Effects of ghrelin administration on decreased growth hormone status in obese animals

Hiroshi Iwakura,1 Takashi Akamizu,1 Hiroyuki Ariyasu,1 Taiga Iraito,1 Kiminori Hosoda,2 Kazuwa Nakao,2 and Kenji Kangawa1,3

1Ghrelin Research Project, Translational Research Center, Kyoto University Hospital; 2Department of Medicine and Clinical Science, Endocrinology and Metabolism, Kyoto University Graduate School of Medicine, Kyoto; and 3Department of Biochemistry, National Cardiovascular Center Research Institute, Osaka, Japan

Submitted 13 December 2006; accepted in final form 22 June 2007

Kazuwa Nakao,2 and Kenji Kangawa1,3

IN HUMANS, OBESITY IS CHARACTERIZED BY MARKEDLY DECREASED GROWTH HORMONE (GH) PRODUCTION AND SECRETION (3, 26). GH STIMULATES LIPOLYSIS AND INCREASES LEAN BODY MASS, WHICH MAY HELP TO COMBAT OBESITY. DECREASED GH SECRETION IN THE CONTEXT OF OBESITY MAY PROMOTE ADDITIONAL FAT DEPOSITION AND PROMOTE WEIGHT GAIN (10). GH, HOWEVER, DOES CONTRIBUTE TO INSULIN RESISTANCE, WHICH CAN WORSEN DIABETES (7).

Ghrelin is a 28-amino acid peptide with unique acylation modification, which is essential for its biological action (14). Ghrelin was originally identified in the rat stomach as an endogenous ligand for an orphan receptor, which so far has been called GH secretagoge receptor (GHS-R) (14). Ghrelin is involved in a wide variety of functions, including regulation of GH release, gastric acid secretion, gastric motility, blood pressure, and cardiac output (4, 8, 18, 19, 23, 28). Ghrelin also has several metabolic functions, including orexigenic action (20, 22), reduction of insulin (5), and control of energy expenditure (24), which are all involved in the pathophysiology of adiposity or diabetes.

Plasma ghrelin level is suppressed in obesity (25), which may compensate for increased body weight by reducing its orexigenic activity, whereas low plasma ghrelin level may contribute to decreased GH secretion in obesity. Furthermore, Poykko et al. (21) reported that low plasma ghrelin level is associated with insulin resistance and incidence of type 2 diabetes.

To elucidate whether ghrelin supplementation can restore decreased GH secretion in obesity, we first determined acute GH and orexigenic responses to ghrelin in three different obese and/or diabetic mouse models: db/db mice, mice on a high-fat diet (HFD mice), and Akita mice for comparison. GH responses to ghrelin were significantly suppressed in db/db, HFD, and Akita mice. Food intake of db/db and Akita mice was basally higher, and further stimulation of food intake by ghrelin was suppressed. Pituitary GH secretagoge receptor mRNA levels in db/db and HFD mice were significantly decreased, which may partly contribute to decreased GH response to ghrelin in these mice. In Akita mice for comparison, decreased hypothalamic GH-releasing hormone (GHRH) mRNA levels may be responsible for decreased GH response, since maximum GH response to ghrelin needs GHRH. When ghrelin was injected into HFD mice with GHRH coadministered, GH responses to ghrelin were significantly emphasized. HFD mice injected with low-dose ghrelin and GHRH for 10 days did not show weight gain. These results indicate that low-dose ghrelin and GHRH treatment may restore decreased GH secretion in obesity without worsening obesity.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS. Eight-week-old male db/db and control mice (misty) were purchased from CLEA Japan, (Tokyo, Japan). As a diet-induced model of obesity, 5-wk-old male C57BL/6J mice, purchased from Japan SLC (Shizuoka, Japan), were maintained on a HFD of 60% fat/kcal (Research Diets, New Brunswick, NJ) for 20 wk. Those maintained on a standard diet were used as control mice for HFD mice. Eight-week-old male Akita mice and C57BL/6J control mice were purchased from Japan SLC. Animals were maintained on standard rat food (CE-2, 352 kcal/100 g; CLEA Japan) with a 12:12-h light-dark cycle unless otherwise indicated. All experimental procedures were approved by the Kyoto University Graduate School of Medicine Committee on Animal Research.

