Evidence That Growth Hormone Exerts a Feedback Effect on Stomach Ghrelin Production and Secretion

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Ghrelin is a recently discovered stomach hormone that stimulates pituitary growth hormone (GH) secretion potently. The purpose of these experiments was to test the hypothesis that a stomach-ghrelin-pituitary-GH axis exists in which either an elevation or reduction in systemic GH levels will exert a negative or positive feedback action, respectively, on stomach ghrelin homeostasis. In rats, GH administration decreased stomach ghrelin mRNA levels and plasma ghrelin levels significantly. In GH-releasing hormone (GHRH) transgenic mice, GHRH overexpression decreased stomach ghrelin peptide levels when compared with control mice. In aged rats (25 months) stomach ghrelin mRNA and peptide levels and plasma ghrelin levels were decreased when compared with young rats (5 months). Because GH secretion is reduced in aged rats, the elevated stomach ghrelin production and secretion may reflect a decreased GH feedback on stomach ghrelin, homeostasis, and secretion. Together, these findings suggest that endogenous pituitary GH exerts a feedback action on stomach ghrelin homeostasis and support the hypothesis that a stomach-ghrelin-pituitary GH axis exists. Exp Biol Med 228:1028–1032, 2003

Key words: rat; mouse; pituitary

Ghrelin, a recently identified stomach hormone, is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) (1, 2). Ghrelin was isolated and characterized from rat stomach extracts based upon its ability to activate intracellular Ca^{++} signaling in a Chinese hamster ovary cell line transfected with the GHS-R (1). The GHS-R was named because a group of synthetic ligands called GH secretagogues (GHSs) stimulated pituitary GH secretion by activation of the GHS-R (3–5). As expected, ghrelin is a potent stimulant of GH release in humans as well as in laboratory animals (1, 6, 7).

An important unanswered question regarding ghrelin physiology is whether a stomach-ghrelin-pituitary-GH axis exists. In such a case, either elevations or reductions in systemic GH levels are expected to impact stomach ghrelin homeostasis (i.e., ghrelin mRNA and peptide levels) and secretion. The purpose of the present study, therefore, was to determine the influence of an elevation or a reduction in systemic GH levels on stomach ghrelin homeostasis and stomach ghrelin secretion. Elevated GH levels were accomplished by administration of GH to rats and use of growth hormone–releasing hormone (GH-RH) transgenic mice, and aged rats were used as a model for decreased systemic GH levels (8, 9).

Materials and Methods

Animals. Adult male Sprague-Dawley (SD) and Fisher-344 rats were maintained in an air-conditioned (24°C ± 2°C) and light-regulated (lights on, 6 AM–6 PM) room. Control and GH-RH transgenic mice were maintained under regulated housing conditions at Southern Illinois University. All animal experiments were conducted in accordance with mandated standards of humane care and were approved by the respective Institutional Animal Care and Use Committees.

Chemicals and Peptides. All chemicals were obtained from Sigma (St. Louis, MO) unless noted otherwise. Synthetic peptides were purchased from Bachem (Torrance, CA) or Phoenix (Belmont, CA). Purified ovine GH was obtained from Dr. A. Parlow (Harbour General Hospital, Torrance, CA) through the National Institutes of Health pituitary hormone distribution program.

Animal Experiments. *Experiment I.* Adult male SD rats (n = 9 rats/group) were given either vehicle or exogenous GH (400 μg, 3×/d for 3 days, sc). GH was prepared in 0.9% saline containing 0.01% bovine serum albumin. Rats were sacrificed in the *ad lib* fed condition and the

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fundal mucosa of the stomach and plasma was harvested for measurement of stomach ghrelin peptide and mRNA levels. The stomach mucosa was homogenized immediately either in an RNA extraction solution or a ghrelin peptide extraction solution (1 *M* acetic acid containing 20 m*M* HCl). Plasma was collected for determination of plasma ghrelin levels.

