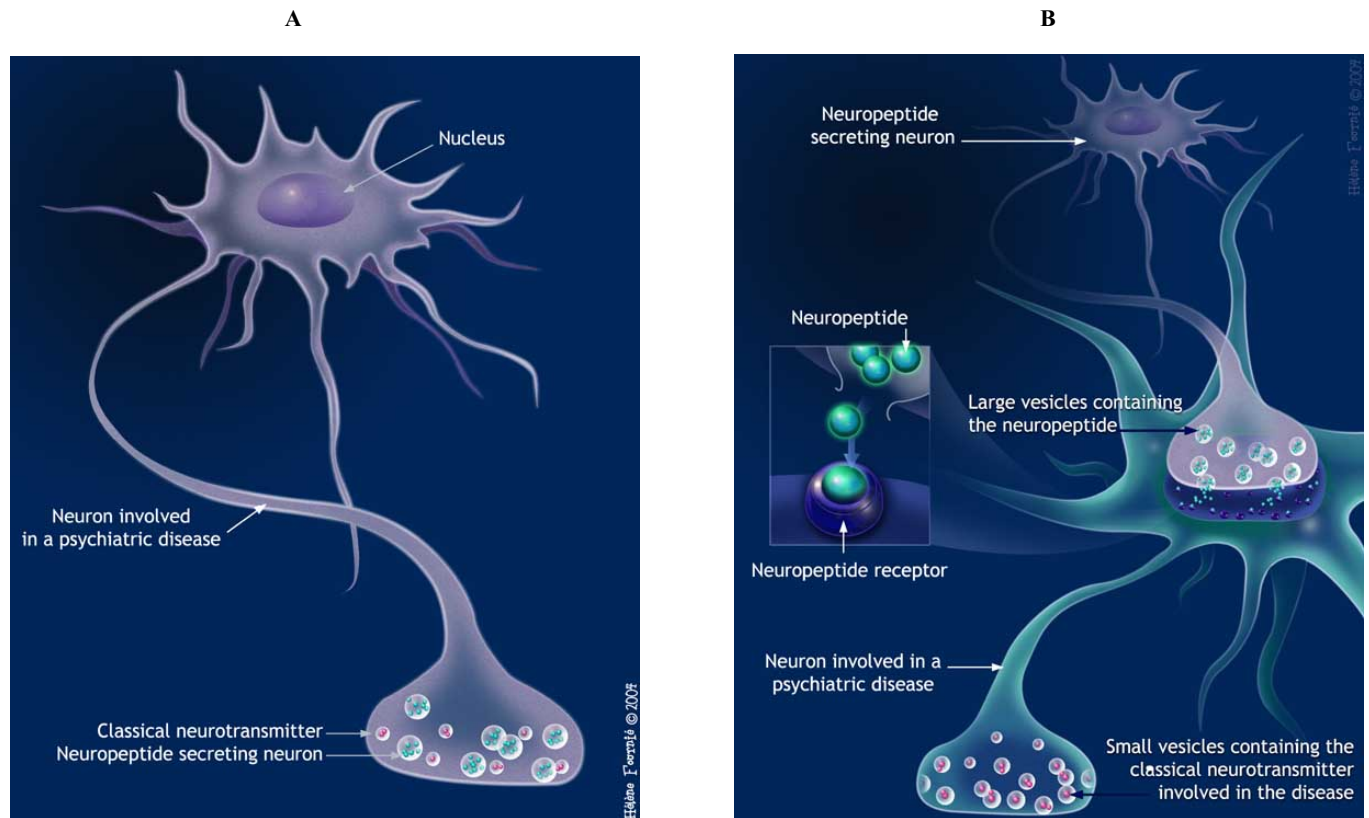


Fig. (1). Different cellular mechanisms tentatively explaining the involvement of neuropeptides in psychiatric diseases: (a) the neuropeptide can be co-localized within neurons with classical neurotransmitters involved in a particular disease; (b) the neuron releasing the neuropeptide controls, *via* specific neuropeptide receptors, a neuron involved in a psychiatric disease.

Mechanisms of Action

The involvement of neuropeptides in psychiatric diseases may result from different non exclusive mechanisms, occurring at both cellular and systemic levels.

