0013-7227/03/$15.00/0
Printed in U.S.A.

Endocrinology 144(11):4729–4733

doi: 10.1210/en.2003-0536

A Role for Neuropeptide W in the Regulation of Feeding Behavior

MUHTASHAN S. MONDAL, HIDEKI YAMAGUCHI, YUKARI DATE, TAKUYA SHIMBARA, KOJI TOSHINAI, YUKIO SHIMOMURA, MASAAKI MORI, AND MASAMITSU NAKAZATO

Department of Internal Medicine, Miyazaki Medical College (M.S.M., H.Y., Y.D., T.S., K.T., M.N.), Miyazaki 889-1692, Japan; and Discovery Research Laboratories, Pharmaceuticals Research Division, Takeda Chemical Industries Ltd. (Y.S., M.M.), Ibaraki 300-4293, Japan

Neuropeptide W (NPW) is a novel hypothalamic peptide that activates the previously described orphan G protein-coupled receptors, GPR7 and GPR8. Two endogenous molecular forms of NPW that consist of 23- and 30-amino acid residues were identified. The localization of GPR7 and GPR8 in some hypothalamic regions of primary importance in the regulation of feeding behavior has provided a springboard for investigation of the role of NPW in the central nervous system. In this study we examined the effects of NPW on feeding and energy expenditure in rats. Single intracerebroventricular (icv) administration of NPW23 and NPW30 to free-feeding rats suppressed dark phase and fasting-induced food intake at similar effective doses. Continuous icv infusion of NPW using an osmotic minipump suppressed feeding and body weight gain over the infusion period. Conversely, icv administration of anti-NPW IgG stimulated feeding. Furthermore, icv administration of NPW increased body temperature and heat production. These data raise the possibility that NPW functions as an endogenous catabolic signaling molecule in the brain. Further investigation of the biochemical and physiological functions of NPW will help us to better understand the hypothalamic regulation of energy homeostasis. (*Endocrinology* 144: 4729–4733, 2003)

GPR7 AND GPR8, two structurally related G protein-coupled receptors (GPCRs), were originally identified by the cloning of opioid-somatostatin-like receptor genes from human genomic DNA (1). GPR7 and GPR8 share 70% nucleotide and 64% amino acid identities with each other. Although orthologs of GPR7 and GPR8 have been isolated by PCR from many species, a rodent GPR8 has not been identified (2). In the rat brain GPR7 mRNA is expressed in the cortex, hippocampus, and some hypothalamic nuclei, including the paraventricular, supraoptic, ventromedial hypothalamic, dorsomedial hypothalamic, suprachiasmatic, and arcuate nuclei (2). Robust expression of GPR7 in the hypothalamus suggests that it may have a role in the modulation of neuroendocrine functions.

We have recently identified a novel neuropeptide, neuropeptide W (NPW), as an endogenous ligand for GPR7 and GPR8 (3). We isolated NPW from porcine hypothalamus using a cAMP accumulation inhibition assay in stable Chinese hamster ovary cell lines expressing human GPR8. cDNA sequences of prepro-NPW were determined in the swine, rat, and human, indicating that NPW is highly conserved among species (3). We identified two mature NPW peptides, NPW23 and NPW30, the former corresponding to the N-terminal region of NPW30. These two peptides are produced by proteolytic processing at two pairs of arginine residues. NPW is named for tryptophan residues at the N and C termini of NPW30. Synthetic NPW23 and NPW30 bind to and activate both GPR7 and GPR8 at similar effective doses.

Central administration of human NPW23 in rats increased serum levels of PRL and corticosterone and stimulated food intake and water drinking (3, 4). In contrast, GPR7 knockout mice were shown to be hyperphagic and became obese (5). To investigate the role of NPW in energy homeostasis, we examined the central effect of NPW23 and NPW30 on feeding by single intracerebroventricular (icv) injection. We also administered NPW23 by continuous icv infusion. We studied the effects of NPW on body temperature and oxygen consumption. Anti-NPW IgG was administered to examine endogenous NPW signaling. Here we report that central administration of NPW suppresses feeding, increases energy expenditure, and possibly plays a role in the central regulation of feeding behavior and energy balance.

