Feeding regulation in the brain: Involvement of Neuropeptide W

Fumiko Takenoya[1,2], Haruaki Kageyama[1], Yukari Date[3], Masamitsu Nakazato[4], Seiji Shioda[1]

[1]Department of Anatomy I, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan
<e-mail>haruaki@med.showa-u.ac.jp, shioda@med.showa-u.ac.jp
[2]Department of Physical Education, Hoshi University School of Pharmacy and Pharmaceutical Science, 2-4-41 Ebara, Shinagawa, Tokyo 142-8501, Japan
<e-mail>kuki@hoshi.ac.jp
[3]Department of Frontier Science Research Center, University of Miyazaki, 5200 Kiyotake, Miyazaki 889-1692, Japan
<e-mail> dateyuka@med.miyazaki-u.ac.jp
[4]Department of Division of Neurology, Respirology, Endocrinology, and Metabolism, Department of Internal Medicine, Faculty of Medicine, University of Miyazaki, 5200 Kiyotake, Miyazaki 889-1692, Japan
<e-mail>nakazato@med.miyazaki-u.ac.jp

Summary. Orphan G protein-coupled receptors (GPCRs) as targets to identify new transmitters have led over the last decade to the discovery of at least twelve novel neuropeptide families. Interestingly, several of these novel neuropeptides have physiological effects that include the modulation of food intake and energy expenditure. Neuropeptide W (NPW) is a novel neuropeptide which was recently isolated from the porcine hypothalamus and shown to be an endogenous ligand for the GPR7 and GPR8 orphan G protein-coupled receptors. NPW is widely distributed in the brain. Infusion of NPW is known to increase food intake in the light phase but inhibit intake in the dark phase. In spite of numerous morphological studies on NPW, its function has yet to be fully elucidated. Moreover, as the distribution of NPW-positive cell bodies in the hypothalamus has not been described, a detailed examination of NPW's distribution and localization in this brain region is required. The expression of NPW mRNA was demonstrated in the hypothalamic paraventricular nucleus (PVN), arcuate nucleus (ARC), ventromedial nucleus (VMH) and lateral hypothalamus (LH). NPW-like immunoreactivity (LI) was dramatically enhanced in animals pretreated with colchicine. At the light microscopic level, NPW-LI cell bodies have been identified in the preoptic areas (POA), PVN, ARC, VMH, LH, periaqueductal gray (PAG), lateral parabrachial nucleus (LPB) and

prepositus nucleus. NPW-LI axon terminals have also been observed in the POA, bed nucleus of the stria terminals, amygdala, PVN, ARC, VMH, LH by electron microscopy. In addition, at the electron microscopic level, NPW-LI cell bodies and dendritic processes were often observed receiving inputs from other unknown neurons in the ARC, PVN, VMH and amygdala. Moreover, double immunostaining experiments showed that NPW-LI axon terminals were in close apposition to orexin-, MCH-, and NPY-containing neurons in the hypothalamus. These morphological and physiological findings strongly suggest that NPW participates in the regulation of feeding behavior in harmony with other feeding-regulating neurons in the hypothalamus.

Key words. Neuropeptide W (NPW), hypothalamus, feeding, neuronal network, Rat,

1 Introduction

It is well known that G protein-coupled receptors (GPCRs) form the largest family of membrane proteins that recognize extracellular messengers (Bockaert and Pin 1999). GPCRs are also known to mediate a variety of intracellular responses leading to the regulation of numerous physiological functions. GPCRs provide enormous potential for new drug development given that they are already the targets of nearly 50% of all prescription drugs including antihistamines, neuroleptics and antihypertensives (Howard et al. 2001). Interestingly, novel neuropeptides such as orexin, ghrelin, melanin-concentrating hormone (MCH), galanin-like peptide (GALP), neuropeptide B (NPB) and neuropeptide W (NPW) have had a strong impact on our understanding of the mechanisms that regulate obesity and energy homeostasis. In this chapter, I will focus on NPW, which is a newly identified GPCR ligand, and review its structure, function, distribution and localization in the brain.

