Z. WARZECHA[1], A. DEMBIŃSKI[1], P. CERANOWICZ[1], M. DEMBIŃSKI[2],
J. CIESZKOWSKI[1], S.J. KONTUREK[1], A. POLUS[3], W.W. PAWLIK[1], A. KUWAHARA[4],
I. KATO[5], P.C. KONTUREK[6]

INFLUENCE OF GHRELIN ON GASTRIC AND DUODENAL GROWTH AND EXPRESSION OF DIGESTIVE ENZYMES IN YOUNG MATURE RATS

[1]Department of Physiology, [2]Second Department of Surgery and [3]Department of Clinical
Biochemistry, Jagiellonian University Medical College, Krakow, Poland;
[4]Laboratory of Physiology, Graduate School of Environmental Sciences and Institute
for Environmental Sciences, University of Shizuoka, Shizuoka, Japan,
[5]Yanaihara Institute Inc., Shizuoka, Japan. [6]Department of Internal Medicine I,
University of Erlangen-Nuremberg, Erlangen, Germany.

Ghrelin, a nature ligand for the growth hormone secretagogue receptor (GHS-R),
stimulates a release of growth hormone, prolactin and adrenocorticotropic hormone.
Also, ghrelin increases food intake in adult rats and humans and exhibits
gastroprotective effect against experimental ulcers induced by ethanol or stress. The
aim of present study was to examine the influence of ghrelin administration on
gastric and duodenal growth and expression of pepsin and enterokinase in young
mature rats with intact or removed pituitary. Methods: Two week after sham
operation or hypophysectomy, eight week old Wistar male rats were treated with
saline (control) or ghrelin (4, 8 or 16 nmol/kg/dose) i.p. twice a day for 4 days.
Expression of pepsin in the stomach and enterokinase in the duodenum was
evaluated by real-time PCR. Results: In animals with intact pituitary, treatment with
ghrelin increased food intake, body weight gain and serum level of growth hormone
and insulin-like growth factor-1 (IGF-1). These effects were accompanied with
stimulation of gastric and duodenal growth. It was recognized as the significant
increase in gastric and duodenal weight and mucosal DNA synthesis. In both organs,
ghrelin administered at the dose of 8 nmol/kg caused maximal growth-promoting
effect. In contrast to these growth-promoting effects, administration of ghrelin
reduced expression of mRNA for pepsin in the stomach and was without effect on
expression of mRNA for enterokinase in the duodenum. Hypophysectomy alone
lowered serum concentration of growth hormone under the detection limit and
reduced serum level of IGF-1 by 90%. These effects were associated with reduction
in daily food intake, body weight gain and gastroduodenal growth. In
hypophysectomized rats, administration of ghrelin was without significant effect on

food intake, body weight gain or growth of gastroduodenal mucosa. Also, serum concentration of growth hormone or IGF-1 was not affected by ghrelin administration in rats with removed pituitary. Conclusion: Administration of ghrelin stimulates gastric and duodenal growth in young mature rats with intact pituitary, but inhibits expression of mRNA for pepsin in the stomach. Growth hormone and insulin-like growth factor-1 play an essential role in growth-promoting effects of ghrelin in the stomach and duodenum.

INTRODUCTION

Ghrelin is a circulating growth hormone-releasing peptide primarily isolated from human and rat stomach. (1, 2). The stomach is a main source of ghrelin, but this peptide has been also detected in other organs such as the bowel, pancreas, kidney, pituitary and hypothalamus (1, 3, 4). Ghrelin is a natural ligand for growth hormone secretagogue receptor (GHS-R) (1). GHS-Rs are predominantly expressed in the pituitary and hypothalamus; however their presence has also been shown in other central and peripheral tissues, but at much lower levels (4). Ghrelin strongly and dose dependently stimulates release of growth hormone from the anterior pituitary (1), as well as promotes a release of adrenocorticotropic hormone, corticosterone, and prolactin (5, 6). Ghrelin stimulates the appetite and fat deposition in rats (7) and humans (8). Plasma ghrelin concentration is decreased in obese subjects (9, 10) and after food intake (2, 10), whereas fasting or anorexia nervosa cause an increase in plasma ghrelin concentration (2, 10). Moreover, fasting plasma level of ghrelin is negatively correlated with body mass index (10).

Role of ghrelin in stomach physiology is not clear. Some studies with anesthetized rats have shown that intravenous (11) or intracerebroventricular (12) administration of ghrelin stimulates gastric acid secretion. Another study with conscious rats has shown that central administration of ghrelin inhibits gastric acid secretion (13); whereas a study performed in rats with chronic gastric fistulas or ligation of pylorus suggests that ghrelin does not affect gastric acid secretion (14). Other reports indicate that ghrelin stimulates gastric motility (11) and protects gastric mucosa against ethanol-induced damage (15, 16). Brzozowski *et al*. (17) have shown that gastroprotective effect of ghrelin co-exists with an increase in gastric acid secretion. This last observation is in agreement with numerous studies which have shown that physiological gastric secretagogues, such as gastrin (18, 19), cholecystokinin (20), acetylcholine (21) or histamine (22-24) enhance gastric defense mechanisms against injury caused by various noxious agents.