Materials and Methods

Animals

Male Wistar rats (Charles River Japan., Inc., Shiga, Japan), weighing 300–350 g, were maintained in individual cages under controlled temperature (21–23 C) and light (lights on, 0800–2000) with *ad libitum* access to food and water. Cannulation and icv administration were performed essentially as previously described (6). Intracerebroventricular cannulas were implanted into the lateral cerebral ventricle under anesthesia by ip injection of sodium pentobarbital (Abbot Laboratories, Chicago, IL), and proper placement of the cannulas was verified at the end of the experiments by the administration of dye. Rats were sham-injected before the study and were weighed and handled daily. Only animals that showed progressive weight gain after the surgery were used in subsequent experiments. All experiments were repeated two or three times. NPW was dissolved in 0.9% saline, and 10 μl solution were administered icv to rats. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care.

Feeding experiments

Human NPW23 and NPW30 were chemically synthesized and purified (3). First, NPW23, NPW30 (1, 3, 5, and 7.5 nmol each), or saline was

Abbreviations: CTA, Conditioned taste aversion; GPC, G protein-coupled receptor; icv, intracerebroventricular; LiCl, lithium chloride; NPB, neuropeptide B; NPW, neuropeptide W; SCN, suprachiasmatic nucleus.

administered icv to free-feeding rats (n = 10/group) before the onset of dark phase (1945 h). The remaining food was weighed at intervals between 1–60 h after administration, as shown in Fig. 1, and food intake was calculated. NPW23 or NPW30 (5 nmol) was administered by icv injection at 0845 h to rats (n = 8) fasting for 10 h. NPW23 (2.5 nmol/25 μl saline·d for 5 d) or vehicle was administered to rats by continuous infusion through osmotic minipumps (n = 7/group). Cannulas implanted into the lateral ventricles were connected to minipumps (type 2001, Alzet, Cupertino, CA) inserted under the skin of the neck. Food consumption and body weight were measured daily at 0900 h.

Conditioned taste aversion (CTA) test

CTA assessment was performed as previously described (7). Briefly, rats were conditioned to 2-h daily access to water from two bottles for 3 d. On the fourth day, rats were given 0.15% saccharin for the 2-h period instead of water, and saccharin consumption was measured. Immediately afterward, five groups of rats (n = 10) were administered NPW23 (5 and 7.5 nmol, icv), saline (icv), lithium chloride (LiCl; Nacalai Tesque, Tokyo, Japan; 0.15 M, 2 ml/kg, ip), or saline (2 ml/kg, ip). LiCl was used as a positive control for the assessment of CTA. On the fifth day, rats were simultaneously presented saccharin and water for 2 h. Two-hour fluid consumption was measured.

Body temperature, oxygen consumption, and locomotor activity

The body temperature of the rats was measured every 15 min from 15 min before to 120 min after an icv administration of 5 nmol NPW23 or saline (n = 8/group) in the early light phase. A sensor tip (measurable range, 25–50 C; measuring error, ±0.02 C) was inserted into the rectum,

