Biochemical and Biophysical Research Communications 430 (2013) 278–283

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Central and peripheral des-acyl ghrelin regulates body temperature in rats

Yoshiyuki Inoue [a], Keiko Nakahara [a], Keisuke Maruyama [a], Yoshiharu Suzuki [a], Yujiro Hayashi [b], Kenji Kangawa [c], Noboru Murakami [a,*]

[a] Department of Veterinary Physiology, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan
[b] Asubio Pharma Co., Ltd., 6-4-3 Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan
[c] Department of Biochemistry, National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan

ARTICLE INFO

Article history:
Received 27 October 2012
Available online 15 November 2012

Keywords:
Des-acyl gherlin
Body temperature
Vasolilation
Parasympathetic nerve

ABSTRACT

In the present study using rats, we demonstrated that central and peripheral administration of des-acyl ghrelin induced a decrease in the surface temperature of the back, and an increase in the surface temperature of the tail, although the effect of peripheral administration was less marked than that of central administration. Furthermore, these effects of centrally administered des-acyl ghrelin could not be prevented by pretreatment with [D-Lys3]-GHRP-6 GH secretagogue receptor 1a (GHS-R1a) antagonists. Moreover, these actions of des-acyl ghrelin on body temperature were inhibited by the parasympathetic nerve blocker methylscopolamine but not by the sympathetic nerve blocker timolol. Using immunohistochemistry, we confirmed that des-acyl ghrelin induced an increase of cFos expression in the median preoptic nucleus (MnPO). Additionally, we found that des-acyl ghrelin dilated the aorta and tail artery in vitro. These results indicate that centrally administered des-acyl ghrelin regulates body temperature via the parasympathetic nervous system by activating neurons in the MnPO through interactions with a specific receptor distinct from the GHS-R1a, and that peripherally administered des-acyl ghrelin acts on the central nervous system by passing through the blood–brain barrier, whereas it exerts a direct action on the peripheral vascular system.

1. Introduction

Ghrelin was originally isolated from rat stomach extracts [1] as an endogenous ligand for the GHS-R. Ghrelin, produced predominantly by endocrine cells of the gastric oxyntic glands [2,3], exists as two major molecular forms: ghrelin and des-acyl ghrelin [4]. Ghrelin is composed of 28 amino acids and is octanoylated at Ser3, an unusual post-translational modification that is catalyzed by the enzyme ghrelin *O*-acyltransferase (GOAT) [5,6]. Des-acyl ghrelin, which lacks the Ser3 residue octanoylation, is unable to release GH or bind to the classic GHS-R1a receptor [4]. These characteristics indicate that octanoic acid plays an important role in physiological activity via GHS-R1a, and des-acyl ghrelin has been considered an inactive form of ghrelin. However, it has recently been reported that des-acyl ghrelin has a variety of functions, such as inhibition of cell death in cardiomyocytes and endothelial cells, induction of adipose tissue production, and enhancement of human osteoblastic cell proliferation through a pathway that does not involve the GHS-R1a [7–9]. These findings suggest the presence of an unidentified receptor specific for des-acyl ghrelin. Thus, although ghrelin and des-acyl ghrelin have similar structures, their actions and the mechanisms involved differ at several levels.

Since its discovery in 1999, it has been clarified that ghrelin acts on energy-metabolic systems by promoting growth hormone secretion and food intake [10–12]. Furthermore, centrally or peripherally administered ghrelin increases the respiratory quotient, indicating that it has an inhibitory action on fat oxidation [13]. Intracerebroventricular (i.c.v.) injection of ghrelin in rats transiently decreases the body core temperature [14], and decreases temperature, sympathetic activity and noradrenalin release in brown adipose tissue (BAT) [15,16]. These findings suggest that ghrelin induces positive energy balance not only by increasing energy intake but also by decreasing energy expenditure.

On the other hand, compared to ghrelin, the effects of des-acyl ghrelin on energy intake are less well characterized [17–20]. Also, the action of des-acyl ghrelin on energy expenditure has not yet been investigated. In the present study, therefore, we examined the effects of central and peripheral injection of des-acyl ghrelin on body temperature, and investigated in detail the mechanisms responsible for this effect.

* Corresponding author. Fax: +81 985 58 7265.
E-mail address: a0d201u@cc.miyazaki-u.ac.jp (N. Murakami).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.10.137

2. Materials and methods

2.1. Animals and i.c.v. injection of des-acyl ghrelin and ghrelin

Male Wistar rats (Charles River Japan, Inc., Yokohama, Japan), weighing 300–350 g, were housed in individual Plexiglas cages in an animal room maintained under a constant light–dark cycle (lights on from 7:00 to 19:00 h) and temperature (22 ± 1 °C) for at least one week. Food and water were provided ad libitum. i.c.v. cannulae were implanted into the lateral cerebral ventricles using a method that has been described previously [21], and after surgery all rats were housed individually in Plexiglas cages. During a 6-day postoperative recovery period, the rats became accustomed to the handling procedure. Des-acyl ghrelin (Peptide Institute, Inc., Osaka, Japan) and ghrelin (Peptide Institute, Inc., Osaka, Japan) was dissolved in saline, and 10 μl of the solution was injected into each free-moving rat through a 27-gauge injection cannula connected to a 50-μl Hamilton syringe. All procedures were performed in accordance with the Japan Physiological Society's guidelines for animal care, and this study was approved by the experimental animal committee of Miyazaki University (authorization number: 2006-052-6).

