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ORIGINAL ARTICLE

Central neuropeptide W has anorexigenic effect in rats

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Summary

Neuropeptide W (NPW) is produced in neurons located in hypothalamus, brain stem and antral G cells and its receptors are present in the hypothalamus, in particular in the paraventricular nucleus (PVN). There are two forms of the peptide, designated as neuropeptide W-23 (NPW23) and neuropeptide W-30 (NPW30). Neuropeptide W is an endogenous ligand for G-protein-coupled receptor, GPR7 and GPR8 receptors (R), which in humans are expressed in the hypothalamus and probably involved in the control of energy homoeostasis and neuroendocrine axes. We conducted this study to investigate the effects of NPW on feeding intake and energy expenditure in Wistar rats. Systemic (icv) injection of both forms of neuropeptide W (NPW23 and NPW30) to *ad libitum* feeding Wistar rats decreased dark feeding and fasting-induced feeding. One week of systemic treatment with NPW23 decreased feeding intake and weight gain during the treatment period. On the other hand, systemic treatment with antineuropeptide W antibody increased feeding intake. Moreover, systemic treatment with neuropeptide W-23 raised body temperature and consequently thermogenesis. These results strongly suggest that neuropeptide W may play an important central role in the feeding intake and energy balance control in mammals.

Keywords NPW, feeding intake, heat production, body temperature

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Introduction

Feeding control requires a complicated interaction of multiple orexigenic and anorectic signals produced in the brain and peripheral tissues. GPR7 and GPR8 are structurally related orphan G-protein-coupled receptors and expressed in the central nervous system, and their endogenous ligands have been recently identified. The Japanese researchers (Shimomura et al., 2002; Mondal et al., 2006) have purified the peptide ligand from porcine hypothalamus and antral G cells of rat, mouse and human stomach and cloned the cDNA encoding its precursor protein. They found that cDNA encodes two forms of the peptide ligand with lengths of 23 and 30 amino acid residues as mature peptides and these two ligands were designated as neuropeptide W-23 (NPW23) and neuropeptide W-30 (NPW30). The shorter form being the N-terminal sequence of the larger peptide (Lee et al., 2002; Shimomura et al., 2002). The amino acid identity of these two peptides is highly conserved among human, porcine, rat and mouse (Brezillon et al., 2003). Synthetic NPW23 and NPW30 activated and bound to both GPR7 and GPR8 at similar doses. It is reported that NPW23 is slightly more potent than NPW30 in binding to GPR7 and GPR8 (Shimomura et al., 2002).

GPR8 is absent in rodents, in which it is replaced by a GPR8-like receptor (Lee et al., 1999). Earlier studies have revealed that GPR7 and GPR8 expressed mainly in brain suggest that the endogenous ligands for the two receptors have multiple roles in the central nervous system. Neuropeptide W immunoreactive cells have been detected in the hypothalamus, hypophyseal gland of rodents and gastric antrum of rodents, mouse and human (Dun et al., 2003; Tanaka et al., 2003; Mondal et al., 2006). These findings indicate that neuropeptide W is the endogenous ligand for both GPR7 and GPR8 and acts as a mediator of the central control of feeding and the neuroendocrine system (Shimomura et al., 2002). Human GPR7 highly resembles human GPR8, with an amino acid identity of 64% (O'Dowd et al., 1995; Siok et al., 2005). Among various families of GPCRs, GPR7 and GPR8 share high similarity to the opioid and somatostatin receptor families.

Moreover, icv injections of synthetic NPW23 stimulated appetite in rats, suggesting the central action of the peptide (Levine et al., 2005). On the other hand, GPR7 or GPR8 knockout mice were shown to be hyperphagic (Ishii et al., 2003). This work was conducted to elucidate the role of neuropeptide W (both forms; NPW23 and NPW30) in feeding intake through performing central administration the neuropeptide W. Moreover, we studied whether the NPW plays a role in energy expenditure by measuring body temperature and gross locomotor activity. Additionally, we elucidated the effect of anti-NPW IgG on food intake to confirm the role of endogenous NPW.

Materials and methods

Animals

The study was performed on male Wistar rats (Shiga, Japan). Rats (weighing 250–300 g) were housed in cages with wire mesh bottoms, at normal room temperature (23 ± 1 °C) and kept under a regimen of 12-h light and 12-h darkness (lights on at 07:00 h) with *ad libitum* access to food and water.