ACUTE GH RESPONSE TO GHRH. Rat ghrelin (40, 120, and 360 µg/kg; Peptide Institute, Osaka, Japan), human GHRH (60 µg/kg; Mecasermin, Astellas Pharma, Tokyo, Japan), or saline was injected subcutaneously into mice on an ad libitum feeding schedule. Blood was collected from retroorbital veins 15 or 30 min after injection. Serum was isolated by centrifugation and stored at −20°C until assayed.

MEASUREMENTS OF HORMONES AND FREE FATTY ACID LEVELS. Serum GH levels were determined by rat growth hormone EIA kit (SPI bio, LC2000).
GCTTGTTCATGA-3

AGGATGCAGCGACACGTAGA-3

GCTTCTTGGATTCCT-3

TCCGATCTGCTCATCTTCCTGTGCATG-3

CGGCAGAAACCGGAAAAACCAAACTAAAT -3

-CGCTGCCTGACCGCTAAGTA-3

GCCCTTGGAACTGTTA-3

-TCTCCCCTT-

AJP-Endocrinol Metab • VOL 293 • SEPTEMBER 2007 • www.ajpendo.org

Fig. 1. Growth hormone (GH) responses to ghrelin in db/db mice. A: serum GH levels 15 or 30 min after sc injection of ghrelin into db/db (db) or control (con) mice. B: serum GH levels 15 min after iv ghrelin injection into db/db or con mice. C: serum GH levels 15 min after sc injection of GH-releasing hormone (GHRH). **P < 0.01.
We first examined acute GH responses to ghrelin in db/db, HFD, and Akita mice. GH responses to ghrelin in db/db mice were markedly lower than those observed in control mice at any dose (40, 120, or 360 μg/kg; Fig. 1A). Thirty minutes after ghrelin injection (40 or 120 μg/kg) of db/db mice, serum GH levels tended to be even lower than those at 15 min (Fig. 1A), indicating that low GH levels at 15 min were not due to delayed response. GH responses at 15 min after intravenous injection of ghrelin were also decreased in db/db mice (Fig. 1B), indicating that the disturbed GH responses observed in db/db mice were not due to the malabsorption of ghrelin caused by fat deposition at the subcutaneous injection site. GH responses to GHRH (60 μg/kg) were also decreased in db/db mice (Fig. 1C). As in db/db mice, GH levels at 15 min after subcutaneous ghrelin injection (40, 120, and 360 μg/kg) in HFD mice were significantly lower than those seen in control mice (Fig. 2A). GH responses to GHRH (60 μg/kg) also tended to be decreased (Fig. 2B). Although GH levels in Akita mice were not significantly different from those in control mice measured at 15 min after 40 μg/kg sc injection of ghrelin, those measured after a higher dose of ghrelin (120 and 360 μg/kg) were significantly lower than those in control mice (Fig. 3A). GH responses to GHRH (60 μg/kg) also tended to be decreased in Akita mice (Fig. 3B).

We then measured 1-h food intake stimulated by ghrelin in db/db and Akita mice. In control mice, a 40 μg/kg sc ghrelin injection evoked about threefold greater food intake than that induced by saline injection (saline vs. ghrelin: 0.09 ± 0.04 vs. 0.28 ± 0.01 g, P < 0.05, n = 7; Fig. 4A). Ghrelin stimulated additional food intake in a dose-dependent manner in control mice, as demonstrated by the ratio of food intake evoked by ghrelin to that induced by saline (Fig. 4A). In db/db mice, basal food intake was higher than that of control mice (0.22 ± 0.05 vs. 0.07 ± 0.02 g, P < 0.05, n = 7). Although sc injection of 40 μg/kg ghrelin into db/db mice did not stimulate food intake significantly (saline vs. ghrelin: 0.30 ± 0.11 vs. 0.18 ± 0.04 g, P = 0.30, n = 7), higher doses of ghrelin (360 μg/kg), however, were able to stimulate food intake (saline vs. ghrelin: 0.14 ± 0.08 vs. 0.39 ± 0.04 g, P < 0.05, n = 7). Although higher ghrelin stimulated food intake in db/db mice, the extent of stimulation as demonstrated by the ratio of ghrelin-induced food intake (40 and 360 μg/kg) to that by saline was significantly smaller than that in control mice (Fig. 4A). In Akita mice, basal food intake was higher than that of control mice (0.28 ± 0.01 vs. 0.15 ± 0.02 g, P < 0.05, n = 7), and no further stimulation of food intake by ghrelin was observed (Fig. 4B).

