

Effects of Ghrelin on circulating Neuropeptide Y levels in humans

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Abstract

OBJECTIVE: Ghrelin is a 28 amino-acid peptide with a strong GH-releasing activity and a complex role in regulation of appetite, fuel utilization, body weight and composition. Neuropeptide Y (NPY) is a well-known stimulator of pathways favouring food intake and energy storage. Recently, studies in rodents suggested a possible mediation of ghrelin action by NPY. In contrast, until now no evidence of ghrelin-NPY interaction in humans has been provided. In the present study, we examined whether ghrelin influences NPY secretion in normal men.

SUBJECTS AND DESIGN: Twelve healthy normal men (aged 24–35 years; body mass index (BMI) 22.3 ± 0.93 kg/m²) were tested twice at 08.00 AM on two different days, in random order at weekly intervals, after an overnight fast and rest in bed.

An intravenous bolus of 1 ug/kg body weight ghrelin (experimental test) or an equal amount of normal saline (control test) was injected at time 0. Blood was taken before and over 90 minutes after injections, and was used for the measurement of plasma NPY levels.

RESULTS: Plasma levels of NPY slightly, but significantly rose in response to ghrelin, with a mean peak level at 15 min after injection, whereas no significant change was observed after saline administration.

MAIN FINDING: Our results show a significant enhancement of plasma NPY levels under ghrelin stimulation.

CONCLUSIONS: To our knowledge, this is the first demonstration of a ghrelin-NPY interaction in humans, which may suggest a possible mediation of ghrelin action by NPY in humans.

Introduction

There is a close relationship between feeding and growth, which does not only depend on availability of the basic nutritional elements to growth, but also on neuroendocrine interactions in the central nervous system; in fact, both appetite and growth are regulated by the brain, particularly by hypothalamic structures. In the present study, we focused our attention on ghrelin and NPY, two important elements involved in the regulation of food intake and growth [4,8]. Ghrelin is a 28 amino-acid peptide with a strong GH-releasing activity. At first it was purified from the stomach; later, it was localized in several hypothalamic areas through the use of immunohistochemical detection methods [3]. The GH-releasing effect of Ghrelin is not mediated by the mechanism activated by GHRH, but follows the peptide binding to receptors mediating the activity of GH secretagogues (GHS), GH-stimulating substances of synthetic origin [9]. Ghrelin has a complex role in regulation of appetite, fuel utilization, body weight and composition, which is complementary to the effect of this hormone as GH stimulating factor [6].

Neuropeptide Y (NPY) is a 36-amino-acid peptide with neurotransmitter and/or neuromodulatory functions. It is widely distributed in the peripheral nervous system (in sympathetic neurons) and in the central nervous system; high concentrations of NPY occur in the hypothalamus, median eminence and neurohypophysis. Furthermore, NPY has been found in the pituitary portal blood, in the anterior pituitary and in the systemic circulation. NPY plays a role in the regulation of various functions, such as blood pressure control, stimulation of drinking, memory retention and hypothalamic-pituitary-adrenal and -ovarian axis activity; but overall, NPY is a well-known stimulator of appetite [7,5].

The role of NPY in the control of the feeding system consists in the stimulation of ways that favour food intake and energy storage [5]. These actions mainly depend on NPY-mediated neurotransmission in the central nervous system, but a role of circulating blood NPY levels both outside and inside the blood-brain barrier (slowly crossed by NPY) cannot be excluded.

In vivo and in vitro studies in rodents [for review see 6] have shown that ghrelin-induced hyperphagia involves NPY mediation. NPY neurons in the arcuate nucleus are located together with GHS-receptors, and intracellular calcium in NPY neurons increases under ghrelin stimulation. Furthermore, intracerebroventricular (ICV) ghrelin injection increases food intake and NPY mRNA in the arcuate nucleus, while ghrelin-stimulated food intake is inhibited by ICV-injected NPY Y1 antagonists. In addition, stimulation of *c-fos* in arcuate NPY neurons has been reported to occur in response to ghrelin, given by either ICV or peripheral injection [6].

In contrast with studies in rodents, experiments carried out in chicks failed to show a central interaction of ghrelin with NPY [6] in this species.

The present study was carried out in order to obtain a better insight into ghrelin-NPY interaction in humans with non-invasive methods. Recently, our measurements of circulating NPY levels provided useful informations about changes in food intake occurring in elderly human subjects [1]. In the present study, we examined whether ghrelin influences NPY secretion in normal men, by measuring plasma NPY levels in response to a systemic administration of ghrelin.

Materials and methods

Twelve healthy normal men (aged 24–35 years; body mass index (BMI) $22.3 \pm 0.93 \text{ kg/m}^2$) participated in this study after giving their informed consent. The study was performed according to the Helsinki declaration of 1982 and was approved by the university ethical commission. All men were of similar body constitution. None of them were taking drugs for at least 3 weeks before the experimental day.

Two tests were performed in random order at weekly intervals in all subjects.

Control test: men were tested with an intravenous bolus injection of normal saline.

Experimental test: an intravenous bolus of 1 $\mu\text{g/kg}$ ghrelin (Clinalfa AG, Laufelfingen, Switzerland) diluted in an equal amount of normal saline was injected.

