Hemodynamic and hormonal effects of human ghrelin in healthy volunteers

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Methods

Study subjects. We studied six healthy male hospital staff members, aged 30 ± 1 yr, who weighed 68 ± 5 kg. None of them had any history of cardiovascular, renal, respiratory, hepatic, or metabolic disease, and none were taking any drugs. Physical examination and electrocardiographic and echocardiographic findings were also normal. The study was approved by the ethics committee of the National Cardiovascular Center, and all subjects gave written informed consent.

Preparation of synthetic human ghrelin. Human ghrelin was obtained from the Peptide Institute, Osaka, Japan. The homogeneity of human ghrelin was confirmed by reverse-phase, high-performance liquid chromatography (RP-HPLC) and amino acid analysis. Ghrelin was dissolved in distilled water with 4% D-mannitol and was sterilized by passage through a 0.22-μm filter (Millipore). Ghrelin was stored as 1 ml volumes (each containing 600 μg ghrelin) at −80°C until the time of preparation for administration.

Study protocol. The subjects were studied on 2 separate days (1 day, ghrelin; 1 day, placebo) 1–2 wk apart in a randomized, crossover fashion. This study was performed after the subjects fasted overnight, because plasma ghrelin level has been shown to be altered by food intake (19). A 7.5 French Swan-Ganz catheter (TOO21H-7.5F, Baxter) was po-

sitioned in the pulmonary artery through a jugular vein to measure pulmonary arterial pressure and pulmonary capillary wedge pressure. Cardiac output was determined by thermodilution method in triplicate (7). A 22-gauge cannula was inserted into a radial artery for measurement of heart rate and systemic arterial pressure. Another 22-gauge cannula was inserted into a forearm vein for infusion of ghrelin. Ghrelin (10 μg/kg) was dissolved in 5 ml saline. After an equilibration period of 60 min, baseline measurements were performed. Then, 5 ml of ghrelin solution or placebo (0.9% saline) was administered as an intravenous bolus. Hemodynamic measurements were repeatedly performed until 120 min after the injection. Blood sampling was repeated at 15 points (−10, 0, 1, 3, 5, 10, 15, 20, 30, 40, 50, 60, 80, 100, and 120 min after ghrelin injection). Plasma half-life of ghrelin was calculated using WinNonlin library (noncompartmental model, Scientific Consulting).

RIA for plasma ghrelin. RIA for plasma ghrelin was performed as described previously (10). In brief, a polyclonal antibody was raised against the COOH terminal fragment (13–28) of rat ghrelin in a rabbit. A maleimide-activated mariculture keyhole limpet hemocyanin-[Cys]-ghrelin-(13–28) conjugate was used for immunization. [Tyr0]-rat ghrelin (13–28) was radiiodinated by the lactoperoxidase method. A moniodinated ligand was purified by RP-HPLC on a µBondasphere C18 column (3.9 × 150 mm, Waters, Milford, MA). The tracer was stable for 3 mo and stored at −20°C in 0.1% bovine serum albumin. The RIA incubation mixture consisted of 100 μl of unknown sample, 100 μl of normal rabbit serum, and 200 μl of antiserum at a dilution of 1:10,000. After 12 h incubation at 4°C, 100 μl of 125I-labeled ligand (15,000 cpm) was added to the mixture. After 36 h incubation at 4°C, 100 μl of anti-rabbit goat antibody was added. Free and bound tracers were separated by centrifugation at 3,000 rpm for 30 min after incubation for 24 h at 4°C. After aspiration of supernatant, radioactivity in the pellet was quantified with a gamma counter (ARC-600, Aloka, Tokyo, Japan). The minimum detectable dose of ghrelin was <6 fmol/tube. The antiserum exhibited 100% cross-reactivity with rat or human ghrelin (13–28). No significant cross-reactivity with other peptides was observed. Intraobserver variability of ghrelin measurement was less than 6%, and its interobserver variability was less than 9%.

Other biochemical measurements. Serum GH was measured with an immunoradiometric assay kit (Ab Bead HGH Eiken, Eiken Chemical, Tokyo, Japan). Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), and thyroid-stimulating hormone (TSH) were measured using immunoradiometric assay kits (SPAC-S FSH, LH, Prolactin, and TSH, Daiichi Radioisotope Laboratories, Tokyo, Japan). Plasma adrenocorticotropic (ACTH) was measured with an immunoradiometric assay (ACTH IRMA Mitsubishi, Mitsubishi Chemical, Tokyo, Japan). Plasma cortisol was measured with a radioimmunoassay kit (Gamma-Coat Cortisol, Date Behring). Serum insulin-like growth factor-1 (IGF-1) was determined by an immunoradiometric assay kit (Somatomedin CII Bayer, Bayer Medical, Tokyo, Japan). Plasma norepinephrine and epinephrine were measured with HPLC. Plasma cAMP and cGMP were determined with specific RIA kits (cAMP assay kit, cGMP assay kit, Yamasa Shoyu, Chiba, Japan).

