Published in final edited form as:

Curr Pharm Des. 2011 ; 17(25): 2626–2631.

Novel Neuropeptides as Ligands of Orphan G Protein-Coupled Receptors

Yan Zhang, Zhiwei Wang, Gregory Scott Parks, and Olivier Civelli*

Department of Pharmacology, University of California Irvine, Irvine, CA 92697, United States

Abstract

Neuropeptides control a wide spectrum of physiological functions. They are central to our understanding of brain functions. They exert their actions by interacting with specific G protein-coupled receptors. We however have not found all the neuropeptides that exist in organisms. The search for the novel neuropeptides is thus of great interest as it will lead to a better understanding of brain function and disorders. In this review, we will discuss the historical as well as the current approaches to neuropeptide discovery, with a particular emphasis on the orphan GPCR-based strategies. We will also discuss two novel peptides, neuropeptide S and neuromedin S, as examples of the impact of neuropeptide discovery on our understanding of brain functions. Finally, the challenges facing neuropeptide discovery will be discussed.

Keywords

Neuropeptide; Orphan GPCR; Neuropeptide S; Neuromedin S

1. INTRODUCTION

Neuropeptides are short-chain amino acid neuromodulators. They often localize in discrete brain regions and influence a wide variety of physiological functions, such as analgesia [1, 2], reward [3, 4], food intake [5], and learning and memory [6, 7]. Similar to classical neurotransmitters (acetylcholine, biogenic amines and amino acids), neuropeptides are produced and released by neurons to regulate intercellular communication. However, neuropeptides are much larger, about 3–100 amino-acid residues long, and are more diverse than classical neurotransmitters since more than 50 neuropeptides are known to exist in humans. Additionally, their *in vivo* concentrations are two to three orders of magnitude lower than most classical transmitters and their affinities at activating receptors *in vivo* are up to 1000 times higher. Several other fundamental differences between neuropeptides and other neurotransmitters exist with respect to their synthesis, storage and inactivation, which will not be discussed in detail within this review.

Neuropeptides initiate their biological effects by interacting with G-protein coupled receptors (GPCRs). GPCRs are membrane-bound receptors that transduce signals by

* Address correspondence to this author at the Department of Pharmacology, School of Medicine UC Irvine 369 Med Surge II Irvine, CA 92697-4625; Tel: (949)824-2522; Fax: (949) 824-4855; ocivelli@uci.edu.

coupling to several proteins, most notably, G proteins which in turn activate effector molecules. GPCRs are the most numerous modulators of intercellular interactions. Of these, 367 are viewed as “transmitter” receptors because they are expected to be activated by opsins, biogenic amines, neuropeptides, glycoprotein hormones, purine ligands, lipid mediators and a few other small molecules such as amino acids [8]. These transmitter GPCRs were the first to be considered potential drug targets. Some 210 of these GPCRs have been matched to known transmitters. This leaves about 150 GPCRs whose natural ligands have not yet been identified -the so-called “orphan GPCRs” [9]. It is estimated that neuropeptides will account for 50% of the orphan GPCR ligands [10].

In this review we will present neuropeptide discovery strategies with an emphasis on the orphan GPCR-based approaches. We will then discuss two novel peptides as examples of the impact that the search for neuropeptides has on our understanding of brain functions. We will finish by discussing the difficulties and the potentials of the current neuropeptide discovery.

2. THE DISCOVERY OF NOVEL NEUROPEPTIDES

Originally, novel neuropeptides were discovered as the components of tissue extracts found active in tissue assays. The first neuropeptide, substance P was discovered in 1931 by Von Euler and Gaddum in extracts of equine brain and intestine through its ability to induce intestinal contraction *in vitro* [11]. However, the chemical composition of substance P was not described until 1971, when Susan Leeman and her collaborators showed that substance P is a C-terminal amidated, 11 amino-acid residue peptide [12]. After substance P was identified, it was recognized that some neuropeptides contain a C-terminal amidation and this distinct chemical feature was used to identify several more neuropeptides in the following decade [13]. In the 1950s, arginine vasopressin (AVP) [14–16] and the related hormone oxytocin (OXT) [17] were characterized when found active in altering blood pressure, and were synthesized shortly thereafter. In 1955, du Vigneaud won the Nobel Prize in chemistry due, in part, to his early descriptions and syntheses of AVP and OXT. This work was followed by the sequencing of the hypothalamic releasing factors, such as thyrotropin-releasing factor (TRF) [18–20], somatostatin (SS) [21] and gonadotropin releasing hormone (GnRH) [22–24] in the 60's by Roger Guillemin and Andrew Schally, who were awarded the Nobel Prize for medicine in 1977 for their discoveries concerning “the peptide hormone production of the brain.”

The adoption of molecular cloning techniques dramatically enhanced neuropeptide discovery. Prominent examples are the discovery of γ -melanocyte-stimulating hormones in the sequence of the proopiomelanocortin precursor [25], calcitonin-gene-related peptide (CGRP) which was found in the calcitonin gene [26] and the discoveries of cortistatin [27] and the hypocretins using mRNA subtraction techniques [28].