2 NPW as a ligand for GPR7 and GPR8

O'Dowd and colleagues originally identified endogenous ligands of the GPCRs GPR7 and GPR8 that were originally identified by cloning the opioid somatostatin-like receptor genes from human genomic DNA (O'Dowd et al. 1995). GPR7 and GPR8 are thus quite closely related to opioid receptors. While GPR7 is found both in humans and rodents, GPR8 is apparently expressed only in humans (Lee et al. 1999). By using RT–PCR analysis, both GPR7 and GPR8 mRNA were detected at abundant levels in

human tissues in the central nervous system (CNS) (Fujii et al. 2002). In particular, high levels of GPR7 mRNA were found in the hippocampus and amygdala (Brezillon et al. 2003). However, in the human brain, GPR7 and GPR8 mRNA is not found in many regions. On the other hand, in the rat, strong GPR7 mRNA expression was detected by RT–PCR analysis in the hypothalamus and amygdala (Fujii et al. 2002). In addition, *in situ* hybridization studies have revealed that GPR7 mRNA is present in the rat hypothalamus, including the ARC, VMH, PVN, dorsomedial nucleus (DMH) and supraoptic nucleus (SON) (Jackson et al. 2006; Lee et al. 1999). These nuclei in the hypothalamus are well known to be involved in feeding regulation and energy homeostasis. However, a detailed study to compare the distributions of both GPR7 and GPR8 in brain has not yet been done. It was reported that GPR7 mRNA is expressed in the hippocampus (Lee et al. 1999), but radio-ligand binding assays were unable to demonstrate the presence of its protein in this region (Singh et al. 2004).

3 Identification of neuropeptide W (NPW)

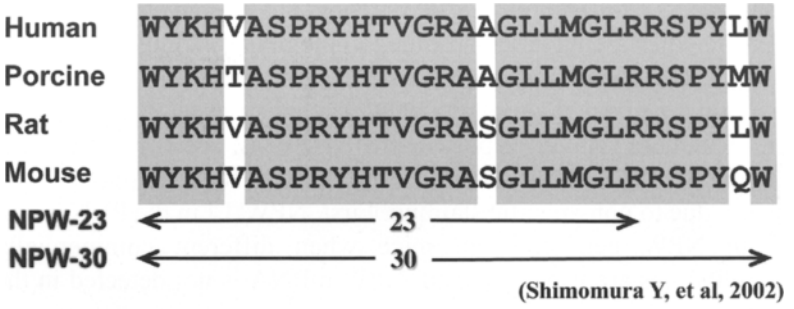

Fig.1. Amino acid sequences of NPW-23 and NPW-30. Shaded areas show similarities in amino acid sequence identities between human, porcine, rat and mouse.

The search for endogenous ligands for GPR7 or GPR8 led to the characterization of new peptides consisting of 23- and 30-amino acid residues (Fig.1). These neuropeptides, termed neuropeptide W-23 (NPW23) and neuropeptide W-30 (NPW30), were isolated from the porcine hypothalamus as endogenous ligands for GPR7 and GPR8 (Shimomura et al. 2002;Tanaka et al. 2003). NPW is named for tryptophan residues at the N and C termini of NPW30. Synthetic NPW23 and NPW30 were shown to bind and activate both GPR7 and GPR8 at similar effective doses (Shimomura et al. 2002; Tanaka et al. 2003). In *in vitro* experiments, human

NPW23 and NPW30 were shown to have a similar potency to activate human GPR7. NPW mRNA expression in the human CNS was strongest in the substantia nigra, amygdala and hippocampus (Fujii et al. 2002). In the mouse brain, NPW mRNA was not detected in the amygdala and hippocampus, whereas moderate expression was detected in the dorsal raphe nuclei (DRN), periaqueductal grey (PAG), and Edinger Westphal nuclei (EW). *In situ* hybridization studies have revealed NPW mRNA in the PAG, ventral tegmental area (VTA) and DRN in the mouse CNS (Tanaka et al. 2003), and in the PAG, EW and VTA in the rat brain (Kitamura et al. 2006).

