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Ghrelin administered spinally increases the blood glucose level in mice

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ABSTRACT

Ghrelin is known as a regulator of the blood glucose homeostasis and food intake. In the present study, the possible roles of ghrelin located in the spinal cord in the regulation of the blood glucose level were investigated in ICR mice. We found that intrathecal (i.t.) injection with ghrelin (from 1 to 10 μg) caused an elevation of the blood glucose level. In addition, i.t. pretreatment with YIL781 (ghrelin receptor antagonist; from 0.1 to 5 μg) markedly attenuated ghrelin-induced hyperglycemic effect. The plasma insulin level was increased by ghrelin. The enhanced plasma insulin level by ghrelin was reduced by i.t. pretreatment with YIL781. However, i.t. pretreatment with glucagon-like peptide-1 (GLP-1; 5 μg) did not affect the ghrelin-induced hyperglycemia. Furthermore, i.t. administration with ghrelin also elevated the blood glucose level, but in an additive manner, in D-glucose-fed model. Our results suggest that the activation of ghrelin receptors located in the spinal cord plays important roles for the elevation of the blood glucose level.

1. Introduction

Ghrelin, an endogenous peptide hormone which is known as a ligand of growth hormone secretagogue receptors (GHS-Rs), is synthesized and released from the stomach [19]. GHS-Rs are existed in various tissues, including the pancreas [16,17]. In general, the blood level of ghrelin is increased during fasting and decreased after food intake [15,27]. In support of this finding, fasting increases ghrlein mRNA levels in the stomach, hypothalamus and the pituitary and ghrelin mRNA levels in those regions are reduced by feeding [14]. The activation of ghrelin receptors produces several pharmacological effects, including the reduction of insulin release from pancreatic β-cells [2]. Peripheral ghrelin is an important signal in meal initiation and food intake stimulation [23]. Sun et al., have shown that the ablation of ghrelin increases secretion of insulin in response to glucose challenge [25]. Furthermore, Bewick et al., have demonstrated that, in ghrelin transgenic mouse, the circulating ghrelin level is elevated [1]. The same group has shown that up-regulation of ghrelin level is associated with hyperphasia and decreased insulin level in response to glucose. In a clinical study, Broglio et al., have reported that intravenous administration of ghrelin decreases insulin and increase blood glucose level [3].

In addition to the involvement of ghrelin receptors in the peripheral system, several lines of evidence have suggested that ghrelin system located in the brain also appears to be associated with the glucose homeostasis and metabolism. For example, Gonzalez et al., have demonstrated that central administration of ghrelin ameliorates the interleukin-1β-induced anorexia [10]. In addition, the lowered fasting glucose level is observed in ghrelin receptor-deficient mice are returned to wild-type levels in the hindbrain region [21]. Furthermore, supraspinal administration with ghrelin increases food intake, water intake, and body weight [24]. Finally, Kim et al., have reported that chronic central administration of ghrelin reverses the effects of leptin by altering food intake [13]. However, acylated ghrelin microinjected into the basolateral nucleus of the amygdala decreases the liquid consumption [26], suggesting that centrally administered ghrelin shows the differential actions in food intake according to the brain regions acted by ghrelin.

Several studies have demonstrated that GHS-R is also found in the spinal cord [9,28]. However, Zigman et al., have reported that no GHS-R mRNA expression is observed in the cervical spinal cord in both rats and mice [29]. Spinal ghrelin is associated with several functions such as antinociception, neuroprotection, and gastrointestinal propulsion [5,8,18,22]. The involvement of ghrelin located peripherally as well as in the brain sites in the regulation of the blood glucose level has been well demonstrated in numerous previous studies. However, the roles of ghrelin located in the spinal

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cord have not been characterized yet. Thus, in the present study, the effects of ghrelin administered spinally on blood glucose level were examined in ICR mice.

2. Materials and methods

These experiments were approved by the Hallym University Animal Care and Use Committee (Registration Number: Hallym 2009-05-01). All procedures were conducted in accordance with the 'Guide for Care and Use of Laboratory Animals' published by the National Institutes of Health.

2.1. Experimental animals

Male Hsd: CD-1 (ICR) [Charles River, USA] mice, weighing 24–26 g, were used for all the experiments. Five mice were housed per cage in a room maintained at 22 ± 0.5 °C with an alternating 12 h light–dark cycle. Food and water were available ad libitum. The animals were allowed to adapt to the laboratory for at least 2 h before testing and were only used once. Experiments were performed during the light phase of the cycle (10:00–17:00).