All tests started at 8.00 a.m. of the experimental days. Two intravenous catheters were placed in antecubital veins of opposite arms in subjects lying in the recumbent position and fasting from the previous evening. One catheter was utilized for blood sampling, the other served for ghrelin or normal saline administration.

Normal saline or ghrelin was injected twenty minutes after catheter insertion (time 0). A basal blood sample was taken at time 0, just before normal saline or ghrelin injection. Further blood samples were taken after 15, 30, 45, 60 and 90 min.

Assay

Plasma NPY levels were measured by RIA, using commercial kits. The intra- and inter-assay coefficients of variation were 3.2 and 11.2%, respectively. The sensitivity of the method was 6 pmol/l for NPY. Statistical analysis was performed with the Student paired and unpaired t-tests, as appropriate. Values are reported as mean \pm S.E.

Results and Discussion

Normal saline injection did not produce hormonal variations in any subject (Figure 1).

Plasma levels of NPY slightly, but significantly rose in response to ghrelin administration (Figure 1). The mean peak level after ghrelin injection was observed at 15 min ($P < 0.001$ vs baseline and vs control test) (Figure 1).

The data reported here show a significant enhancement of plasma NPY levels under ghrelin stimulation.

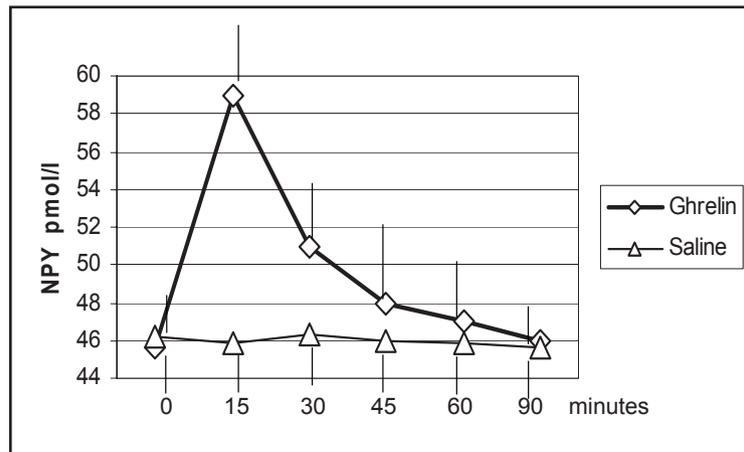


Figure 1. Effect of an intravenous bolus injection of normal saline or ghrelin on plasma NPY levels in 12 normal men. Each point represents the mean \pm SE of the observations.

To our knowledge, this is the first demonstration of a ghrelin-NPY interaction in humans.

The peripheral injection of ghrelin has been reported to activate NPY neurons in the rat hypothalamus [6]. In agreement with this observation we have found an increase of NPY production (shown by increased circulating NPY levels) after peripheral ghrelin administration in humans.

It is possible that in men, like in rodents [6], ghrelin-induced stimulation of food intake involves NPY mediation. Ghrelin has been shown to stimulate GH secretion at hypothalamic level [9]. At this site, ghrelin may coordinate food intake with GH secretion [9]. Particularly, Ghrelin has been supposed to have the specific role to ensure calory provisions needed by GH for growth and repair [2]. From this point of view, it is possible that Ghrelin-NPY interaction is part of a wider mechanism by which Ghrelin stimulates appetite and influences the central regulation of energy balance and growth. Further studies are needed to substantiate this hypothesis.

REFERENCES

- Chiodera P, Volpi R, Pilla S, Cataldo S, Coiro V. Decline in circulating neuropeptide Y levels in normal elderly human subjects. *European J Endocrinol.* 2000; **143**:715–6.
- Druce M, Bloom SR. Central regulators of food intake. *Curr Opin Clin Nutr Metab Care.* 2003; **6**:361–7.
- Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature.* 1999; **402**:656–60.
- Nakazato ML, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for Ghrelin in the central regulation of feeding. *Nature.* 2001; **409**:194–8.
- Pavia JM, Hastings JA, Morris MJ. Neuropeptide Y potentiation of potassium-induced nor-adrenaline release in the hypothalamic paraventricular nucleus of the rat in vivo. *Brain Research.* 1995; **690**:108–11.
- Saito ES, Kaiya H, Tachibana T, Tomonaga S, Denbow DM, Kangawa K, Furuse M. Inhibitory effect of ghrelin on food intake is mediated by the corticotropin-releasing factor system in neonatal chicks. *Regulatory Peptides.* 2005; **125**:201–8.
- Torda T, Saavedra JM. Determination of guanine nucleotide sensitivity of (125J)-Neuropeptide Y binding in the rat pituitary gland by quantitative autoradiography. *Neuroendocrinology.* 1980; **52**:361–7.
- Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, Guan JL, Wang QP, Funahashi H, Sakurai T, Shioda S, Matsukura S, Kangawa K, Nakazato M. Ghrelin-induced food intake is mediated via the orexin pathway. *Endocrinology.* 2003; **144**:1506–12.
- Wren AM, Small CJ, Fribbens CJ, Neary NM, Ward HL, Seal LJ, Ghatei MA, Bloom SR. The hypothalamic mechanisms of the hypophysiotropic action of Ghrelin. *Neuroendocrinology.* 2002; **76**:316–24.