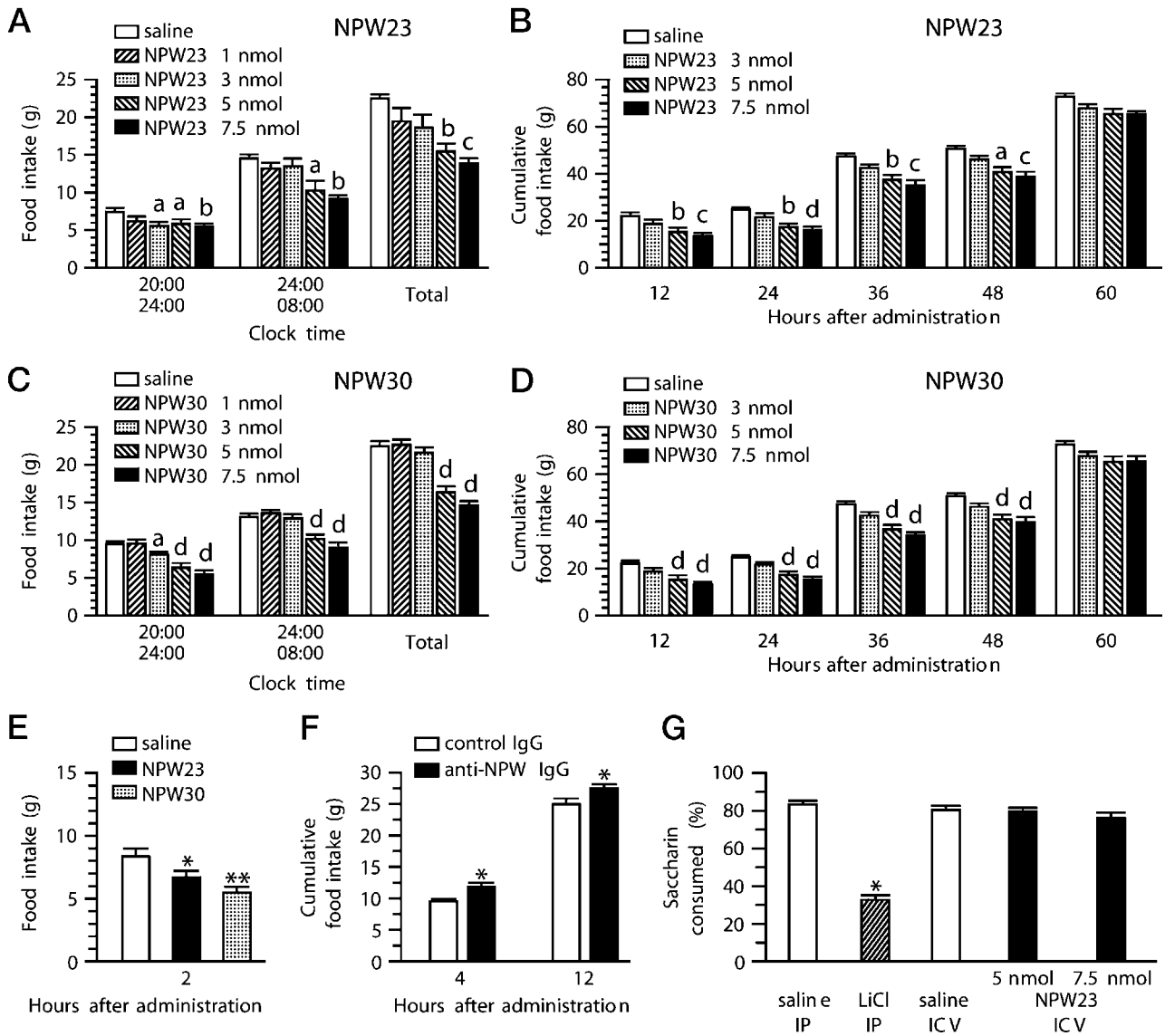

FIG. 1. Effects of NPW on feeding. A–D, Suppression of food intake in free-fed rats (n = 10/group) by icv administration of NPW23 (A and B) and NPW30 (C and D; 3, 5, and 7.5 nmol/10 μl) at 1945 h. Control rats were given 0.9% saline. The data in A and C represent food intake between the indicated times, and those in B and D represent cumulative food intake after icv administration. a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$; d, $P < 0.0001$ (*vs.* saline controls). E, Two-hour food intake of 10-h fasted rats (n = 8/group) receiving icv NPW23 (5 nmol) or NPW30 (5 nmol) at 0845 h. *, $P < 0.05$; **, $P < 0.005$ (*vs.* saline controls). F, Anti-NPW IgG increases dark phase food intake. Free-feeding rats (n = 10/group) received icv administration at 1800 h of 0.1 μg anti-NPW IgG or control IgG. Food intake was measured 4 and 12 h after the start of dark phase. *, $P < 0.01$ *vs.* control IgG. G, CTA test. Conditioned rats (n = 10/group) received icv administration of NPW (5 and 7.5 nmol) or saline and ip administration of LiCl or saline. *, $P < 0.001$ (*vs.* ip saline controls).

and the digital signal was transferred to Thermometer MT-1 (Senko Co. Ltd., Tokyo, Japan). Oxygen consumption and heat production were measured in other rats with an O_2/CO_2 Analyzer MM202R apparatus (Muromachi Co. Ltd., Tokyo, Japan) (8) in the dark phase. Rats (n = 5/group) were given 5 nmol NPW23 or saline, icv, and then individually returned to a sealed chamber with an air flow of 1.75 liters/min for 4 h. Thereafter, oxygen consumption and heat production were measured for 150 min. The locomotor activity of the rats was measured after icv administration of 5 nmol NPW23 or saline (n = 10/group) in the early light or dark phase using a Rat Locomotor Activity Recording Systems device (Muromachi Co. Ltd.) as described previously (9). We made locomotor activity counts every 15 min and summed them for the dark and light phases.

Immunoneutralization

