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Expression of neuropeptide W in rat stomach mucosa: Regulation by nutritional status, glucocorticoids and thyroid hormones

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Abstract

Neuropeptide W (NPW) is a recently identified neuropeptide that binds to G-protein-coupled receptor 7 (GPR7) and 8 (GPR8). In rodent brain, NPW mRNA is confined to specific nuclei in hypothalamus, midbrain and brainstem. Expression of NPW mRNA has also been confirmed in peripheral organs such as stomach. Several reports suggested that brain NPW is implicated in the regulation of energy and hormonal homeostasis, namely the adrenal and thyroid axes; however the precise physiological role and regulation of peripheral NPW remains unclear. In this study, we examined the effects of nutritional status on the regulation of NPW in stomach mucosa. Our results show that in this tissue, NPW mRNA and protein expression is negatively regulated by fasting and food restriction, in all the models we studied: males, females and pregnant females. Next, we examined the effect of glucocorticoids and thyroid hormones on NPW mRNA expression in the stomach mucosa. Our data showed that NPW expression is decreased in this tissue after glucocorticoid treatment or hyperthyroidism. Conversely, hypothyroidism induces a marked increase in the expression of NPW in rat stomach. Overall, these data indicate that stomach NPW is regulated by nutritional and hormonal status.

Keywords: Neuropeptide W; Body weight; Nutritional status; Glucocorticoids; Thyroid hormones

1. Introduction

Neuropeptide W (NPW) is a recently discovered peptide that activates the previously described orphan G-protein-coupled receptors, GPR7 and GPR8 [1,2]. Two endogenous molecular forms of NPW that consist of 23- and 30-amino acid residues have been identified [1,2]. NPW mRNA in rodent brain is confined to specific nuclei in hypothalamus, midbrain and brainstem [1,3,4]. This pattern of expression, alongside the localization of GPR7 and GPR8 in some hypothalamic nuclei, such as the paraventricular, supraoptic, ventromedial, dorsomedial, suprachiasmatic and arcuate [5,6], initially suggested that NPW may be implicated in feeding regulation. However, the reported results are contradictory. Firstly, Mondal et al. observed a mild anorectic effect (associated to increased body temperature and heat production) after NPW central administration [7]. On the other hand, Levine et al. have demonstrated an orexigenic action for NPW [8]. Whatever the case, this evidence suggests the possibility that NPW may act as an endogenous feeding–regulating molecule in the brain.

Besides this role in body weight homeostasis, NPW may play a role in the regulation of hypothalamic–pituitary–adrenal (HPA) and thyroid (HPT) axes. It has been reported that central

Abbreviations: AMT, aminotriazole; BAT, brown adipose tissue; BBS, bombesin; CCK, cholecystokinin; CNS, Central Nervous System; HPA axis, hypothalamic–pituitary–adrenal axis; HPT axis, hypothalamic–pituitary–thyroid axis; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; GPR7, G-protein-coupled receptor 7; GPR8, G-protein-coupled receptor 8; NPW, neuropeptide W; OXM, oxyntomodulin; PYY, Peptide YY; T4, L-thyroxine; WAT, white adipose tissue.

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Table 1
Corporal and plasma parameters in fed, fasted and refed rats

	Fed	Fast 24 h	Fast 48 h	Refed
Body weight change (g)	20.0±1.5	−31.6±1.1***	−41.6±1.8***	2.2±1.3*** (*vs.* fed)
Food intake (g)	29.3±0.5	NFI	NFI	38.5±0.6*** (*vs.* fed)
Glucose (mmol/l)	7.8±0.3	5.3±0.5***	5.3±0.8***	8.0±0.4
Insulin (ng/ml)	2.2±0.4	0.4±0.1***	0.2±0.08***	2.0±0.3
Leptin (ng/ml)	5.5±0.4	0.9±0.2***	0.7±0.1***	5.1±0.8

***: $P<0.001$ *vs.* fed and refed. In order to simplify the table, comparisons between Fast 24 hours vs. Fast 48 hours and refed vs. fast (24/48) have been omitted.
NFI: non-food intake.

NPW administration increases plasmatic levels of corticosterone [9,10]. Moreover, a direct effect of NPW on adrenocortical cells has been also described in rodents [11,12] and humans [13]. On addition, both NPW receptors, GPR7 and GPR8, are highly expressed in the thyroid gland [14], which suggest the existence of direct effect of NPW on thyroid function.

