Neuropeptide W is present in antral G cells of rat, mouse, and human stomach

M S Mondal, H Yamaguchi, Y Date, K Toshinai, T Kawagoe, T Tsuruta, H Kageyama[1], Y Kawamura[1], S Shioda[1], Y Shimomura[2], M Mori[2] and **M Nakazato**

Department of Internal Medicine, Miyazaki Medical College, University of Miyazaki, Kiyotake, Miyazaki 889-1692, Japan

[1]Department of Anatomy, Showa University School of Medicine, Tokyo 142-8555, Japan

[2]Discovery Research Laboratories, Pharmaceuticals Research Division, Takeda Chemical Industries, Ibaraki 300-4293, Japan

(Requests for offprints should be addressed to M Nakazato; Email: nakazato@med.miyazaki-u.ac.jp)

Abstract

Neuropeptide W (NPW) is a 30-amino-acid peptide initially isolated from the porcine hypothalamus as an endogenous ligand for the G protein-coupled receptors GPR7 and GPR8. An intracerebroventricular administration of NPW increased serum prolactin and corticosterone concentrations, decreased dark-phase feeding, raised energy expenditure, and lowered body weight. Peripherally, GPR7 receptors are abundantly expressed throughout the gastrointestinal tract; the presence of NPW in the gastrointestinal endocrine system, however, remains unstudied. Using monoclonal and polyclonal antibodies raised against rat NPW, we studied the localization of NPW in the rat, mouse, and human stomach by light and electron microscopy. NPW-immunoreactive cells were identified within the gastric antral glands in all three species. Double immunohistochemistry and electron-microscopic immunohistochemistry studies in rats demonstrated that NPW is present in antral gastrin (G) cells. NPW immunoreactivity localized to round, intermediate-to-high-density granules in G cells. NPW-immunoreactive cells accounted for 90% chromagranin A- and 85% gastrin-immunoreactive endocrine cells in the rat gastric antral glands. Using reversed-phase HPLC coupled with enzyme immunoassays specific for NPW, we detected NPW30 and its C-terminally truncated form, NPW23, in the gastric mucosa. Plasma NPW concentration of the gastric antrum was significantly higher than that of the systemic vein, suggesting that circulating NPW is derived from the stomach. Plasma NPW concentration of the gastric antrum decreased significantly after 15-h fast and increased after refeeding. This is the first report to clarify the presence of NPW peptide in the stomachs of rats, mice, and humans. In conclusion, NPW is produced in gastric antral G cells; our findings will provide clues to additional mechanisms of the regulation of gastric function by this novel brain/gut peptide.

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Introduction

Two structurally related G protein-coupled receptors (GPCRs), GPR7 and GPR8, were identified by cloning opioid-somatostatin-like receptor genes from human genomic DNA (O'Dowd *et al.* 1995). GPR7 and GPR8 share 70% nucleotide and 64% amino acid identity (O'Dowd *et al.* 1995). While orthologs of GPR7 and GPR8 have been found in multiple species by PCR, a rodent GPR8 has yet to be identified (Lee *et al.* 1995). Rat GPR7 mRNA is widely expressed throughout the brain, including the paraventricular, supraoptic, ventromedial hypothalamic, dorsomedial hypothalamic, suprachiasmatic, and arcuate nuclei (Lee *et al.* 1995). This abundant GPR7 expression in the hypothalamus suggests a role in the modulation of neuroendocrine function. Recently, we isolated an endogenous peptide ligand for GPR7 and GPR8, named neuropeptide W (NPW), from the porcine hypothalamus using a cAMP accumulation inhibition assay of Chinese hamster ovary cell lines stably expressing human GPR8 (Shimomura *et al.* 2002). Using the porcine cDNA sequence for prepro-NPW, we isolated swine, rat, and human cDNA fragments, indicating that NPW is highly conserved among species (Shimomura *et al.* 2002). Human NPW differs from the rat form by only one amino acid at position 17. Two mature NPW peptides have been identified, NPW23 and NPW30, the former corresponding to a C-terminal truncation of NPW30. NPW23 and NPW30 are produced by proteolytic processing at two pairs of arginine residues at positions 24 and 25, and 31 and 32. NPW is named after the tryptophan residues (single-letter code W) at the

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N- and C-termini of NPW30. Synthetic NPW23 and NPW30 both bind to and activate GPR7 and GPR8 at similar effective doses. A single intracerebroventricular (icv) administration of NPW23 to rats increased serum corticosterone levels and augmented water drinking (Baker *et al.* 2003). We showed that icv administration of either NPW23 or NPW30 to free-feeding rats suppressed dark-phase and fasting-induced food intake at similar effective doses (Mondal *et al.* 2003*b*). Continuous icv infusion of NPW23 for 5 days using an osmotic minipump suppressed feeding and body-weight gain over the infusion period. Conversely, icv administration of anti-NPW IgG increased food intake. Icv administration of NPW increased body temperature and heat production. GPR7-deficient mice are hyperphagic and develop obesity (Ishii *et al.* 2003). These results indicate that NPW may function as an endogenous catabolic signaling molecule in the brain.