To generate a monoclonal antibody against the N-terminal region of NPW, synthetic [^{14}Cys]human NPW-[1–13] was conjugated to porcine thyroglobulin with sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1 carboxylate (Sulfo-SMCC, Pierce Chemical Co., Rockford, IL). The antigenic conjugate was injected into mice. The resulting antibody recognized both human and rat NPW23 and NPW30, which differ by only one amino acid. The antibody was subjected to Affi-Gel Protein A affinity chromatography (Bio-Rad Laboratories, Hercules, CA). To analyze the effect of immunoneutralization of endogenous NPW on feeding, a 0.1 μg/10 μl saline solution of anti-NPW IgG or normal mouse IgG was administered icv at 1800 h to free-feeding rats (n = 10/group). Food intake was measured 4 and 12 h after IgG administration.

Statistical analysis

Groups of data (mean ± SEM) were compared using ANOVA and *post hoc* Fisher's test. $P < 0.05$ was considered significant.

Results

Feeding experiments

Intracerebroventricular administrations of both NPW23 and NPW30 significantly reduced the food intake of free-feeding rats in a dose-dependent manner (Fig. 1, A–D). Neither of these two peptides changed dark phase food intake in the first 1 or 2 h after administration (data not shown). Both peptides reduced feeding 4 h after administration. The anorectic effect of 3 nmol of either NPW23 or NPW30 was maintained for less than 12 h post injection, whereas that of 5 and 7.5 nmol of both peptides was maintained for 48 h post injection (Fig. 1). A higher dose (7.5 nmol) of both NPW reduced food intake relative to a dose of 5 nmol. In rats that had fasted for 10 h before injection, NPW reduced the 2-h food intake compared with that of the saline-injected group (Fig. 1E). NPW-treated rats were alert and showed no prominently unusual behavior relative to controls.

To determine whether endogenous NPW signaling is present in the brain, we investigated the effect of anti-NPW IgG on feeding behavior. This was done in a cross-over experiment in which all animals received a central injection of anti-NPW IgG or control IgG on separate days. In comparison with the control IgG, anti-NPW IgG significantly increased dark phase food intake at 4 and 12 h post injection in free-feeding rats (Fig. 1F).

Nonspecific, toxic, or aversive effects of NPW were ruled out by a CTA test. Saccharin intake was measured after the administration of NPW or LiCl, a toxin that causes rats to avoid saccharin. LiCl caused taste aversion, whereas NPW (5 and 7.5 nmol) did not reduce saccharin intake (Fig. 1G).

