Suppression of Intestinal Mucosal Apoptosis by Ghrelin in Fasting Rats

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Ghrelin is mainly produced in the stomach and has several physiologic functions. The aim of this study was to investigate whether ghrelin regulates apoptosis in the small intestinal mucosa of fasting rats. Intestinal mucosal apoptosis was evaluated as the percentage of fragmented DNA, villus height, and terminal deoxynucleotidyl transferase–mediated dUDP-biotin nick end-labeling (TUNEL) staining and by Western blot analysis of caspase-3 in 48-hr fasting rats. Crypt cell proliferation was evaluated by counting the number of 5-bromo-2-deoxyuridine (BrdU) positive cells. Ghrelin was administered intraperitoneally at dosages of 2.5, 25, and 250 μg/kg per 48 hrs by continuous infusion via an Alzet micro-osmotic pump or injections at 12-hr intervals. Ghrelin was also infused in rats that underwent truncal vagotomy. The lowest dosage of ghrelin (2.5 μg/kg per 48 hrs) was administered into the third cerebroventricle. Ghrelin treatment attenuated the percentage of fragmented DNA in the small intestinal mucosa in 48-hr fasting rats in a dose-dependent manner. Continuous infusion of ghrelin and injections of ghrelin at 12-hr intervals suppressed intestinal apoptosis almost equally. This effect on apoptosis was not attenuated by truncal vagotomy. Cerebroventricular infusion of ghrelin also attenuated intestinal apoptosis. The antiapoptotic effect of ghrelin was confirmed by decreased TUNEL staining, recovery of the villus height, and decreased expression of caspase-3. BrdU uptake indicated that ghrelin enhanced cell proliferation in the intestinal crypt. Taken together, these data indicate that ghrelin enhanced intestinal growth with the suppression of small intestinal mucosal apoptosis in 48-hr fasting rats, suggesting that ghrelin controls intestinal function through the regulation of intestinal apoptosis. Exp Biol Med 233:48–56, 2008

Key words: small intestine; cell proliferation; intraperitoneal infusion; vagotomy

Introduction

Ghrelin, a 28–amino acid gastric hormone, was originally discovered as a ligand for the growth hormone (GH) secretagogue receptor and exists as two major forms, the acylated and unacylated ghrelin peptides (1). Ghrelin undergoes post-translational modification with covalent attachment of a medium-chain fatty acid, typically octanoic acid, to the serine-3 residue. This acylation is required for ghrelin to have an effect on the brain from the systemic circulation, activating its classic receptor, the GH secretagogue receptor (GHS-R) type 1a (1). Des-acyl ghrelin does not activate the GHS-R type 1a receptor (1). Ghrelin is mainly produced in the stomach and is detected in the duodenum, jejunum, ileum, and colon (2). The GHS-R type 1a is prominently expressed in the brain, especially in the arcuate nucleus, ventromedial nucleus, and hippocampus (3). The receptor has also been detected in peripheral tissues, including the gastrointestinal tract, endocrine system, kidney, placenta, lymphatic tissue, myocardium, adipose tissue, and bone (4, 5). This widespread distribution is likely to explain the many endocrine and nonendocrine effects of ghrelin. Another GHS-R, type 1b, is produced by an alternative splicing mechanism, a COOH-terminal–truncated form of the type 1a receptor, and this receptor might be pharmacologically inactive (1, 6).

In addition to its well-known effects on appetite regulation and GH secretion, ghrelin also has pleiotropic effects on gastrointestinal functions (7). The intravenous administration of ghrelin dose-dependently increases gastric acid secretion and stimulates gastric motility (8, 9), whereas the intracerebroventricular administration of ghrelin increases gastric acid secretion in a dose-dependent manner