Several gut/brain peptides exhibit diverse effects that include the regulation of feeding via the gut/brain axis in addition to gastrointestinal functions. GPR7 is robustly expressed in various peripheral tissues of rats, including the stomach and intestine (Fuji *et al.* 2002). NPW mRNA is expressed in the stomach, but a cellular source has yet to be identified (Tanaka *et al.* 2003). In this study, we investigated the cellular source of NPW in the rat, mouse, and human stomach by immunohistochemistry, immunohistochemical double-staining, and immunoelectron microscopy. Characterization of the NPW molecular forms present in the stomach was performed by reversed-phase HPLC (RP-HPLC) coupled with enzyme immunoassay (EIA). We determined plasma NPW concentrations in the gastric antral vein and systemic vein. Finally, we studied the effects of fasting and high-lipid diet and high-carbohydrate diet on plasma NPW concentration. Here, we report that the NPW-producing cells are the gastrin (G) cells in the gastric antrum.

Materials and Methods

Animals

Male Wistar rats, weighing 250 – 300 g (Charles River Japan, Shiga, Japan), were used in all the experiments. Animals were given standard laboratory chow and water *ad libitum*. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of the Miyazaki Medical College, University of Miyazaki.

Peptide synthesis

Human NPW23 and NPW30, rat NPW23 and NPW30, human [[14]Cys]NPW-[1–13], human [[1]Cys]NPW-[11–23], and rat [[1]Cys]NPW-[16–30] were synthesized using an ABI 433A peptide synthesizer (Applied Biosystems, Foster City, CA, USA) following an Fmoc (*N*-(9-fluorenyl)methoxycarbonyl) strategy. Amino acid sequences of human and rat NPW are shown in Fig. 1. After de-blocking all the protecting groups by treatment with a mixture of trifluoroacetic acid (TFA)/thioanisole/*m*-cresol/tri-isopropylsilane/1,2-ethane-dithiol (85:5:5:2·5:2·5), peptides were purified by RP-HPLC. Cys-extended peptides were used for the immunizations described below. The validity of the synthesis was confirmed by amino acid analysis, sequencing, and spectrometric analysis.

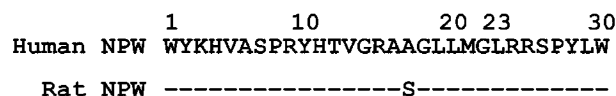

Figure 1 Alignment of the amino acid sequences of human and rat NPW. Numbers indicate amino acid positions from the N-terminus. Dashed lines indicate same amino acids.

Preparation and characterization of antisera

To obtain monoclonal antibodies recognizing the N-terminal region of NPW, the C-terminal region of NPW23, and the C-terminal region of NPW30, we conjugated human [[14]Cys]NPW-[1–13] and human [[1]Cys]NPW-[11–23] with porcine thyroglobulin and rat [[1]Cys]NPW-[16–30] to keyhole limpet hemocyanin using sulfosuccinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1 carboxylate (Sulfo-SMCC; Pierce Chemical Co., Rockford, IL, USA). BALB/c mice (female, 6–8 weeks old) were immunized with each immunogen. The spleen cells isolated from the immunized mice 3 days after intravenous immunogen injection were fused with mouse myeloma cells, P3X63Ag8·653, as described previously (Suzuki *et al.* 1989). Three monoclonal antibodies, ANPW-N (IgG1, κ) for the N-terminal region of NPW, ANPW23-C (IgG1, κ) for the C-terminal region of NPW23, and ANPW30-C (IgG2b, κ) for the C-terminal region of NPW30, were purified from ascites fluids using an immobilized protein A column (Seikagaku Co., Tokyo, Japan). To raise polyclonal anti-NPW antisera, synthetic human [[14]Cys]NPW-[1–13] was conjugated to maleimide-activated mariculture keyhole limpet hemocyanin (Sulfo-SMCC). The antigenic conjugate solution (1·5–3 ml) was administered to New Zealand white rabbits. The resulting rabbit antihuman NPW-[1–13] antiserum recognized both human NPW23 and NPW30. The antisera exhibited 100% cross-reactivity with rat NPW23 and NPW30.

EIA procedure for NPW23 and NPW30

The ANPW23-C and ANPW30-C antibodies were immobilized to 96-well microtest plates. ANPW-N was

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conjugated to horseradish peroxidase (HRP) as described previously (Ichimori *et al.* 1987); the HRP-labeled ANPW-N was used to detect NPW23 and NPW30 trapped by immobilized antibody. The two-site EIA for NPW was performed essentially as described by Ichimori *et al.* (1987) with the following minor modification. The immobilized antibody plates were prepared by adding 20 μg/ml of either ANPW23-C or ANPW30-C followed by 300 μl Block Ace (Snow Brand Milk Products Co., Sapporo, Japan). Rat NPW23 and NPW30 standard peptides or unknown test samples in 100 μl buffer C (20 mM phosphate buffer (pH 7·0), 1% BSA, 0·4 M NaCl, and 2 mM EDTA) were added to the antibody-immobilized plates and incubated at 4 °C for 16 h. After washing with PBS, plates were treated with 100 μl HRP-labeled ANPW-N at a 1000-fold dilution in buffer C at 4 °C for an additional 16 h. After washing with PBS, bound enzyme activity was measured using a TMB microwell peroxidase system (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA).