RT-PCR analysis. mRNA expression of GHS-R, a specific receptor for ghrelin, was examined in the descending aorta, the left ventricle, and the left atrium of male Wistar rats. Total RNA was extracted from the tissues and was converted to cDNA by reverse transcription (RT). PCR was performed for 35 cycles (5 s at 98°C, 10 s at 65°C, and 1 min at 72°C) with specific primers (sense: GAGATGCCTCACAGTCCAGCCAG-ATGAC and antisense, TAATGCCCAAACTGAGGTCTGCG). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was amplified as the control.

All animal experiments were conducted in accordance with the principles and procedures outlined in the National Cardiovascular Center Guide for the Care and Use of Laboratory Animals, which adheres strictly to the National Institutes of Health animal experimental guidelines, with the approval of the National Cardiovascular Center Animal Experimental Committee.

Statistical analysis. Data were expressed as means ± SE. Comparisons of the time course of parameters between two groups were made by two-way ANOVA for repeated measures, followed by Newman-Keuls test. A P value < 0.05 was considered statistically significant.

RESULTS

All study subjects tolerated this study protocol, although ghrelin caused a slight warm feeling and sleepiness in four subjects.

Hormonal response to ghrelin. Plasma ghrelin level increased about 61-fold of baseline value ~1 min after ghrelin injection. The plasma ghrelin level disappeared with half-lives of 10 min (Fig. 1A). Injection of ghrelin elicited a marked increase in circulating GH, with the peak level (15× baseline value) occurring 20 min after

Fig. 1. Circulating ghrelin level after a single injection of ghrelin (A). Effects of ghrelin on circulating growth hormone (GH) (B). Data are means ± SE. *P < 0.05 vs. placebo group. The arrow indicates an intravenous injection of ghrelin or placebo.
administration (Fig. 1B). The elevation of GH level lasted >60 min after the bolus injection. Ghrelin significantly increased circulating levels of PRL, ACTH, and cortisol, whereas it did not significantly alter FSH, LH, or TSH level (Table 1). No significant change in serum IGF-1 was observed throughout the study protocol. Ghrelin significantly increased plasma epinephrine but not norepinephrine. Ghrelin significantly increased plasma cAMP but not plasma cGMP. These hormonal parameters remained unchanged during placebo injection.

Hemodynamic response to ghrelin. Injection of ghrelin significantly decreased mean arterial pressure to a minimum level (−12 mmHg, \( P < 0.05 \); Fig. 2) without a significant change in heart rate (−4 beats/min, \( P = 0.39 \)). The hypotensive effect of ghrelin lasted for 100 min after the injection. No significant change in mean pulmonary arterial pressure or pulmonary capillary wedge pressure was observed (data not shown). Cardiac index significantly rose to the maximum level 15 min after injection of ghrelin (+16%, \( P < 0.05 \)) and returned to near preinjection level at 60 min. Stroke volume index also rose to the maximum level 15 min after injection (+22%, \( P < 0.05 \)). Consequently, ghrelin markedly decreased systemic vascular resistance (−24%, \( P < 0.05 \)). These hemodynamic parameters remained unchanged after placebo injection.

Detection of GHS-R expression. GHS-R mRNA was detectable by RT-PCR in the rat aorta as well as the left ventricle and left atrium (Fig. 3).

**DISCUSSION**

This is the first study to examine hemodynamic and hormonal effects of ghrelin in humans. In the present study, we demonstrated that 1) intravenous injection of ghrelin elicited a potent, long-lasting GH release in healthy volunteers and 2) ghrelin decreased mean arterial pressure and increased cardiac output but did not increase heart rate.

Ghrelin is a novel GH-releasing peptide, isolated from the stomach, that acts through a mechanism independent from that of hypothalamic GHRH (12). The GH-releasing effects of ghrelin are thought to be mediated by specific receptors, GHS-R, mainly present at the pituitary and hypothalamic level. Taken together with the fact that intravenously administered ghrelin induced a potent GH release in humans, the data suggest that this molecule is produced in and secreted from the stomach and circulates in the bloodstream to act on the pituitary. A single injection of ghrelin resulted in a relatively long-lasting elevation of circulating GH, although plasma ghrelin level decayed soon after a single intravenous administration.