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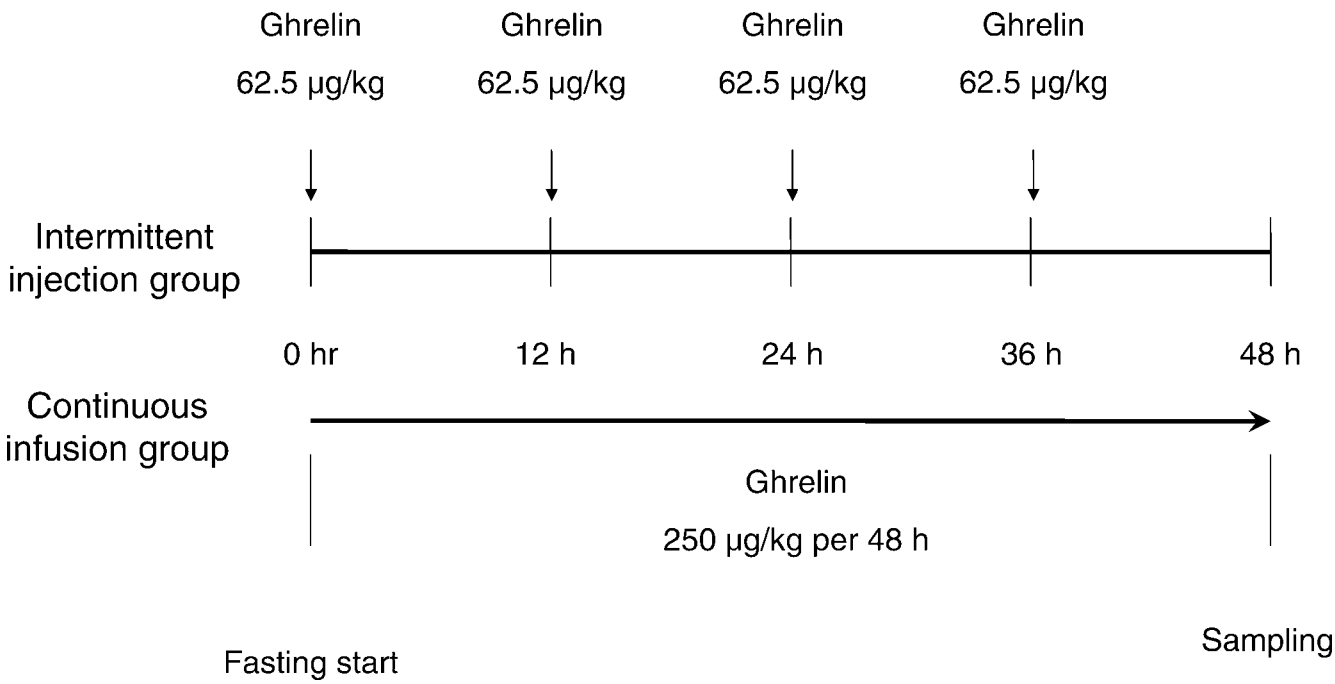

Figure 1. Experimental design of ghrelin treatment. Ghrelin was administered ip in rats during 48 hrs of fasting. One group was treated with continuous ghrelin infusion by using an Alzet micro-osmotic pump (250 μg/kg per 48 hrs), and the other group received ghrelin injections at 12-hr intervals (62.5 μg/kg per 48 hrs). Control rats were treated with the same volume of vehicle. After 48 hrs of fasting, intestinal mucosal tissues were collected between 08:00 and 09:00. Six rats were tested in each group.

(10). Furthermore, *Helicobacter pylori* infection is associated with reduced circulating levels of ghrelin, and this association is independent of the body mass index (11).

It is well established that the central nervous system regulates appetite in rats and humans (12, 13). In contrast to the anorectic effect of leptin, ghrelin enhances appetite. Ghrelin interacts with the leptin hypothalamic network in the arcuate nucleus, and the opposite effects of leptin and ghrelin on neurons in the arcuate nucleus may maintain the neurophysiologic balance of the orexigenic and anorectic effects of ghrelin and leptin (14).

Homeostasis of intestinal epithelial architecture in the small intestine is very important for nutrient absorption. To maintain the physiologic state, the intestinal epithelium keeps steady-state turnover of enterocytes. Our previous studies showed that fasting induces mucosal cell apoptosis, which occurs via a receptor-mediated type I apoptotic pathway in the rat small intestine (15, 16). We also previously demonstrated that the exogenous administration of leptin enhances mucosal apoptosis in the small intestine, thereby decreasing the efficiency of nutrient absorption (17). However, the role of ghrelin in the small intestinal mucosa has not yet been clarified. Hypothetically, ghrelin may facilitate a positive energy balance not only by stimulating appetite but also by affecting intestinal mucosal growth. The aim of this study was to investigate the influence of ghrelin administration on intestinal apoptosis and cell proliferation in fasting rats.

Materials and Methods

Animals. The experimental protocol and design were approved by the Animal Experimentation Committee of Saga University and performed according to the Saga University Guidelines for Animal Experimentation. Male Sprague Dawley rats, each weighing approximately 200–250 g, were used in this study. The rats were housed individually in wire-bottomed cages to prevent coprophagia and placed in a room illuminated from 08:00 to 20:00 (12 hr:12 hr light:dark cycle) at a constant temperature (22° ± 1°C). The animals were allowed free access to water and food until the beginning of the intervention at 7 weeks of age.