Reverse transcription PCR (RT-PCR) for NPW

Total RNA was extracted from the mucosal and muscular layers of antral stomachs of three Wistar rats using the acid guanidinium thiocyanate/phenol/chloroform (AGPC) method (Chomczynski & Sacchi 1987). First-strand cDNA was synthesized from 2·5 μg RNA with 7 μM oligo-(dT)18 primer and ReverTra Ace-α-™ reverse transcriptase (Toyobo Co., Osaka, Japan). The resulting cDNA was subjected to PCR amplification using 2 μM of each sense and antisense primer and 2·5 units Pyrobest DNA polymerase (Takara Shuzo Co., Shiga, Japan). PCR primers for NPW were 5′-CCAACCTGAGCAGTCG CTAAG-3′ (sense) and 5′-TCGGTTCTTGAGACGGT CGT-3′ (antisense), corresponding to nucleotide numbers 486 − 506 and 636 − 655 (Shimomura *et al.* 2002). PCR was conducted in a 25 μl reaction volume for 35 cycles, comprising denaturation for 5 s at 94 °C, annealing for 10 s at 65 °C, and extension for 1 min at 72 °C. PCR products were electrophoresed on a 2% agarose gel (FMC BioProducts, Rockland, ME, USA).

Quantification of immunoreactive NPW in rat stomach

Immediately after decapitation, stomachs were resected from ten 12-week-old male Wistar rats fed *ad libitum*. The whole gastric antrum and the mucosal layer from the glandular part of the stomach were removed from five and ten rats, respectively. Tissues were heated at 95 − 100 °C for 10 min in a 10-fold volume of water to inactivate intrinsic proteases. After cooling to 4 °C, ethanoic acid and HCl were added to final concentrations of 1 M and 20 mM, respectively. The tissues were then homogenized in a Polytron for 10 min. Homogenates were centrifuged at 11 500 ***g*** for 30 min. After applying the supernatants to Sep-Pak C-18 cartridges (Waters, Milford, MA, USA), bound peptides were eluted in 60% acetonitrile containing 0·1% TFA. The antrum eluates were quantified by EIAs for NPW23 and NPW30. Some portions of the stomach mucosa eluates were quantified by two EIAs, while the remaining portions were subjected to RP-HPLC. All HPLC fractions were quantified by EIAs. Authentic rat NPW23 and NPW30 were chromatographed by the same HPLC system.

Quantification of plasma NPW in the gastric antral vein and systemic vein, after a 15 h fast

For the determination of NPW30 concentration in the systemic vein and gastric antral vein, three groups of rats which had been fed *ad libitum* ($n=9$), fasted for 15 h from 19:00 to the next morning at 10:00 ($n=9$) or fasted for 15 h (19:00–10:00) and then refed for 2 h ($n=9$) were studied. The stomach was exposed after anesthesia with sodium pentobarbital. The gastroesophageal and the gastroduodenal junctures were ligated by grade 4·0 silk. Blood was obtained from the gastric antral vein. Since the plasma volume obtained from the gastric antrum of one rat was 30 μl, plasma samples from three rats were pooled for the determination of NPW. The plasma of the jugular vein was obtained from the same rats. The plasma samples were diluted 1:1 with 0·9% saline and applied to Sep-Pak C-18 cartridges pre-equilibrated with 0·9% saline. The adsorbed peptides were eluted with a 60% acetonitrile solution containing 0·1% TFA, and then subjected to EIA for NPW30.

Effects high-lipid diet and high-carbohydrate diet on plasma NPW concentration

Rats were first fed standard laboratory diets for 7 days. They next were given high-lipid diet (40·1% kcal derived from carbohydrate, 34·7% kcal derived from fat, and 25·2% derived from protein; KBT Oriental Co., Saga, Japan) or high-carbohydrate diet (65·2% kcal derived from carbohydrates, 9·6% kcal derived from fat, and 25·2% kcal derived from protein; $n=7$ per group) for 2 weeks. Plasma samples from the gastric antral vein of rats fed *ad libitum* were pooled and then subjected to EIA for NPW30.