The study performed on fetal and neonatal rats has shown that ghrelin-immunoreactive cells are present in the stomach starting from the 18th day of pregnancy and the number of these cells increases in an age-dependent manner from the neonatal to adult stage (25). Influence of exogenous ghrelin on gastric and pancreatic growth in young animals is age-dependent. Ghrelin reduces gastric and pancreatic growth in suckling rats; whereas in peribubertal animals, treatment with ghrelin increases gastric (26) and pancreatic growth (27).

The aim of present study was to investigate the influence of ghrelin administration on gastric and duodenal mucosal growth in young mature eight week old rats with intact or removed pituitary.

MATERIALS AND METHODS

Animals and treatment

Studies were performed on male eight week old Wistar rats weighing 180-200g. Experimental protocol was approved by the Committee for Research and Animal Ethics of Jagiellonian University. Animals were housed in cages with wire mesh bottoms at normal room temperature in 12-h light-dark cycle and supplied with standard laboratory chow and water, available *ad libitum*. Two weeks before final experiments with ghrelin, six week old rats were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Vetoquinol Biowet, Gorzów Wielkopolski, Poland) and sham-operated or hypophysectomized via the transauricular approach according to a method described previously (28). After two weeks recovery, rats with intact or removed pituitary were treated for 4 days with saline or ghrelin (4, 8 or 16 nmol/kg/dose) given intraperitoneally twice a day, last injection took place 1 h before the end of the experiment. Eight animals were used- in each experimental group. Food intake was recorded once a day. Rat ghrelin was synthesized in Yanaihara Institute Inc. by a solid phase methodology with Fmoc-strategy using peptide synthesizer (Applied Biosystem 9030 Pioneer, Foster, CA, USA).

Determination of gastric and duodenal blood flow

At the end of experiment, animals were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Vetoquinol Biowet, Gorzów Wlkp., Poland) and weighed. Abdominal cavity was opened and gastric and duodenal blood flow was measured by a laser Doppler flowmeter using PeriFlux 4001 Master monitor (Perimed AB, Järfälla, Sweden), as described previously (29). Data were presented as percent of control value obtained in rats injected with saline.

Determination of serum concentration of growth hormone and insulin-like growth factor-1

Immediately after measurement of gastric and duodenal mucosal blood flow, the abdominal aorta was exposed and blood was taken for determination of serum growth hormone and insulin-like growth factor-1 concentration. Serum level of growth hormone was determined by radioimmunoassay, using Rat Growth Hormone RIA Kit (LINCO Research, St. Charles, Missouri, USA). Serum insulin-like growth factor concentration was measured by radioimmunoassay, using Mouse/Rat IGF-1 RIA Kit (Diagnostic System Laboratories, Inc., Webster, Texas, USA).

Determination of gastric and duodenal mucosa weight, mucosal DNA synthesis and gene expression for pepsinogen and enterokinase

After blood withdrawal, the stomach and duodenum were dissected out of the body, opened and washed using a solution of 0.9% NaCl. The oxyntic gland area and a 2 cm segment of the upper duodenum were dissected out and weighed. Gastric and duodenal mucosa was scraped and collected for determination of DNA synthesis and expression of mRNA for pepsin and enterokinase. The rate of DNA synthesis in gastric and duodenal mucosa was determined by measurement of [³H]thymidine incorporation ([6-3H]-thymidine, 20-30 Ci/mmol, Institute for Research, Production and Application of Radioisotopes, Prague, Czech Republic) into DNA as described previously (30). DNA synthesis was expressed as [³H]thymidine disintegrations per minute per microgram DNA (dpm/µg DNA).

Expression of mRNA for pepsinogen and enterokinase was determined in RNA isolated from homogenized mucosa by the guanidine thiocyanate-caesium chloride method (31). The quality of RNA was confirmed by denaturing gel electrophoresis. The quantitative gene expression analysis was performed by real-time PCR using GAPDH as the reference gene. For the cDNA synthesis 1 µg of total RNA was reversely transcribed at 42°C for 50 min. in a total volume of 40 µL reaction buffer containing 5 × First Strand Buffer, DTT, oligo(dT) (Sigma), deoxy-NTPs (Promega), and 200 units of SUPERSCRIPT II reverse transcriptase (Invitrogen Life Technologies). The reaction mixture was heated to 70°C for 15 min and immediately chilled on ice. Subsequently, cDNA was subjected to real-time PCR in a reaction mixture containing QuantiTect SYBR Green PCR (Qiagen) mix and primers. The sequences of the primers used in this study: GAPDH sense 5'GCAAGTTCAACGGCACAGT3' antisense 5'GGTGAAGACGCCAGTAGACTC3'; pepsinogen sense 5'CCCCTCTTCGGAGTCTACCT3' antisense 5'CGCCTCCAAACACAATCTG3'; enterokinase sense 5'GAGCCCTGGACCACACAA3' antisense 5'CCACCTCCAGTAGATAGTATTG3'. The primers were designed to include an intervening intron between the sense and antisense primers, thereby eliminating the possibility of amplifying any genomic DNA, and checked for specificity by BLAST searches. All real-time PCR reactions were performed on the DNA Engine Opticon II (MJ Research). The thermal profile included initial denaturation for 15 min. at 95°C, followed by 40 amplification cycles: of denaturation for 30 sec. at 94°C, annealing for 30 sec. at 60°C, and elongation for 30 sec at 72°C. Following PCR amplification, melting curve analysis was performed with a temperature profile slope of 1°C/s from 35°C to 95°C. A negative control without cDNA template was run with every assay to ensure overall specificity. The expression rates were calculated as the normalized C_τ difference between a control probe and sample with the adjustment for the amplification efficiency relative to the expression level of the housekeeping gene GAPDH. Calculation of gene expression data was performed using the program Calculation Matrix for PCR Efficiency REST-XL (gene.quantification@wzw.turn.de). This program tests the significance of differences between groups by Pair Wise Fixed Reallocation Randomization Test (32).