Intracerebroventricular cannulation

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (Abbott Laboratories, North Chicago, USA; 45 mg/kg b.wt.) and were then mounted in a Narusige brain stereotaxic instrument (Narusige, Tokyo). A stainless steel cannula (guide cannula, 23 gauge; insert, 27 gauge; outer diameter, OD: 550 μm) was then implanted into the lateral left ventricle. The cannula tip was placed at stereotaxic coordinates: 8 mm anterior to interaural; 1.5 mm lateral to the midline; 3.0 mm below the dura. The guide cannula was anchored to the skull with machine screws and dental acrylic. After surgery, animals were housed individually and allowed to recover for 4 days before injection of NPW (Peptide Institute, Osaka, Japan). Rats were saline-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 rats were handled daily to habituate and minimize any stress. Neuropeptide W was dissolved in 0.9% saline, and 10 μl of solution was administered icv to rats using 100 μl Hamilton syringe. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care. At the end of the experiments, proper placement of the cannulae was verified by administering Evans Blue dye (10 μl), followed by sacrifice and brain sectioning (20-μm intervals). Data for rats lacking dye in the lateral left ventricle were excluded from the analysis.

Feeding intake and body weight gain measurement

Neuropeptide W was purchased from Peptide Institute, Osaka, Japan. Both neuropeptide W forms (NPW23 and NPW30) were of human type. The doses of NPW (NPW23 or NPW30) used were 2, 4, 6 and 8 nmol each. Control group was administered saline and all animals were free-feeding (n = 8/group) and administered icv before the beginning of dark period (1845 h). Feeding intake was measured at 4 and 12 h from administration after weighing the remaining food at the time of measurement. Cumulative food intake was measured at 12, 24, 36, 48 and 60 h after administration. Moreover, both forms of NPW (NPW23 or NPW30) (6 nmol each) were icv administered at 0745 h fasted for 10 h (n = 8) and feeding intake was measured 2 h after administration. NPW23 at a dose of 3 nmol/10 μl saline for long term (1 week) or saline was administered to Wistar rats (n = 8). Feeding intake and cumulative body weight gain were measured everyday at 0800 h for 1 week. To confirm the role of endogenous NPW in feeding control, 0.2 μg/10 μl saline solution of antineuropeptide W IgG or normal mouse IgG was administered icv at 1800 h to free-feeding rats (n = 8/group). Cumulative feeding intake was measured four and 12 h after IgG administration.

We performed conditioned taste aversion (CTA) test according to the method described previously by Howard et al. (2000). Shortly, the experimental rats were allowed to 3 h daily access to water from three bottles for 5 days. On the sixth day, rats were given 0.15% saccharin for the 3-h period instead of water, and saccharin consumption was measured. Immediately thereafter, five groups of rats (n = 8) were administered NPW23 (6 and 8 nmol, icv), saline (icv), lithium chloride (Nacalai Tesque, Osaka, Japan; 0.15 M, 2 ml/kg, ip) or saline (2 ml/kg, ip). Lithium chloride was used as a positive control for the estimation of conditioned taste aversion (CTA) test (lithium chloride, a toxin that causes rats to avoid saccharin). On the 7th day, two-hour fluid consumption was measured after rats were simultaneously presented saccharin and water for 2 h.

Preparation and characterization of antisera

To produce a monoclonal antibody against the N-terminal region of neuropeptide W, synthetic [14Cys] human NPW-[1–13] was bound to porcine thyroglobulin

with sulfosuccinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1 carboxylate (Seikagaku, Tokyo, Japan). The antigenic bounded product was injected into mice. The obtained 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 analyse the effect of immunoneutralization of endogenous neuropeptide W on feeding, a 0.2 μg/10 μl saline solution of anti-NPW IgG or normal mouse IgG was administered icv at 1800 h to free-feeding rats (n = 8/group). At 4 and 12 h after IgG icv injection, feeding intake was measured.

Measurement of energy expenditure parameters

Measurement of gross locomotor activity

The gross locomotor activity of the rats was measured using a Rat Locomotor Activity Recording Systems device (Muromachi, Tokyo, Japan) comprising infrared sensors, an interface and a computer (Marumato et al., 1996; Murakami et al., 1997) after icv administration of 6 nmol NPW23 or saline (n = 8/group) in the early light (30 lux) or dark phase. After injection, the rats were immediately returned to the individual cages. The infrared sensors were placed above the cages and measured all locomotions such as eating and movement around within the cage. Each cage with its infrared sensor was placed in an isolated chamber box with a controlled light/dark cycle. The data were collected at 15-min intervals and analysed by CompactACT AMS software (Muromachi). Gross locomotor activity counts every 15 min and then summed them for each the dark and light phases.