Light-microscopic immunostaining

Three Wistar rats were anesthetized by intraperitoneal injection of sodium pentobarbital (75 mg/kg body weight). Animals were then perfused transcardially for 10 min with 100 ml 0·1 M phosphate buffer (pH 7·4) containing heparin (100 U/100 ml), then for an additional 15 min with 150 ml fixative containing 4% paraformaldehyde. After removal, rat stomachs were postfixed with

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fixative for 24 h at 4 °C, and then incubated for 24 h in 0·1 M PBS (pH 7·4) containing 30% sucrose. Glandular stomachs were quickly frozen in dry ice and stored at –80 °C. Mouse gastric antrums (n=3) were obtained from C57B6/J mice. Human gastric antrums were obtained at autopsy from three patients who had died of cardiovascular disease. Written consent from the deceased relatives was taken prior to the removal of tissues and the study was approved by the Ethics Committee of Human Research of Miyazaki Medical College. Samples were cut serially at –20 °C with a cryostat into slices 7 µm thick, then thaw-mounted on silane-coated slides and stored at –80 °C. Serial sections were treated with 0·3% hydrogen peroxide to inactivate endogenous peroxidase activity, and then incubated with normal goat serum to block non-specific binding. These sections were incubated overnight at 4 °C with anti-NPW-[1–13] antiserum (final dilution 1:5000), anti-NPW antiserum (ANPW-N; final dilution 1:5000), anti-chromogranin A antiserum (DAKO Corp., Glostrup, Denmark; final dilution 1:500), anti-gastrin antiserum (DAKO Corp.; final dilution 1:5), anti-somatostatin antiserum (DAKO Corp.; final dilution 1:200), or anti-serotonin antiserum (DAKO Corp.; final dilution 1:5). All sections were stained using the avidin–biotin complex method described previously (Date *et al.* 2000). We examined three immunostained slides for analytical purposes. Control studies were done with normal rabbit serum or anti-NPW-[1–13] antiserum that had been pre-absorbed with 10 µg rat NPW.

Electron-microscopic immunohistochemistry

Three Wistar rats were perfused with 2% paraformaldehyde and 2·5% glutaraldehyde in 0·1 M PBS. Electron microscopy imunnohistochemistry was performed as described previously (Date *et al.* 2000) with minor modifications. Stomachs were excised and fixed with the above fixative overnight at 4 °C, then postfixed at 4 °C for 90 min with 1% osmium tetroxide in 0·1 M PBS. Samples were then dehydrated using a graded ethanol series and embedded in Epon. Ultrathin sections of the specimens were cut and treated for 30 min with 5% sodium metaperiodate. Samples were immersed for 10 min in 5% normal goat serum in PBS supplemented with 1% BSA, then incubated overnight at 4 °C with rabbit polyclonal anti-NPW-[1–13] antiserum (dilution 1:5000). Next, sections were incubated with 8 nm colloidal gold-conjugated anti-rabbit goat IgG (Jackson British Bio Cell International, Cardiff, UK; dilution 1:50) and counterstained with uranyl acetate and lead citrate. As controls, anti-NPW antiserum was either omitted or replaced by normal rabbit serum. Sections were examined using an Hitachi H-7000 electron microscope (Ibaraki, Japan). The size of 200 NPW-containing granules was measured.

Figure 2 Representative agarose gel showing the RT-PCR product corresponding to NPW mRNA amplified from the mucosal layer of the rat gastric antrum.

Results

RT-PCR amplification of NPW transcript

Using NPW-specific primers, we amplified an RT-PCR product corresponding to the predicted size of the NPW transcript (347 bp) from an RNA sample derived from the mucosal layer of the rat stomach, but did not detect it in the sample from the muscle layer (Fig. 2).

Identification of NPW molecules and NPW level in rat gastric antrum

RP-HPLC, coupled with two separate EIAs, was used to analyze NPW molecules from the rat gastric mucosa (Fig. 3). In the EIA for NPW23 we detected one major peak,

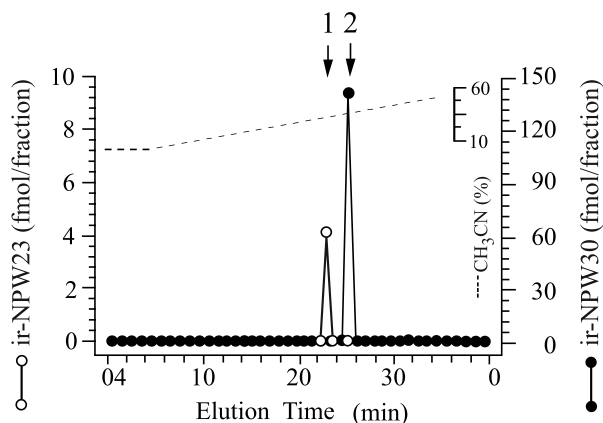

Figure 3 Representative RP-HPLC profile of NPW23 and NPW30 immunoreactivities in rat stomach. Gastric mucosa (50 mg) was subjected to chromatography on a TSK ODS SIL 120A column (4·6 × 150 mm). RP-HPLC was performed for 40 min at 1·0 ml/min with a linear gradient of 10–60% acetonitrile (CH_3CN) containing 0·1% TFA. Arrows indicate the elution positions of authentic NPW23 (1) and NPW30 (2). NPW23 and NPW30 values are represented by the left and right scales, respectively.

