Peptic ulcer healing requires the reconstitution of epithelial structures and underlying connective tissue through cellular proliferation, migration, and differentiation. Previous studies have shown that administration of growth hormone (1) and other growth factors, such as: epidermal growth factor (2), platelet-derived growth factor (3) or fibroblast growth factor-2 (4) accelerates the healing of experimental gastroduodenal ulcers.

Ghrelin, a 28-residue peptide, was primarily isolated from the human and rat stomach (5, 6), and the stomach is a main endogenous source of circulating ghrelin (6). Ghrelin is a natural ligand of growth hormone secretagogue receptor (GHS-R).

Recent studies have shown that ghrelin exhibits gastroprotective effects. The aim of present study was to examine the influence of ghrelin administration on the healing of chronic gastric and duodenal ulcers and to evaluate the role of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) in this process. In pituitary-intact or hypophysectomized rats, chronic gastric and duodenal ulcers were induced by acetic acid. After induction of ulcers, rats were treated intraperitoneally twice a day with saline, ghrelin (4, 8 or 16 nmol/kg/dose) or IGF-1 (20 nmol/kg/dose) for six or ten days. In animals with intact pituitary, treatment with ghrelin increased serum level of GH and IGF-1. These effects were accompanied by the increase in mucosal cell proliferation, mucosal blood flow and healing rate of gastric and duodenal ulcers. After hypophysectomy, the significant increase in serum level of endogenous ghrelin was observed, but the healing of gastric and duodenal ulcers was delayed. This effect was accompanied by a significant decrease in serum concentration of endogenous GH and IGF-1, and reduction in mucosal blood flow and DNA synthesis. In hypophysectomized rats, administration of exogenous ghrelin was without any effect on serum level of GH and IGF-1, healing rate of gastroduodenal ulcers or mucosal cell proliferation. In contrast to this effect, administration of IGF-1 increased mucosal cell proliferation, healing rate of gastroduodenal ulcers and mucosal blood flow in hypophysectomized rats. Conclusion: Treatment with ghrelin accelerates healing of chronic gastroduodenal ulcers and this effect is mediated by the release of endogenous GH and IGF-1.

Keywords: ghrelin; gastric and duodenal ulcer; hypophysectomy; growth hormone; insulin-like growth factor-1; mucosal blood flow; mucosal cell proliferation
type 1a, and acting on this receptor, ghrelin strongly and dose dependently stimulates growth hormone release from the anterior pituitary (5). Apart from the release of growth hormone, ghrelin exerts several biological activities including the increase in intestinal motility (7) and stimulation of appetite and fat deposition in rats and humans (8-10). It has been demonstrated that food intake and obesity decrease plasma concentration of ghrelin (6, 11, 12); whereas fasting or anorexia nervosa cause an increase in plasma ghrelin concentration (6, 12).

Ghrelin affects the development and maturation of digestive tract organs and this effect is age-dependent. In young suckling rats administration of ghrelin reduces gastric and pancreatic growth; whereas in young seven week old rats, treatment with ghrelin stimulates gastric and pancreatic growth and increases pancreatic activity of amylase (13-16).

Previous studies have shown that treatment with ghrelin protects the heart (17), kidney (18) and brain (19) against ischemic injury and attenuates sepsis-induced lung injury and mortality (20). In the gut, pretreatment with ghrelin reduces gastric mucosal damage induced by ethanol (21, 22), stress (23) or alendronate (24). Also, administration of ghrelin inhibits the development of cerulein- or ischemia/reperfusion-induced acute pancreatitis (25, 26). The last study has shown that protective effect of ghrelin on the pancreas is mediated by release of endogenous growth hormone and insulin-like growth factor-1 (IGF-1) (26). However, the protective effect of ghrelin requires administration of this peptide before exposure to damaging factors and therefore has minimal clinical value. The role of ghrelin in the healing of gastric and duodenal ulcer is unknown. Therefore, the aim of our present investigation was to examine whether ghrelin administration exhibits any effect on the healing of chronic gastric and duodenal ulcers and, if so, what is the role of endogenous growth hormone and IGF-1 in this effect.