Experiment II. The stomach was harvested from control and GH-RH transgenic female mice (n = 9 mice/group) and frozen immediately for shipment to the Galveston laboratory. Samples were shipped on dry ice. GH-RH transgenic mice were selected as an additional model of increased systemic GH levels. In Galveston, the fundus was separated from the stomach antrum and rumen. The full thickness of the fundus was homogenized in a ghrelin peptide extraction solution and processed for measurement of ghrelin peptide levels by a ghrelin radioimmunoassay (RIA).

Experiment III. Young (5-month-old) and aged (25-month-old male) Fisher-344 rats (n = 8 rats/group) were sacrificed in the *ad lib* fed condition. Fisher-344 rats are the accepted rat model of aging. Plasma was collected for measurement of plasma ghrelin levels. The stomach fundus was harvested for extraction of total cellular RNA and ghrelin peptide. Ghrelin mRNA and peptide levels were measured by Northern blotting and RIA.

RNA Purification and Northern Blotting Analysis. In rat experiments, tissues were homogenized immediately upon harvest in 4 *M* guanidinium isothiocyanate containing 25 m*M* sodium citrate, pH 7.0, 0.5% sodium lauroylsarcosine, and 0.1 *M* β-mercaptoethanol. Extracts were frozen at −80°C until purification by ultracentrifugation over a cesium chloride cushion (2 ml, 5.7 *M*) as described previously (10, 11). For Experiment III, stomach poly [A+] RNA was prepared as described previously (12). Either total cellular RNA (Experiments I and II) or poly [A+] RNA samples (Experiment III) were then separated on a 1% agarose gel (~10 μg/lane) in a 20-m*M* 3-[N-morpholino]propanesulfonic acid running buffer system and then transferred to a nylon membrane and subjected to Northern hybridization. ^{32}P-labeled riboprobes prepared from Strip-EZ RNA kits (Ambion, Inc., Austin, TX) were used for Northern hybridizations. Complementary RNA for rat ghrelin was a gift of M. Kojima (1). Either rat ribosomal 18S or IB15 was used to normalize for variations in RNA loading and transfer. Expression levels of ghrelin, ribosomal 18S, or IB15 genes were quantitated by phosphoimaging.

Ghrelin Radioimmunoassay. Stomach tissue and plasma ghrelin levels were measured using a ghrelin RIA as described previously (10). Ghrelin peptide was extracted from the mouse and rat stomach fundus by homogenizing tissues in approximately 10 vol 1 *M* acetic acid containing 20 m*M* HCl. Homogenates were then boiled for 20 min and supernatants were lyophilized and resuspended in ghrelin assay buffer for the ghrelin RIA. Plasma for ghrelin RIA was collected into glass tubes containing EDTA (1 mg/ml blood) and a protease inhibitor (aprotinin [Trasylol, Bayer AG, Munich, Germany], 70 μg/ml blood). Plasma (1.2 ml/rat) was extracted by use of a C_{18} Sep Paks (Waters, Milford, MA) as reported earlier (10).

Statistics. Results are shown as means ± SE. Data were analyzed by a one-way or two-way analysis of variance followed by the Newman-Keuls test where pertinent.