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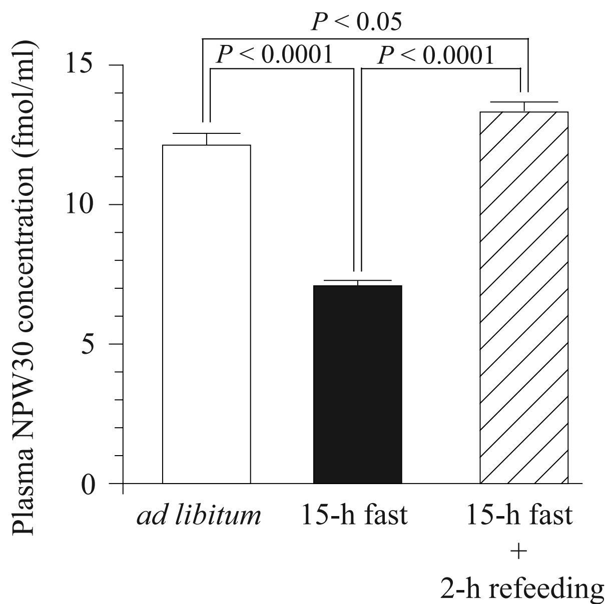

Figure 4 Plasma NPW30 concentrations in the gastric antrum in rats fed *ad libitum*, fasted for 15 h, or fasted for 15 h and then fed for 2 h.

eluting at a position consistent with authentic NPW23 (peak 1). EIA for NPW30 also revealed one major NPW-immunoreactive peak eluting at position consistent with authentic NPW30 (peak 2). Respective contents of NPW23 and NPW30 in the rat gastric antrum were 32·3 ± 0·8 and 142·7 ± 14·7 fmol/g tissue weight (means ± S.E., n=3).

Plasma NPW concentrations after 15-h fast and high-lipid and high-carbohydrate diet

The plasma concentration of NPW30 in the gastric antral vein in rats fed *ad libitum* was 12·0 ± 0·4 fmol/ml and that in the systemic vein 0·42 ± 0·7 fmol/ml. Plasma NPW30 concentration of the gastric antrum decreased significantly after fast and increased upon refeeding (Fig. 4). No significant difference in plasma NPW30 concentration of the gastric antrum was observed between rats fed with high-lipid diet and with high-carbohydrate diet for 2 weeks (high-fat, 9·6 fmol/ml, versus high-carbohydrate, 9·7 fmol/ml).

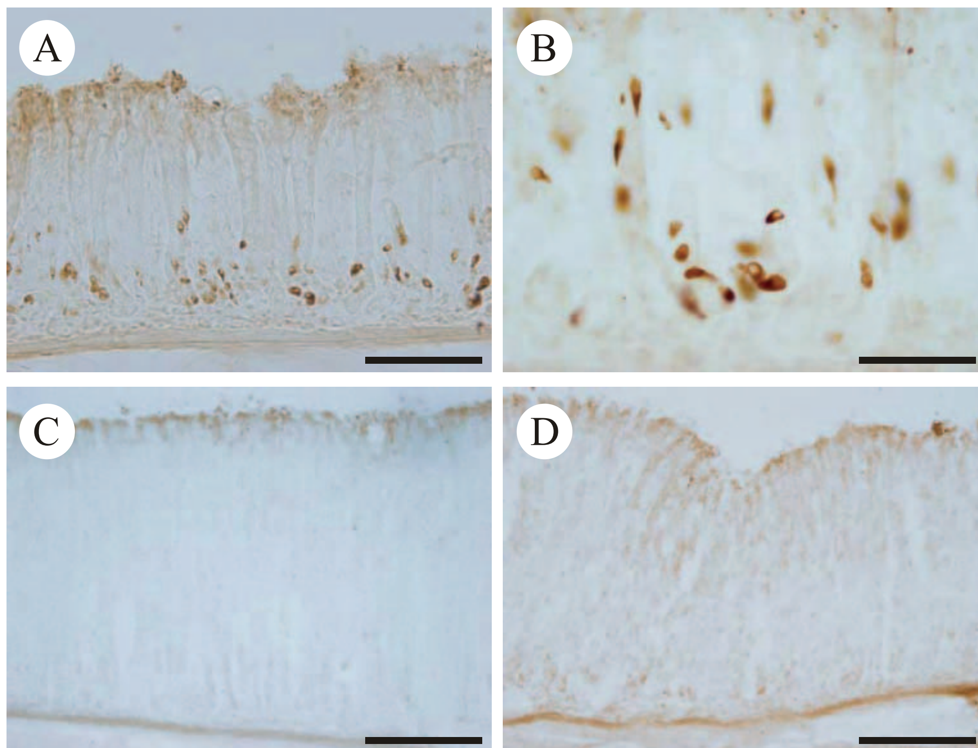

Figure 5 Immunohistochemical localization of NPW in the rat stomach. (A) NPW-immunoreactive cells in the gastric antrum. Scale bar, 100 μm. (B) Higher magnification of NPW cells. Scale bar, 50 μm. (C) No NPW-immunoreactive cells in the gastric fundus. (D) Absorption test. (C, D) Scale bars, 100 μm.