4 Distribution of NPW in the brain

In immunohistochemical studies, we first tested the effect of colchicine treatment of animals prior to brain fixation. We demonstrated that NPW-LI was dramatically enhanced when animals were pre-treated with colchicine compared to untreated controls (Takenoya et al. 2008). Dan et al have already reported that NPW-LI was dramatically enhanced in colchicine-treated rats (Dun et al. 2003). They and we have reported that NPW-LI cell bodies are widely distributed in the brain, including the POA, PVN, SON, ARC, dorsal and lateral hypothalamic areas and anterior and posterior pituitary gland (Dun et al. 2003; Takenoya et al. 2008) (Fig.1). However, Kitamura et al. reported that NPW-LI cell bodies were identifiable in the PVN in rats and mice, but, for two separate reasons, this was probably due to non-specific staining. First, NPW-LI in the PVN is still observed in NPW gene-deficient mice when different commercially available antibodies are used. Second, NPW mRNA is not detected in the PVN of rats or mice by *in situ* hybridization (Hondo et al. 2008; Kitamura et al. 2006). In contrast, using RT-PCR, we identified NPW-LI cell bodies in the PVN as well as in the ARC, LH and VMH (data not shown). Recently, we observed NPW-LI axon terminals have also been observed in the POA, bed nucleus of the stria terminals (BST), amygdala, PVN, ARC, VMH, LH by electron microscopy. We also found that NPW-LI cell bodies were present in the EW, PAG, lateral parabrachial nucleus (LPB), medial parabrachial nucleus (MPB) in the rat brain as reported by Kitamura et al. (Kitamura et al. 2006).

In contrast, we observed NPW-LI processes in several other regions such as the lateral septum, BST, dorsomedial and posterior hypothalamus, central amygdaloid nucleus (Ce), hippocampus, interpeduncular nucleus, inferior colliculus, lateral parabrachial nucleus, facial nucleus and hypoglossal nucleus.