2.2. Drugs

D-Glucose was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ghrelin, glucagon-like peptide-1 (GLP-1) and ghrelin receptor antagonist (YIL781) were purchased from Tocris Bioscience Co. (Minneapolis, MN, USA). YIL781 was prepared with the following steps: (A) 1 g of YIL781 was dissolved in 0.5 ml of ethanol, plus 0.5 ml of polyethylene glycol 400. (B) Separately, 100 mg of sodium carboxymethylcellulose was dissolved in 9 ml of distilled water. (C) Finally, solution (A) and solution (B) were vigorously mixed. The polyethylene glycol 400, ethanol and sodium carboxymethylcellulose mixture (PEC), excluding YIL781, was used as the vehicle control. All drugs were prepared just before use. Blood glucose meter, lancing device and strips were purchased from Roche Diagnostics (Sandhofer Strasse, Mannheim, Germany). The mouse insulin ELISA kit was purchased from Shibayagi Co. (Shibukawa, Japan).

2.3. Intrathecal (i.t.) injection

I.t. administration was performed in conscious mice, following the method of Hylden and Wilcox, using a 30-gauge stainless-steel needle attached to a 25 μl Hamilton microsyringe which was inserted into the tissue to one side of the L5 or L6 spinous process so that it slipped into the groove between the spinous and transverse processes [12]. The i.t. injection volume was 5 μl and the injection site was verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the spinal cord. The dye injected i.t. was distributed both rostrally and caudally but with short distance (about 0.5 cm) and no dye was found in the brain.

2.4. Measurement of blood glucose level

Blood glucose measurements were obtained using blood samples collected by lateral tail vein laceration. A minimum volume (1 μl) of blood was collected as quickly as possible. Glucose level was measured using Accu-Chek Performa blood glucose monitoring system (Sandhofer Strasse, Mannheim, Germany).

2.5. Insulin ELISA assay

Measurement of serum levels of insulin was performed according to the manufacturer's manual. The levels of insulin in the serum were evaluated by measuring the absorbance at 450 nm using a microplate spectrophotometer Epoch (Biotek, Winooski, VT).

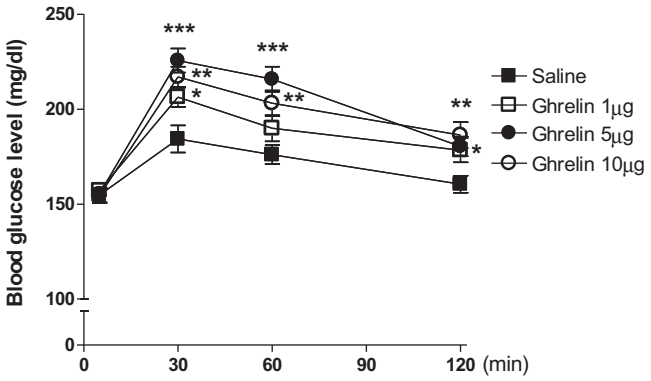

Fig. 1. The effect of ghrelin (1–10 μg/5 μl) administered i.t. on the blood glucose level. The blood glucose level was measured at 30, 60 and 120 min after ghrelin injection. The blood was collected from tail-vein. The vertical bars indicate the standard error of mean. Each quantified result was analyzed by one-way ANOVA with a Bonferroni post hoc test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$; compared to saline group). The number of animal used for each group was 8–10.

2.6. Statistical analysis

Statistical analysis was carried out by a Student *t* test GraphPad Prism Version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). *P*-values of less than 0.05 were considered to indicate statistical significance. All values were expressed as the mean ± S.E.M. In our study, we established the mean blood glucose value of the control group through many experiments under matching conditions. Selected mice of the established blood glucose level were then used in replication experiments.

3. Results

3.1. The effect of ghrelin administered i.t. on the blood glucose level

Mice were treated i.t. with ghrelin (from 1 to 10 μg/5 μl). As shown in Fig. 1 ($F = 0.8373$; $P = 0.4990$), i.t. administration of ghrelin at the dose of 1, 5 or 10 μg caused an elevation of the blood glucose level at 30 min and the up-regulated ghrelin-induced response was maintained up to 120 min after ghrelin administration (1 μg +11.9%, 5 μg +22.4%, 10 μg +17.7% at 30 min; 1 μg +7.9%, 5 μg +22.6%, 10 μg +15.4% at 60 min).

3.2. The effect of YIL781 pretreated i.t. on the blood glucose level

To examine whether the ghrelin-induced response is mediated by activation of GHS-R, the effect of YIL781 (a GHS-R antagonist) on the ghrelin-induced hyperglycemic effect was investigated. We found in the present study that i.t. pretreatment with YIL781 (from 0.1 to 5 μg/5 μl) attenuated ghrelin-induced up-regulation of the blood glucose level as shown in Fig. 2B ($F = 0.7506$; $P = 0.5729$) (YIL781 5 μg + ghrelin 5 μg −9.1% at 30 min; −14.9% at 60 min). The i.t. treatment with YIL781 alone did not affect the blood glucose level ($F = 0.8160$; $P = 0.5095$) (Fig. 2A).

3.3. Effect of ghrelin administered i.t. on the plasma insulin level

To examine if the ghrelin-induced response is due to the changes of plasma insulin level, we investigated effect of i.t. administered ghrelin on the plasma insulin level. The plasma insulin level was measured at 30 min after ghrelin injection. As shown in Fig. 2C