Results Probl Cell Differ (46)
O. Civelli, Q.-Y. Zhou: Orphan G Protein-Coupled Receptors and Novel Neuropeptides
DOI 10.1007/400_2007_054/Published online: 24 January 2008

Neuromedin S: Discovery and Functions

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Abstract Neuromedin S, a novel neuropeptide of 36 amino acids, was isolated from rat brain as an endogenous ligand for the orphan G protein-coupled receptors FM-3/GPR66 and FM-4/TGR-1, identified to date as type-1 and type-2 neuromedin U (NMU) receptors, respectively. The peptide was designated neuromedin S (NMS) because it is specifically expressed in the suprachiasmatic nucleus of the hypothalamus. NMS is structurally related to NMU; these peptides share a C-terminal core structure. In this review, we will outline the recent discoveries regarding the structure, cognate receptors, distribution, and possible physiological functions of NMS.

1 Discovery of Neuromedin S

Neuropeptides have been implicated in a wide range of physiological processes. Previous identifications of novel neuropeptides have revealed novel regulatory mechanisms in physiological processes; thus, researchers have continued to search for novel neuropeptides. Although a variety of neuropeptides have been isolated based on their functionality, a powerful survey method to isolate novel neuropeptide with unknown functions was developed in the 1990s (Civelli 1998). Human genomic sequencing revealed the existence of several hundred orphan G protein-coupled receptors (GPCRs), for which ligands have remained unidentified (Vassilatis et al. 2003). As dozens of these orphan GPCRs exhibit sequence similarity to GPCRs with known neuropeptide ligands, these orphan GPCRs have been used as tools to identify novel neuropeptides. This survey method has the advantage of identifying both the neuropeptide and its cognate receptor simultaneously. The rate of neuropeptide discovery increased considerably with this strategy. Over the course of a decade, use of a reverse-pharmacological technique has led to the discovery of approximately ten novel neuropeptides, which have been discovered as endogenous ligands of orphan GPCRs (Civelli et al. 2006).

Neuromedin U (NMU), originally isolated from porcine spinal cord, is a brain–gut peptide with a potent activity to cause uterine smooth muscle contraction (Minamino et al. 1985). As the receptor had not been identified, however, the physiological roles of NMU were poorly understood. In 2000, NMU was found to be an endogenous ligand for two orphan GPCRs, FM-

3/GPR66 and FM-4/TGR-1, which were renamed the NMU receptor type-1 and type-2, respectively (Fujii et al. 2000; Hedrick et al. 2000; Hosoya et al. 2000; Howard et al. 2000; Kojima et al. 2000; Raddatz et al. 2000). FM-3/GPR66 mRNA is widely distributed throughout peripheral tissues. In contrast, FM-4/TGR-1 mRNA is predominantly expressed in the central nervous system. The unique distributions of NMU receptors have provided novel insights into the function of NMU. Peripherally, NMU induces smooth muscle contraction (Minamino et al. 1985), elevates blood pressure (Minamino et al. 1985), modifies intestinal ion transport (Brown and Quito 1988), and promotes inflammation (Moriyama et al. 2005). Centrally, NMU functions in the regulation of feeding behaviors (Howard et al. 2000; Kojima et al. 2000), energy homeostasis (Nakazato et al. 2000; Hanada et al. 2003), stress responses (Hanada et al. 2001), circadian rhythms (Nakahara et al. 2004a), and nociceptive responses (Yu et al. 2003; Nakahara et al. 2004b).

In cases apart from ours, NMU was identified as an endogenous ligand for FM-3/GPR66 and FM-4/TGR-1 from a library of bioactive molecules, including synthetic peptides. In contrast, we purified natural ligands from tissue extracts. Thus, we were able to succeed in the isolation of neuromedin S (NMS) (Mori et al. 2005). Gel filtration of rat small intestine extracts revealed a single agonist activity capable of increasing intracellular calcium ion con-