Measurement of oxygen consumption and heat production

An O_2/CO_2 Analyzer MM202R apparatus (Muromachi, Tokyo, Japan) (Nakazato et al., 2000) was used to measure oxygen consumption and heat production in rats during the dark phase. A 6 nmol NPW23 or saline was administered to rats (n = 8/group) icv and then individually returned to a sealed chamber with an air flow of 1.5 litres/min for 4 h. Then, oxygen consumption and heat production were measured for 2 h.

Measurement of body temperature

Thermometer MT-1 (Senko, Tokyo, Japan) was used to measure the body temperature of the experimental animals as well as rats. The sensor tip (measurable range, 25–50 °C; measuring error, ± 0.02 °C) was inserted into the rectum, and the digital signal was transferred to Thermometer MT-1. The body temperature was measured every 15 min from 15 min before icv administration to 2 h after an icv administration of 6 nmol NPW23 or saline (n = 8/group) in the early light phase.

Statistical analysis

All results are expressed as means ± SEM. Data were analysed by analysis of variance and the *post hoc* Fisher's test. A difference with p value less than 0.05 was considered statistically significant.

Results

Effect of NPW on food intake

Two types of feeding intake were measured: dark feeding intake at 4 and 12 h after administration and cumulative feeding intake at 12, 24, 36, 48 and 60 h after administration as shown in both Figs 1 and 2. The obtained results revealed that central injection of neuropeptide W significantly decreased feeding intake in *ad libitum* feeding rats. Central injection of neuropeptide W-23 (NPW23) and neuropeptide W-30

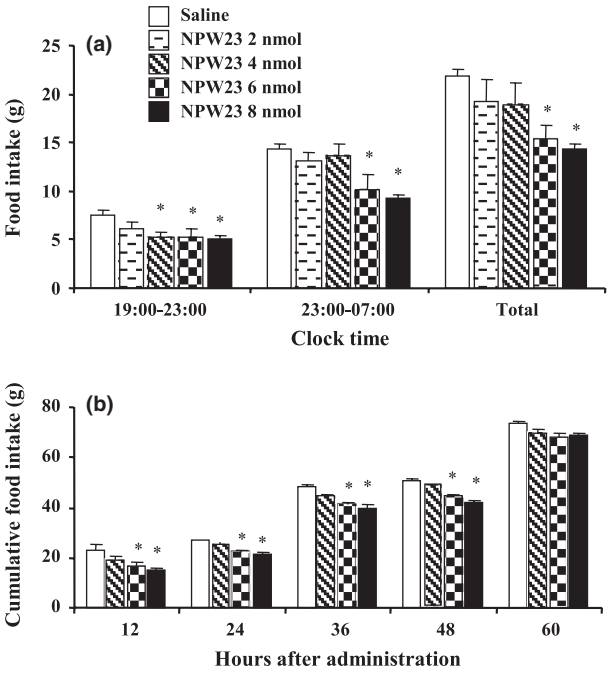

Fig. 1 Effect of NPW23 on (a) feeding intake and (b) cumulative feeding intake in free-feeding rats (n = 8/group) by icv administration of NPW23 (2, 4, 6 and 8 nmol/10 μl) at 1845 h. Control rats were given 0.9% saline. The data in A represent feeding intake between the time indicated and those in B represent cumulative feeding intake after icv administration. *p < 0.05 (vs. saline controls).