MATERIALS AND METHODS

Animals and treatment

Studies were performed on male Wistar rats weighing 200-220 g and were conducted following the experimental protocol approved by the Local Commission of Ethics for the Care and Use of Laboratory Animals. Animals were housed in cages with wire mesh bottoms, in normal room temperature (22 ± 1 °C) and a 12-h light-dark cycle.

After fasting for 16 h, rats were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Vetoquinol Biowet, Gorzow Wielkopolski, Poland) and sham-operated or hypophysectomized via the transauricular approach according to a method described previously (27). Two weeks later, after fasting for 16 h with the unlimited access to water, rats were reanesthetized and chronic gastric and duodenal ulcers were induced using our modification (2) of acetic acid method originally described by Okabe et al. (28). Briefly, the abdomen was opened and the stomach and duodenum were exposed. A plastic tube of 4.2 mm inner diameter was applied tightly to the serosal surface of the anterior wall of the distal portion of the stomach, proximal to the pylorus, and to the wall of the duodenum about 5 mm beyond the pylorus. About 70 µl of 100% acetic acid was applied for 20 s through the tube on the serosal surface of the stomach and 70 µl of 80% acetic acid was applied for 10 s on the serosal surface of duodenum. After removal of the acetic acid, the abdomen was closed by sutures. This method was found to result in the formation of chronic ulceration of mucosa and submucosa within the area of acetic acid application. At the ulcer induction day (day 0), the area of ulcer was 13.8 mm². All rats were fasted with the unlimited access to water at the day 0 and then had free access to food and water.

Studies were performed on pituitary-intact or hypophysectomized rats. Rats with intact pituitary gland were treated with saline (control group) or ghrelin (4, 8 or 16 nmol/kg/dose) given intraperitoneally twice a day for six or ten day (the first injection at the day of ulcer induction, the last dose was injected 1 h before the end of experiment). Hypophysectomized rats were treated with saline, ghrelin (8 nmol/kg/dose) or IGF-1 (Pro-Spec-Tany TechnoGene Ltd., Rehovot, Israel; 20 nmol/kg/dose) given intraperitoneally twice a day for the same time period as rats with intact pituitary gland. In hypophysectomized rats, we used ghrelin at the dose of 8 nmol/kg/dose because this dose caused the best therapeutic effect in the first series of studies with pituitary-intact rats. The dose of IGF-1, 20 nmol/kg/dose was chosen because this amount caused a similar increase in serum concentration of IGF-1, as administration of ghrelin at the dose of 8 nmol/kg/dose in rats with intact pituitary. Experiments were repeated to obtain ten observations in each experimental group and each time of observation.

Active N-octanoyl rat ghrelin was synthesized in Yanaihara Institute by a solid phase methodology with Fmoc-strategy using automated peptide synthesizer (Applied Biosystem 9030 Pioneer, Foster, CA, USA). An esterification of the Ser3 hydroxyl group was achieved with n-octanoic acid using dicyclohexylcarbodiimine and 4-
dimethylaminopyridine for 18 hours at 4°C. The crude peptide was purified by reverse phase HPLC on column of YMC-Pack D-ODS-5 (30×250 mm) with a solvent system of TPA/CH₃CN) (72/28/57/43, v/v) over 30 min at a flow rate 16 ml/min. Analytical HPLC and MALDI-TOF MS confirmed the homology of the product.

**Determination of gastric and duodenal blood flow, and mucosal lesions**

Six or ten days after induction of chronic gastric and duodenal ulcers, rats were anesthetized again with ketamine and the abdomen was opened by a midline incision. The stomach and duodenum were exposed and the gastric and duodenal mucosal blood flow was measured using laser Doppler flowmeter (PeriFlux 4001 Master monitor, Perimed AB, Jarfalla, Sweden). Blood flow was measured in five areas of gastric and duodenal mucosa, and mean value of five recordings was presented as percent of mucosal blood flow recorded in saline-treated rats with intact pituitary gland. After measurement of mucosal blood flow, the area of ulcerated mucosa was measured, using computerized planimeter (Morphomat, Carl Zeiss, Berlin, Germany) as described previously (29). The measurement was made by person blinded to the origin of coded specimens.

