available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/peptides

Different responses of circulating ghrelin, obestatin levels to fasting, re-feeding and different food compositions, and their local expressions in rats

Zhi-Fu Guo [a,1,*], An-Jing Ren [b,1], Xing Zheng [a,*], Yong-Wen Qin [a], Fang Cheng [c], Jing Zhang [d], Hong Wu [a], Wen-Jun Yuan [b], Lin Zou [e]

[a] Department of Cardiovascular Diseases, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
[b] Department of Physiology, Basic Medical College, Second Military Medical University, Shanghai 200433, China
[c] Department of Rheumatology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China
[d] First Department of Geriatrics, General Hospital of Jinan Military Command, Jinan 250031, China
[e] Department of Orthopaedics, General Hospital of Jinan Military Command, Jinan 250031, China

ARTICLE INFO

Article history:
Received 8 January 2008
Received in revised form
24 February 2008
Accepted 26 February 2008
Published on line 7 March 2008

Keywords:
Ghrelin
Obestatin
Food intake
Food composition
Rat

ABSTRACT

Obestatin, a sibling of ghrelin derived from preproghrelin, opposes several physiological actions of ghrelin. Our previous study has demonstrated that both plasma ghrelin and obestatin levels were decreased significantly 2 h after food intake in human. To further expand current knowledge, we investigated the temporal profiles of their levels in *ad libitum* fed rats, 48 h fasted rats and 48 h fasted rats refed 2 h with a standard chow, crude fiber, 50% glucose or water, and their expressions in stomach, liver and pancreatic islets immunohistochemically. Plasma ghrelin and obestatin levels were measured by EIA. Plasma leptin, insulin and glucose levels were also evaluated. Both plasma ghrelin and obestatin levels increased significantly in fasted rats compared with *ad libitum* fed rats. The ingestion of standard chow produced a profound and sustained suppression of ghrelin levels, whereas plasma obestatin levels decreased significantly but recovered quickly. Intake of crude fiber or 50% glucose, however, produced a more profound and sustained suppression of obestatin levels, though they had relatively less impact on ghrelin levels. Plasma glucose was the only independent predictor of ghrelin levels, obestatin levels, and ghrelin to obestatin ratios. Obestatin immunoreactivity was detected in the fundus of stomach, liver and pancreatic islets, with roughly similar patterns of distribution to ghrelin. These data show quantitative and qualitative differences in circulating ghrelin and obestatin responses to the short-term feeding status and nutrient composition, and may support a role for obestatin in regulating metabolism and energy homeostasis.

1. Introduction

Ghrelin, a 28-amino-acid peptide secreted primarily by the gastrointestinal tract [23], is an orexigenic hormone that plays important roles in the regulation of short- and long-term energy homeostasis. Recently Zhang et al. [49] reported that GHRL (ghrelin/obestatin preprohormone) gene also encodes another 23-amino-acid secreted peptide, termed ''obestatin''.

* *Corresponding authors*. Tel.: +86 21 2507 0540; fax: +86 21 5522 5366.
E-mail addresses: guozhifu@126.com (Z.-F. Guo), zhengxing57530@163.com (X. Zheng).
[1] These authors contributed equally to this work.
0196-9781/$ – see front matter
doi:10.1016/j.peptides.2008.02.020

The biological activity of obestatin depended on the amidation at its carboxyl terminus, and what's surprising was that obestatin, though derived from the same peptide precursor, suppressed food intake, inhibited jejunal contraction, decreased body-weight gain and antagonized the actions of ghrelin when both peptides were co-administered [6,20,24,31,42,51], with still many other functions being also suggested now [9,10,14,19,22,27,38,44,48]. These facts, though with some controversies [3,13,17,18,32,41], may suggest that the intricate balance of ghrelin and obestatin is critical to the regulation of energy homeostasis.

However, to the best of our knowledge, no report has been issued on the temporal profiles of plasma obestatin levels in relation to feeding and that of ghrelin, a critical problem as to the fully understanding of its function in regulation of energy homeostasis. Our previous study has demonstrated that both plasma ghrelin and obestatin levels were decreased significantly 2 h after food intake in human [21]. To further expand current knowledge, we investigated the temporal profiles of plasma obestatin levels, and their relationship to ghrelin, leptin, glucose and insulin, in *ad libitum* fed, fasted and fasted-refed rats.

2. Materials and methods

2.1. Animals

For all experiments, male Sprague Dawley rats (Slac Laboratory Animal Co. Ltd., Shanghai, China) weighing about 250 g at the onset of the experiments were used. One week before experimental procedures, animals were housed in stainless steel cages in an air-conditioned (24 ± 2 °C) and light-regulated (lights on, 06:00–18:00 h) room with *ad libitum* access to tap water and a standard laboratory chow (Slac Laboratory Animal Co. Ltd., Shanghai, China) containing 52.5% Nitrogen Free Extract, 20.5% protein, 4.62% fat, 4.35% fiber, 9.7% moisture, and 6.2% ash residue of total mass. All experimental protocols were approved by the Second Military Medical University Animal Care and Use Committee.