The advent of the genomic era and the emergence of bioinformatics have offered new approaches to neuropeptides discovery. The peptide QRFP/P52 was found *in silico* by searching for a C-terminal motif in a virtual protein transcript database [29, 30].

Technological advancements in mass spectrometry and the use of genome sequences have made it possible to study the peptidome. This approach has led to the description of a number of previously uncharacterized peptides [31, 32] including five novel peptides with the same precursor proSAAS [33] and katalcalcin, a novel peptide derived from the calcitonin precursor [34].

2.1. THE DISCOVERY OF NEUROPEPTIDES AS LIGANDS OF ORPHAN GPCRS

The fact that nearly all neuropeptides activate G protein coupled receptors led to the development of a different approach to searching for novel neuropeptides in the mid 90's, orphan receptor strategy. This strategy relies on using of orphan GPCRs as targets to identify novel neuropeptides from tissue extracts. Orphan GPCRs which are identified on the basis of their sequences as putative neuropeptide binding GPCRs are expressed into suitable cells systems which are exposed to tissue extracts. The presence of the natural ligand is recognized by monitoring the cellular changes accompanied by the signal transduction, which in most cases is the changes of second messenger levels, cAMP and Ca^{2+} [35], but sometimes is the release of arachidonic acid [36] or the extracellular acidification rate, a rarely used method that was successfully used in the case of discovery of apelin [37]. Nociceptin/orphanin FQ (N/OFQ) was the first novel peptide discovered using this strategy [38, 39]. Since then, 9 novel mammalian neuropeptides have been identified: orexins/hypocretins [40, 41], PrRP [36], apelin [37], ghrelin [42], kisspeptin [43], neuropeptide B (NPB) and W (NPW) [44–46], neuropeptide S (NPS) [47, 48] and neuromedin S (NMS) [49]. In addition, five peptides which although previously known, have been paired to specific GPCRs [melanin concentrating hormone (MCH) [50–53], urotensin II (UII) [54], neuromedin U (NMU) [55–58], relaxin 3 [59, 60], and prokineticin 1 and 2 [61]. Detailed information on these bioactive peptides is listed in Table 1. This strategy is expected to lead to most future neuropeptide discoveries. In this review, we will discuss two novel neuropeptides discovered using the orphan receptor strategy, the NPS and NMS systems, in order to illustrate the success of this strategy and to exemplify the recent relevant findings on these neuropeptide systems.

3. TWO EXAMPLES OF GPCR DEORPHANIZATIONS

3.1. THE NPS SYSTEM

NPS was isolated from the rat whole brain extracts and identified as the natural ligand of the orphan G protein-coupled receptor GPR154 [62]. The name of NPS comes from its conserved N-terminal serine residue and accordingly its receptor is now referred to as the NPS receptor (NPSR). Some recent comprehensive reviews on NPS system have been published [63–65].

NPS is a 20 amino acids long peptide that is encoded by a relatively short precursor protein. The NPS precursor shares the typical structural characteristics of other neuropeptides: a hydrophobic signal peptide immediately follows the initiation of translation, and the immature peptide is preceded by a pair of basic amino acids (Lys-Arg) that are presumed processing sites for proteolytic cleavage. The amino acid sequences for NPS and its precursor protein are shown in Fig. (1A, C).

The anatomical distribution of NPS and NPSR has been studied in detail by *in situ* hybridization [48, 66]. In the rat brain, expression of NPS precursor is restricted to three brainstem structures: the locus coeruleus area, the principle 5 sensory nucleus and the lateral parabrachial nucleus of the brainstem. In contrast to the restricted distribution pattern of the NPS precursor, NPSR mRNA is widely expressed throughout the nervous system with the highest expression levels found in the cortex, thalamus, hypothalamus, amygdala, parahippocampal regions including the subiculum, while only low levels are detectable in the brainstem. Using double *in situ* hybridization NPS was found to co-localize with excitatory neurotransmitters, such as glutamate, acetylcholine, or corticotropin-releasing hormone, while never co-localizing with markers for GABAergic, noradrenergic, or dopaminergic neurons. The pattern of NPS and NPSR distribution in the CNS suggests the system may be involved in emotional and sensory processing, arousal, stress, energy homeostasis, endocrine regulation, learning and memory. The mRNAs of both the NPS precursor and NPSR have also been found in peripheral tissues, including thyroid, salivary, mammary glands [48, 66, 67] and it has recently been shown that the NPS and NPSR transcripts are expressed in muscle tissue, skin, and respiratory tract in pigs [67].

Pharmacological studies have shown that nanomolar levels of NPS can activate NPSR and induce mobilization of intracellular Ca^{2+} and stimulate cAMP synthesis while a radiolabeled analog of NPS shows high affinity saturable and displaceable binding ($K_d=0.3$ nM) [48]. Multiple single nucleotide polymorphisms (SNPs) and several splice variants have been identified in human NPSR. Genetic linkage studies have suggested that some SNPs may be the risk factors for the development of asthma or the allergic diseases [68], although this has been questioned in another analysis [69]. Pharmacological analysis has shown that the Asn¹⁰⁷Ile polymorphism in human NPSR results in a gain-of-function by increasing agonist potency up to ten fold. Furthermore, this variant associates with panic disorder in male patient [70], and circadian phenotypes, such as “average bedtime”[71]. The pharmacological and biochemical consequences of mutations near position 107 have recently been investigated and suggest that N-linked glycosylation is not important for NPSR biogenesis or function, but residue D105 may be critical for ligand binding because removing the charged side chain of D105 may either inhibit ligand binding and/or signal transduction [72].