Explanations related to the cellular level are illustrated on Fig. 1. Either the neuropeptide neuron is directly involved in the disease, or it is involved because of its interaction with neurons expressing a classical neurotransmitter known to be involved in the disease. In this latter case, explanations are based on the co-localization of the neuropeptide with a classical neurotransmitter (Fig. 1a), or on interactions between the neuron expressing the peptide and other targets (Fig. 1b).

In the case of co-localization, if a given disease results in an alteration in the number of neurons expressing a classical neurotransmitter or in a modification of their function, this may induce an alteration in the number or in the function of the neurons expressing the neuropeptide. The induced symptoms can thus either be related to the modification of the peptide or to the alteration of the neurotransmitter. Indeed, several neuropeptides are co-transmitters of classical neurotransmitters well known for their involvement in psychiatric illnesses. For example, co-localization of neurotensin and dopamine has been described in mesocorticolimbic dopaminergic neurons originating from the ventral tegmental area of the mesencephalon and projecting to the prefrontal cortex, the entorhinal cortex, the nucleus accumbens, the basolateral nucleus of the amygdala

and the lateral septum [82,83]. Interestingly, such a co-localization is not found for other dopaminergic fibres, such as the nigro-striatal pathway. The mesocorticolimbic dopaminergic neurons are well known for their involvement in schizophrenia.

In the case of a neuronal interaction between peptide- and neurotransmitter-containing neurons, the alteration of the function of a neurotransmitter-containing neuron can be due to a dysfunction of the peptidergic neuron. This is the case for substance P, a neuropeptide preferentially binding to NK1 receptors and localized on serotonergic neurons. Indeed, the firing rate of the rat dorsal raphé serotonergic neurons after a 2-day treatment with the NK1 antagonist CP-96,345 is increased by 50%, an effect accompanied by a desensitization of the 5-HT_{1A} autoreceptor [84]. This effect is further increased after a 14-day treatment, in this case the firing rate of 5-HT neurons is increased by 90% and the degree of desensitization of the 5-HT_{1A} autoreceptor is greater than in the 2-day treated group. It has been suggested that this probably occurs *via* the NK1 receptors present on GABAergic neurons that tonically inhibit dorsal raphé serotonergic neurons. The involvement of serotonergic transmission in major depression is well documented and NK1 receptor antagonists have been studied successfully for their efficacy in this disease, although more recent clinical studies failed to replicate the initial findings [85-87]. Finally, Weiss and coll. [88] proposed that galanin released during periods of elevated activity in locus coeruleus neurons projecting to the ventral tegmental area (VTA) may lead to