Statistical analysis

Results are expressed as mean ± S.E.M. Statistical analysis of data was carried out by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPadPrism (GraphPad Software, San Diego, CA, USA). Differences were considered to be statistically significant when P was less than 0.05.

RESULTS

In young mature eight week old rats with intact pituitary (control), daily food intake was 29.9 ± 1.8 g, whereas total body weight gain during 4 days of the study

was 32.6 ± 1.5 g (*Fig. 1*). In these rats, treatment with ghrelin for 4 days at all doses used significantly increased daily food intake and body weight. Maximal stimulatory effect on food intake and body weight gain was observed after ghrelin administered at the dose of 8 nmol/kg/dose. In saline treated rats, hypophysectomy significantly reduced daily food intake and total body weight gain by 21 and 45%, respectively. Administration of ghrelin was without effect on appetite or body weight gain in hypophysectomized rats.

In control rats with intact pituitary and treated with saline, weight of the gastric oxyntic gland area and a 2 cm duodenal segment reached 1115 ± 29 and 336 ± 15 mg, respectively (*Fig. 2*). Treatment with ghrelin increased weight of the stomach and duodenum in rats with intact pituitary. In the stomach, this effect was statistically significant after ghrelin administered at the dose of 4 and 8 nmol/kg, whereas in the duodenum only after the dose of 8 nmol/kg. Hypophysectomy alone significantly reduced weight of the duodenum and oxyntic gland area of the stomach. In these rats, treatment with ghrelin did not significantly affect gastric and duodenal weight.

In control saline-treated rats with intact pituitary, DNA synthesis in gastric and duodenal mucosa was 50.8 ± 2.4 and 61.5 ± 2.0 dpm/µg DNA, respectively
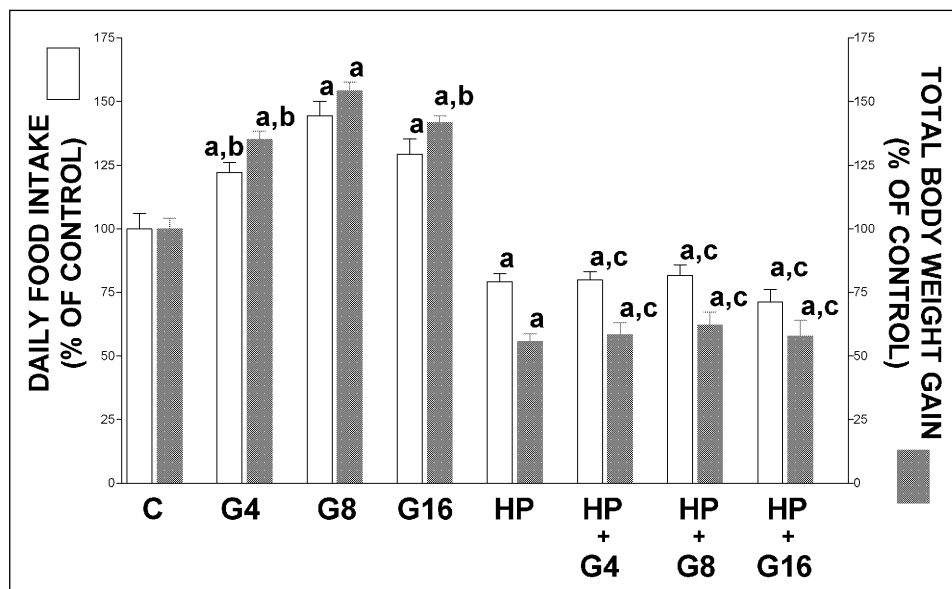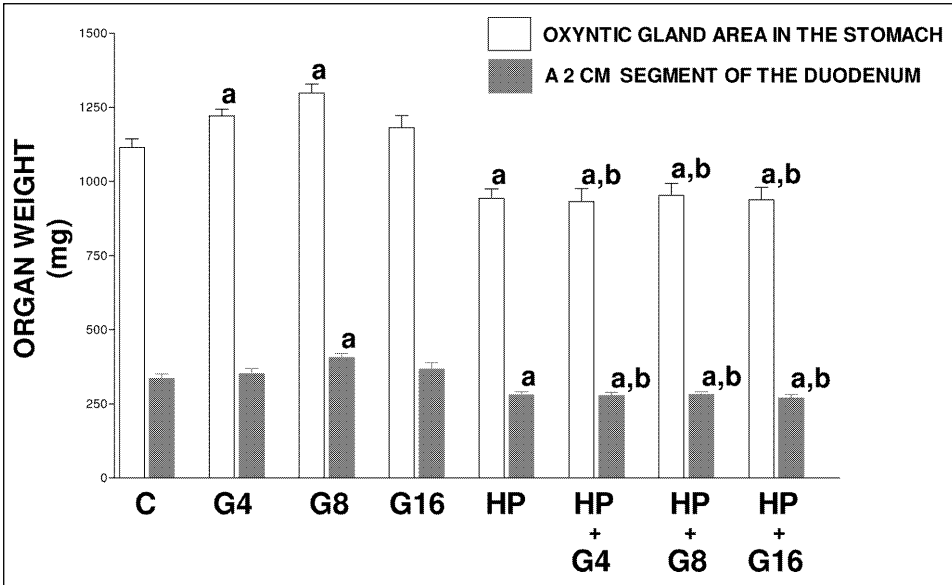