2.2. Measurement of body temperature

We removed food and water from each cage at 18:00 h and switched off ambient illumination at 19:00 h. Subsequently, during the dark period, we started infrared thermographic imaging of the back (back surface temperature) and tail (caudal surface temperature) of the rats at 19:30 h, and saved images taken at 1 min intervals during the following 30 min (FLIR SC620, FLIR Systems, Danderyd, Sweden). Thereafter, des-acyl ghrelin was administered by i.c.v. (0.01, 0.1, 0.5 nmol/10 μl) or intraperitoneal (i.p.; 0.01, 0.03, 0.1 mg/kg body weight) injection, and measurements were conducted for the following 60 min. The camera was newly calibrated and fixed in a standardized position 1.30 m vertically above each rat. The FLIR SC620 has a thermal resolution of <0.04 °C, an accuracy of ±2%, and a picture resolution of 640 × 480 pixels. The average value during the 10 min before administration was assumed to be 0, and the values obtained thereafter were indicated as increases or decreases. Moreover, i.c.v. injection of ghrelin (0.5 nmol/10 μl) was also performed under the same conditions.

2.3. Influence of [D-Lys3]-GHRP-6 on the effects of des-acyl ghrelin on body temperature

Rats were subjected to implantation of a stainless steel cannula into the lateral left ventricle using the method described above. The GHS-R1a antagonist [D-Lys3]-GHRP-6 (Sigma–Aldrich Co., St. Louis, USA; 10 nmol/5 μl) was dissolved in saline and i.c.v. injected at 19:30 h, and then 30 min later, saline, ghrelin (0.5 nmol/5 μl) and des-acyl ghrelin (0.5 nmol/5 μl) were injected into the lateral ventricle using a 50-μl Hamilton syringe. After injection, measurements were performed every 1 min for 60 min.

2.4. Influence of an autonomic nerve blocker on the effects of des-acyl ghrelin on body temperature

Rats were subjected to implantation of a stainless steel cannula into the lateral left ventricle using the method described above. The muscarinic receptor antagonist methylscopolamine (Sigma–Aldrich Co., St. Louis, USA; 0.5 mg/kg body weight) or the adrenergic receptor antagonist timolol (Sigma–Aldrich Co., 0.5 mg/kg body weight) was dissolved in saline and i.p. injected at 19:30 h, and then 30 min later, saline and des-acyl ghrelin (0.5 nmol/10 μl) were injected into the lateral ventricle using a 50-μl Hamilton syringe. After injection, measurements were performed every 1 min for 60 min. Moreover, peripheral administration of des-acyl ghrelin (0.03 mg/kg body weight) was also performed under the same conditions.

2.5. Immunofluorescence staining of cFos in the MnPO

Immunohistochemical staining for cFos was performed 90 min after i.c.v. (0.5 nmol/10 μl) and i.p. (0.03 mg/kg body weight) injection of des-acyl ghrelin during the dark period. Frozen brain sections were cut with a cryostat at a thickness of 10 μm. The cFos staining was performed with a Vectastain ABC kit (Vector Laboratories, Burlingame, CA) according to the method described. Briefly, after blocking the sections with diluted normal goat serum, they were incubated at 4 °C for 24 h with anti-cFos antiserum (Santa Cruz Biotechnology, Santa Cruz, CA) in 0.3% Triton X-100/phosphate-buffered saline (PBS). After being washed for 30 min with 0.1% tween 20/PBS, the sections were incubated for 2 h with a biotinylated second antibody and for an additional 2 h with avidin–biotin–peroxidase complex. The sections were then stained with 0.02% 3,3′-diaminobenzidine and 0.05% hydrogen peroxide in Tris buffer.

2.6. Measurement of perfusion pressure

After the rats had been killed, tail arteries and the aorta were rapidly removed and suspended from two L-shaped stainless steel wires in an organ bath (LABO Support Co., Ltd., Osaka, Japan) filled with Tyrode solution and maintained at 37 °C. The bath solution was continuously bubbled with 95% O_2 and 5% CO_2. Isometric tension was recorded using a PowerLab/8SP computerized data acquisition system (AD Instruments, Castle Hill, NSW, and Australia). When the contraction reached a plateau upon treatment with phenylephrine, des-acyl ghrelin (0.2, 2, 20 μM) was added to the bath cumulatively. Additionally, to study endothelium-dependent vasodilation via the NO-cGMP pathway, rings with intact endothelium that had been pretreated with N^G-nitro-L-arginine methyl ester (L-NAME: 100 μM) for 30 min were contracted by treatment with phenylephrine. When the contraction reached a plateau, des-acyl ghrelin (2 μM) was added to the bath employing the same schedule as that described above.

2.7. Statistical analysis

All results are presented as mean ± S.E.M. Effects of des-acyl ghrelin and ghrelin on body temperature were analyzed using repeated-measures ANOVA. Furthermore, AUC values were analyzed by Student's *t* test. Perfusion pressure was analyzed using Tukey's multiple comparison test. Values were considered to differ significantly at $p < 0.05$.

3. Results

3.1. Effects of central and peripheral administration of des-acyl ghrelin on body temperature

To evaluate the effects of des-acyl ghrelin on thermogenesis, we performed infrared thermographic imaging of the back (back surface temperature) and tail (caudal surface temperature) after i.c.v. injection of des-acyl ghrelin. The back surface temperature after i.c.v. injection of des-acyl ghrelin was significantly decreased at a concentration of 0.1 and 0.5 nmol compared with the saline group for at least 1 h (Fig. 1B). Moreover, i.c.v. injection of 0.1, 0.5 nmol des-acyl ghrelin acutely increased the caudal surface