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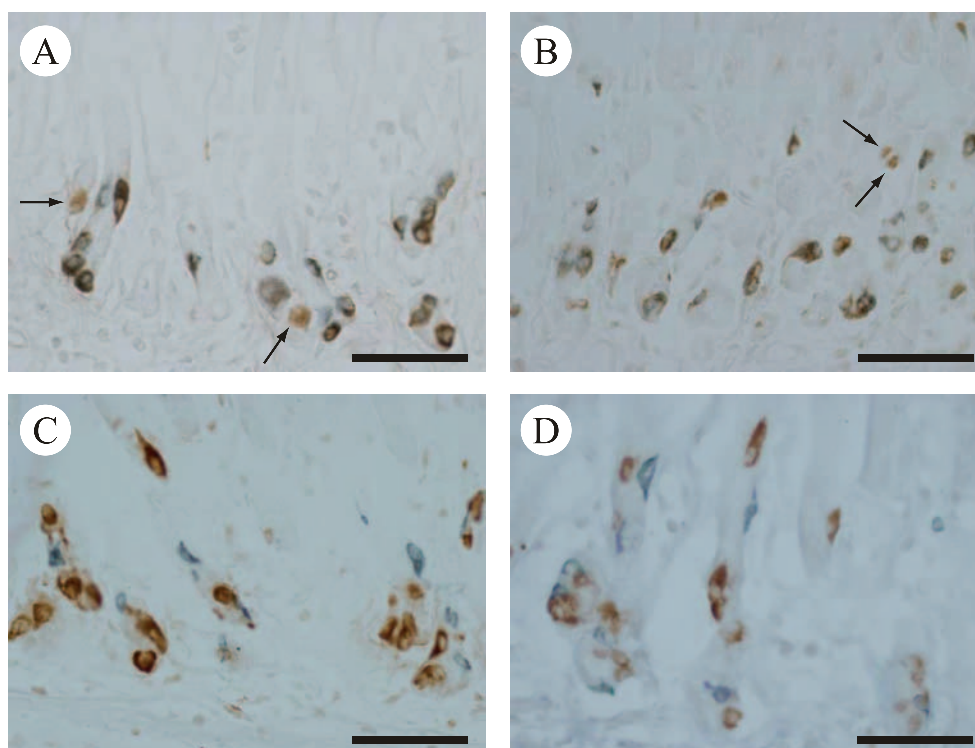

Figure 6 Immunohistochemical localization of NPW in the rat gastric antrum. NPW (blue-black in A and B; brown in C and D) co-localizes with (A) chromogranin A (brown) and (B) gastrin (brown), but not with (C) somatostatin (blue-black) or (D) serotonin (blue-black). The majority of (A) chromogranin A cells and (B) G cells have NPW immunoreactivity, whereas some chromogranin A cells and G cells do not have it, as indicated by the arrows. Scale bars, 50 µm.

Immunohistochemistry

NPW-immunoreactive cells were scattered throughout the mucosal layer of rat gastric antrum (Fig. 5A and B). NPW cells were present in the basal part of the mucosal layer. No NPW-positive cells, however, could be detected in the gastric fundus (Fig. 5C). No specific immunoreactivity was observed in the rat gastric antrum when either normal rabbit serum or antiserum pre-absorbed with excess NPW was used (Fig. 5D). Double staining of NPW-immunoreactive cells in the rat gastric antrum is shown in Fig. 6. NPW-immunoreactive cells accounted for 90% (90 of 100) of chromogranin A-immunoreactive cells (Fig. 6A) and for 85% (85 of 100) of gastrin-immunoreactive endocrine cells (Fig. 6B), but did not have somatostatin or serotonin immunoreactivity (Fig. 6C and D). NPW immunoreactivity was also present in the mouse gastric antrum (Fig. 7A and B). NPW-immunoreactive cells were abundant in the middle layer of the mucosa of the human antral glands (Fig. 7C). NPW immunoreactivity was found in 89% (89 of 100) of human antral G cells (Fig. 7D). No specific immunoreactivity was observed in the mouse or human gastric antrum when normal rabbit serum or antisera pre-absorbed with excessive NPW was used (data not shown).

Immunoelectron microscopy

Immunoelectron microscopy showed the typical morphology of granules in rat antral G cells (Fig. 8A). Immunogold particles indicative of NPW immunoreactivity were observed in multiple, round, intermediate-to-high-density granules (Fig. 8B). The storage granules were clustered at the cell base and 189 ± 14 nm (mean ± S.E.) in diameter.