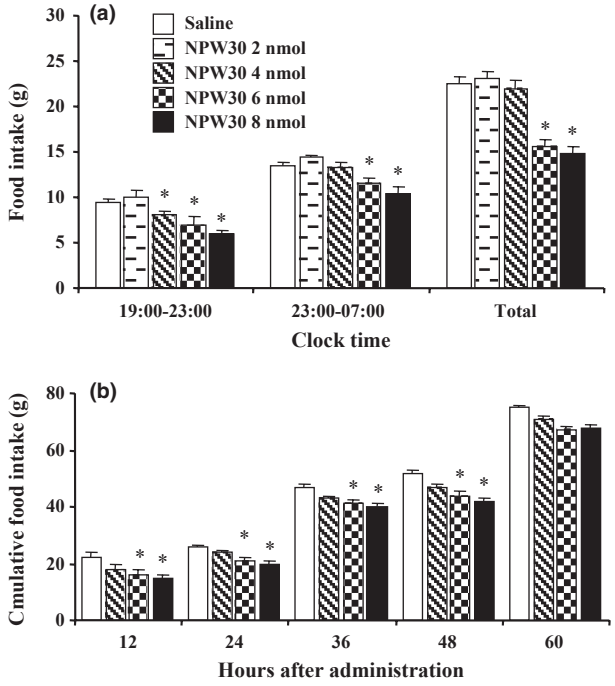

Fig. 2 Effect of NPW30 on (a) feeding intake and (b) cumulative feeding intake in free-feeding rats (n = 8/group) by icv administration of NPW30 (2, 4, 6 and 8 nmol/10 μl) at 1845 h. Control rats were given 0.9% saline. The data in A represent feeding intake between the time indicated and those in B represent cumulative feeding intake after icv administration. *p < 0.05 (vs. saline controls).

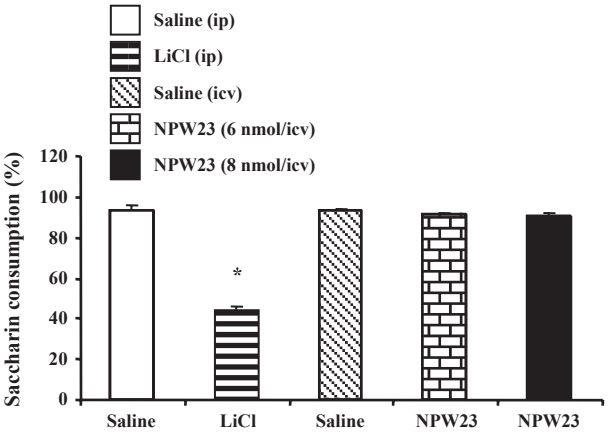

Fig. 3 Conditioned taste aversion test, conditioned rats (n = 8/group) received icv administration of NPW (6 and 8 nmol) or saline and ip administration of lithium chloride or saline. *p < 0.001 (vs. ip saline controls).

(NPW30) decreased feeding intake dose dependently (Figs 1a and 2a respectively). Also both peptides did not affect feeding intake at a dose of 2 nmol or less. However, the two peptides (NPW23 and NPW30) decreased the feeding intake 4 h after central injection (Figs 1a and 2a). Also, the significant anorectic effect of both forms of NPW at 4 nmol was only observed at 12 h post-injection, while at 6 nmol and 8 nmol, the effect was existed till 48 h post-injection (Figs 1b and 2b). To determine whether neuropeptide W-23 reduced eating effect was specific, we performed a conditioned taste aversion test. The obtained results revealed that lithium chloride caused taste aversion (rats avoided saccharin); however, NPW23 (6 and 8 nmol) did not reduce saccharin intake (did not cause taste aversion), indicating that NPW's effect on eating may in fact be specific (Fig. 3).

In group that was fasted for 10 h before injection, both forms of NPW (NPW23 and NPW30) significantly decreased feeding intake (Fig. 4a). To confirm the role of endogenous NPW in feeding control, a group of rats was injected centrally with 0.2 μg/10 μl saline solution of anti-NPW IgG or normal control IgG. The results revealed that anti-NPW IgG significantly stimulated feeding intake both at four and 12 h after administration (Fig. 4b). We conducted an experiment to investigate the effect of chronic or long-term (for 1 week) central injection of NPW23 (3 nmol/day or saline at 0800 h). The results revealed a significant decrease in feeding intake and cumulative body weight gain (Figs 5a and 5b respectively) in treated group along the injection period in comparison with saline-injected group.

Effect of NPW on gross locomotor activity

Icv administration of NPW23 did not affect gross locomotor activity (95 $\pm$ 9.5% of control group; 99 $\pm$ 1.9% of treated group).

Effect of NPW on body temperature, oxygen consumption and heat production

Icv administration of NPW23 significantly increased body temperature (Fig. 6a). There were no signs of disturbance observed on the animals due to body temperature increasing effect of NPW23. Also icv administration of NPW23 significantly increased oxygen consumption (Fig. 6b) and heat production (Fig. 6c) up to 120 min after administration.