**Biochemical analysis**

After measurement of gastric and duodenal blood flow, blood samples were collected from the aorta and allowed to clot. Serum was collected and frozen at -60°C. In the case of serum collected for determination of ghrelin, before cool storage, 1 ml of serum was acidified with 50 µl of 1 N HCl and 10 µl of phenylmethylsulfonyl fluoride (PMSF) solution (10 mg of PMSF/ml of methanol) was added.

Serum ghrelin concentration was determined by radioimmunoassay, using a commercially available kit from Peninsula Laboratories, Inc. (San Carlos, CA, USA), division of BACHEM. This kit is specific for the biologically active form of ghrelin with the octanoyl group on Serine 3, without cross-reactivity with des-octanoyl ghrelin. The sensitivity of this assay was 15 pg/ml, the inter- and intra-assay coefficients of variation were ≤ 15 and 5%, respectively.

Serum growth hormone concentration was determined by radioimmunoassay, using a commercial Rat Growth Hormone RIA Kit (LINCO Research, St. Charles, Missouri, USA). Sensitivity of the assay was 0.5 ng/ml, the inter- and intra-assay coefficients of variation were ≤ 15.4%.

Serum IGF-1 concentration was measured by radioimmunoassay, using a commercial Mouse/Rat IGF-1 RIA Kit (Diagnostic System Laboratories, Inc., Webster, Texas, USA). Before measurement of serum IGF-1 concentration, samples were treated with extraction solution (an ethanolic solution of HCl). After that, extracts were neutralized according to the assay protocol. The sensitivity of the assay was 21 ng/ml without cross-reactivity with human IGF-1. Intra- and inter-assay coefficients of variation were ≤ 6.1 and 9.7%, respectively.

Biopsy samples from the gastric and duodenal mucosa were taken for determination of mucosal DNA synthesis, as an index of mucosal cell proliferation. DNA synthesis 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 mucosal DNA as described previously (30). The incorporation of labeled thymidine into DNA was determined by counting 0.5 ml DNA-containing supernatant in a liquid scintillation system. DNA synthesis was expressed as tritium disintegrations per minute per µg DNA (dpm/µg DNA).

**Statistical analysis**

Results were expressed as mean ± SEM. Statistical analysis 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**

**Healing of gastric and duodenal ulcers**

Figures 1 and 2 show the results of healing of chronic gastric and duodenal ulcers in pituitary-intact or hypophysectomized rats treated with saline, ghrelin or IGF-1. In control animals with intact pituitary, the ulcer area showed progressive decrease at 6th and 10th day after induction of ulcers. In the stomach, ulcer area was 7.8 ± 0.5 at the 6th day after induction of ulcers and 1.6 ± 0.2 mm² at the 10th day. In the duodenum in control rats, the ulcer area was 7.0 ± 0.4 mm² and 2.3 ± 0.2 4 mm², respectively.

In pituitary-intact rats, intraperitoneal administration of ghrelin caused a significant acceleration of healing rate of both, gastric and duodenal ulcers at the 6th and 10th day after induction of
Fig. 1. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose), hypophysectomy (HP) and treatment with IGF-1 on the area of chronic gastric ulcers at the 6th and 10th day after induction of these ulcers. Mean ± SEM. N= 10 in each group of animals. aP<0.05 compared to saline-treated rats with intact pituitary (control) at the 0 day; bP<0.05 compared to saline-treated rats with intact pituitary (control) at the 6th day of observation; cP<0.05 compared to G8 in pituitary-intact rats at the same day of observation; dP<0.05 compared to hypophysectomized saline-treated rat (HP) at the same day of observation; eP<0.05 compared to saline-treated rats with intact pituitary (control) at the 10th day of observation.