Discussion

Using cell-based reporter systems, searches for endogenous ligand for orphan GPCRs have led to the discovery of multiple novel peptides (Kalra *et al.* 1999, Schwartz *et al.* 2000, Spiegelman & Flier 2001, Ahima & Osei 2001). NPW was isolated from the porcine hypothalamus as a peptide ligand for the heretofore called ‘orphan’

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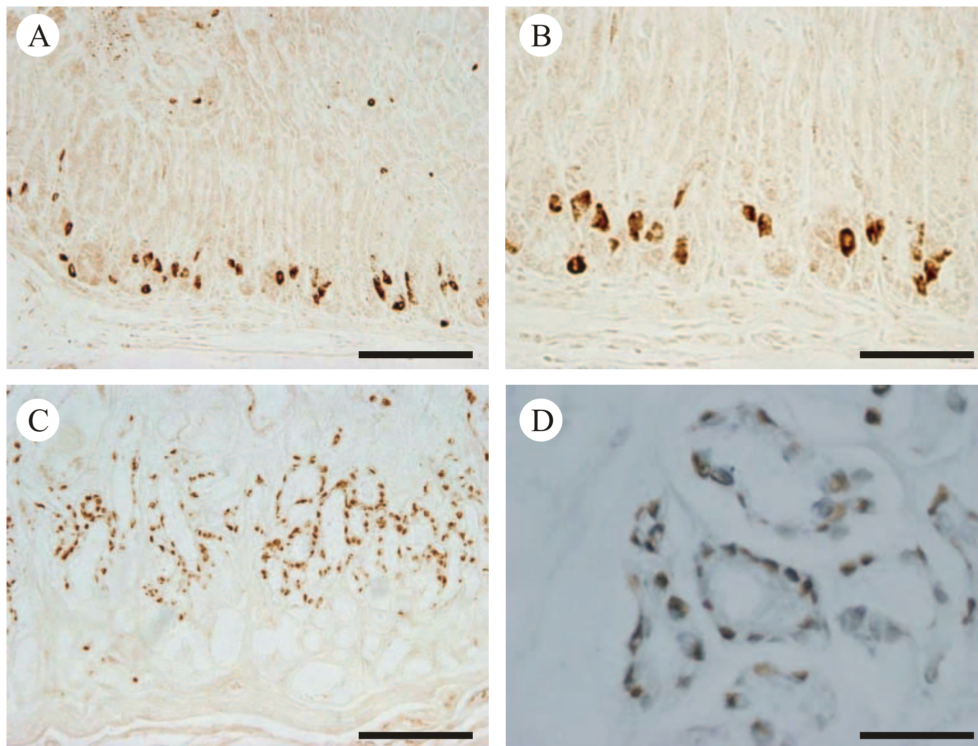

Figure 7 Immunohistochemical localization of NPW in the mouse and human gastric antrum. (A) NPW-immunoreactive cells in mouse gastric antrum. Scale bar, 100 μm. (B) Higher magnification of (A). Scale bar, 50 μm. (C) NPW-immunoreactive cells in human gastric antrum. Scale bar, 100 μm. (D) NPW (blue-black) co-localizes with gastrin (brown) in human antrum. Scale bar, 50 μm.

GPCRs, GPR7 and GPR8, using this application system (Shimomura *et al.* 2002). Two mature endogenous molecular forms of NPW, NPW23 and NPW30, were identified (Shimomura *et al.* 2002). Central administration of NPW suppressed dark-phase and fasting-induced feeding, increased energy expenditure, and augmented serum prolactin and corticosterone (Baker *et al.* 2003, Mondal *et al.* 2003*b*). Chronic administration of NPW decreased food intake and caused weight loss (Mondal *et al.* 2003*b*). These findings suggest that NPW may play a role in feeding behavior and energy homeostasis. Gut/brain peptides involved in the regulation of feeding behavior, such as somatostatin, cholecystokinin, orexin-A, neuromedin U, and ghrelin, also contribute to the regulation of gastric acid secretion, gastric mucosal cell proliferation, and neural regulation of the enteric nervous system (Polak *et al.* 1975*a*, 1975*b*, Moran and McHugh 1982, Aponte *et al.* 1984, Sakurai *et al.* 1998, Kirchgessner & Liu 1999, Date *et al.* 2000, Nakazato *et al.* 2001, Wren *et al.* 2002, Mondal *et al.* 2003*a*). Rat GPR7 mRNA is expressed in the stomach and intestine (Tanaka *et al.* 2003), but the presence of NPW in the gastrointestinal tract has yet to be identified. Our RT-PCR analysis revealed that NPW mRNA is present in the rat gastric antrum. Using the combination of two EIAs with HPLC, we first detected NPW23 and NPW30 in the rat stomach mucosa. Our present study showed that the plasma NPW concentration of the gastric antrum was higher than that of the systemic vein, suggesting that circulating NPW is derived from the stomach. Plasma NPW concentration did not change after high-lipid diet or high-carbohydrate diet, suggesting that dietary nutrients do not influence NPW secretion from the gastric antrum. Plasma NPW concentration of the gastric antrum decreased upon fast and increased after refeeding. An icv administration of NPW to rats decreased food intake and body-weight gain (Mondal *et al.* 2003*b*). Further investigations are needed to clarify the functional relationship between central and peripheral NPW in the regulation of feeding.