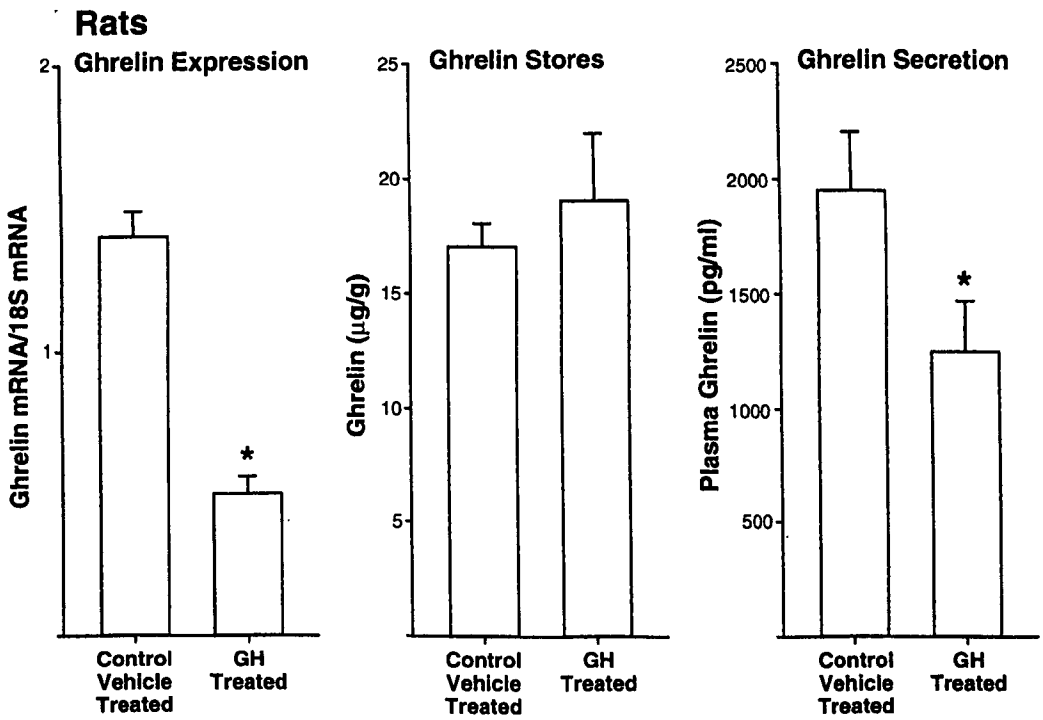

Figure 1. Exogenous GH decreases stomach ghrelin expression and secretion in the male rat. Exogenous GH does not affect stomach ghrelin stores in the rat. n = 6–8 rats/group. *P < 0.05 versus controls.

Differences with a value of $P < 0.05$ were considered significant.

Results

GH Treatment Decreases Stomach Ghrelin Expression and Secretion in Rats. Administration of GH to rats decreased stomach mRNA levels ($P < 0.05$ versus vehicle-treated rats) (Fig. 1). In GH-treated rats, stomach ghrelin mRNA levels were approximately one-third of control ghrelin mRNA levels. Administration of GH also decreased plasma ghrelin levels ($P < 0.05$). In GH-treated rats, plasma ghrelin levels were reduced approximately 40%. Stomach ghrelin peptide stores were unaffected by increased GH levels.

Elevated Endogenous GH Decreases Stomach Ghrelin Peptide Stores in Mice. In GH-RH transgenic female mice, elevation of systemic GH levels resulted in a reduction ($P < 0.05$) of stomach ghrelin peptide levels (Fig. 2).

Reduced GH Secretion in Aged Rats Results in Elevated Stomach Ghrelin Production and Secretion. In aged male rats (25 months), stomach ghrelin mRNA and peptide levels were increased when compared with young rats (5 months) ($P < 0.05$) (Fig. 3). Stomach ghrelin mRNA and peptide levels were elevated approximately 4- and 0.5-fold, respectively, when compared with ghrelin mRNA and peptide levels in young rats. Plasma ghrelin levels in aged rats were elevated 2-fold when compared with young rats.

Discussion

Ghrelin is a recently discovered stomach hormone that stimulates GH secretion in laboratory animals and in humans (1, 6, 7). Ghrelin is as potent as, if not more potent than, GH-RH in the stimulation of pituitary GH secretion (13, 14). Ghrelin can be thought of conceptually as a releasing factor produced in the stomach that stimulates a pituitary hormone. Whether alterations in systemic GH levels influence stomach ghrelin homeostasis (i.e., stomach ghrelin mRNA and peptide levels) and secretion is not known and an important physiologic question because it introduces the concept of a stomach-ghrelin-pituitary-GH axis. The purpose of the present study therefore was to test the hypothesis that either an elevation or a reduction in systemic GH levels will result in corresponding changes in stomach ghrelin homeostasis and ghrelin secretion. In other words, GH is expected to exert a negative or positive feedback effect on stomach ghrelin production and secretion. A positive feedback action occurs during reduced systemic GH levels.