Fig. 2. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose), hypophysectomy (HP) and treatment with IGF-1 on the area of chronic duodenal ulcers at the 6th and 10th day after induction of these ulcers. Mean ± SEM. N= 10 in each group of animals. aP<0.05 compared to saline-treated rats with intact pituitary (control) at the 0 day; bP<0.05 compared to saline-treated rats with intact pituitary (control) at the 6th day of observation; cP<0.05 compared to G8 in pituitary-intact rats at the same day of observation; dP<0.05 compared to hypophysectomized saline-treated rat (HP) at the same day of observation; eP<0.05 compared to saline-treated rats with intact pituitary (control) at the 10th day of observation.
these lesions. Maximal therapeutic effect was observed after ghrelin used at the dose of 8 nmol/kg/dose.

Hypophysectomy delayed the healing of gastric and duodenal ulcers. Either at the 6th or 10th day after induction of mucosal lesion, the area of both gastric and duodenal ulcers was significantly more extensive in hypophysectomized rats than in pituitary-intact rats.

In contrast to pituitary-intact rats, administration of ghrelin was without any effect on the healing rate of gastric and duodenal ulcers in hypophysectomized rats. The area of ulcers in this group of rats was similar to that observed in hypophysectomized rats treated with saline.

The mean area of both, gastric and duodenal ulcers was significantly decreased in hypophysectomized rats treated with IGF-1. The ulcer area was reduced below that observed in saline-treated hypophysectomized rats, as well as saline treated pituitary-intact rats. This effect was statistically significant at the 6th and 10th day after induction of ulcers and similar to that observed in pituitary-intact rats treated with ghrelin at the dose of 8 nmol/kg/dose.

**Gastric and duodenal mucosal DNA synthesis**

*Figures 3 and 4 demonstrate the effect of treatment with saline, ghrelin or IGF-1 on gastric and duodenal mucosal DNA synthesis in pituitary-intact or hypophysectomized rats. In control animals with intact pituitary gland, gastric mucosal DNA synthesis reached 44.3 ± 2.4 dpm/µg DNA at the 6th day after induction of ulcers and 40.2 ± 3.6 dpm/µg DNA at the 10th day Fig. 3. In the duodenum of this group of animals, the rate of DNA synthesis was 50.5 ± 2.1 dpm/µg DNA and 48.7± 1.8 dpm/µg DNA at the 6th and 10th day after ulcer induction, respectively (Fig. 4).

Administration of ghrelin in pituitary-intact rats caused an increase in gastric and duodenal mucosal DNA synthesis, and this effect was significant at both time points of observation, at the 6th and 10th day after induction of ulcers. Moreover, maximal stimulatory effect of ghrelin on mucosal DNA synthesis was obtained after ghrelin used at the dose of 8 nmol/kg/dose.

Hypophysectomy significantly reduced mucosal DNA synthesis in the stomach and duodenum by about 30 and 20%, respectively. This effect was found in both periods of observation, at the 6th and 10th day after induction of ulcers.

Administration of ghrelin was without effect on gastric and duodenal mucosal DNA synthesis in hypophysectomized rat. In contrast to that, administration of IGF-1 increased gastric and duodenal mucosal DNA synthesis in hypophysectomized rats to a similar value as that in pituitary-intact ghrelin-treated rats.

![Fig. 3. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose), hypophysectomy (HP) and treatment with IGF-1 on mucosal DNA synthesis in the stomach at the 6th and 10th day after induction of ulcers. Mean ± SEM. N= 10 in each group of animals. aP<0.05 compared to saline-treated rats with intact pituitary (control) at the same day of observation; bP<0.05 compared to HP alone at the same day of observation.](image-url)
Fig. 4. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose), hypophysectomy (HP) and treatment with IGF-1 on mucosal DNA synthesis in the duodenum at the 6th and 10th day after induction of ulcers. Mean ± SEM. N= 10 in each group of animals. aP<0.05 compared to saline-treated rats with intact pituitary (control) at the same day of observation; bP<0.05 compared to G4 in rats with intact pituitary at the same day of observation; cP<0.05 compared to HP alone at the same day of observation.