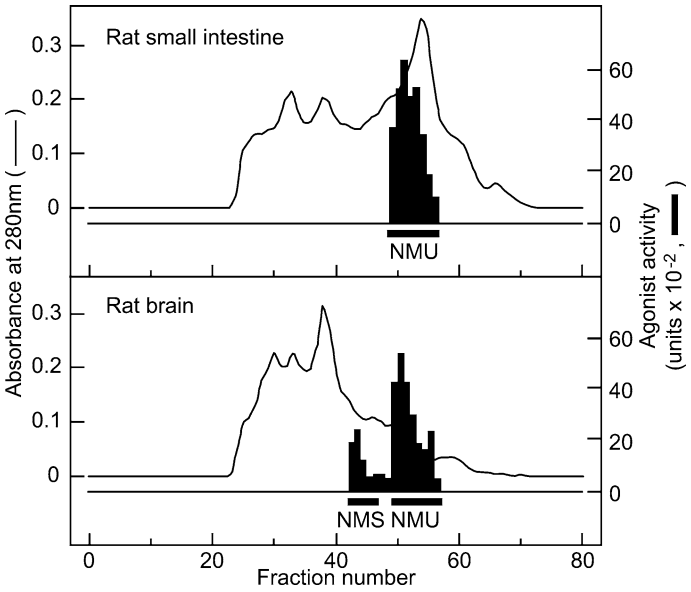

Fig. 1 Discovery of neuromedin S. Gel filtration on a Sephadex-G50 column of tissue extracts from rat small intestine (*upper panel*) and rat brain (*lower panel*). The *black bars* indicate agonist activity for FM-4/TGR-1 expressed recombinantly in CHO cells. Active fractions containing NMS and NMU are indicated

centrations in CHO cells expressing FM-4/TGR-1 (Fig. 1). In contrast, two agonist activities were found following gel filtration of rat brain extracts, indicating the presence of two endogenous ligands. The activity that eluted in the fractions at a smaller molecular mass corresponded to the activity found in the small intestine. NMU had previously been purified from rat small intestine as an endogenous ligand for FM-3/GPR66 (Kojima et al. 2000). Therefore, the second isolated activity likely corresponded to a novel neuropeptide. This ligand was designated neuromedin S (NMS) because of its specific expression in the suprachiasmatic nucleus (SCN) of the hypothalamus (Mori et al. 2005).

2 Structure of Neuromedin S

Rat NMS is a C-terminal amidated neuropeptide of 36 amino acid residues. NMS homologs have been identified to date in humans, rats, mice, and frogs (Fig. 2) (Mori et al. 2005; Chen et al. 2006). NMS is structurally related to NMU. The seven-residue C-terminal amidated sequence of NMS is identical to that of NMU; this structure is essential for NMU receptor binding (Minamino et al. 1985). The N-terminal portion of NMS, however, has no sequence homology to any known peptides or proteins. NMS is not a splice variant of NMU, because the human *NMS* and *NMU* genes map to chromosomes 2q11.2 and 4q12, respectively.

The NMS pro-protein and gene are structurally similar to those of NMU (Mori et al. 2005). The NMS pre-pro-protein contains four potential processing sites cleavable by subtilisin-like pro-protein convertases. These four sites are conserved in the NMU pre-pro-protein, indicating similar domain structures. NMS and NMU are produced from precursor proteins by proteolytic processing at the third and fourth of these sites. The amino acid sequences between the first and second processing sites exhibit homology to each other. As with the *NMU* gene, the *NMS* gene is composed of ten exons; the exon–intron boundaries in the NMS and NMU pre-pro-proteins are comparably conserved.

Fig. 2 Structure of NMS. The amino acid sequences of NMS are compared to those of NMU. Residues that are identical between the peptides are *shaded*

NMS was also isolated from the dermal venom of Eurasian bombinid toads, indicating that the *NMS* and *NMU* genes had diverged at the level of the Amphibia during evolution (Chen et al. 2006). A high degree of splice variations were observed in the NMS transcripts of toads. Differential splicing was highly conserved throughout tetrapod vertebrates. Alternative splice variants of NMS mRNA have also been cloned from mammals (unpublished data from the author's laboratory).

3
Receptors for Neuromedin S

NMS and NMU have the same core structure that is required for binding to their cognate receptors (see Sect. 2), suggesting that NMS shares at least two receptors, FM-3/GPR66 and FM-4/TGR-1, with NMU. In 1998, FM-3/GPR66 was cloned as an orphan GPCR similar to the neurotensin and growth hormone secretagogue receptor families (Tan et al. 1998). Subsequent homology search identified FM-4/TGR-1, which is similar to FM-3/GPR66 (Hosoya et al. 2000; Howard et al. 2000). Human FM-3/GPR66 exhibits 52% amino acid identity with human FM-4/TGR-1. Expression of FM-3/GPR66 mRNA is widely distributed throughout various tissues; high levels of expression are found in peripheral tissues (Fujii et al. 2000; Raddatz et al. 2000). In contrast, FM-4/TGR-1 mRNA is primarily expressed in the central nervous system (Hosoya et al. 2000; Raddatz et al. 2000). In rat brain, FM-4/TGR-1 mRNA expression is clearly detected in the paraventricular nucleus (PVN), the wall of the third ventricle in the hypothalamus, and the CA1 region of the hippocampus (Howard et al. 2000).