We identified NPW-immunoreactive endocrine cells in the rat, mouse, and human stomachs. NPW-immunoreactive cells are abundant in the mucosal layer of the antrum in these three species. The antral mucosa has roughly equal numbers of three endocrine cell types – G cells, enterochromaffin cells (EC), and somatostatin (D) cells – which respectively secrete gastrin, serotonin, and somatostatin (Solcia *et al.* 1987, Walsh 1994). We showed that 85% of NPW-immunoreactive cells were identifiable as antral G cells. G cells are most abundant in the rat gastric antrum mucosa where they may number $5 \times 10^5/cm^2$

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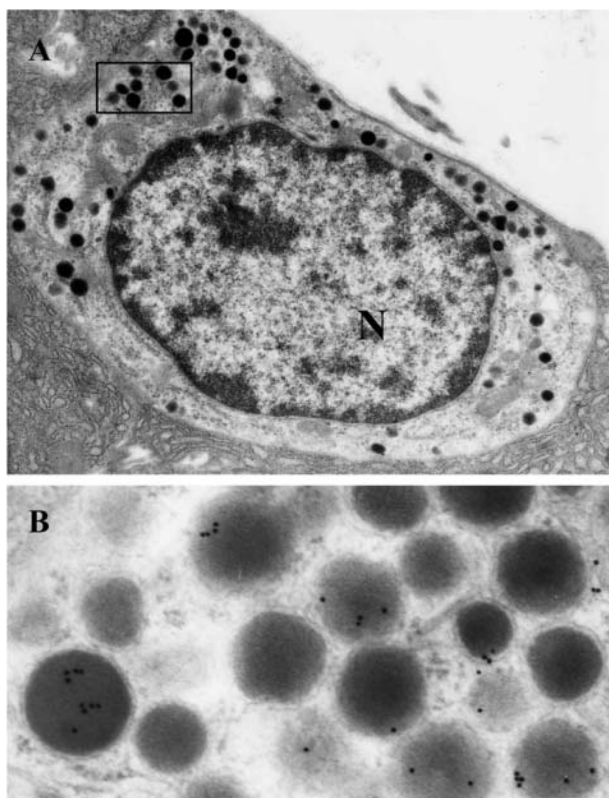

Figure 8 Representative immunoelectron micrographs of NPW cells in rat gastric antral gland. (A) The antral gastrin G cell contains multiple, round, intermediate-to-high-density granules. N, nucleus. Scale bar, 0·5 µm (original magnification ×7000). (B) Higher magnification of (A). Granules in the cytoplasm are labeled with immunogold staining for NPW. Scale bar, 100 nm (original magnification ×36 000).

(Fawcett 1994). The G cells are flask-shaped cells with a varying distribution throughout the mucosal levels of different species (Solcia *et al.* 1987). We observed that G cells predominate in the basal part of the mucosal glands in rats, and in contrast they are found in the middle layer of the mucosa in humans. This study showed that NPW immunoreactivity is present in multiple, round, intermediate-to-high-density granules within G cells. G cells express an impressive number of peptides, including polypeptide Y, neurotensin, vasointestinal peptide, ACTH, thyrotropin-releasing hormone, pro-enkephalin-gene-derived peptides, xenopsin, and human chorionic gonadatrophin α (Sundler & Håkanson 1991, Capella *et al.* 1991). The physiological significance of expression of many peptides in G cells is presently less well understood. The functional significance of co-expression of NPW with gastrin is unclarified.

In our on-going study, one finding showed that an intraperitoneal administration of NPW to rats increased the gastric acid secretion. Additional studies would be necessary to determine the role of NPW in the regulation of motility of the gut wall, gastric acid secretion, and the renewal of the gut epithelium.

G cells, which also have NPW, belong to the 'open' type cells which have apical cytoplasmic extensions that project to the glandular lumen with short microvilli and pinocytotic vesicles (Fujita & Kobayashi 1977). This structure is regarded as representing the anatomical basis for the cell response to physical and/or chemical variations of the gastric contents. The secretory granules in 'open' type cells usually exhibit clear-cut polarization in the basal cytoplasmic pole or in basal cytoplasmic processes. NPW-containing granules are clustered at the cell base. In response to vagal stimulation or distension of the stomach, G cells secrete gastrin, a peptide hormone that stimulates gastric motility and acid secretion from the oxyntic cells. Gastrin also acts on the stem cells in the gastric glands to stimulate cell proliferation and differentiation. NPW30 was found in the gastric vein circulation. Identification of NPW immunoreactivity within G cells suggests a feasible mechanism for delivering NPW to other tissues that express GPR7. The morphological features of NPW-immunoreactive cells suggest that NPW may function in an endocrine fashion.

Future determination of NPW peptide and mRNA contents in the stomach under various physiological and pathophysiological conditions should provide additional information concerning the mechanisms by which the biosynthesis and secretion of this peptide are governed. The findings presented here will help establish new ways to clarify the additional, as yet undefined, physiological functions of this novel brain/gut hormone, NPW.

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