Fig. 5. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose), hypophysectomy (HP) and treatment with IGF-1 on gastric mucosal blood flow at the 6th and 10th day after induction of ulcers. Mean ± SEM. N= 10 in each group of animals. aP<0.05 compared to saline-treated rats with intact pituitary (control) at the same day of observation; bP<0.05 compared to G8 in pituitary-intact rats at the same day of observation; cP<0.05 compared to HP alone at the same day of observation.
Gastric and duodenal mucosal blood flow

In pituitary intact rats, administration of ghrelin increased gastric (Fig. 5) and duodenal (Fig. 6) mucosal blood flow. In the stomach, this effect was statistically significant in both time points of observation after ghrelin administered at the dose of 4 and 8 nmol/kg/dose. Gastric mucosal blood flow was maximally increased after treatment with ghrelin at the dose of 8 nmol/kg, reaching at the 6th and 10th day about 140 and 130% of control value, respectively (Fig. 5). In this group of animals, duodenal mucosal blood flow was significantly increased at the 6th day after ghrelin administered at the dose of 8 nmol/kg/dose; whereas at the 10th day, significant increase of duodenal mucosal blood flow was found after treatment with ghrelin at the dose of 4 and 8 nmol/kg/dose.

Hypophysectomy caused insignificant decrease in gastric and duodenal blood flow, and administration of ghrelin was without any effect on gastric and duodenal blood flow in hypophysectomized rats.

In hypophysectomized rats, treatment with IGF-1 increased gastric and duodenal mucosal blood flow to a value as that observed in pituitary-intact rats treated with ghrelin at the dose of 8 nmol/kg/dose.

Serum concentration of ghrelin

In pituitary-intact rats treated with saline serum concentration of ghrelin reached a value 223.2 ± 12.6 pg/ml at the 6th day and 232.1 ± 19.6 pg/ml at the 10th day (Fig. 7). Treatment with exogenous ghrelin at the dose of 4, 8 or 16 nmol/kg/dose increased serum level of ghrelin in rats with intact pituitary by about 69, 170 or 270%, respectively. Hypophysectomy increased serum level of endogenous ghrelin by 60% and administration of exogenous ghrelin at the dose of 8 nmol/kg/dose caused an additional increase in serum concentration of this peptide by about 125%. In hypophysectomized rats, administration of IGF-1 was without effect on serum concentration of ghrelin.

Serum concentration of growth hormone

In control pituitary-intact rats treated with saline, serum growth hormone concentration reached a value 148.6 ± 8.1 ng/ml at the 6th day after induction of ulcers and 136.2 ± 9.3 ng/ml at the 10th day (Fig. 8). Administration of ghrelin significantly increased serum level of growth hormone in rats with intact pituitary gland and maximal increase by about 70% was observed after ghrelin used at the dose of 8 nmol/kg/dose. Effect of ghrelin given at the dose of 4

---

Fig. 6. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose), hypophysectomy (HP) and treatment with IGF-1 on duodenal mucosal blood flow at the 6th and 10th day after induction of ulcers. Mean ± SEM. N = 10 in each group of animals. *P<0.05 compared to saline-treated rats with intact pituitary (control) at the same day of observation; **P<0.05 compared to G8 in pituitary-intact rats at the same day of observation; ***P<0.05 compared to HP alone at the same day of observation.
Fig. 7. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose), hypophysectomy (HP) and treatment with IGF-1 on serum concentration of ghrelin at the 6th and 10th day after induction of ulcers. Mean ± SEM. N= 10 in each group of animals. *P<0.05 compared to saline-treated rats with intact pituitary (control) at the same day of observation; †P<0.05 compared to G8 in pituitary-intact rats at the same day of observation; ‡P<0.05 compared to HP alone at the same day of observation.