Pharmacological characteristics of NMS were examined using recombinant receptors exogenously expressed in CHO cells (Mori et al. 2005). Both NMS and NMU induce robust increases in intracellular calcium ion concentrations in CHO cells expressing either FM-3/GPR66 or FM-4/TGR-1. NMS and NMU possess similar efficacy and potency at these receptors. Competitive radioligand binding analysis demonstrated high-affinity binding of NMS to these receptors. NMS and NMU display similar inhibition constants for FM-3/GPR66. Interestingly, NMS has a higher binding affinity for FM-4/TGR-1 than NMU.

4
Distribution of Neuromedin S

The tissue distribution of NMS mRNA in rats was investigated by quantitative reverse transcription–polymerase chain reaction (Mori et al. 2005). NMS mRNA is primarily expressed in the central nervous system, spleen, and

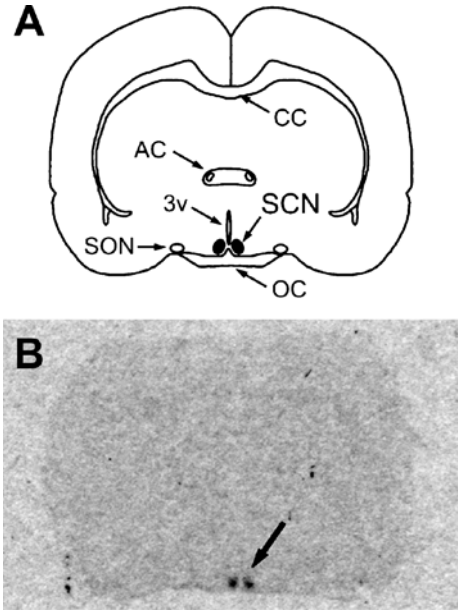

Fig. 3 Specific expression of NMS mRNA in the SCN of the hypothalamus. **A** Schematic representation of a coronal rat brain section containing the SCN. *3v* third ventricle, *AC* anterior commissure, *CC* corpus callosum, *OC* optic chiasm, *SCN* suprachiasmatic nucleus, *SON* supraoptic nucleus. **B** In-situ hybridization analysis of NMS mRNA expression in a coronal section of rat brain. Specific expression in the SCN is indicated by the *arrow*

testis. The highest levels of NMS mRNA are detected in the hypothalamus. In rat brain, NMS mRNA is predominantly expressed in the SCN of the hypothalamus; only minimal expression is found in other areas of the brain. The SCN, which is comprised of a pair of structures that contain about 20 000 neurons, is located on either side of the third ventricle, superior to the optic chiasma (Fig. 3a) (Reppert and Weaver 2001). In-situ hybridization histochemistry revealed that NMS mRNA expression is restricted to the SCN in rat brain (Fig. 3b) (Mori et al. 2005). The SCN is divided into ventrolateral and dorsomedial portions, in which the neuropeptides vasoactive intestinal polypeptide (VIP) and arginine vasopressin (AVP) are expressed, respectively. NMS mRNA is expressed in the ventrolateral SCN in a similar distribution to VIP mRNA.

5 Functions of Neuromedin S

Intracerebroventricular (ICV) administration of NMS to rats has primarily been used to investigate its central functions, as one of the NMS receptors,

Table 1 The central effects of NMS

Biological function	Comment
Circadian oscillator system	Induce phase shift of circadian rhythm
Feeding regulation	Decrease food intake
Gonadotropic axis	Increase LH release
Urinary output	Increase AVP release

AVP arginine vasopressin, *LH* luteinizing hormone

FM-4/TGR-1, is expressed within the central nervous system (see Sect. 3). These functions, summarized in Table 1, are outlined in this section.

5.1 Circadian Oscillator System

The SCN of the hypothalamus contains the master circadian pacemaker that governs the circadian rhythms underlying behavioral and physiological processes in mammals. Therefore, specific expression of NMS mRNA within the SCN strongly suggests a role for NMS in the circadian oscillator.