Early studies on the NPS system provided evidence that NPS promotes arousal and wakefulness by suppressing all stages of sleep [48]. Central administration of NPS has been reported to have anxiolytic-like effects and to acutely reduce fear responses and modulate long-term aspects of fear memory [48, 66, 73, 74]. Additionally, recent studies have demonstrated that NPS modulates energy and endocrine homeostasis [75–77] and that NPS and NPSR transcripts are altered with caffeine or nicotine treatment, suggesting complex interactions with adenosine and cholinergic system [78, 79].

In summary, a substantial body of work has established that NPS is a potent modulator of arousal, sleep and wakefulness, anxiety behaviors and food intake, but owing to its novelty, more studies are still needed to characterize the NPS system and understand its physiological functions. In this respect, the availability of mice devoid of NPS or its receptor [64] and of a chemical antagonist [80] raises hope for our understanding of the NPS system.

3.2. THE NMS SYSTEM

NMS is the most recently discovered peptide found using the orphan receptor strategy. It was discovered to be a natural ligand for FM-3/GPR66/NMU1R and FM-4/TGR-1/NMU2R receptors in 2005 from rat brain extracts [49].

Rat NMS is a C-terminal amidated neuropeptide of 36 amino acid residues. NMS is structurally related to neuromedin U (NMU) [Fig. (1C)] [49]. The seven-residue C-terminal amidated sequence of NMS is identical to that of NMU; this structure is essential for NMU receptor binding. Both NMS and NMU act on the same receptors, NMU receptors type-1 (NMU1R) and type-2 (NMU2R) [49]. The N-terminal portion of NMS, however, has no sequence homology to any known peptide or proteins. The NMS pre-proprotein (Fig. 1B) contains four potential processing sites for cleavage by convertases [81]. NMS is produced from precursor proteins by proteolytic processing at the third and fourth of these sites. The fourth processing site contains Gly 145, which presumably serves as an amide donor for C-terminal amidation [81].

NMS mRNA is primarily expressed in the CNS but is also present in the spleen and testis [49] and has recently been identified in the human heart [82]. In the CNS, NMS mRNA is predominantly found in the suprachiasmatic nucleus (SCN) of the hypothalamus and is minimally expressed in other areas of the brain [49].

The NMS system has been pharmacologically characterized using recombinant receptors heterologously expressed in CHO cells [49]. NMS and NMU show similar potency for inducing the intra-cellular release of calcium in cells expressing NMU1R or NMU2R. Competitive radioligand binding analysis has demonstrated that NMS binds with high-affinity to NMU receptors and that NMS and NMU display similar binding affinity for NMU1R, while NMS has a higher binding affinity for NMU2R than NMU.

Functionally, intracerebroventricular injection (ICV) of NMS decreases food intake and body weight in a dose-dependent manner [83], which suggests that NMS may be involved in feeding regulation. Highlighting the functional importance of the NMS system, both NMS and NMU have been found to act centrally on the NMU2R to exert anorectic and weight-reducing effects [84]. In rats, ICV injection of NMS induces a rapid release of vas AVP, followed by a decrease of nocturnal urinary output, which suggests that NMS may exert a physiological antidiuretic action by altering AVP release [85]. NMS is important in oxytocin release response to the suckling stimulus in rats [86]. NMS also has roles in circadian rhythms [49], the HPA axis [87] and LH secretion [88].

Due to its recent discovery, characterization of the NMS system and its physiological functions are still in its infancy, though NMU which was discovered 20 years earlier, is somewhat better characterized [89, 90]. Neuroanatomical and ICV injection studies indicate that the NMS system may modulate several important physiological processes, particularly the interplay between circadian rhythms and energy balance homeostasis [91]. Because NMS and NMU work on the same receptor, genetically modified mouse models and the development of specific pharmacological probes will be critical to understanding the functions of the NMS system.

4. CONCLUSIONS AND PERSPECTIVES

Orphan GPCR research has had a broad impact on our understanding of neuropeptide mediated responses and has thus far led to the discovery of 10 novel neuropeptides. The search for new neuropeptides is an ongoing effort. The rate at which neuropeptide GPCRs are being deorphanized has slowed considerably in recent years. 15 neuropeptide GPCRs were deorphanized, between 1998 and 2005, while none have been in the last 5 years. There are several issues that account for this.

