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Effect of ghrelin on autonomic activity in healthy volunteers

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ABSTRACT

Ghrelin is a novel growth hormone (GH)-releasing peptide originally isolated from the stomach. Recently, we have shown that ghrelin suppresses cardiac sympathetic activity and prevents early left ventricular remodeling in rats with myocardial infarction. In the present study, we evaluated the effect of ghrelin on autonomic nerve activity in healthy human subjects. An intravenous bolus of human synthetic ghrelin (10 μg/kg) was administered to 10 healthy men (mean age, 33 years). Holter monitoring assessment was performed before and during 2 h after the ghrelin therapy. The standard deviation of normal RR intervals (SDNN), square root of the mean of the sum of the squares of differences between adjacent RR intervals (rMSSD), high-frequency power (HF), and low-frequency power (LF) were analyzed. Blood samples were also obtained before and after the therapy. A single administration of ghrelin decreased both heart rate and blood pressure. Interestingly, ghrelin significantly decreased the LF and LF/HF ratio of heart rate variability and increased the SDNN, rMSSD, and HF. Ghrelin also elicited a marked increase in circulating GH, but not insulin-like growth factor-1. These data suggest that ghrelin might suppress cardiac sympathetic nerve activity and stimulate cardiac parasympathetic nerve activity.

Introduction

Ghrelin is a growth hormone (GH)-releasing peptide originally isolated from the stomach that serves as an endogenous ligand for the GH secretagogue receptor (GHS-R) [6]. Beside its potent GH-releasing activity, ghrelin induce a positive energy balance by stimulating food intake and inducing adiposity through growth hormone independent mechanisms [15]. Ghrelin also has several cardiovascular effects, as suggested by the presence of the receptor on blood vessels and in the cardiac ventricles [5]. We previously demonstrated that ghrelin suppresses cardiac sympathetic nervous activity and prevents early left ventricular remodeling in rats after myocardial infarction [19]; administration of ghrelin immediately after myocardial infarction prevents the early increases in cardiac sympathetic nerve activity, resulting in improved survival [17]. Furthermore, we have recently reported that ghrelin administration reduced ventricular arrhythmias concomitant with prevention of the loss of connexin 43 during acute myocardial ischemia at least in part by modulation of cardiac autonomic nerve activity [20].

In human, Nagaya et al. investigated the effects of intravenous bolus ghrelin administration to patients with congestive heart failure for 3 weeks and found that repeated ghrelin injection improves left ventricular (LV) function in these patients [14]. In fact, ghrelin increased the LV ejection fraction, which was associated with an increase in LV mass and a decrease in LV end-systolic volume. Although these results suggest that ghrelin has therapeutic potential in cardiac dysfunction, how ghrelin exerts its beneficial action on cardiac function remained to be elucidated.

Abbreviations: GH, growth hormone; GHS-R, growth hormone secretagogue receptor; LV, left ventricular; ECG, electrocardiography; SDNN, standard deviation of normal RR intervals; rMSSD, square root of the mean of the sum of the squares of differences between adjacent R-R intervals; CVRR, coefficient of variance of R-R interval; HF, high-frequency power; LF, low-frequency power; IGF-1, insulin like growth factor-1.

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At present, the effect of ghrelin on human autonomic activity remains unknown. Therefore, in the present study, we evaluated the effect of ghrelin on cardiac autonomic activity in healthy human subjects.

Methods

Preparation of human ghrelin

Human synthetic ghrelin was obtained from the Peptide Institute Inc. (Osaka, Japan). This peptide is not commercially available. Ghrelin was dissolved in distilled water with 4% D-mannitol and sterilized by passage through a 0.22-μm filter (Millipore Corp., Bedford, MA). Ghrelin was stored in 2-mL volumes, each containing 700 μg ghrelin. The chemical nature and content of the human ghrelin in vials were verified by high-performance liquid chromatography and radioimmunoassay. All vials were stored frozen at −80 °C from the time of dispensing until the time of preparation for administration.

Study subjects

This study included 16 healthy men (mean age 33 years) who had no history of any mental or medical disorders. All the subjects gave written informed consent to participate to the study, which had been approved by the Institutional Review Board of The University of Tokushima Clinical Research Center. The study protocol was in accordance with the Declaration of Helsinki Principles. The subjects randomly received either ghrelin (ghrelin group, $n = 10$) or placebo (placebo group, $n = 6$).