We then measured the mRNA expression of ghrelin-GH system in pituitaries and hypothalami of db/db, HFD, and Akita mice (Fig. 5). Pituitary mRNA levels of GHS-R were significantly lower in db/db and HFD mice, whereas those in Akita mice were significantly higher compared with their
control mice (Fig. 5A). Pituitary mRNA levels of ghrelin were significantly lower in db/db mice, whereas those in Akita mice were significantly higher (Fig. 5A). Pituitary mRNA levels of GH were significantly lower in db/db and HFD mice and tended to be lower in Akita mice (Fig. 5A). Pituitary mRNA levels of SSTR2 were significantly higher in db/db mice, whereas they were not significantly changed in HFD and Akita mice (Fig. 5A). Pituitary mRNA levels of SSTR5 were significantly lower in Akita mice, whereas those levels were not significantly changed in db/db and HFD mice (Fig. 5A). There were no significant changes in the expression levels of GHRH-R in pituitaries of these mice (Fig. 5A). In hypothalamus, GHS-R and GHRH mRNA levels were significantly higher and lower, respectively, in Akita mice (Fig. 5B). Ghrelin mRNA levels were significantly lower in hypothalamus of HFD mice (Fig. 5B).

Finally, we examined the effect of chronic ghrelin injection to HFD mice. To maximize GH-stimulating activity of ghrelin and to minimize orexigenic action of ghrelin, we first examined GH responses to low-dose ghrelin with GHRH coadministration in HFD mice. Acute GH responses to ghrelin were significantly potentiated by coadministration of GHRH even at the lowest dose in HFD mice (Fig. 6A). By 10 days of twice daily injections of saline or ghrelin and GHRH, both control and HFD mice lose weight by ~6–8%. This weight reduction might be due to stress of twice daily injection, which is usually covered by growth in younger mice. Control mice treated with ghrelin and GHRH tended to take in more food than those with saline (Fig. 6C). Fat masses were more preserved in the ghrelin- and GHRH-treated groups than in the saline-treated group in control mice (Fig. 6B, D, and E), although percent body weight and percent lean body mass changes were comparable between the saline-treated and the ghrelin- and GHRH-treated group in control mice. In HFD mice, food intake, percent body weight change, and percent lean body mass change were comparable between the saline-treated group and the ghrelin- and GHRH-treated groups (Fig. 6B, D, and E). In contrast to control mice, fat mass tended to even be decreased in the ghrelin and GHRH group than in the saline-treated group in HFD mice (Fig. 6D). In both control and HFD mice, blood glucose, serum insulin, and serum IGF-I levels of the ghrelin- and GHRH-treated group were not significantly different from those of the saline-treated group (Fig. 6).

**DISCUSSION**

We have demonstrated that GH responses to ghrelin are decreased in both genetic and diet-induced mouse models of obesity. Recently, Luque and Kineman (15) reported that plasma GH levels acquired by random sampling without stimulation in ob/ob mice and HFD mice tended to be lower than...
those seen in control mice. Because GH secretion is pulsatile, it is difficult to compare GH values obtained by random sampling in the absence of stimulation. For this reason, we could not detect any significant difference in basal GH values between \(db/db\) or HFD and control mice. Following ghrelin or GHRH stimulation, however, we clearly observed a severe impairment in GH secretions by obese mice. Alvarez-Castro et al. (2) previously reported that GH responses to ghrelin were decreased in obese human subjects compared with those seen in normal controls; although reduced, these responses to ghrelin were greater in magnitude than those observed following GHRH treatment in obese human subjects. Our observations in mice were consistent with these data obtained in humans, which verify the use of \(db/db\) and HFD mice as experimental animal models for ghrelin treatment for obesity.

We also demonstrated that GH responses to ghrelin are decreased in Akita mice. As far as we know, this is the first report on the GH responses to ghrelin in insulin-deprived mice. In humans with insulin-deprived diabetes, it is well known (11) that basal GH is elevated and that GH response to provocative tests, including GHRH or GHS administration, is exaggerated. Thus discrepancy between human and mouse GH response to ghrelin in insulin-deprived status exist.