Fig. 1. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose; twice a day for 4 days) and hypophysectomy (HP) applied alone or in their combination on daily food intake and total body weight gain in eight week old rats. Mean ± S.E.M. [a]P<0.05 compared to control with intact pituitary; [b]P<0.05 compared to animals with the intact pituitary and treated with ghrelin at the dose 8 nmol/kg/dose; [c]P<0.05 compared to animals with the intact pituitary and treated with the same dose of ghrelin.

Fig. 2. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose; twice a day for 4 days) and hypophysectomy (HP) applied alone or in their combination on the weight of oxyntic gland area in the stomach and a 2 cm duodenal segment of the duodenum in eight week old rats. Mean ± S.E.M. [a]P<0.05 compared to control with intact pituitary; [b]P<0.05 compared to animals with the intact pituitary and treated with the same dose of ghrelin.

(*Fig. 3*). Treatment with ghrelin caused a significant increase in gastric and duodenal mucosal DNA synthesis and in both organs maximal value of mucosal DNA synthesis was observed, when ghrelin was administered at the dose of 8 nmol/kg. Hypophysectomy markedly reduced DNA synthesis in gastric and duodenal mucosa and this effect was not reversed by administration of ghrelin.

Treatment with ghrelin significantly increased gastric and duodenal mucosal blood flow in rats with intact pituitary (*Fig. 4*). Hypophysectomy alone tended to reduce mucosal blood flow in the stomach and duodenum but this effect was statistically insignificant. In these rats, administration of ghrelin was without effect on gastric and duodenal mucosal blood flow.

In the stomach, expression of mRNA for pepsinogen was significantly reduced by ghrelin administered at the dose of 8 nmol/kg, whereas expression of mRNA for enterokinase in the duodenum was not affected by treatment with any dose of ghrelin (*Fig. 5*).

In rats with intact pituitary, serum concentration of growth hormone and IGF-1 was 128.5 ± 9.3 and 436.5 ± 21.9 ng/ml (*Fig. 6*). Administration of ghrelin significantly increased serum level of growth hormone and IGF-1 in these rats. Maximal stimulatory effect was observed after ghrelin administered at the dose of 8 nmol/kg. Hypophysectomy reduced serum level of growth hormone below
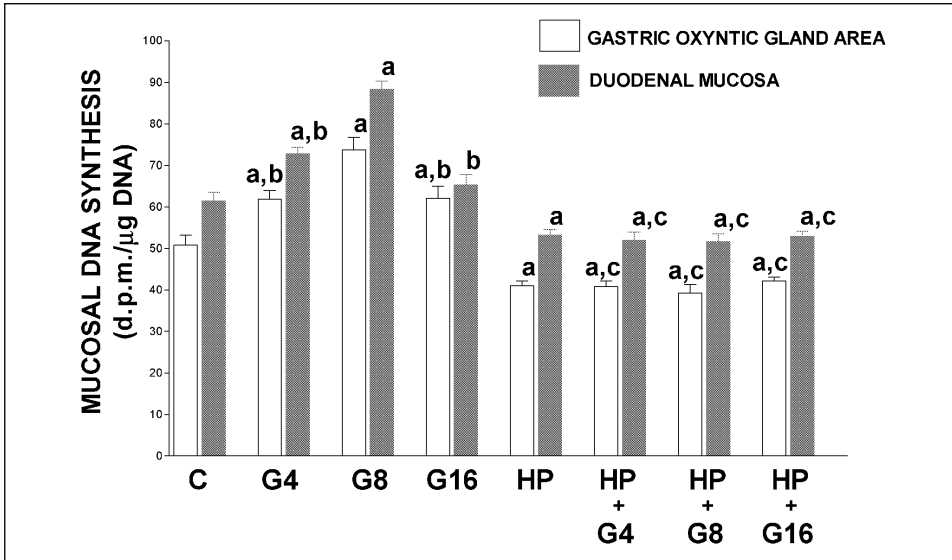
Fig. 3. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose; twice a day for 4 days) and hypophysectomy (HP) applied alone or in their combination on gastric and duodenal mucosal DNA synthesis in eight week old rats. Mean ± S.E.M. [a]$P<0.05$ compared to control with intact pituitary; [b]$P<0.05$ compared to animals with the intact pituitary and treated with ghrelin at the dose 8 nmol/kg/dose; [c]$P<0.05$ compared to animals with the intact pituitary and treated with the same dose of ghrelin.
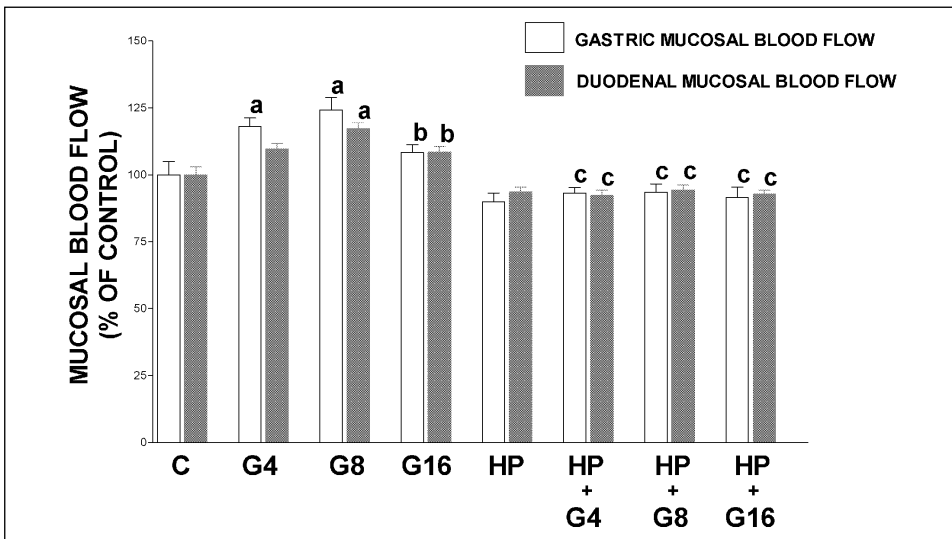