The results of the present experiments indicate that an elevation in circulating GH levels causes a reduction in stomach ghrelin production and secretion. In rats, GH administration decreased stomach ghrelin expression and secretion significantly, and in GH-RH transgenic mice, an elevation in systemic GH levels (15) lowered stomach

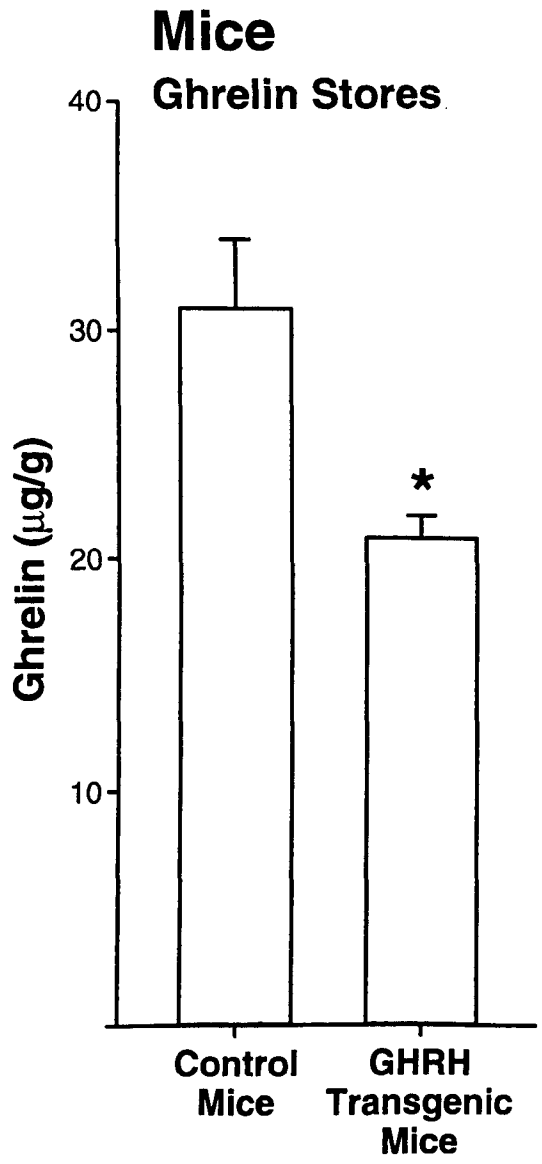

Figure 2. Overexpression of GHRH decreases stomach ghrelin stores in the mouse; $n = 8–9$ mice/group. $^*P < 0.05$ versus controls.

ghrelin peptide stores. In GH-RH transgenic mice, plasma GH levels are approximately 10-fold higher when compared with wild-type mice (15, 16). Administration of exogenous GH did not affect stomach ghrelin peptide stores in the rat. Although speculative, the inability of increased systemic GH levels to lower stomach ghrelin levels may be attributed to the idea that caloric intake also affects stomach ghrelin homeostasis (10), which may be difficult to separate from the feedback effect of GH.

In aged rats, stomach ghrelin expression and peptide stores and plasma ghrelin levels increase significantly when compared with young rats. GH secretion is reduced with aging in rodents (8, 9). In view of our findings showing that exogenous GH exerts a negative feedback action on stomach ghrelin, the increased stomach ghrelin production and secretion in aged rats are most likely due to a reduction in the negative feedback action of endogenous GH. GH exerts