One issue is the recent discovery that many GPCRs exist not as monomers, as was originally believed, but as dimers or higher order oligomers [92]. It is now accepted that many GPCRs interact not only with themselves but also with other GPCRs to activate their signaling cascade and thus have a different pharmacological profile than monomers. It is possible that some of the remaining orphan GPCRs do not act alone but can only be activated in conjunction with other GPCRs and would require this second GPCR to induce a second messenger pathway response. Indeed such a possibility has been described in the case of orphan GPR50 which has been shown to heterodimerize with MT1 and MT2 melatonin receptors and antagonize the function of MT1 receptor [93]. If orphan GPCRs heterodimerize with other GPCRs, determining their natural ligand becomes exponentially more difficult as they could associate with any of the 210 deorphanized GPCRs. Related to this issue is also the possibility that some orphan GPCRs may require the expression of accessory proteins for their activity. This has been shown in the case of the CGRP receptor which can function as either a CGRP receptor or an adrenomedullin receptor depending on which receptor-activity-modifying proteins (RAMPs) are expressed [94].

Another issue is the fact that current reverse pharmacological techniques rely on monitoring changes in second messenger level. While we would have expected that GPCRs induce second messenger responses via G proteins, some may instead couple to alternate signal transduction pathways, some of which may be undiscovered [95]. If this is the case, deorphanization of the remaining GPCRs that utilize these alternate signaling mechanisms will not be possible until these pathways have been defined and a method exists to monitor activation of these pathways.

Another persistent hurdle facing continued receptor deorphanization is the potentially low concentration of the undiscovered neuropeptides in their natural environment. It is possible that some transmitters are only expressed at particular times in the life of the organism or under specific conditions. In these cases, it is likely that some knowledge of biology of these transmitters will be required before matching them to their orphan GPCRs, or a theoretical approach, such as genomics based approach, for identifying potential ligands may need to develop.

In spite of these obstacles facing deorphanization research, the search for ligands of orphan GPCRs will continue to serve as an important approach in discovering neuropeptides because successfully deorphanizing any one of neuropeptide receptors opens entirely new avenues of research which can profoundly improve understanding of physiological responses of the system and may result in uncharted therapeutic developments.

Acknowledgments

This work was supported by National Institute of Health Grants MH60231, DA024746 and an Established Investigator Award from the National Alliance for Research on Schizophrenia and Depression (NARSAD) to Civelli Olivier.

ABBREVIATIONS

GPCR	G protein-coupled receptor
AVP	Arginine vasopressin
OXT	Oxytocin
SS	Somatostatin
GnRH	Gonadotropin releasing hormone
N/OFQ	Nociceptin/orphanin FQ
MCH	Melanin concentrating hormone
Ull	Urotensin II
CGRP	Calcitonin-gene-related peptide
NMU	Neuromedin U
PrRP	Prolactin-releasing peptide
NPB	Neuropeptide B
NPW	Neuropeptide W
NPS	Neuropeptide S
NMS	Neuromedin S
NPSR	NPS receptor
NMU1R	NMU receptors type-1
NMU2R	NMU receptors type-2
SNP	Single nucleotide polymorphism
RAMPs	Receptor-activity-modifying proteins

References

1. Cheng HY, Penninger JM. Transcriptional mechanisms underlying neuropathic pain: DREAM, transcription factors and future pain management? *Expert Rev Neurother.* 2002; 2:677–89. [PubMed: 19810984]
2. Roques BP, Noble F. Dual inhibitors of enkephalin-degrading enzymes (neutral endopeptidase 24.11 and aminopeptidase N) as potential new medications in the management of pain and opioid addiction. *NIDA Res Monogr.* 1995; 147:104–45. [PubMed: 8742783]