Fig. 4. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose; twice a day for 4 days) and hypophysectomy (HP) applied alone or in their combination on gastric and duodenal mucosal blood flow in eight week old rats. Mean ± S.E.M. [a]$P<0.05$ compared to control with intact pituitary; [b]$P<0.05$ compared to animals with the intact pituitary and treated with ghrelin at the dose 8 nmol/kg/dose; [c]$P<0.05$ compared to animals with the intact pituitary and treated with the same dose of ghrelin.
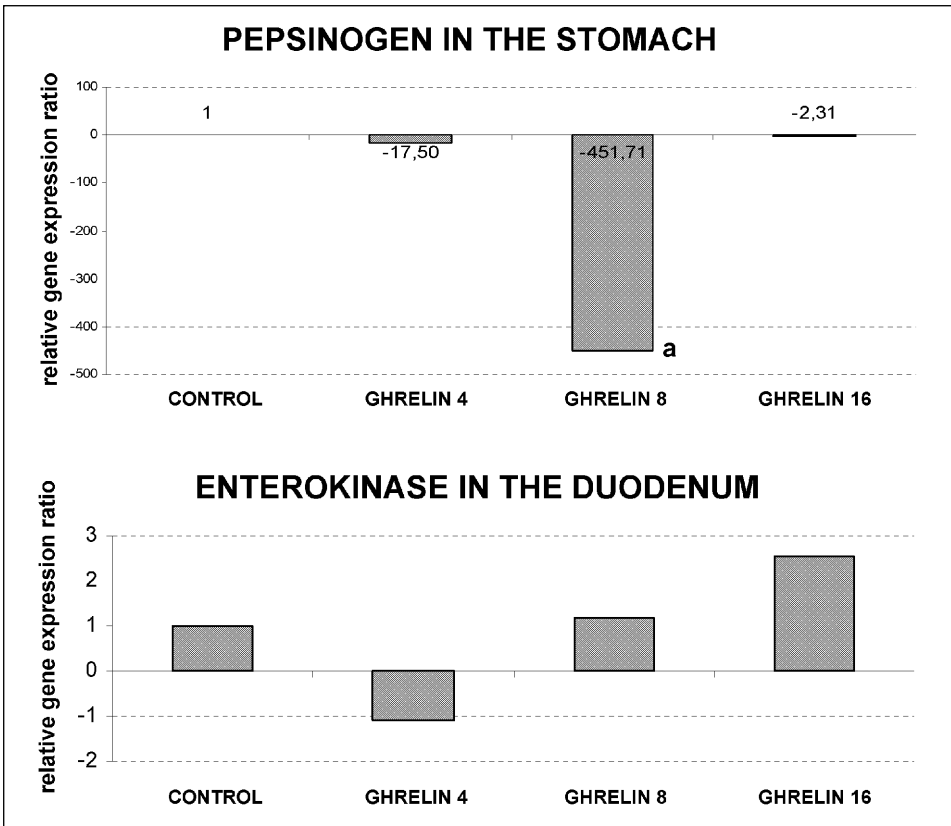
Fig. 5. Influence of ghrelin administration at the dose of 4, 8 or 16 nmol/kg/dose on relative gene expression for pepsinogen in gastric mucosa (A) and enterokinase in duodenal mucosa (B) analyzed by quantitative real-time PCR. Mean ± S.E.M. [a]$P<0.05$ compared with control.

detection limit, whereas serum level of IGF-1 was reduced by 90%. Administration of ghrelin was without effect on serum concentration of growth hormone or IGF-1 in hypophysectomized rats.