Discussion

Currently, over 40 neuropeptide precursors are known, and the definition of neuropeptides has extended. Neuropeptides are peptides or fragments of peptides, which are synthesized in cells via large

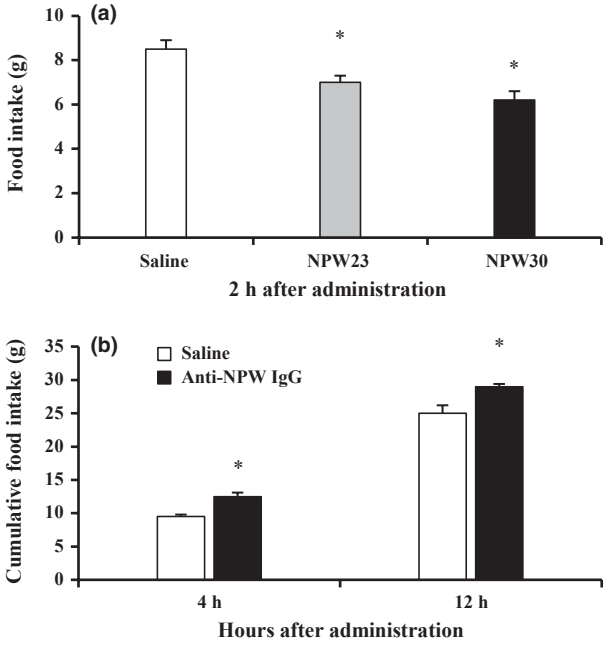

Fig. 4 (a) Effect of NPW23 and NPW30 on 2-h feeding intake in rats after 10-h fasting (n = 8/group) receiving icv NPW23 (6 nmol) or NPW30 (6 nmol) at 0745 h. *p < 0.05 (vs. saline controls). (b) Effect of anti-NPW IgG on dark-phase feeding intake. Free-feeding rats (n = 8/group) received icv administration at 1745 h of 0.2 μg anti-NPW IgG or control IgG. Feeding intake was measured four and 12 h after the start of dark phase. *p < 0.01 (vs. control IgG).

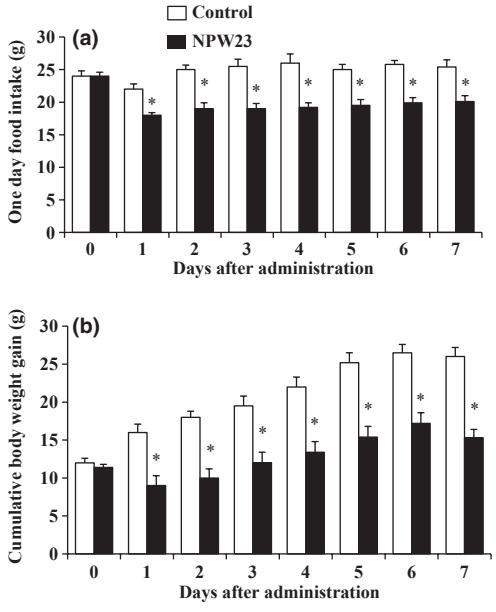

Fig. 5 Effect of long-term injection (1 week) of NPW23 at 3 nmol/10 μl saline/day for 1 week on (a) One-day feeding intake and (b) cumulative body weight gain. *p < 0.01 (vs. saline controls).

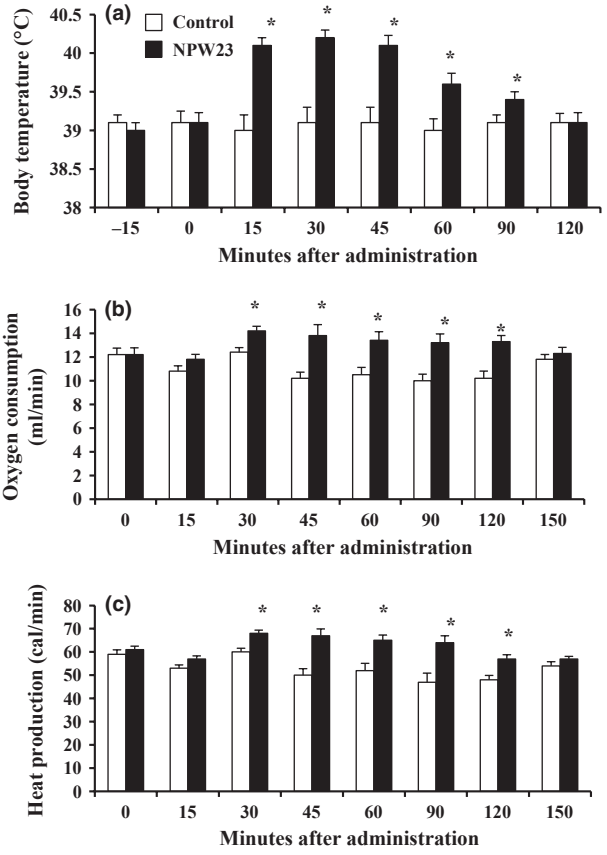

Fig. 6 Effects of icv administration of NPW23 (6 nmol) or saline on (a) body temperature, (b) oxygen consumption and (c) heat production (n = 8/group). *p < 0.05 (vs. saline controls).