3. Van Ree JM, Niesink RJM, Van Wolfswinkel L, et al. Endogenous opioids and reward. *Eur J Pharmacol.* 2000; 405:89–101. [PubMed: 11033317]
4. Kreek MJ, LaForge KS, Butelman E. Pharmacotherapy of addictions. *Nat Rev Drug Discov.* 2002; 1:710–26. [PubMed: 12209151]
5. Hosoda H, Kojima M, Kangawa K. Ghrelin and the regulation of food intake and energy balance. *Mol Interv.* 2002; 2:494–503. [PubMed: 14993401]
6. Mamiya T, Noda Y, Nishi M, Takeshima H, Nabeshima T. Nociceptin system plays a role in the memory retention: involvement of naloxone benzoylhydrazone binding sites. *Neuroreport.* 1999; 10:1171–5. [PubMed: 10363919]
7. Manabe T, Noda Y, Mamiya T, et al. Facilitation of long-term potentiation and memory in mice lacking nociception receptors. *Nature.* 1998; 394:577–81. [PubMed: 9707118]
8. Civelli O. GPCR deorphanizations: the novel, the known and the unexpected transmitters. *Trends Pharmacol Sci.* 2005; 26:15–9. [PubMed: 15629200]
9. Wise A, Jupe SC, Rees S. The identification of ligands at orphan G-protein coupled receptors. *Annu Rev Pharmacol Toxicol.* 2004; 44:43–66. [PubMed: 14744238]
10. Ozawa ALI, Roth B, Kroeze WK. Deorphanization of Novel Peptides and Their Receptors. *AAPS J.* 2010:378–84. [PubMed: 20446073]
11. von Euler US, Gaddum JH. An unidentified depressor substance in certain tissue extracts. *Journal of Physiology-London.* 1931; 72:74–87.
12. Chang MM, Leeman SE, Niall HD. AMINO-ACID SEQUENCE OF SUBSTANCE P. *Nature-New Biology.* 1971; 232:86–7. [PubMed: 5285346]
13. Tatemoto K, Mutt V. Isolation of 2 novel candidate hormones using a chemical method for finding naturally-occurring polypeptides. *Nature.* 1980; 285:417–8. [PubMed: 6892950]
14. Turner RA, Pierce JG, du VV. The purification and the amino acid content of vasopressin preparations. *J Biol Chem.* 1951; 191:21–8. [PubMed: 14850440]
15. Acher R, Chauvet J. La structure dela vasopressine de boeuf. *Biochim Biophys Acta.* 1953; 12:487–8. [PubMed: 13115463]
16. Duvinéaud V, Lawler HC, Popenoe EA. Enzymatic cleavage of glycinamide from vasopressin and a proposed structure for this pressor-antidiuretic hormone of the posterior pituitary. *J Am Chem Soc.* 1953; 75:4880–1.
17. Tuppy H. The amino-acid sequence in oxytocin. *Biochim Biophys Acta.* 1953; 11:449–50. [PubMed: 13093758]
18. Nair RM, Barrett JF, Bowers CY, Schally AV. Structure of porcine thyrotropin releasing hormone. *Biochemistry (Mosc).* 1970; 9:1103–6.
19. Schally AV, Redding TW, Bowers CY, Barrett JF. Isolation and properties of porcine thyrotropin-releasing hormone. *J Biol Chem.* 1969; 244:4077–88. [PubMed: 4979236]
20. Burgus R, Dunn JF, Desiderio D, Guillemin R. Derives polypeptidiques de synthèses doues d'activite hypophysiotropic TRF. *Acad Sci (Paris).* 1969; 269:1870–77.
21. Burgus R, Ling N, Butcher M, Guillemin R. Primary structure of somatostatin, a hypothalamic peptide that inhibits the secretion of pituitary growth hormone. *Proc Natl Acad Sci USA.* 1973; 70:684–8. [PubMed: 4514982]
22. Baba Y, Matsuo H, Schally AV. Structure of porcine lh-releasing and fsh-releasing hormone. 2. Confirmation of proposed structure by conventional sequential analyses. *Biochem Biophys Res Commun.* 1971; 44:459–63. [PubMed: 4946067]
23. Matsuo H, Baba Y, Nair RMG, Arimura A, Schally AV. Structure of porcine LH- and FSH-releasing hormone. 1. Proposed amino acid sequence. *Biochem Biophys Res Commun.* 1971; 43:1334–9. [PubMed: 4936338]
24. Amoss M, Burgus R, Blackwel R, et al. Purification, amino acid composition and n-terminus of hypothalamic luteinizing hormone releasing factor (LRF) of ovine origin. *Biochem Biophys Res Commun.* 1971; 44:205–10. [PubMed: 4940370]
25. Nakanishi S, Inoue A, Kita T, et al. Nucleotide-sequence of cloned cdna for bovine corticotropin-beta-lipotropin precursor. *Nature.* 1979; 278:423–7. [PubMed: 221818]

26. Rosenfeld MG, Mermod JJ, Amara SG, et al. Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific rna processing. *Nature*. 1983; 304:129–35. [PubMed: 6346105]
27. deLecea L, Criado JR, ProsperoGarcia O, et al. A cortical neuropeptide with neuronal depressant and sleep-modulating properties. *Nature*. 1996; 381:242–5. [PubMed: 8622767]
28. Gautvik KM, deLecea L, Gautvik VT, et al. Overview of the most prevalent hypothalamus-specific mRNAs, as identified by directional tag PCR subtraction. *Proc Natl Acad Sci USA*. 1996; 93:8733–8. [PubMed: 8710940]
29. Jiang Y, Luo L, Gustafson EL, et al. Identification and characterization of a novel RF-amide peptide ligand for orphan G-protein-coupled receptor SP9155. *J Biol Chem*. 2003; 278:27652–7. [PubMed: 12714592]
30. Fukusumi S, Yoshida H, Fujii R, et al. A new peptidic ligand and its receptor regulating adrenal function in rats. *J Biol Chem*. 2003; 278:46387–95. [PubMed: 12960173]
31. Schrader M, Schulz-Knappe P. Peptidomics technologies for human body fluids. *Trends Biotechnol*. 2001; 19:S55–S60. [PubMed: 11780972]
32. Minamino N, Tanaka J, Kuwahara H, et al. Determination of endogenous peptides in the porcine brain: possible construction of Peptidome, a fact database for endogenous peptides. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2003; 792:33–48.
33. Fricker LD, McKinzie AA, Sun JL, et al. Identification and characterization of proSAAS, a granin-like neuroendocrine peptide precursor that inhibits prohormone processing. *J Neurosci*. 2000; 20:639–48. [PubMed: 10632593]
34. Sasaki K, Satomi Y, Takao T, Minamino N. Snapshot Peptidomics of the Regulated Secretory Pathway. *Mol Cell Proteomics*. 2009; 8:1638–47. [PubMed: 19339239]
35. Civelli O. Functional genomics: the search for novel neurotransmitters and neuropeptides. *FEBS Lett*. 1998; 430:55–8. [PubMed: 9678594]
36. Hinuma S, Habata Y, Fujii R, et al. A prolactin-releasing peptide in the brain. *Nature*. 1998; 393:272–6. [PubMed: 9607765]
37. Tatemoto K, Hosoya M, Habata Y, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem Biophys Res Commun*. 1998; 251:471–6. [PubMed: 9792798]
38. Reinscheid RK, Nothacker HP, Bourson A, et al. Orphanin-FQ - a neuropeptide that activates an opioid-like g-protein-coupled receptor. *Science*. 1995; 270:792–4. [PubMed: 7481766]
39. Meunier JC, Mollereau C, Toll L, et al. Isolation and structure of the endogenous agonist of opioid receptor-like ORL(1) receptor. *Nature*. 1995; 377:532–5. [PubMed: 7566152]
40. De Lecea L, Kilduff TS, Peyron C, et al. The hypocretins: Hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci USA*. 1998; 95:322–7. [PubMed: 9419374]
41. Sakurai T, Amemiya A, Ishii M, et al. Orexins and orexin receptors: A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*. 1998; 92:573–85. [PubMed: 9491897]
42. Kojima M, Hosoda H, Date Y, et al. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999; 402:656–60. [PubMed: 10604470]
43. Ohtaki T, Shintani Y, Honda S, et al. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature*. 2001; 411:613–7. [PubMed: 11385580]
44. Fujii R, Yoshida H, Fukusumi S, et al. Identification of a neuropeptide modified with bromine as an endogenous ligand for GPR7. *J Biol Chem*. 2002; 277:34010–6. [PubMed: 12118011]
45. Shimomura Y, Harada M, Goto M, et al. Identification of neuropeptide W as the endogenous ligand for orphan G-protein-coupled receptors GPR7 and GPR8. *J Biol Chem*. 2002; 277:35826–32. [PubMed: 12130646]
46. Brezillon S, Lannoy V, Franssen JD, et al. Identification of natural ligands for the orphan G protein-coupled receptors GPR7 and GPR8. *J Biol Chem*. 2003; 278:776–83. [PubMed: 12401809]
47. Sato, S., Shintani, Y., Miyajima, N., Yoshimura, K. Novel G protein-coupled receptor protein and DNA thereof. World patent application. WO 02/31145 A1. 2002.