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Data are means ± SE. \*P < 0.05 vs. placebo group. FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin; TSH, thyroid-stimulating hormone; IGF-1, insulin-like growth factor-1.
placement therapy with GH has been reported to have beneficial effects on myocardial structure and function in patients with chronic heart failure (6, 20). Thus it may be interesting to investigate whether ghrelin administration is beneficial in patients with heart failure by inducing potent, long-lasting GH release.

Ghrelin also exhibited stimulatory effects on PRL, ACTH, and cortisol secretion, which is consistent with the effects of hexarelin (HEX), a synthetic GH-releasing peptide (14). GHS-R, a specific receptor for ghrelin and HEX, has been shown to exist abundantly in the hypothalamus and pituitary (11). Thus the hormonal effects of ghrelin may be due to direct effects at the pituitary level as well as in the hypothalamus. The stimulatory effect of ghrelin on cortisol secretion seems to be due to its ACTH-releasing property, because the cortisol-releasing activity of HEX is lost in the presence of hypothalamo-pituitary disconnection (17). Ghrelin significantly increased plasma epinephrine, but not norepinephrine, level. Considering the presence of GHS-binding sites in the adrenal (8), the epinephrine-releasing effect of ghrelin may be due to direct effects on the adrenal.

To our knowledge, GHRH, another physiological GH-releasing factor, has no direct cardiovascular effects because the GHRH receptor is restricted to specific tissues including pituitary membranes (15). In contrast, ghrelin peptide and its specific binding sites are detected in a variety of tissues including the heart and blood vessels (8, 10, 12). In the present study, we first demonstrated that ghrelin induced a marked, long-lasting hypotensive effect, although activation of the hypothalamic-pituitary-adrenal (HPA) axis is known to be associated with hypertension. A single injection of ghrelin did not significantly increase circulating IGF-1, which has been shown to cause vasodilation through a direct stimulatory effect on nitric oxide synthesis (3). In addition, we recently found that ghrelin induced hypotension in hypophysectomized rats in which ghrelin could not stimulate the release of either GH or IGF-1 (unpublished data). Furthermore, we showed that the GHS-R gene was abundantly expressed in the rat aorta. Ghrelin injection significantly increased plasma cAMP level in association with decreases in mean arterial pressure. These findings raise the possibility that ghrelin has a direct vasorelaxant effect. Further studies are necessary to elucidate the relationship between ghrelin and the HPA axis. Surprisingly, the hypotensive effect of ghrelin was not associated with an increase in heart rate or plasma norepinephrine. It is interesting to speculate that ghrelin may inhibit activation of the sympathetic nervous system during hypotension, which may be beneficial in treating patients with congestive heart failure. Unlike ghrelin, HEX, a GHS, does not induce a hypotensive effect, although HEX has a positive inotropic effect in humans (2). It is interesting to speculate that different affinities for GHS-R in the vasculature may contribute to the differential vascular effects of ghrelin and HEX.

This study also demonstrated that ghrelin significantly increased cardiac index and stroke volume index. Considering the hypotensive effect of ghrelin, we believe that a decrease in cardiac afterload may be responsible for the increased cardiac index. Alternatively, because GH upregulates sarcoplasmic Ca\(^{2+}\)-ATPase and thereby enhances myocardial contractility (18), part of the cardiac effects of ghrelin may be mediated by GH. Interestingly, the GHS-R gene was abundantly expressed in the myocardium. Thus it is possible that ghrelin may have some direct actions on myocardium. It is also possible that increased plasma
epinephrine by ghrelin might indirectly increase cardiac index and stroke volume index. Further studies are necessary to examine the potential mechanisms responsible for these cardiovascular effects of ghrelin.

Study limitations. In the present study, hormonal responses to ghrelin were examined after the subjects fasted overnight, because plasma ghrelin level has been shown to be altered by food intake (19). On the other hand, fasting may alter GH responses to ghrelin and contribute to the uncoupling of the GH/IGF-1 axis in study subjects (4, 9). Further studies are necessary to investigate the effects of food intake on GH/IGF-1 responses to ghrelin.

Summary. Human ghrelin elicited a potent, long-lasting GH release and had beneficial hemodynamic effects via reducing cardiac afterload and increasing cardiac output without increasing heart rate. Based on the results of this preliminary study, we believe that the long-term effects of ghrelin in patients with chronic heart failure should be examined in a randomized, large-scale study.

Perspectives

GH supplement has been reported to improve myocardial structure and function in patients with idiopathic dilated cardiomyopathy, a condition in which compensatory cardiac hypertrophy is believed to be deficient (6). Given the potent GH-releasing activity and direct cardiovascular effects of ghrelin, it may be interesting to investigate whether ghrelin administration is beneficial in patients with intractable chronic heart failure.

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REFERENCES