Examination of the time-dependent profile of NMS expression (Mori et al. 2005) revealed that NMS mRNA levels fluctuate rhythmically within the SCN in rats under 12-hour light/dark cycles; expression is high during the daytime and low at night. In contrast, NMS mRNA expression is stable when animals were maintained under conditions of constant darkness, indicating that the rhythmic expression of NMS within the SCN is not generated spontaneously. The intrinsic circadian rhythmicity of gene expression within the SCN is generated by clock-gene families of transcription factors that act at CACGTG E-box elements (Reppert and Weaver 2001). No CACGTG E-box element is present in the promoter region of the *NMS* gene (Mori et al. 2005), which is consistent with the observation that NMS mRNA expression levels do not oscillate under conditions of constant darkness. These data indicate that the expression of NMS is not under the control of clock-gene family proteins.

ICV administration of NMS affects the circadian rhythms in rats maintained under constant darkness (Mori et al. 2005). ICV injection of NMS during the subjective day elicits phase advance of the circadian rhythm of locomotor activity, while administration at the end of the subjective night induces a phase delay. The phase-response curve for NMS, the interaction between the circadian time of treatment and the magnitude of the phase advance/delay, is very similar to that for nonphotic stimuli. In the SCN, two receptors for NMS are expressed; FM-4/TGR-1 is expressed at higher levels than FM-3/GPR66 (Nakahara et al. 2004a; Mori et al. 2005). These data strongly

suggest that NMS functions to regulate the circadian pacemaker in an autocrine and/or paracrine manner within the SCN.

The pacemaker located in the SCN independently generates a near-24-hour circadian rhythm via an autoregulatory transcription/translation feedback loop composed of clock-gene families. This rhythm is entrained to the 24-hour daily cycle by periodic environmental cues, such as light and temperature, typical photic and nonphotic signals, respectively (Lowrey and Takahashi 2000; Reppert and Weaver 2001, 2002). The circadian rhythm can also be phase-shifted by photic and nonphotic stimuli (Mrosovsky 1996; Lowrey and Takahashi 2000). The ventrolateral SCN receives and integrates photic and nonphotic signals from the retina and other brain areas to entrain the circadian rhythm (Reppert and Weaver 2001). Several neuropeptides are implicated in circadian entrainment. VIP, which is expressed in the ventrolateral SCN, plays a role in photic entrainment of the circadian rhythm (Piggins and Cutler 2003). No SCN-intrinsic neuropeptide involved in the nonphotic circadian entrainment, however, has been identified. NMS mRNA is expressed in the ventrolateral portion of the SCN. As ICV administration of NMS induces a nonphotic-type phase shift in the circadian rhythm, NMS is a candidate for a nonphotic entrainment factor intrinsic to the SCN.

ICV administration of NMU also induces a nonphotic-type phase shift of the circadian rhythm (Nakahara et al. 2004a). NMS and NMU, however, appear to play distinct roles in the regulation of the circadian oscillator system. In contrast to NMS mRNA, NMU mRNA is expressed in the dorsomedial SCN (Graham et al. 2003), which is involved in the spontaneous generation of a strong rhythm. Moreover, its expression shows a circadian rhythm in rats maintained in constant darkness (Nakahara et al. 2004a), indicating that NMU, unlike NMS, is controlled by the circadian pacemaker. Therefore, NMU may act either as a part of the central clock mechanism or as an output signal of the SCN.

5.2 Feeding Regulation

NMU, an anorexigenic neuropeptide, functions in the central regulation of feeding behaviors (Howard et al. 2000; Kojima et al. 2000). Deficiency of NMU in mice leads to hyperphagia and obesity (Hanada et al. 2004). The *NMS* gene locus in humans is consistent with the location of a quantitative trait locus implicated in obesity (Mori et al. 2005).

Central NMS injection was used to investigate the role of NMS in feeding regulation (Ida et al. 2005). ICV administration of NMS decreases 12-hour food intake during the dark (night-equivalent) period in a dose-dependent manner. This anorexigenic effect is more potent than that induced by similar doses of NMU. The amount of food intake induced by ghrelin, neuropep-

tide Y, and agouti-related protein is reduced by co-administration of NMS. The PVN and arcuate nucleus (Arc) of the hypothalamus regulate feeding through a complex neuronal network of orexigenic and anorexigenic neuropeptides. Two anorexigenic neuropeptides, α-melanocyte-stimulating hormone (α-MSH) and corticotropin-releasing hormone (CRH), are necessary for NMS actions on feeding mediated by these nuclei. ICV administration of NMS increases proopiomelanocortin, precursor of α-MSH, and CRH mRNA levels in the Arc and PVN, respectively. The suppression of food intake by NMS can be attenuated by pretreatment with both SHU9119 and α-hCRF, antagonists of α-MSH and CRH, respectively.