<div align="center">DISCUSSION</div>

Previous studies have shown that effect of ghrelin on food intake is age dependent. In adult rats and humans, administration of ghrelin increases food intake (7, 8, 33), whereas in young chicks (34) and weaned rats (27) treatment with ghrelin decreases appetite. Our present study has shown that ghrelin administered in young mature rats evokes the same food intake-stimulating effect as in adult rats and humans. This orexigenic effect of ghrelin in mature young rats seems to be mainly dependent on possibility of ghrelin to stimulate the release of
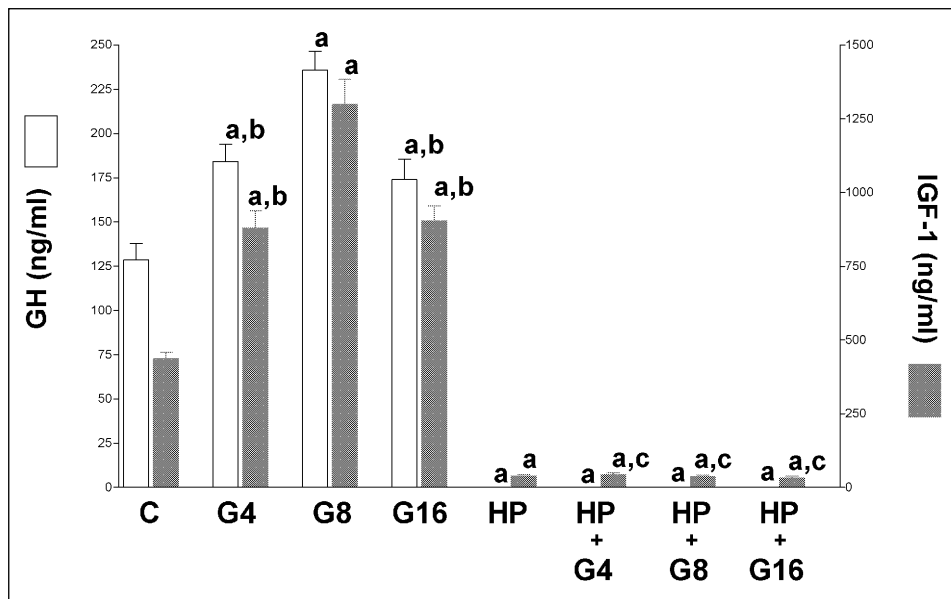
Fig. 6. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose) twice a day for 4 days and hypophysectomy (HP) applied alone or in their combination on serum concentration of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) in eight week old rats. Mean ± S.E.M. [a]P<0.05 compared to control with intact pituitary; [b]P<0.05 compared to animals with the intact pituitary and treated with ghrelin at the dose 8 nmol/kg/dose; [c]P<0.05 compared to animals with the intact pituitary and treated with the same dose of ghrelin.

growth hormone and IGF-1. This thesis is supported by our present observations that: (a) administration of ghrelin increases growth hormone and IGF-1 release and this effect is associated with increase in appetite; (b) hypophysectomy eliminates growth hormone from the circulation and reduced serum level of IGF-1 by 90%, and this effect is associated with reduction in food intake; (c) administration of ghrelin is without effect on serum concentration of growth hormone and IGF-1, as well as does not stimulate food intake in hypophysectomized mature young rats.

Ingestion of food affects body weight gain and plays the most important role in the regulation of gastrointestinal mucosa growth (35, 36). Constituents of the diet in the gastrointestinal tract may directly (37) and indirectly stimulate growth of the mucosa. Indirect growth-promoting effect of food on gastrointestinal mucosa involves a variety of evens, such as the release of hormones, stimulation of nerves, and activation of exocrine secretion, motility and absorption (38). On the other hand, fasting (36, 39) or total intravenous alimentation (40) produces atrophy of gastric and intestinal mucosa. These data are in agreement with our present study and explain, at least in part, the mechanism of growth-promoting

effect of ghrelin on gastroduodenal mucosal in rats with intact pituitary. In these rats, administration of ghrelin stimulated food intake and this effect was associated with an increase in body weight gain, gastric and duodenal weight, and mucosal cell proliferation in the stomach and duodenum. Additionally, our results bring a direct evidence that growth-promoting effect of ghrelin on gastric and duodenal mucosa is mediated by growth hormone and IGF-1. Both hormones, especially IGF-1 exhibit anabolic effects and can directly increase the rate of protein synthesis in cells of the body (41, 42). In our present study, we have found that administration of ghrelin stimulates growth hormone and IGF-1 release in rats with intact pituitary, and this effect is connected with an increase in food intake and stimulation of growth of mucosa in the stomach and duodenum. On the other hand, in hypophysectomized rat, treatment with ghrelin was without any effect on serum level of growth hormone or IGF-1, as well as on gastroduodenal mucosa growth.

In the present study, administration of ghrelin significantly increased gastric and duodenal mucosal blood flow in eight week old rats with intact pituitary. In contrast, treatment with ghrelin was without effect on mucosal blood flow in the stomach and duodenum in hypohysectomized rats. These differences are most likely related to the influence of ghrelin on food intake in these two groups of rats. In rats with intact pituitary, treatment with ghrelin, increases food intake leading to the stimulation of gastric and duodenal exocrine secretion, motility and metabolism of the mucosa. The final effect of these changes is gastric and duodenal hyperemia. Other explanation of hyperemic activity of ghrelin in rats with intact pituitary may be effect of ghrelin on the release of IGF-1. Previous *in vivo* and *in vitro* studies have shown that IGF-1 causes vascular relaxation and increases organ blood flow (43, 44). In hypophysectomized rats, treatment with ghrelin was without effect on food intake and serum level of IGF-1, and for this reason administration of ghrelin did not affect gastric and duodenal mucosal blood flow.