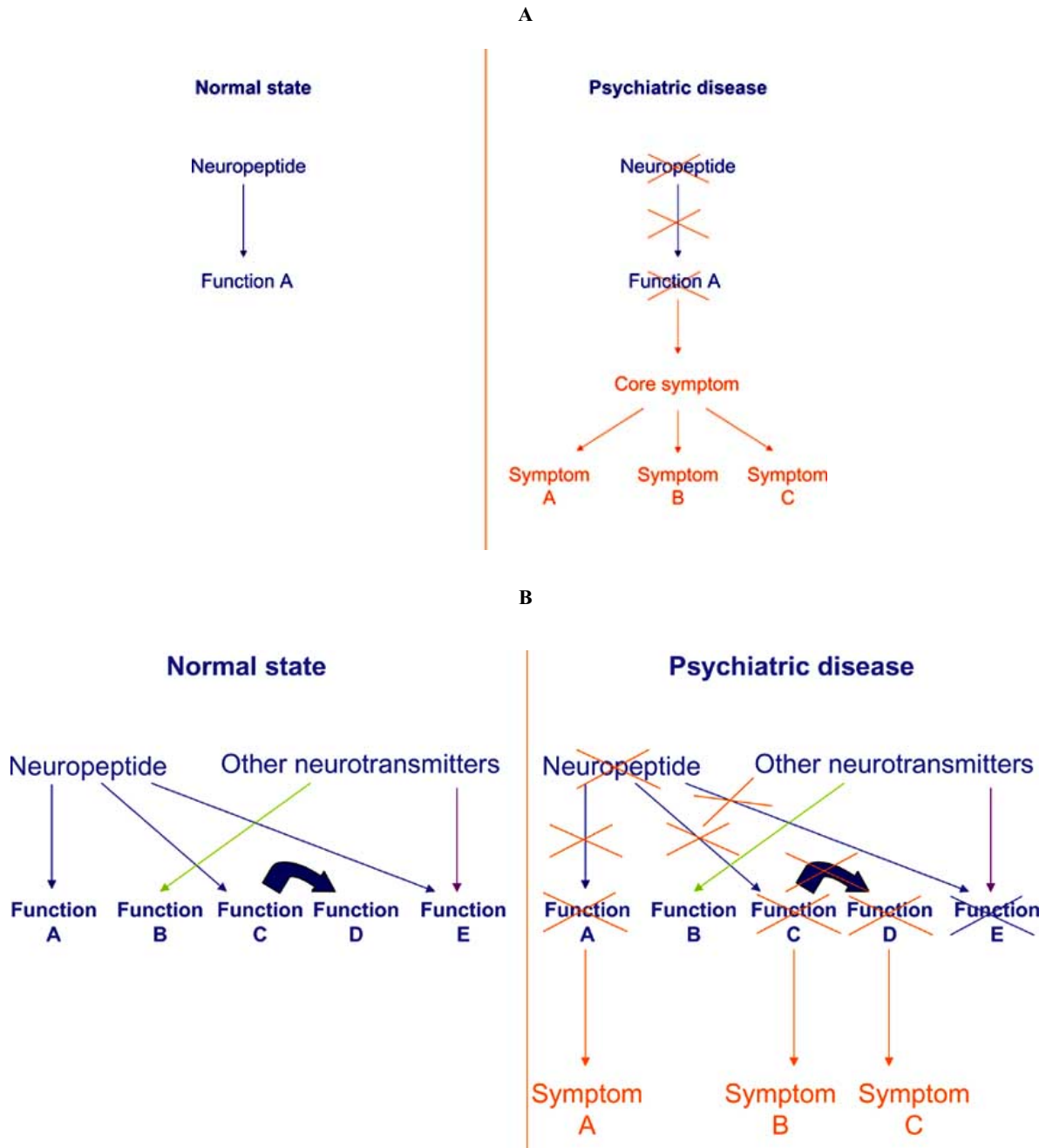


Fig. (2). Systemic mechanisms explaining the involvement of neuropeptides in psychiatric disease. The orange colour indicates processes induced by the dysfunction of the neuropeptide. (a) Dysfunctions of the neuropeptide induce a core symptom leading to several abnormalities. (b) Dysfunctions of the neuropeptide can act on some but not all of the symptoms of the disease. The symptom is either directly (symptom A and B) or indirectly (symptom C) the consequence of the neuropeptide dysfunction. In some cases, no symptom appears as a consequence of a compensatory mechanism (see for example function E).

the occurrence of a depressive-like state through inhibition of dopaminergic VTA cells. This idea fits well with the observation of increased depressive-like symptoms in the forced-swim test after galanin injections in the VTA, but not in the hypothalamus or the midbrain reticular formation.

Explanations at the systemic level are detailed below and are illustrated on Fig. 2.

- a) The neuropeptide is directly responsible for the disease, either because it participates in its etiology,

or because its dysregulation induces a core symptom of the psychiatric disease. Abnormalities in the neuropeptide may produce a core symptom, leading to a given psychiatric illness including several symptoms (Fig. 2a). For example, abnormalities in feeding behaviour are a core symptom of pathologies such as bulimia or anorexia nervosa. Leptin or neuropeptide Y are abundant in the hypothalamus, a brain area involved in the control of feeding behaviour. Abnormal levels of these molecules have

been reported in eating disorders such as anorexia nervosa or bulimia nervosa [89]. Wakefulness dysfunction is a core symptom of narcolepsy. Dense projections from hypocretin (hcrt) neurons to monoaminergic nuclei including tuberomammillary nucleus, locus coeruleus (LC), dorsal raphe, VTA and pedunculopontine tegmental nucleus provide anatomical evidence for the role of hypocretins in sleep/wakefulness regulation [90,91]. Injection of hcrt in the rat brain increases wakefulness and decreases REM sleep as well as the number and duration of slow-wave sleep episodes [92,93]. This arousal effect of hcrt is related to the activation of the tuberomammillary histaminergic system [94,95], the activation of adrenergic cells from LC and the dopaminergic cells from the VTA [96,97]. On the basis of these findings, hcrt neurons have been suggested to be involved in narcolepsy. Interestingly, in a canine model displaying mutations of the hcrt2-R gene [98] and in mice deficient in prepro-hypocretin, a phenotype very similar to human narcolepsy has been described [99]. In addition, narcoleptic patients exhibit a 80 to 100 % reduction in the number of hcrt-containing neurons and a low or undetectable concentration of this neuropeptide in the cerebrospinal fluid [100-102], suggesting an important role of this peptide in the regulation of arousal and vigilance state.