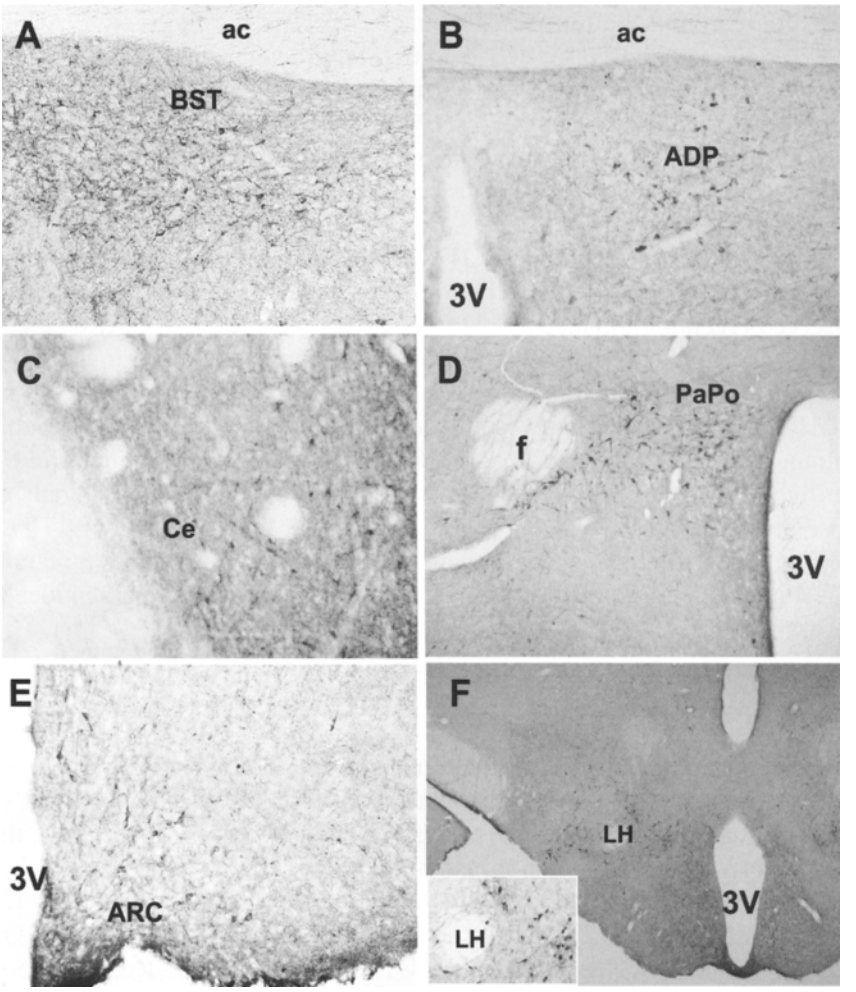

Fig. 2. Photomicrographs showing the distribution of NPW-LI in the rat brain. NPW-LI processes are seen in the bed nucleus of the stria terminalis (BST) (A), and the central amygdaloid nucleus (Ce)(C). NPW-LI cell bodies are seen in the anterodorsal preoptic nucleus (ADP)(B), posterior part (PaPo) of the paraventricular nucleus (D), arcuate nucleus (ARC)(E), and lateral hypothalamic area (LH)(F). ac: anterior commissure; f: fornix; 3V: 3rd ventricle.

In addition, we found that many NPW-LI processes could be observed in the central amygdaloid nucleus and BST (Fig2-A, C). Given the location of neurons with NPW-LI, these findings strongly suggest that NPW is

involved in the emotive responses and regulation of reproduction in addition to feeding regulation. Interestingly, a high density of NPW-LI processes was found around the fornix in the anterior part of the LH region. At this level, very few NPW-LI cell bodies were detected. However, many NPW-LI cell bodies were observed in the region where the mammillary recess of the 3rd ventricle appears. NPW-LI cell bodies in the LH were thus observed within a narrow space in the caudal hypothalamus (Fig2-F). In addition, large numbers of neurons containing feeding regulating peptides such as orexin (Sakurai et al. 1998) and MCH (Kawauchi et al. 1983) have been shown to be present in the LH. We have also observed, at the electron microscopic level, NPW-LI cell bodies and dendritic processes were often observed receiving inputs from other unknown neurons in the ARC, PVN, VMH and amygdala (data not shown). In addition very close interactions between NPW-containing nerve processes and orexin- and MCH-containing neuronal cell bodies and processes by double immunostaining (Takenoya et al. 2008). Furthermore, we have identified at the light and electron microscope level, NPY-positive axon terminals in close apposition to NPW-LI neurons in the PVN (data not shown). These morphological findings suggest that NPW has neuromodulatory functions in feeding behavior in conjunction with other feeding regulating peptides in the brain.

5 Feeding regulation induced by NPW

In rat, GPR7 is expressed in the hypothalamus, including its feeding centers, suggesting it probably has a modulatory role for NPW in the control of feeding regulation. Some physiological studies have shown that the intracerebroventricular (icv) infusion of NPW affects food intake. For example, Shimomura et al. (Shimomura et al. 2002) reported that icv administration of NPW23 induced acute food in take on the light phase. On the other hand, in the dark phase, the icv infusion of NPW decreases food intake in the first 48 h (Mondal et al. 2003) even though the first 2 h following NPW injection is characterized by a hyperphagic state (Tanaka et al. 2003). GPR7 is expressed in the suprachiasmatic nucleus which is considered to control the circadian rhythm (Lee et al. 1999; Singh et al. 2004), hence the effect of the light/dark cycle in modulating NPW's action. It has also been suggested that chronic infusion of NPW reduces body weight and increases body temperature, heat production and oxygen consumption. Furthermore, by using GPR7 knockout mice, administration of NPW has hyperphagic and decreased energy expenditure effects. These results suggest that NPW is an anorexigenic peptide. It should be noted that these effects are evident in males but not on females, so the effects of NPW

on energy balance may be sexually dimorphic (Ishii et al. 2003). In addition, icv infusion of NPW in rats initially provokes acute food intake (Mondal et al. 2003), while icv infusion of NPW in free-feeding rats suppresses feeding for an extended time (Shimomura et al. 2002).

Other reports have shown that administration of NPW increases c-Fos expression in the LH and PVN (Levine et al. 2005). These data suggest an appetite-regulating function of NPW in the hypothalamus and other brain regions. Interestingly, NPW is expressed in the stomach in addition to the CNS. The presence of NPW has been demonstrated in rat stomach antral cells and a decreased level of NPW has been reported in fasted animals; upon re-feeding, levels of NPW increase to normal in these animals (Mondal et al. 2006). NPW has been reported to lower blood leptin concentrations, with this mechanism possibly inferring the existence of an NPW-mediated regulation of energy homeostasis (Rucinski et al. 2007)

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