Another important finding of our present study is the observation that administration of ghrelin inhibits gene expression for pepsinogen in gastric mucosa. Previous studies have shown ghrelin protects gastric mucosa against damage evoked by ethanol or stress (15-17). Reduction in pepsinogen production may, at least in part, explain the gastroprotective effect of ghrelin on gastric mucosa.

In contrast to pepsinogen, gene expression of enterokinase in duodenal mucosa was not affected by ghrelin administration. Enterokinase activates inactive pancreatic trypsinogen to active proteolytic enzyme, trypsin, and subsequently trypsin activates other pancreatic protelytic enzymes, as well as changes procolipase to colipase. Our observation indicates that ghrelin administration does not affect activation of pancreatic proteolytic enzymes in the duodenum.

Finally, our present study demonstrates that: (1) treatment with ghrelin stimulates food intake, body weight gain and gastroduodenal growth in young mature eight week old rats with intact pituitary; (2) in these rats, administration of ghrelin inhibits expression of mRNA for pepsin in the stomach, but does not affect gene expression for enterokinase in the duodenum; (3) growth-promoting effect of ghrelin on gastric and duodenal growth seems to be mainly dependent on the release of growth hormone and IGF-1.

REFERENCES

1. Kojima M, Hosoda H, Date Y, *et al.* Ghrelin is a growth hormone-releasing acylated peptide from stomach. *Nature* 1999; 402: 656-660.
2. Ariyasu H, Takaya K, Tagami T, *et al.* Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J Clin Endocrinol Metab* 2001; 86: 4753-4758.
3. Date Y, Kojima M, Hosoda H, *et al.* Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000; 141: 4255-4261.
4. Gnanapavan S, Kola B, Bustin SA, *et al.* The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *J Clin Endocrinol Metab* 2002; 87: 2988-2991.
5. Takaya K, Ariyasu H, Kanamoto N, *et al.* Ghrelin strongly stimulates growth hormone release in humans. *J Clin Endocrinol Metab* 2000;85:4908-4911.
6. Broglio F, Benso A, Castiglioni C, *et al.* The endocrine response to ghrelin as a function of gender in humans in young and elderly subjects. *J Clin Endocrinol Metab* 2003;88:1537-1542.
7. Wren AM, Small CJ, Abbott CR, *et al.* Ghrelin causes hyperphagia and obesity in rats. *Diabetes* 2001; 50: 2540-2547.
8. Wren AM, Seal LJ, Cohen MA, *et al.* Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 2001; 86: 5992-5995.
9. Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 2001; 50: 707-709.
10. Shiiya T, Nakazato M, Mizuta M, *et al.* Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab* 2002; 87: 240-244.
11. Masuda Y, Tanaka T, Inomata N, *et al.* Ghrelin stimulates gastric acid secretion and motility in rats. *Biochem Biophys Res Commun* 2000; 276: 905-908.
12. Date Y, Nakazato M, Murakami N, Kojima M, Kangawa K, Matsukura S. Ghrelin acts in the central nervous system to stimulate gastric acid secretion. *Biochem Biophys Res Commun* 2001; 280: 904-907.
13. Sibilia V, Pagani F, Guidobono F, *et al.* Evidence for a central inhibitory role of growth hormone secretagogues and ghrelin on gastric acid secretion in conscious rats. *Neuroendocrinology* 2002; 75: 92-97.
14. Dornonville de la Cour C, Lindstrom E, Norlen P, Hakanson R. Ghrelin stimulates gastric emptying but is without effect on acid secretion and gastric endocrine cells. *Regul Pept* 2004; 120: 23-32.

15. Sibilia V, Rindi G, Pagani F, *et al.* Ghrelin protects against ethanol-induced gastric ulcers in rats: studies on the mechanisms of action. *Endocrinology* 2003; 144: 353-359.

16. Konturek PC, Brzozowski T, Pajdo R, *et al.* Ghrelin-a new gastroprotective factor in gastric mucosa. *J Physiol Pharmacol* 2004; 55: 325-336.

17. Brzozowski T, Konturek PC, Konturek SJ, *et al.* Exogenous and endogenous ghrelin in gastroprotection against stress-induced gastric damage. *Regul Pept* 2004; 120; 39-51.

18. Nishizaki Y, Guth PH, Kim G, Wayland H, Kaunitz JD. Pentagastrin enhances gastric mucosal defenses in vivo: luminal acid-dependent and independent effects. *Am J Physiol* 1994; 267: G94-G104.

19. Tanaka S, Skiba Y, Kaunitz JD. Pentagastrin gastroprotection against acid is related to H2 receptor activation but not acid secretion. *Gut* 1998; 43: 334-341.

20. West SD, Helmer KS, Chang LK, Cui Y, Greeley GH, Mercer DW. Cholecystokinin secretagogue-induced gastroprotection: role of nitric oxide and blood flow. *Am J Physiol Gastrointest Liver Physiol* 2003; 284: G399-G410.