When rat NMS and NMU were administered to avian species, an interesting phenomenon is observed (Shousha et al. 2005). ICV administration of rat NMS into adult Japanese quail results in the expected suppression of feeding behavior. In contrast, ICV-administered rat NMU increases food intake. Although both peptides decrease food intake in the rat, rat NMU exhibits an opposing effect to rat NMS on feeding regulation in Japanese quail, despite the fact that both peptides share the C-terminal core structure required for receptor binding (see Sect. 2).

While exogenously administered NMS strongly suppresses food intake, the physiological role of NMS in feeding regulation is not completely elucidated. Further investigation of the physiological significance of NMS is required. It is interesting how satiety signals affect NMS expression in the PVN and Arc of the hypothalamus, key centers of feeding regulation, as NMS mRNA is usually expressed at only low levels in these nuclei (Mori et al. 2005). NMS projections from the SCN to the PVN and/or Arc may be important in the regulation of feeding behavior. Multiple reports have linked the development of obesity to disruption of the circadian rhythm; mice deficient in *Clock*, a transcription factor that is an essential component of master circadian pacemaker in the SCN (Reppert and Weaver 2001), are hyperphagic and obese (Turek et al. 2005). The interaction between molecular control of the circadian rhythm and energy homeostasis remains unclear.

5.3 Gonadotropic Axis

NMS has also been implicated in the central regulation of the female gonadotropic axis (Vigo et al. 2007). In female rats, hypothalamic NMS mRNA is only minimally expressed during the neonatal period, but increases during the late-infantile and juvenile stages of postnatal development. NMS mRNA expression decreases again around puberty and is then restored upon reaching adulthood. In the hypothalamus of adult females, NMS expression fluctuates significantly throughout the estrous cycle; maximum expression is detected at proestrus. NMS mRNA levels in the hypothalamus are de-

creased after ovariectomy and rescued by progesterone, but not estradiol, supplementation.

ICV administration of NMS into pubertal female rats results in significant increases in serum luteinizing hormone (LH) levels. In adult females, the magnitude of the NMS-induced stimulatory effect on LH release is affected by the stage of the estrous cycle. When administered at estrus, NMS induces a potent increase in circulating LH level, whereas only modest LH secretion is induced by ICV-administered NMS at diestrus. Robust increases in LH secretion are also elicited by NMS in female rats during a short-term fast. In contrast, central administration of NMS reduces the elevated serum LH concentrations of ovariectomized rats.

5.4 Antidiuretic Action

ICV administration of NMS increases the plasma concentrations of AVP and decreases nocturnal urine volume (Sakamoto et al. 2007). NMS induces a more rapid release of AVP than NMU. A tenfold higher dose of NMU is necessary to exert the same effect as NMS. AVP is synthesized in the PVN and supraoptic nucleus (SON); FM-4/TGR-1 is expressed in these nuclei. Activation of a subset of AVP-producing neurons in the PVN and SON are indicated by the enhanced expression of c-Fos following ICV administration of NMS. These data suggest that NMS may function in the regulation of urinary output by altering AVP release from the PVN and SON.

6 Summary

Identification and examination of NMS has implicated several novel regulatory mechanisms functioning in physiologic processes. Current evidence suggests potential roles for NMS in the central regulation of circadian rhythms, feeding behaviors, LH secretion, and urinary output. The specific expression pattern of NMS in peripheral tissues, however, suggests tissue-specific physiological functions. High levels of NMS mRNA are observed in the spleen; the NMS receptor, FM-3/GPR66, is also expressed in the spleen and on immune cells (Hedrick et al. 2000), suggesting that NMS may play a role in immune responses.

NMS is a new bioactive peptide identified as a ligand for orphan GPCR, whose physiologic and pathologic roles are not fully understood. Engineering mice deficient in NMS and further detailed anatomical localization analysis will greatly improve our understanding of the functions of this peptide. Further characterization of NMS will hopefully provide novel insight into the regulatory mechanisms of physiological phenomena.

Acknowledgements We thank Drs. Masayasu Kojima and Noboru Murakami for their collaboration on our studies. Studies in the authors' laboratory are supported in part by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO) and by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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