To determine the longer-term effect of NPW on feeding behavior, a chronic icv infusion of NPW (2.5 nmol/d) or saline solution was administered for 5 d using an osmotic minipump. Rats treated with NPW demonstrated significantly decreased food intake and body weight gain over the infusion period compared with control animals (Fig. 2).

Energy expenditure

To determine the effect of NPW on energy expenditure, rats were monitored for changes in body temperature, oxygen consumption, heat production, and locomotor activity after injection. Intracerebroventricular administration of NPW23 significantly increased body temperature from 15–90 min after injection (Fig. 3A). NPW administration also increased both oxygen consumption and heat production from 30–135 min after injection (Fig. 3, B and C). NPW did not affect locomotor activity in either the light or dark phase (dark phase, 93 ± 8% of vehicle-injected group; light phase, 97 ± 1%; not significant).

Discussion

Quantum leaps in our understanding of the mechanisms involved in energy homeostasis have resulted from the recent identification of neuronal circuits that control food intake. A large array of hypothalamic neuropeptides participates in the central regulation of feeding and energy expenditure (10–13). We undertook a systematic biochemical search for endogenous peptide ligands of multiple orphan GPCRs using a cell-based reporter system. These screening

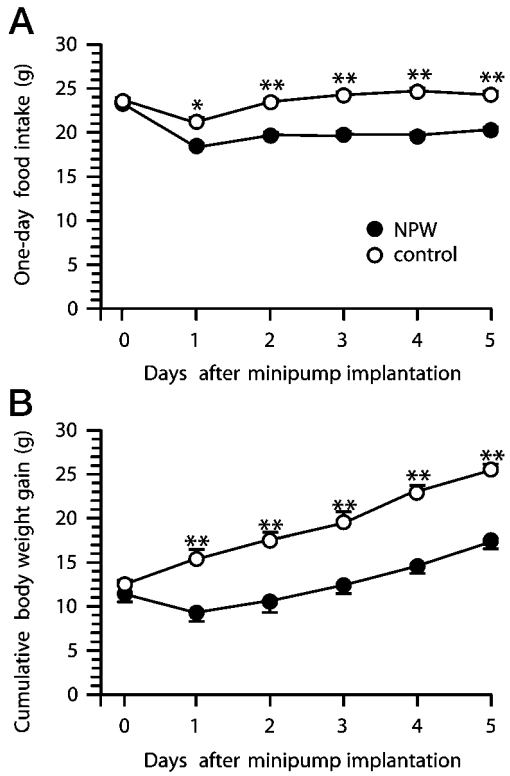

FIG. 2. Effect of chronic NPW23 icv administration on rats. One-day food intake (A) and cumulative body weight gain (B) during an icv infusion of 2.5 nmol/d for 5 d. Alzet minipumps were implanted on d 0. *, $P < 0.01$; **, $P < 0.0001$ (*vs.* saline controls).