inactive precursor proteins. There are many hypothalamic neuropeptides participate in the central regulation of feeding and energy expenditure (Howard et al., 2000; Ahima and Osei, 2001; Date et al., 2010). Recently, a Japanese researcher identified a novel peptide ligand for GPR7 and GPR8 from porcine hypothalamus, and this peptide called NPW has two endogenous forms, NPW23 and NPW30. Moreover, GPR7, the target of NPW in the rat, is expressed in many hypothalamic central nuclei as paraventricular and ventromedial as well as in the arcuate nucleus, which plays important roles in feeding regulation (Lee et al., 1999; Schwartz et al., 2000; Ahima and Osei, 2001; Takenoya et al., 2010). We here investigated whether NPW has a role in feeding intake control in rodents.

Regarding our results, we investigated the effect of neuropeptide W-23 and neuropeptide W-30 on dark period feeding as the central injection of both forms of neuropeptide W was performed at 1900 h. We found that both forms of neuropeptide W significantly decreased feeding intake during dark period. Earlier

studies revealed that NPW increased appetite on light time in rodents (Lee et al., 2002; Baker et al., 2003; Beck et al., 2010; Jennifer and Stephen, 2012). Our results and the earlier results suggest that both forms of NPW may have divergent action on feeding intake (suppress dark feeding and stimulate light feeding). Lee et al. (1999), and Uchio et al. (2009) have reported that GPR7 receptors are expressed in suprachiasmatic nucleus (SCN) that is responsible for circadian rhythm. This important finding suggests that NPW has diurnal effects on feeding that is controlled by suprachiasmatic nucleus.

Lee et al. (2002) reported that both NPW23 and NPW30 bind to and activate the target receptors (GPR7) at similar effective doses explaining why the suppressing effect of both NPW23 and NPW30 are of the same potency. Also, our results revealed that both forms of NPW have suppressed feeding intake either in free-feeding rats or in fasting-induced rats. Moreover, our results revealed that long-term central injection of NPW23 for 1 week suppressed both feeding intake and body weight gain. In addition, we found that central injection of anti-NPW IgG stimulated appetite. These findings together with the earlier studies (Mondal et al., 2003; Levine et al., 2005; Niimi and Murao, 2005; Taylor et al., 2005; Date et al., 2010) suggest that NPW may play an important role in feeding intake control.

Regarding to whether NPW plays a role in energy expenditure, we investigated the effect of NPW23 on gross locomotor activity, body temperature, oxygen consumption and heat production in free-feeding Wistar rats. Central injection of NPW23 increased thermogenesis (increase in body temperature, heat production and oxygen consumption) without affecting gross locomotor activity. These results suggest that NPW23 stimulates energy expenditure without affecting locomotor activity. It seems that NPW23 decreased weight gain by decreasing feeding intake and stimulating lipolysis (Skrzypski et al., 2012), while has thermogenic effect, that is, maybe chemical thermogenesis due to absence of the effect of NPW23 on locomotor activity. The effect of NPW23 on body temperature was only less than 2 h, while it was for up to 12 h on feeding intake. We do not know why the effect of NPW23 on body temperature is shorter than its effect on feeding intake. After icv administration of NPW23, changes in feeding may produce a transient change in body temperature. The mechanism of this transient thermal change is still unclear, but may be related to the changes in metabolism observed after NPW23 administration, because the regulatory centre of body temperature is located close to feeding centre in same hypothalamus. Two-hour food restriction every day in rats induced anticipatory increase in body temperature (Boulos and Terman, 1980), suggesting that both regulatory mechanisms for temperature and feeding may link each other.

In conclusion, endogenous NPW may play an important role in the control of food intake and body temperature as it is considered as catabolic regulator for energy metabolism. Further studies are required to elucidate the exact mechanisms of actions of neuropeptide W and any further physiological and biochemical functions that it may have.

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