2.2. Experimental protocol

After 1 week of chow, rats were assigned to one of six weight-matched groups at 07:00 h, and then housed individually in plastic cages. In the *ad libitum* fed group ($n = 10$), rats were given free access to water and the standard chow for 48 h; in the fasted group ($n = 10$), animals were deprived of food for 48 h with free access to water; in the fasted-refed groups ($n = 30$ per group), animals from the fasted group were allowed a 2 h *ad libitum* re-feeding period with different foodstuffs [standard chow plus water, or pelleted crude fiber (Slac Laboratory Animal Co. Ltd., Shanghai, China) and water, or 50% glucose, or water alone]. Animals in the *ad libitum* fed and fasted group were killed at 07:00 h, and different cohorts (assigned to groups of comparable body weight) in the refed groups were killed every 30 min ($n = 6$ per refed group per time point) beginning at 09:00 h.

Rats were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg). After the abdomen was opened, blood samples for the determination of ghrelin, obestatin, leptin, insulin and glucose levels were collected from the inferior vena cava and immediately transferred to chilled polypropylene tubes containing EDTA-2Na (1 mg/ml) and aprotinin (Phoenix Pharmaceuticals, Belmont, CA, USA: 100 μl containing 0.6 trypsin inhibitor unit per milliliter of blood), centrifuged at 4 °C, 1600 g for 15 min, and then plasma samples were stored at −80 °C until assayed. The fundus of stomach, liver and pancreas were rapidly removed, rinsed with saline, and immersion fixed in 10% neutral phosphate-buffered formalin for 24 h. Samples were then rinsed and stored in 70% alcohol before processing and embedding into paraffin wax.

2.3. Plasma glucose and hormone measurements

The plasma glucose levels were measured by an automated glucose oxidase method (Automatic Analyzer 7600-020, Hitachi, Tokyo, Japan). Plasma ghrelin and obestatin levels were measured using commercial EIA kits (Phoenix Pharmaceuticals, Belmont, CA, USA) according to the manufacturer's instructions. The sensitivity of the assay was 0.1 ng/ml. Intra- and inter-assay coefficients of variation (CV) reported by the manufacturer were <5% and <14%, respectively. Plasma leptin levels were measured using an ELISA kit (Phoenix Pharmaceuticals, Belmont, CA, USA). The sensitivity of the assay was 62.5 pg/ml. Intra- and inter-assay CV were <10% and <15%, respectively. Plasma insulin levels were measured using an ELISA kit (Mercodia AB, Uppsala, Sweden). The sensitivity of the assay was 0.07 μg/L. Intra- and inter-assay CV were <3.4% and <2.2%, respectively.

2.4. Immunohistochemistry

Immunohistochemical study was performed using the DAKO Envision method (EnVision™+ System, Dako A/S, Glostrup, Denmark) according to the manufacturer's instructions. Ghrelin antiserum is a rabbit polyclonal directed against the rat/mouse ghrelin (GSSFLSPEHQKAQQRKESKKPPAKLQPR-NH2; Phoenix Pharmaceuticals, Belmont, CA, USA; dilution 1:1000), and obestatin antiserum is a rabbit polyclonal directed against the rat/mouse obestatin (FNAPFDVGIKLSGAQYQQHGRAL-NH2; Phoenix Pharmaceuticals, Belmont, CA, USA; dilution 1:1000). The obestatin antiserum reacts 100% with rat/mouse obestatin and rat/mouse Des (1–10) obestatin, and does not cross-react with human/monkey obestatin, mouse/rat or human ghrelin, human preproghrelin (86–117), preproghrelin (52–75) or preproghrelin (101–117). Ghrelin antiserum recognizes both the acylated and desacylated form of ghrelin, reacts 100% with rat/mouse ghrelin and human ghrelin, and does not cross-react with rat/mouse obestatin. Briefly, sections (5 μm) were cut and mounted on polylysine coated glass slides and dried overnight at 42 °C prior to immunohistochemical analysis. Then the sections were deparaffinized in xylene and rehydrated in graded alcohol. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 20 min. After three times of rinsing with 0.01 mol/L phosphate-buffered saline (PBS), the slides were incubated for 60 min at 37 °C with the primary antibodies described above. After rinsed in PBS three times, they were incubated with Envision complex for 30 min at 37 °C, and stained with diaminobenzidine (DAB) for 8–12 min after washed