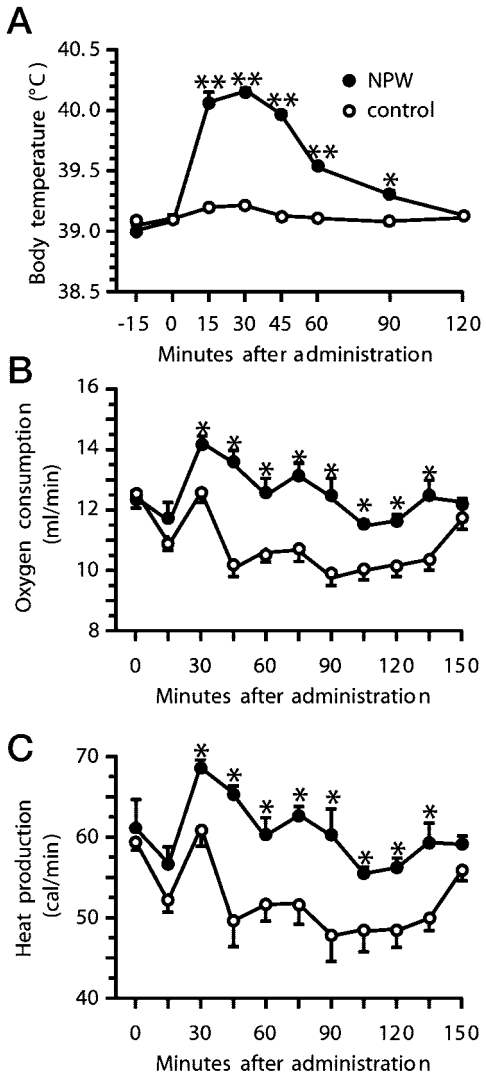

FIG. 3. Effects of icv administration of NPW23 (5 nmol) on energy expenditure. A, Body temperature was measured 15 min before 120 min after administration of NPW23 or saline to rats (n = 8/group) at 1000 h. *, $P < 0.05$; **, $P < 0.001$ (*vs.* saline controls). Oxygen consumption (B) and heat production (C) were measured 15 min before and 120 min after icv administration of NPW23 (5 nmol) or saline to rats (n = 5/group) at 1945 h. *, $P < 0.05$ (*vs.* saline controls).

experiments led to the identification of novel peptides that bind to previously described orphan GPCRs (14–17). We recently identified a novel peptide ligand for GPR7 and GPR8 from porcine hypothalamus. This peptide, called NPW, was isolated as two endogenous molecular forms, NPW23 and NPW30. Porcine hypothalamus has nearly equal amounts of NPW23 and NPW30, but it has not yet been determined which is the major molecular form of NPW in rat brain. GPR7, the target of NPW in the rat, is expressed in paraventricular and ventromedial hypothalamic nuclei as well as in the lateral part of the arcuate nucleus. These nuclei primarily function to reduce feeding behavior (2, 10–13). In this study we examined the effects of NPW23 and NPW30.

An icv administration of both NPW peptides dose-dependently suppressed dark phase (feeding phase) feeding. By contrast, NPW23 stimulated feeding during the light phase in rats (3, 4). NPW may have a diurnally divergent effect on feeding behavior. Such diurnal variation of feeding also has been shown for GHRH and norepinephrine (18, 19). There is a functional relationship between GHRH neurons and the suprachiasmatic nucleus (SCN), an area critical for the regulation of circadian rhythm. The diurnal variation in feeding behavior caused by GHRH is thought to be related to this circadian oscillation. The GPR7 receptors are robustly expressed in the SCN of the rat (2). This suggests that diurnal variation of feeding effects of NPW may be regulated by the SCN. The minimally active dose was 3 nmol of either NPW peptide. Anorectic potency and active duration of NPW23 and NPW30 are similar, which is consistent with the findings that they bound to and activated GPR7 at similar effective doses (3). Reduction of food intake after the administration of 5 and 7.5 nmol of both NPW23 and NPW30 was 20–38% at the time points examined, being moderate compared with other potent anorectic peptides. NPW23 and NPW30 also suppressed fasting-induced food intake. The persistent anorectic effect of NPW was evident in continuous icv infusion over a 5-d period. Body weight gain was also suppressed by NPW (2.5 nmol/d) infusion, but this effect was moderate compared with that of other anorectic peptides. The icv administration of anti-NPW IgG, in turn, stimulated feeding.