Study protocol

After overnight fasting, the study was begun in the morning at 0830–0930. An intravenous bolus of 0.9% saline, with or without human synthetic ghrelin (10 μg/kg), was administered. Holter monitoring assessment was performed before and during 2 h after the ghrelin therapy using two-channel Holter electrocardiography (ECG) recorder (SM-50; Fukuda Denshi Co., Ltd., Tokyo, Japan). The standard deviation of normal R-R intervals (SDNN), square root of the mean of the sum of the squares of differences between adjacent RR intervals (rMSSD), coefficient of variance of R-R interval (CVRR), high-frequency power (HF, 0.15–0.40 Hz), and low-frequency power (LF, o.o4–0.15 Hz) were analyzed using a time-series data-analysis software (Fukuda Denshi Co., Ltd., Tokyo, Japan). Blood samples were also obtained before and after the therapy (at 30 min and at 120 min). The blood was immediately transferred into a chilled glass tube containing disodium ethylenediaminetetraacetic acid (1 g/L) and aprotinin (500 U/mL) and centrifuged immediately at 4 C. The plasma samples were frozen and stored at −80 C until the measurements. Serum growth hormone (GH) and insulin like growth factor-1 (IGF-1) concentrations were measured by enzyme immunoassay kits (R&D Systems, Inc. Quantikine Human IGF-I Immunoassay, Quantikine Human Growth Hormone Immunoassay).

Statistical analysis

Data are expressed as the mean ± S.D. Differences among the groups were evaluated by two-way analysis of variance for repeated measurements. When a statistical difference was detected by analysis of variance, the Bonferroni method of adjusting for multiple pairwise comparisons was used. A value of $P < 0.05$ was considered statistically significant.

Results

Sixteen healthy men were randomly divided into 2 groups who receive ghrelin or placebo. There were no significant difference in age (33 ± 5 vs 34 ± 8 years). A single administration of ghrelin decreased both heart rate and blood pressure (Fig. 1). The hypotensive effect of ghrelin lasted for 30 min and the bradycardia effect of ghrelin lasted for 60 min after the ghrelin infusion. On the other hand, the administration of placebo did not change heart rate and blood pressure (Fig. 1). Interestingly, the administration of ghrelin increased the HF, reflecting the parasympathetic activity. Ghrelin also decreased LF and LF/HF ratio, reflecting the sympathetic activity while placebo did not (Fig. 2). The effect of ghrelin on HF lasted longer than 60 min and the effect of ghrelin on LF/HF ratio lasted longer than 120 min after the infusion of ghrelin (Fig. 2). In addition, ghrelin increased the SDNN, rMSSD, and CVRR, which effect lasted for 60 min after the ghrelin administration (Fig. 3). These effects of ghrelin on the autonomic parameters were not found in placebo group. Furthermore, ghrelin also elicited a marked increase in circulating GH 30 min after the infusion (Fig. 4) whereas placebo did not. On the other hand, no significant change in serum IGF-1 level was observed throughout the study period after the infusion of ghrelin (Fig. 4). In this study, we saw some side effects of ghrelin such as perspiration (8/10), drowsiness (5/10), hunger (3/10), general fatigue (1/10), and mild diarrhea (1/10). However, these side effects were transient and not severe.

Discussion

The main novel findings of the present study are that ghrelin significantly decreased the LF and LF/HF ratio of heart rate variability and increased the SDNN, rMSSD, CVRR, and HF in human subjects. Although some previous studies showed that ghrelin decreased blood pressure or heart rate in human subjects [7,13], there were no studies investigating the effect of ghrelin on the autonomic activities in human subjects. Then, the present study is the first research to demonstrate that ghrelin might suppress cardiac sympathetic

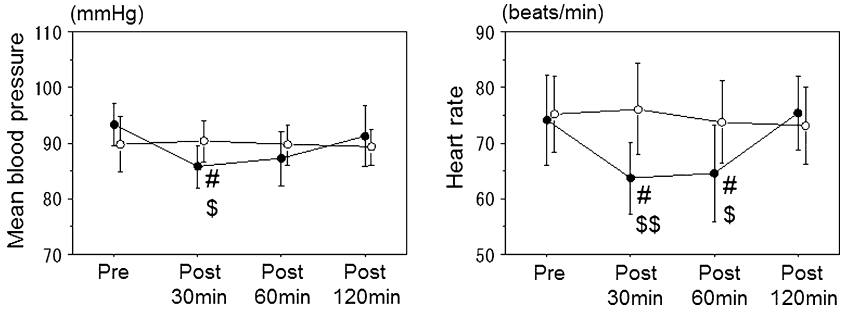

Fig. 1. Changes in mean arterial pressure and heart rate during infusion of ghrelin (closed circle) or placebo (open circle). Intravenous infusion of ghrelin but not placebo significantly decreased both mean blood pressure and heart rate. $^{\#}P < 0.05$ vs pre-infusion; $^{\$\$}P < 0.01/^{\$}P < 0.05$ vs respective placebo group.