Fig. 8. Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose), hypophysectomy (HP) and treatment with IGF-1 on serum concentration of growth hormone at the 6th and 10th day after induction of ulcers. Mean ± SEM. N= 10 in each group of animals. *P<0.05 compared to saline-treated rats with intact pituitary (control) at the same day of observation; †P<0.05 compared to G8 in pituitary-intact rats at the same day of observation.
Hypophysectomy caused total elimination of growth hormone from the serum and administration of ghrelin was without any effect on serum concentration of growth hormone in hypophysectomized rats. Also, administration of IGF-1 failed to affect serum level of growth hormone in hypophysectomized rats. Serum concentration of IGF-1 in saline-treated control rats with intact pituitary, serum concentration of IGF-1 was 415.6 ± 19.7 ng/ml at the 6th day after induction of ulcers and 472.6 ± 34.8 ng/ml at the 10th day (Fig. 9). In pituitary-intact rats, treatment with ghrelin significantly increased serum level of IGF-1 and maximal effect (by about 170%) was observed after ghrelin given at the dose of 8 nmol/kg/dose. Either the effect of lower or higher dose of ghrelin on serum concentration of IGF-1 was significantly weaker. In hypophysectomized saline-treated rats, serum concentration of IGF-1 was reduced and reached about 10% of control value. Administration of ghrelin did not affect serum concentration of IGF-1 in hypophysectomized rats. Treatment with IGF-1 at the dose of 20 nmol/kg/dose increased serum concentration of this peptide in hypophysectomized rats to a value similar to that as in pituitary-intact rats treated with ghrelin at the dose of 8 nmol/kg/dose.

DISCUSSION

This study is the first paper, except for our reports presented during annual meetings of the American Gastroenterological Association (31, 32), showing that treatment with ghrelin accelerates the healing of experimental chronic gastric and duodenal ulcers. Healing of mucosa damage occurs by at least two different mechanisms. Initially, the rapid process of mucosal restitution or reepithelization takes place by migration of surrounding epithelial cells from the ulcer margin to cover the denuded area (33). Secondary to that is the replacement of lost cell by cell proliferation. This last process starts after 12-16 h from ulcer development and is required for complete regeneration of damaged mucosa. DNA synthesis precedes cell division and the rate of mucosal DNA synthesis is an index of mucosal cell proliferation. In our present study, treatment with ghrelin has increased

---

**Fig 9.** Effect of ghrelin (G) administration (4, 8 or 16 nmol/kg/dose), hypophysectomy (HP) and treatment with IGF-1 on serum concentration of IGF-1 at the 6th and 10th day after induction of ulcers. Mean ± SEM. N= 10 in each group of animals. aP<0.05 compared to saline-treated rats with intact pituitary (control) at the same day of observation; bP<0.05 compared to G8 in pituitary-intact rats at the same day of observation; cP<0.05 compared to HP alone at the same day of observation.
DNA synthesis in gastric and duodenal mucosa in pituitary-intact rats. This observation indicates that therapeutic effect of ghrelin administration in the healing of gastric and duodenal ulcers involves the stimulation of epithelial cell proliferation.

Other mechanism involved in the healing effect of ghrelin administration seems to be related to the ghrelin-induced increase in mucosal blood flow. Mucosal blood flow plays an important role in the protection of gastro-duodenal mucosa against damage. In the stomach, numerous experimental studies have shown that exposure of gastric mucosa to potentially noxious environment results in little or no damage, as long as adequate blood flow is maintained; whereas reduction in mucosal blood flow leads to severe gastric injury (34). Protective effect of adequate blood flow depends on supplying the mucosa with oxygen, bicarbonate and nutritious substances, and removing carbon dioxide, hydrogen ions and toxic agents diffusing from the gastric lumen (34). Gastric hypoxia, resulting in accumulation of H+ within gastric mucosa leads to mucosal acidification and development of gastric ulcers (35). In ulcers induced by stress, the fall of gastric intramucosal pH is an important predictor of risk of mucosal bleeding (36) and a reduction in gastric blood flow leads to the increase in gastric mucosa damage (37). In the duodenum, mucosal hypoxia inhibits the healing of ulcers (38).

Another important finding of our present study is the observation that therapeutic effect of ghrelin in the healing of gastric and duodenal ulcers is indirect and depends on the release of IGF-1. This conclusion is supported by results obtained in pituitary-intact and hypophysectomized rats.