We demonstrated that GHS-R mRNA levels were decreased in the pituitaries of \(db/db\) and HFD mice. Ghrelin does stimulate GH release from rat pituitary in vitro (14), but maximal

![Graphs and tables showing GH responses to ghrelin and GHRH stimulation in mice on a HFD.](Fig. 6. Chronic treatment of ghrelin and GHRH on mice on a HFD. A: serum GH levels 15 or 30 min after sc injection of 40 \(\mu\)g/kg ghrelin or 60 \(\mu\)g/kg GHRH or ghrelin and GHRH (60, 180, 540 \(\mu\)g/kg) into HFD or con mice. #Body weight (B), fat mass (D), and lean body mass (E) changes before and after treatment of 40 \(\mu\)g/kg ghrelin and 60 \(\mu\)g/kg GHRH or saline for 10 days in control mice (open bars) or mice on a HFD (filled bars). C: cumulative food intake for 10 days. Blood glucose (F), serum insulin level (G), and serum IGF-I level (H) after 10 days administration of ghrelin and GHRH, *P < 0.05; **P < 0.01 compared with control; #P < 0.05; ##P < 0.01 compared with ghrelin; +P < 0.05 compared with saline; n = 7.)
response of GH to ghrelin requires the existence of GHRH (9). Kamegai et al. (13) reported that pituitary ghrelin regulates GH secretion by modulating pituitary response to GHRH. The decreased expression of GHS-R in pituitary in db/db and HFD mice might contribute to suppressed GH response to ghrelin by attenuating the pituitary response to GHRH. Of course, decreased mRNA levels of GH in pituitary or, as reported in human (6, 16), elevated serum IGF-I or FFA levels in these mice might also contribute to suppressed GH responses.

Although GH responses to ghrelin were also decreased in Akita mice, the pituitary mRNA levels of GHS-R were significantly higher than those seen in control mice, indicating that pituitary GHS-R did not contribute to decreased GH responses. GHRH mRNA expression levels in hypothalamus of Akita mice were significantly lower compared with those of control mice. This reduction of GHRH mRNA levels may be responsible for decreased GH responses to ghrelin in Akita mice. Ghrelin stimulates GHRH secretion from the hypothalamus (27). And recently, Mano-Otagiri et al. (17) reported that GHS-R signaling upregulates hypothalamic GHRH expression. Although plasma ghrelin levels were significantly higher in Akita mice than those displayed by control mice, GHRH mRNA expression levels in hypothalamus of Akita mice were significantly decreased. In addition, the food intake of Akita mice was significantly elevated at baseline and was not stimulated by ghrelin any further. These results indicate the existence of ghrelin unresponsiveness in postreceptor level in hypothalamus.

In the chronic treatment experiment, ghrelin and GHRH treatment for 10 days tended to stimulate food intake and showed fat-sparing effect in control mice. In contrast, HFD mice injected with ghrelin and GHRH tended to decrease more fat mass compared with those treated with saline, which may be due to restored GH secretion and suppressed orexigenic response to ghrelin. In this setting, blood glucose and serum insulin levels did not change by ghrelin and GHRH treatment in HFD mice. This may be explained by the fact that the change in fat mass was only subtle and that lean body mass did not change by ghrelin and GHRH treatment. These results indicate that low-dose ghrelin and GHRH supplementation at least do not worsen obesity and metabolic status and that it may at least partially restore suppressed GH secretion.

In the current experiment, IGF-I levels were higher in HFD mice after chronic treatment of ghrelin and GHRH. Since IGF-I levels of the saline-treated group of HFD mice were also higher than those of control mice, this elevation seems to reflect nutritional status between HFD and control mice.

In conclusion, we demonstrated that acute GH responses to ghrelin were suppressed in both genetic and diet-induced mouse models of obesity. The decreased pituitary levels of GHS-R mRNA may contribute to suppression of GH response. We also demonstrated that acute GH responses to ghrelin were suppressed in Akita mice, an insulin-deprived diabetic mouse model. Decreased GHRH mRNA levels in hypothalamus and the lack of stimulation of food intake by ghrelin indicate the involvement of hypothalamus in the mechanism of suppressed GH response to ghrelin in Akita mice. These results indicate that suppressed GH response to ghrelin has a different mechanism in obese and insulin-resistant mice and insulin-deprived diabetic animals. In addition, HFD mice injected with ghrelin and GHRH showed potentiated GH responses. Chronic treatment of low-dose ghrelin and GHRH did not promote fat deposition in HFD mice. These results indicate that low-dose ghrelin and GHRH administration at least does not worsen obesity and that it may restore suppressed GH secretion.

ACKNOWLEDGMENTS

We thank Hitomi Hiratani, Chieko Ishimoto, Naoko Takehisa, Kozue Fukuda, and Chimiya Shiraiwa for excellent technical assistance.

GRANTS

This study was supported by funds from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and the Ministry of Health, Labour, and Welfare of Japan.

REFERENCES