These findings together with the lack of an NPW-induced taste aversion at doses that reduce food intake suggest that NPW may be a homeostatic regulator of food intake. Occurring concomitant with a reduction of food intake after NPW administration are increased body temperature, oxygen consumption, and heat production, implying that NPW increases energy expenditure. NPW did not affect locomotor activity, suggesting that oxygen consumption increased by NPW is not related to locomotor activity. NPW produces weight loss by decreasing food intake while at the same time revving up metabolic rates. Metabolic heat production can be increased by either shivering or sympathetic excitation in brown adipose tissue and skeletal muscle. The latter mechanism, also called chemical thermogenesis, appears to be the primary mechanism of NPW-induced thermogenesis, because shivering was not observed in rats administered NPW. Based on our data, NPW possibly meets the criteria for a catabolic signaling molecule.

Recently, two research groups discovered another neuropeptide, named neuropeptide B (NPB), as an endogenous ligand for GPR7 (20, 21). NPB consists of 29 amino acids with bromine modification at the N-terminal tryptophan. NPB is 61% identical to NPW. Rat NPB mRNA was detected in the ventromedial hypothalamic nucleus and lateral hypothalamic area, which are implicated in the regulation of feeding. The distribution of NPW-expressing neurons in the rat brain has not yet been elucidated; however, Tanaka *et al.* (21) recently reported that mouse NPW-expressing neurons are expressed in periaqueductal gray matter, ventral tegmental area, Edinger-Westphal nucleus, and dorsal raphe nucleus by *in situ* hybridization. The presence of two peptide ligands for GPR7 will stimulate further research into the neuroendocrine and brain functions of the GPR7 system. Additional studies would be necessary to determine the role of GPR7 in the response to administration of NPW.

Feeding is finely and redundantly regulated by the com-

plicated interaction of many orexigenic and anorectic signals produced in the brain and peripheral tissues. Central NPW may serve as an endogenous catabolic signaling molecule in the regulation of energy homeostasis. The identification and localization of NPW-producing neurons, the neuronal network by which the NPW system exerts its effects, and biochemical mechanisms governing the biosynthesis and release of this peptide are fascinating areas requiring future studies. Further investigations of NPW functions will help our understanding of weight control mechanisms and should facilitate the study of eating disorders.

Acknowledgments

Received April 28, 2003. Accepted August 1, 2003.

Address all correspondence and requests for reprints to: Masamitsu Nakazato, M.D., Ph.D., Third Department of Internal Medicine, Miyazaki Medical College, Kiyotake, Miyazaki 889-1692, Japan. E-mail: nakazato@post.miyazaki-med.ac.jp.

This work was supported in part by grants-in-aid from the 21st Century Center of Excellence Program of the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