21. Ichikawa T, Ishihara K, Kusakabe T, *et al.* Distinct effects of tetragastrin, histamine, and CCh on rat gastric mucin synthesis and contribution of NO. *Am J Physiol* 1998; 274: G138-G146.

22. Warzecha Z, Dembiński A, Brzozowski T, *et al.* Gastroprotective effect of histamine and acid secretion on ammonia-induced gastric lesion in rats. *Scan J Gastroenterol* 2000; 35: 916-924.

23. Warzecha Z, Dembiński A, Brzozowski T, Ceranowicz P, Stachura J, Konturek SJ. Histamine in stress ulcer prophylaxis in rats. *J Physiol Pharmacol* 2001; 52: 407-421.

24. Dembiński A, Warzecha Z, Ceranowicz P, *et al.* Role of capsaicin-sensitive nerves and histamine H_1, H_2, and H_3 receptors in the gastroprotective effect of histamine against stress ulcers in rats. *Eur J Pharmacol* 2005; 508: 211-221.

25. Hayashida T, Nakahara K, Mondal MS, *et al.* Ghrelin in neonatal rats: distribution in stomach and its possible role. *J Endocrinol* 2002; 173: 239-245.

26. Warzecha Z, Dembiński A, Ceranowicz P, *et al.* Dual age-dependent effect of ghrelin administration on serum level of insulin-like growth factor-1 and gastric growth in young rats. *Eur J Pharmacol* 2006; 529: 145-150.

27. Dembiński A, Warzecha Z, Ceranowicz P, *et al.* Variable effect of ghrelin administration on pancreatic development in young rats. Role of insulin-like growth factor-1. *J Physiol Pharmacol* 2005; 56: 555-570.

28. Falconi G, Rossi GL. Transauricular hypophysectomy in rats and mice. *Endocrinology* 1964; 74: 301-303.

29. Dembiński A, Warzecha Z, Ceranowicz P, Konturek SJ. The role of capsaicin-sensitive sensory neurons and nitric oxide in regulation of gastric mucosal growth. *J Physiol Pharmacol* 1995; 46: 351-362.

30. Warzecha Z, Dembiński A, Ceranowicz P, *et al.* Immunohistochemical expression of FGF-2, PDGF-A, VEGF and TGFβ RII in the pancreas in the course of ischemia/reperfusion-induced acute pancreatitis. *J Physiol Pharmacol* 2004; 55: 791-810.

31. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Annal Biochem* 1987; 162: 156-159.

32. Pfaffl MW. W A new mathematical model for relative quantification in real-time RT-PCR *Nucleic Acids Res* 2001; 29: e45.

33. Shimbara T, Mondal MS, Kawagoe T *et al.* Central administration of ghrelin preferentially enhances fat ingestion. *Neurosci Lett* 2004; 369: 75-79.

34. Saito ES, Kaiya H, Takagi T *et al.* Chicken ghrelin and growth hormone-releasing peptide-2 inhibit food intake of neonatal chicks. *Eur J Pharmacol* 2002; 453: 75-79.

35. Tabata K, Johnson LR. Ornithine decarboxylase and mucosal growth in response to feeding. *Am J Physiol* 1986; 251: G270-G274.

36. Dembiński A, Warzecha Z, Ceranowicz P, *et al.* Influence of central and peripheral administration of pancreatic polypeptide on gastric mucosa growth. *J Physiol Pharmacol* 2004; 55: 223-237.

37. Dembinski AB, Yamaguchi T, Johnson LR Stimulation of mucosal growth by a dietary amine. *Am J Physiol* 1984; 247: G352-G356.

38. Johnson LR. New aspects of the trophic action of gastrointestinal hormones. *Gastroenterology* 1977; 72: 788-792.

39. Steiner M, Bourges HR, Freedman LS, Gray SJ. Effect of starvation on the tissue composition of the small intestine in the rat. *Am J Physiol* 1968; 215: 75-77.

40. Johnson LR, Copeland EM, Dudrick SJ, Lichtenberger LM, Castro GA. Structural and hormonal alterations in the gastrointestinal tract of parenterally fed rats. *Gastroenterology* 1975; 68: 1177-1183.

41. Ho KK, O'Sullivan AJ, Hoffman DM. Metabolic actions of growth hormone in man. *Endocr J* 1996; 43 Suppl: S57-S63.

42. Zapf J, Froesch ER. Insulin-like growth factors/somatomedins: structure, secretion, biological actions and physiological role. *Horm Res* 1986; 24: 121-130.

43. Walsh MF, Barazi M, Pete G, Muniyappa R, Dunbar JC, Sowers JR. Insulin-like growth factor I diminishes in vivo and in vitro vascular contractility: role of vascular nitric oxide. *Endocrinology* 1996; 137: 1798-1803.

44. Hasdai D, Rizza RA, Holmes DR Jr, Richardson DM, Cohen P, Lerman A. Insulin and insulin-like growth factor-1 cause coronary vasorelaxation in vitro. *Hypertension* 1998; 32: 228-234.

Author's address: Professor Artur Dembinski, MD, PhD, Department of Physiology, Jagiellonian University Medical College, ul. Grzegorzecka 16, 31-531 Kraków, Poland; Telephone: +48-12-4211006; Fax: +48-12-4225478; E-mail: mpdembin@cyf-kr.edu.pl