In addition to the central nervous system (CNS), NPW expression has been also detected in peripheral tissues. Recent reports have demonstrated the presence of NPW in multiple endocrine glands of the rat, including pituitary, thyroid, parathyroid, pancreas, adrenal gland (cortex and medulla), ovary and testis [3,12,14,15], as well as in antral gastrin (G) cells of rat, mouse, and human stomach [16]. However, data are lacking about the specific regulation of NPW expression in stomach. This issue is particularly relevant considering that other gut-derived molecules regulating food intake, such as leptin, ghrelin, bombesin (BBS), cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and -2 (GLP-2), oxyntomodulin (OXM) and peptide YY (PYY) have been shown to be sensitive to the nutritional state, being rapidly mobilized in response to food intake following fasting, or after the administration of satiety factors [17–21]. This suggests a role for gastric-derived peptides in the short-term regulation of feeding, giving information to the brain on the availability of external (food) energy resources, thus participating in short- and long-term satiation [18–21].

In this study we aim to characterize the effects of nutritional status on NPW expression in rat stomach mucosa. In addition, given the possible involvement of NPW on the regulation of HPA and HPT axes, we studied the effect on glucocorticoids and thyroid hormone status on stomach NPW expression.

2. Materials and methods

2.1. Animals

Adult Sprague–Dawley rats (Animalario General USC, Spain) were housed in a temperature-controlled room, with a 12-hour light/dark cycle (light: 8:00–20:00). Food and water were available *ad libitum* prior to the initiation of the experiment. All experiments were conducted in accordance with the Ethics Committee of the University of Santiago de Compostela. The experiments were performed in agreement with the Rules of Laboratory Animal Care and International Law on Animal Experimentation.

2.2. Fasting–refeeding experiments

Male rats ($n=8$–10 per experimental group) were deprived of food for 24 or 48 h and refed *ad libitum* during 24 h [22,23]. Body weights, food intake (refeeding), leptin, insulin and glucose levels were measured in all the animals to check the effects of fasting–refeeding (Table 1).

2.3. Chronic food restriction

Food restriction was performed as described previously [24,25]. Briefly, male rats ($n=8$–10 per experimental group) were fed 30% of the daily *ad libitum* intake during 12, 16 or 21 days. Control rats were fed *ad libitum*. To determine the pattern of rat NPW mRNA expression throughout pregnancy ($n=8$–10 animals per experimental group) and analyze the food restriction effect on stomach tissue at different times during pregnancy, rats on gestational days 12, 16 and 21 were obtained [24,25]. All animals were fed every day at 18:00 h.

2.4. Treatments with dexamethasone

Male rats ($n=8$–10 animals per experimental group) received a daily subcutaneous (SC) administration of dexamethasone (DX) (Fortecortin®, Merck; Barcelona, Spain) in a dose of 40 μg/day, dissolved in 200 μl of saline for 8 and 15 days. Control animals were treated with the same SC volume of vehicle. All the treatments began at 9:00 h.

2.5. Induction of hypo- and hyperthyroidism

Hypothyroidism was induced as previously described [26,27] by administration of 0.1% aminotriazole (Sigma, Poole, UK) in drinking water for a period of 8 days. Hyperthyroidism was induced by chronic subcutaneous administration of L-thyroxine (100 μg/day, dissolved in 200 μl of saline; Sigma, Poole, UK). Control animals and hypothyroid animals were treated with the same subcutaneous volume of vehicle. Ten male rats per experimental group were used. To confirm the hyperthyroid status we studied plasma T3, T4 and TSH levels (Table 2).

Table 2
Corporal and plasma parameters in euthyroid, hypothyroid and hyperthyroid rats

	Euthyroid	Hypothyroid	Hyperthyroid
TSH (ng/ml)	3.4±0.2	27.2±1.8***	0.4±0.1***
T4 (nmol/l)	80.2±7.2	ND***	321.6±33.3***
T3 (nmol/l)	3.2±0.2	0.9±0.1***	5.1±0.6***

***: $P<0.001$ *vs.* euthyroid. In order to simplify the table, comparisons between hypothyroid *vs.* hyperthyroid have been omitted.
ND: non-detected.