References

1. **O'Dowd BF, Scheideler MA, Nguyen T, Cheng R, Rasmussen JS, Marchese A, Zastawny R, Heng HH, Tsui LC, Shi X** 1995 The cloning and chromosomal mapping of two novel human opioid-somatostatin-like receptor genes, GPR7 and GPR8, expressed in discrete areas of the brain. Genomics 28:84–91
2. **Lee DK, Nguyen T, Porter CA, Cheng R, George SR, O'Dowd BF** 1995 Two related G protein-coupled receptors: the distribution of GPR7 in rat brain and the absence of GPR8 in rodents. Mol Brain Res 71:96–103
3. **Shimomura Y, Harada M, Goto M, Sugo T, Matsumoto Y, Abe M, Watanabe T, Asami T, Kitada C, Mori M, Onda H, Fujino M** 2002 Identification of neuropeptide W as the endogenous ligand for orphan G-protein-coupled receptors GPR7 and GPR8. J Biol Chem 277:35826–35832
4. **Baker JR, Cardinal K, Bober C, Taylor MM, Samson WK** 2003 Neuropeptide W acts in brain to control prolactin, corticosterone, and growth hormone release. Endocrinology 144:2816–2821
5. **Ishii M, Fei H, Friedman JM** 2003 Targeted disruption of GPR7, the endogenous receptor for neuropeptides B and W, leads to metabolic defects and adult-onset obesity. Proc Natl Acad Sci USA 100:10540–10545
6. **Ida T, Nakahara K, Murakami N, Nakazato M** 1999 Effects of lateral cerebroventricular injection of the appetite-stimulating neuropeptides, orexin and neuropeptide Y, on the various behavioral activities of rats. Brain Res 821: 526–529
7. **Howard AD, Wang R, Pong SS, Mellin TN, Strack A, Guan XM, Zeng Z, Williams Jr DL, Feighner SD, Nunes CN, Murphy B, Stair JN, Yu H, Jiang Q, Clements MK, Tan CP, McKee KK, Hreniuk DL, McDonald TP, Lynch KR, Evans JF, Austin CP, Caskey CT, Van der Ploeg LH, Liu Q** 2000 Identification of receptors for neuromedin U and its role in feeding. Nature 406: 70–74
8. **Nakazato M, Hanada R, Murakami N, Date Y, Mondal MS, Kojima M, Yoshimatsu H, Kangawa K, Matsukura S** 2000 Central effects of neuromedin U in the regulation of energy homeostasis. Biochem Biophys Res Commun 277:191–194
9. **Murakami N, Marumoto N, Nakahara K, Murakami T** 1997 Daily injections of melatonin entrain the circadian activity rhythms of nocturnal rats but not diurnal chipmunks. Brain Res 775:240–243
10. **Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra PS** 1999 Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr Rev 20:68–100
11. **Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG** 2000 Central nervous system control of food intake. Nature 404:661–667
12. **Spiegelman BM, Flier JS** 2001 Obesity and the regulation of energy balance. Cell 104:531–543
13. **Ahima RS, Osei SY** 2001 Molecular regulation of eating behavior: new insights and prospects for therapeutic strategies. Trends Mol Med 7:205–213
14. **Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S** 2001 A role for ghrelin in the central regulation of feeding. Nature 409:194–198
15. **Kojima M, Haruno R, Nakazato M, Date Y, Murakami N, Hanada R, Matsuo H, Kangawa K** 2000 Purification and identification of neuromedin U as an endogenous ligand for an orphan receptor GPR66 (FM3). Biochem Biophys Res Commun 276:435–438
16. **Mori M, Sugo T, Abe M, Shimomura Y, Kurihara M, Kitada C, Kikuchi K, Shintani Y, Kurokawa T, Onda H, Nishimura O, Fujino M** 1999 Urotensin II is the endogenous ligand of a G-protein-coupled orphan receptor, SENR (GPR14). Biochem Biophys Res Commun 265:123–129
17. **Shimomura Y, Mori M, Sugo T, Ishibashi Y, Abe M, Kurokawa T, Onda H, Nishimura O, Sumino Y, Fujino M** 1999 Isolation and identification of melanin-concentrating hormone as the endogenous ligand of the SLC-1 receptor. Biochem Biophys Res Commun 261:622–626
18. **Feifel D, Vaccarino FJ** 1989 Feeding effects of growth hormone-releasing factor in rats are photoperiod sensitive. Behav Neurosci 103:824–830
19. **Margules DL, Lewis MJ, Dragovich JA, Margules AS** 1972 Hypothalamic norepinephrine: circadian rhythms and the control of feeding behavior. Science 178:640–642
20. **Fujii R, Yoshida H, Fukusumi S, Habata Y, Hosoya M, Kawamata Y, Yano T, Hinuma S, Kitada C, Asami T, Mori M, Fujisawa Y, Fujino M** 2002 Identification of a neuropeptide modified with bromine as an endogenous ligand for GPR7. J Biol Chem 277:34010–34016
21. **Tanaka H, Yoshida T, Miyamoto N, Motoike T, Kurosu H, Shibata K, Yamanaka A, Williams SC, Richardson JA, Tsujino N, Garry MG, Lerner MR, King DS, O'Dowd BF, Sakurai T, Yanagisawa M** 2003 Characterization of a family of endogenous neuropeptide ligands for the G protein-coupled receptors GPR7 and GPR8. Proc Natl Acad Sci USA 100:6251–6256