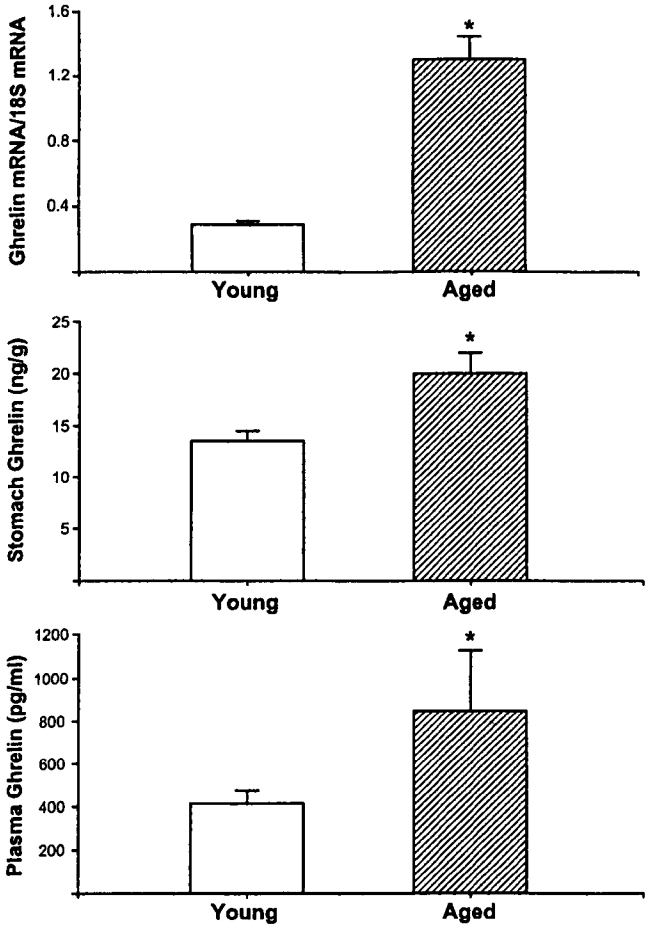

Figure 3. Stomach ghrelin mRNA and peptide levels and secretion are elevated in aged male Fisher-344 rats (25 months) when compared with young rats (5 months). $^*P < 0.05$ versus young rats. n = 8 rats/group.

a positive feedback effect on stomach ghrelin homeostasis and secretion. These findings support the hypothesis that a stomach-ghrelin pituitary GH axis exists and that stomach ghrelin is responsive to changes in systemic GH levels. Other findings suggest that insulin-like growth factor-1 exerts a feedback action on ghrelin secretion, because ghrelin secretion is increased significantly in rats fed a low-protein diet (10). Rats maintained on a low-protein diet have low systemic insulin-like growth factor-1 levels (17, 18).

The mechanism underlying the feedback actions of systemic GH on ghrelin homeostasis and secretion is not known. GH receptors are found in the stomach (19); however, whether GH receptors exist on stomach ghrelin cells has not been explored. A demonstration of GH receptors on ghrelin cells will support the idea that GH exerts a direct influence on stomach ghrelin homeostasis and secretion. Alternatively, stomach somatostatin (SRIF) may mediate the effects of systemic GH on stomach ghrelin cells. SRIF exerts an inhibitory effect on many gastrointestinal hormones (20) and acute changes in stomach somatostatin tone may affect stomach homeostasis and secretion.

The importance of the present findings is that they support the hypothesis that a stomach-ghrelin-pituitary-GH axis exists that may exert widespread effects on body metabolism. Exogenous administration of ghrelin has been shown to influence food intake, growth, adiposity, insulin secretion, and gastrointestinal function as well as GH secretion (1, 6, 10, 21, 22). In humans, ghrelin can also increase blood levels of somatostatin and pancreatic polypeptide, two peptides with metabolic actions (23, 24). The stomach-ghrelin-pituitary-GH axis links nutritional intake to regulation of GH secretion, growth, and metabolism and would be the first such demonstration of a gastrointestinal-pituitary axis. Although the primary production site of ghrelin is the stomach, ghrelin is also produced in the hypothalamus (1) and may interact with stomach ghrelin as well as hypothalamic GH-RH and SRIF in the regulation of pituitary GH secretion (25).

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