In many instances, a neuropeptide can be involved in the regulation of factors contributing to the etiology of the disorder. This has been shown for example with CRF and stress, this latter being a key factor underlying major depression and several anxiety disorders. Numerous acute and chronic stress paradigms have been shown to increase CRF concentration and expression in the hypothalamus, but also in various extrahypothalamic areas [103-107]. It is well established that CRF influences numerous behaviors and functions linked to central and endocrine responses to stress: anxiety, emotional and cognitive processes, regulation of autonomic nervous system, behavioural arousal, motor activity, feeding behaviour, regulation of homeostasis, energy resources, thermogenesis, GI functions and immune system [108-110]. It is therefore not surprising that when the CRF system is inhibited by a CRF antibody or an anti-sense RNA, the stress effects are blocked and sometimes an anxiolytic-like profile is even observed [111-113]. Further, CRF₁-R deficient mice display a blunted HPA axis response to stress and decreased anxiety-like behaviours [114,115]. Moreover, recent non-peptide CRF₁-R antagonists have been shown to exhibit anxiolytic-like effects and reduce CRF- or stress-induced overactivity of the HPA axis without major disruption of the basal functioning of this axis [116-121]. In humans, the most abundant literature on the role of CRF in psychiatric diseases concerns major depression (for review see [122,123]). Indeed, a major HPA system dysregulation occurs in this disorder which is characterized by an hypercortisolemia [124], an elevated CSF CRF level [125], an increase of CRF

neurons into limbic structures [126], a blunted pituitary response to CRF challenge and a disruption of the negative feedback on the HPA axis highlighted by the dexamethasone suppression test (DST) [123,127-128]. Furthermore, it has been shown that the clinical remission requires a normalisation of the HPA axis functioning [129]. Recently, the antidepressant potential of CRF₁-R antagonists was established in human by encouraging preliminary clinical trials [130,131].

- b) Neuropeptides may not only modulate the core symptom per se or the etiology of the disease but some specific related symptoms, such as sleep, motivation or pain (Fig. 2b). For example, orexin is involved in sleep and may therefore be involved in depression, a disease characterized by sleep abnormalities.

It is noteworthy that in some cases, a given function may also be controlled by other neurotransmitters, so that the neuropeptide dysfunction can lead to no apparent symptoms because of compensatory mechanisms related to the action of the classical neurotransmitters. In other cases, a given symptom can give rise to other symptoms in a non specific way. For example, absence of sleep induces irritability; therefore, if a given neuropeptide is involved in the control of sleep, its dysfunction can mechanically induce irritability, even if it is not directly involved in aggressive behaviour.

CONCLUSION

This brief overview on the involvement of neuropeptides in stress-related disorders underlines the importance of these molecules in psychiatric diseases. As a consequence, drug discovery is more and more focusing on the discovery of small and brain-penetrant compounds that target several of these neuropeptides in order to provide innovative avenues for the treatment of these disorders.

ABBREVIATIONS

5-HT	= 5-Hydroxytryptamine (serotonin)
ACTH	= Adrenocorticotrophic hormone
AROM	= Antisense-RNA-overlapping-MCH
AVP	= Arginine Vasopressin
CCK	= Cholecystokinin
CSF	= Cerebrospinal fluid
CNS	= Central nervous system
CRF	= Corticotropin releasing factor
DEX	= Dexamethasone
DST	= Dexamethasone suppression test
FMRF amide	= Phe-Met-Arg-Phe-amide
GABA	= Gamma-amino-butyric acid
GAL	= Galanin
hcrt	= Hypocretin
GI	= Gastro-intestinal
HPA	= Hypothalamic-pituitary-adrenal axis

LC	=	Locus coeruleus
MCH	=	Melanin-concentrating hormone
MGOP	=	Mch-gene-overprinted-polypeptide
NEI	=	Neuropeptide-Glu-Iso
NGE	=	Neuropeptide-Gly-Glu
NK	=	Neurokinin
OCD	=	Obsessive-compulsive disorder
OT	=	Oxytocin
POMC	=	Proopiomelanocortin
PTSD	=	Post-traumatic stress disorders
VIP	=	Vasoactive intestinal peptide
VTA	=	Ventral tegmental area

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