In our present study, administration of ghrelin has increased the serum concentration of growth hormone and IGF-1 in pituitary intact rats, and this effect was associated with the acceleration of healing rate of gastric and duodenal ulcers. It is well known that ghrelin stimulates the release of growth hormone from the anterior pituitary, leading to activation of the hormonal axis, growth hormone - IGF-1. Growth hormone can directly stimulate the rate of protein synthesis in cells of the body; however the most of growth hormone-induced anabolic effects is indirect and mediated through the release of IGF-1 from the liver (39-41).

Our observation that healing effect of ghrelin is related to growth hormone and IGF-1 release is in harmony with findings that administration of IGF-1 (42) and growth hormone-releasing hormone (GH-RH) (43) accelerates the healing of gastric ulcers. GH-RH and somatostatin are released by the hypothalamus and play the essential role in the regulation of growth hormone release from the anterior pituitary. Somatostatin inhibits the release of growth hormone, whereas growth GH-RH stimulates the release of growth hormone. Stimulatory effect of ghrelin and GH-RH on the release of growth hormone from somatotroph cells of the anterior pituitary is mediated by binding with different types of receptors and involves different intracellular signaling pathways. Ghrelin acts on the growth hormone secretagogue receptor (GH-SR), leading to activation of phospholipase C and increase in production of the second messengers, calcium and diacylglycerol. (5, 44, 45); whereas GH-RH binds to GH-RH receptor (GH-RHR), leading to activation of phospholipase C and cAMP production (45). However, there is a functional interaction between activation of growth hormone secretagogue receptor 1a and growth hormone-releasing hormone receptor (45, 46). It has been shown that ghrelin and growth hormone secretagogues potentiate the GH-RH-induced cyclic adenosine 3',5'-monophosphate production in cells expressing transfected GH-RH and GHS receptors (45).

Strong evidences that healing effect of ghrelin depends on IGF-1 release have been reached in our experiments with hypophysectomized rats. First of all, hypophysectomy has increased serum concentration of endogenous ghrelin but inhibited mucosal DNA synthesis, mucosal blood flow and serum concentration of IGF-1, leading to the delay in the healing of gastric and duodenal ulcers. Next support for the conclusion that growth-promoting and healing effect of ghrelin depends on the release of IGF-1 brings the observation that administration of ghrelin in hypophysectomized rats failed to affect serum concentration of IGF-1 and for this reason was without effect on mucosa cell proliferation, mucosal blood flow and healing of chronic gastric and duodenal ulcers. In harmony with these findings is the last our observation that treatment with IGF-1 hypophysectomized rats stimulates mucosal cell proliferation, leading to acceleration the healing of gastric and duodenal ulcers.

On the other hand, there are studies showing direct effect of ghrelin on peripheral tissues. In vitro study performed by Rak and Gregoraszczuk (47) has shown that ghrelin directly increases expression and activity of aromatase in prepubertal porcine ovarian granulose and theca interna cells, leading to an increase in synthesis of estradiol. Also they have found that ghrelin stimulates cell proliferation and reduces apoptosis of these cells. Similar direct of ghrelin was observed by Zwirska-Korczala et al. (48). They have found, using preadipocytes cell culture, that ghrelin increases proliferation of these cells and reduces culture medium concentration of malondialdehyde by an increase in activity of antioxidative enzymes.
These data are in contrast with our present finding. The difference between their and our results is probably dependent on type of study. They have performed in vitro study, whereas our investigation was in vivo study. It is most likely that strong effect of ghrelin on the release of growth hormone hides eventual direct effect of ghrelin on the stomach and duodenum. On the other hand, we have not observed any beneficial effect of ghrelin in hypophysectomized rats. This observation indicates that ghrelin has not any direct effect on gastric and duodenal mucosa regeneration.

Finally, we conclude that treatment with ghrelin accelerates healing of chronic gastric and duodenal ulcers. However, this effect is indirect and depends on the release of growth hormone and IGF-1.

REFERENCES

45. Cunha SR, Mayo KE. Ghrelin and growth hormone (GH) secretagogues potentiate GH-releasing hormone (GHRH)-induced cyclic adenosine 3',5'-monophosphate production in cells expressing transfected GHRH and GH secretagogue receptors. Endocrinology 2002; 143: 4570-4582.

Received: March 28, 2008
Accepted: February 20, 2009

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