48. Xu YL, Reinscheid RK, Huitron-Resendiz S, et al. Neuropeptide S: A neuropeptide promoting arousal and anxiolytic-like effects. *Neuron*. 2004; 43:487–97. [PubMed: 15312648]
49. Mori K, Miyazato M, Ida T, et al. Identification of neuromedin S and its possible role in the mammalian circadian oscillator system. *EMBO J*. 2005; 24:325–35. [PubMed: 15635449]
50. Chambers J, Ames RS, Bergsma D, et al. Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1. *Nature*. 1999; 400:261–5. [PubMed: 10421367]
51. Lembo PMC, Grazzini E, Cao J, et al. The receptor for the orexigenic peptide melanin-concentrating hormone is a G-protein-coupled receptor. *Nat Cell Biol*. 1999; 1:267–71. [PubMed: 10559938]
52. Shimomura Y, Mori M, Sugo T, et al. Isolation and identification of melanin-concentrating hormone as the endogenous ligand of the SLC-1 receptor. *Biochem Biophys Res Commun*. 1999; 261:622–6. [PubMed: 10441476]
53. Saito Y, Nothacker HP, Wang ZW, et al. Molecular characterization of the melanin-concentrating-hormone receptor. *Nature*. 1999; 400:265–9. [PubMed: 10421368]
54. Nothacker HP, Wang ZH, McNeil AM, et al. Identification of the natural ligand of an orphan G-protein-coupled receptor involved in the regulation of vasoconstriction. *Nat Cell Biol*. 1999; 1:383–5. [PubMed: 10559967]
55. Fujii R, Hosoya M, Fukusumi S, et al. Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3. *J Biol Chem*. 2000; 275:21068–74. [PubMed: 10783389]
56. Howard AD, Wang R, Pong SS, et al. Identification of receptors for neuromedin U and its role in feeding. *Nature*. 2000; 406:70–4. [PubMed: 10894543]
57. Hedrick JA, Morse K, Shan L, et al. Identification of a human gastrointestinal tract and immune system receptor for the peptide neuromedin U. *Mol Pharmacol*. 2000; 58:870–5. [PubMed: 10999960]
58. Szekeres PG, Muir AI, Spinage LD, et al. Neuromedin U is a potent agonist at the orphan G protein-coupled receptor FM3. *J Biol Chem*. 2000; 275:20247–50. [PubMed: 10811630]
59. Liu CL, Eriste E, Sutton S, et al. Identification of relaxin-3/INSL7 as an endogenous ligand for the orphan G-protein-coupled receptor GPCR135. *J Biol Chem*. 2003; 278:50754–64. [PubMed: 14522968]
60. Liu CL, Chen JC, Sutton S, et al. Identification of relaxin-3/INSL7 as a ligand for GPCR142. *J Biol Chem*. 2003; 278:50765–70. [PubMed: 14522967]
61. Masuda Y, Takatsu Y, Terao Y, et al. Isolation and identification of EG-VEGF/prokineticins as cognate ligands for two orphan G-protein-coupled receptors. *Biochem Biophys Res Commun*. 2002; 293:396–402. [PubMed: 12054613]
62. Mori, MHK., Miya, H., Sato, S., Kitada, C., Matsumoto, H., Nagi, T., Shimomura, Y. Human, rat, and mouse G protein-coupled receptor TGR23 and identification of protein ligands. *PCT Int Appl*. WO 2003025179. 2003.
63. Okamura N, Reinscheid RK. Neuropeptide S: A novel modulator of stress and arousal. *Stress*. 2007; 10:221–6. [PubMed: 17613937]
64. Duangdao DM, Clark SD, Okamura N, Reinscheid RK. Behavioral phenotyping of Neuropeptide S receptor knockout mice. *Behav Brain Res*. 2009; 205:1–9. [PubMed: 19646487]
65. Reinscheid RK, Xu YL. Neuropeptide S and its receptor: A newly orphanized G protein-coupled receptor system. *Neuroscientist*. 2005; 11:532–8. [PubMed: 16282594]
66. Xu YL, Gall CM, Jackson VR, Civelli O, Reinscheid RK. Distribution of neuropeptide S receptor mRNA and neurochemical characteristics of neuropeptide S-expressing neurons in the rat brain. *J Comp Neurol*. 2007; 500:84–102. [PubMed: 17099900]
67. Yao Y, Lin XQ, Su J, et al. Cloning and distribution of neuropeptide S and its receptor in the pig. *Neuropeptides*. 2009; 43:465–81. [PubMed: 19854507]
68. Laitinen T, Polvi A, Rydman P, et al. Characterization of a common susceptibility locus for asthma-related traits. *Science*. 2004; 304:300–4. [PubMed: 15073379]
69. Shin HD, Park KS, Park CS. Lack of association of GPRA (G protein-coupled receptor for asthma susceptibility) haplotypes with high serum IgE or asthma in a Korean population. *J Allergy Clin Immunol*. 2004; 114:1226–7. [PubMed: 15536436]

70. Okamura N, Hashimoto K, Iyo M, et al. Gender-specific association of a functional coding polymorphism in the Neuropeptide S receptor gene with panic disorder but not with schizophrenia or attention-deficit/hyperactivity disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007; 31:1444–8. [PubMed: 17669576]
71. Gottlieb DJ, O'Connor GT, Wilk JB. Genome-wide association of sleep and circadian phenotypes. *Bmc Medical Genetics*. 2007; 8(Suppl 1):S9. [PubMed: 17903308]
72. Clark SD, Tran HT, Zeng J, Reinscheid RK. Importance of extracellular loop one of the neuropeptide S receptor for biogenesis and function. *Peptides*. 2009; 31:130–8. [PubMed: 19874863]
73. Jungling K, Seidenbecher T, Sosulina L, et al. Neuropeptide S-mediated control of fear expression and extinction: Role of intercalated GABAergic neurons in the amygdala. *Neuron*. 2008; 59:298–310. [PubMed: 18667157]
74. Leonard SK, Dwyer JM, Rizzo SJS, et al. Pharmacology of neuropeptide S in mice: therapeutic relevance to anxiety disorders. *Psychopharmacology (Berl)*. 2008; 197:601–11. [PubMed: 18311561]
75. Yoshida K, Kim J, Nakajima K, et al. Electrophysiological effects of neuropeptide S on rat ventromedial hypothalamic neurons *in vitro*. *Peptides*. 31:712–9.
76. Beck B, Fernet B, Stricker-Krongrad A. Peptide S is a novel potent inhibitor of voluntary and fast-induced food intake in rats. *Biochem Biophys Res Commun*. 2005; 332:859–65. [PubMed: 15919054]
77. Smith KL, Patterson M, Dhillon WS, et al. Neuropeptide S stimulates the hypothalamo-pituitary-adrenal axis and inhibits food intake. *Endocrinology*. 2006; 147:3510–8. [PubMed: 16574794]
78. Lage R, Gonzalez CR, Dieguez C, Lopez M. Nicotine treatment regulates neuropeptide S system expression in the rat brain. *Neurotoxicology*. 2007; 28:1129–35. [PubMed: 17645946]
79. Lage R, Dieguez C, Lopez M. Caffeine treatment regulates neuropeptide S system expression in the rat brain. *Neurosci Lett*. 2006; 410:47–51. [PubMed: 17055161]
80. Fukatsu, KNY., Tarui, N., Mori, M., Matsumoto, H., Kurasawa, O., Banno, H. Preparation of oxazolo[3,4-a]pyrazine derivatives as TGR23 ligand antagonists. World patent. WO2005021555. 2005.
81. Rouille Y, Duguay SJ, Lund K, et al. Proteolytic processing mechanisms in the biosynthesis of neuroendocrine peptides: the subtilisin-like proprotein convertases. *Front Neuroendocrinol*. 1995; 16:322–61. [PubMed: 8557169]
82. Mitchell JD, Maguire JJ, Kuc RE, Davenport AP. Expression and vasoconstrictor function of anorexigenic peptides neuromedin U-25 and S in the human cardiovascular system. *Cardiovasc Res*. 2009; 81:353–61. [PubMed: 18987052]
83. Ida T, Mori K, Miyazato M, et al. Neuromedin S is a novel anorexigenic hormone. *Endocrinology*. 2005; 146:4217–23. [PubMed: 15976061]
84. Peier A, Kosinski J, Cox-York K, et al. The Antiobesity Effects of Centrally Administered Neuromedin U and Neuromedin S Are Mediated Predominantly by the Neuromedin U Receptor 2 (NMUR2). *Endocrinology*. 2009; 150:3101–9. [PubMed: 19324999]
85. Sakamoto T, Mori K, Nakahara K, et al. Neuromedin S exerts an antidiuretic action in rats. *Biochem Biophys Res Commun*. 2007; 361:457–61. [PubMed: 17645872]
86. Sakamoto T, Mori K, Miyazato M, et al. Involvement of neuromedin S in the oxytocin release response to suckling stimulus. *Biochem Biophys Res Commun*. 2008; 375:49–53. [PubMed: 18675786]
87. Jaszberenyi M, Bagasi Z, Thurzo B, Foldesi I, Telegdy G. Endocrine and behavioral effects of neuromedin S. *Horm Behav*. 2007; 52:631–9. [PubMed: 17900576]
88. Vigo E, Roa J, Lopez M, et al. Neuromedin S as novel putative regulator of luteinizing hormone secretion. *Endocrinology*. 2007; 148:813–23. [PubMed: 17110433]
89. Minamino N, Kangawa K, Matsuo H. Neuromedin-U-8 and neu-romedin-U-25 - novel uterus stimulating and hypertensive peptides identified in porcine spinal-cord. *Biochem Biophys Res Commun*. 1985; 130:1078–85. [PubMed: 3839674]
90. Budhiraja S, Chugh A. Neuromedin U: physiology, pharmacology and therapeutic potential. *Fundam Clin Pharmacol*. 2009; 23:149–57. [PubMed: 19645813]

91. Novak CM. Neuromedin S and U. *Endocrinology*. 2009; 150:2985–7. [PubMed: 19549882]
92. Bulenger S, Marullo S, Bouvier M. Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. *Trends Pharmacol Sci*. 2005; 26:131–7. [PubMed: 15749158]
93. Levoe A, Dam J, Ayoub MA, et al. The orphan GPR50 receptor specifically inhibits MT1 melatonin receptor function through heterodimerization. *EMBO J*. 2006; 25:3012–23. [PubMed: 16778767]
94. McLatchie LM, Fraser NJ, Main MJ, et al. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature*. 1998; 393:333–9. [PubMed: 9620797]
95. Marinissen MJ, Gutkind JS. G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol Sci*. 2001; 22:368–76. [PubMed: 11431032]

A)
MISSVKLNILVLSLSTMHVFWCYPVPSSKVSGDYFLILLNSCPTRLDRSKEL
 AFLKPILEKMFV**KRS**SFRNGVGTGMKKTSFQRAKS

B)
MKHLRPQFPLILAIYCFCM**LQIPSSGFPQ**PLADPSDGLDIVQLEQLAYCLSQWA
 PLSRQPKDNQDIY**KR**FLFHYSRTQEATHPVKTGFPPVHPLMHLAAKLANRR**MR**
LQRGSGTAAVDFTKKDHTATWGRPFFLFRPRNGRNIEDEAQIQW

C)

SFRNGVGTGMKKTSFQRAKS	NPS
ILQRGSGTAAVDFTKKDHTATWGRPFFLFRPRN-NH ₂	NMS
FRVDEEFQSPFASQSRGYFLFRPRN-NH ₂	NMU

Fig. (1).

Structures of NPS, NMS and NMU. (A) NPS precursor sequence. The signal peptide is shown in bold. The mature peptide is shown with underline and the endoprotease cleavage site (KR) which releases the peptide is shown in italic. (B) NMS precursor sequence. The signal peptide is shown in bold. The mature peptide is shown with underline and the endoprotease cleavage sites (KR, RR and R) which release the peptide are shown in italic. The glycine (shown in small cap) serves as an amide donor for C-terminal amidation. (C) The sequences of mature human peptides NPS, NMS and NMU

Table 1

Novel Neuropeptides Identified Using Orphan GPCR Strategy

Neuropeptide Ligand	Orphan Receptor	Ligand Source	Activity Monitored	Year Found	Ref.
N/OFQ	ORL-1	Brain	cAMP	1995	[38, 39]
Orexins/Hypocretins	HFGAN72	Brain	[Ca ²⁺]	1998	[40, 41]
PrRP	GPR10	Brain	Arachidonic acid	1998	[36]
Apelin	APJ	Stomach	Extracellular acidification rate	1998	[37]
Ghrelin	GHSR	Stomach	[Ca ²⁺]	1999	[42]
Kisspeptin	GPR54	Brain	[Ca ²⁺]	2001	[43]
NPB and NPW	GPR 7/8	Brain	GTPγS, cAMP,	2002–2003	[44–46]
NPS	NPS receptor	Brain	cAMP, [Ca ²⁺]	2003	[47, 48]
NMS	NMU receptor	Brain	[Ca ²⁺]	2005	[49]