Experimental Design. Ghrelin (Peptide Institute Inc., Osaka, Japan) was dissolved in distilled water and administered intraperitoneally (ip) during a 48-hr fast. This study used two groups of rats because ghrelin has a short half-life (30 mins) when injected intravenously (18). As indicated in Figure 1, one group was treated with continuous ghrelin (250 μg/kg per 48 hrs) ip infusion during the 48-hr fast by using an Alzet micro-osmotic pump (Model 1003D; Durect Co., Cupertino, CA), and the other group received ghrelin (62.5 μg/kg per 48 hrs) ip injections at 12-hr intervals during the 48-hr fast. We used the doses of ghrelin on the basis of previous reports regarding the induction of hyperphagia (13), the stimulatory effect of intestinal motility (19), and the anti-inflammatory effect on colitis (20). In the corresponding control groups, the same volume of distilled

water was administered by the same method. For the continuous ghrelin infusion into the peritoneum, an Alzet micro-osmotic pump was implanted into the abdominal cavity through a 2-cm incision in rats under halothane anesthesia. After the 48-hr fast, each animal under halothane anesthesia was killed between 08:00 and 09:00 to collect the intestinal tissues. Six rats were tested in each group. Additional rats were given infusions via an Alzet micro-osmotic pump after truncal vagotomy 1 week before the experiment (six rats in each group). Truncal vagotomy was performed as follows: the esophagus was gently lifted just below the liver and diaphragm, and the mesentery and visible vagal fibers were cut within 2 cm of the esophagus (21, 22).

In addition to ip injections, ghrelin was infused into the third cerebroventricle in another group of rats by using an Alzet micro-osmotic pump. The infusion dosage of ghrelin into the third cerebroventricle was 2.5 μg/kg per 48 hrs, which was 100-fold lower than the ip infusion dosage of 250 μg/kg per 48 hrs. Control rats were given infusions of vehicle. Cannulation into the third cerebroventricle was performed 1 week before the experiment, as described previously (17, 23). A hole that was 3 mm in diameter was drilled in the skull on the midline at 6.0 mm anterior to the ear bar zero. A 23-gauge, 13-mm-long stainless steel cannula was inserted into the third ventricle to a depth of 7.8 mm from the cortical surface. At the time of the experiment, the food intake and body weight were ascertained to have returned to the presurgery levels. Six rats were tested in each group.

Collection of Intestinal Mucosa. After the 48-hr fast, the animals were anesthetized and then killed. The entire small intestine was carefully removed and placed on ice. The oral 10-cm part of the intestine was regarded as the duodenum, and the rest of the intestine was divided into two equal segments representing the proximal (jejunum) and distal (ileum) segments. Some pieces approximately 2 cm in length were resected from the middle portion of each segment and fixed in 10% neutral-buffered formalin for the measurement of the mucosal height and immunohistochemical analysis with the terminal deoxynucleotidyl transferase (TdT)-mediated dUDP-biotin nick end-labeling (TUNEL) assay. Each segment was rinsed thoroughly with physiologic saline and opened longitudinally to expose the intestinal epithelium. The mucosal layer was harvested by gentle scraping of the epithelium with a glass slide, as previously described (16, 24). Half of each specimen was subjected to a DNA fragmentation assay, and the remaining half was used for the evaluation of apoptotic proteins by Western blot analysis.

DNA Fragmentation Assay. Mucosal scrapings were processed immediately after collection to minimize nonspecific DNA fragmentation. The amount of fragmented DNA was determined as previously described (25). Briefly, the mucosal scrapings were first homogenized in 10 volumes of lysis buffer that consisted of 5 m*M* Tris-HCl (pH 8.0), 20 m*M* EDTA, and 0.5% Triton X-100. Aliquots (1 ml) of each sample were then centrifuged at 27,000 *g* for 20 mins at 4°C to separate the intact chromatin (pellet) from the fragmented DNA (supernatant) (26). The supernatant was decanted and saved, and the pellet was resuspended in 1 ml of Tris buffer that consisted of 10 m*M* Tris-HCl (pH 8.0) and 1 m*M* EDTA. The pellet and supernatant fractions were assayed for their DNA contents by using the diphenylamine reaction (27). The results were expressed as the percentage of fragmented DNA relative to the total DNA.

Mucosal Height. Tissue samples were removed from the jejunum, immediately fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned. The sections were stained with hematoxylin and eosin. The mucosal lengths (villus height and crypt depth) in 48-hr fasting rats after treatment with distilled water or ghrelin were measured under a light microscope by using micrometer scale standards.

Imumunohistochemical Staining. Apoptotic cells were stained by the TUNEL method as previously described (28), with some modifications, by using the ApopTag Peroxidase In Situ Apoptosis Detection Kit (Oncor, Gaithersburg, MD). Briefly, sections were dewaxed and immersed in phosphate-buffered saline (PBS) that contained 0.3% hydrogen peroxide for 10 mins at room temperature and then incubated with 20 mg/ml proteinase K for 15 mins at room temperature. Next, 75 ml of equilibration buffer was directly applied to the specimens for 10 mins at room temperature, and incubation in 55 ml of TdT enzyme at 37°C for 1 hr followed. The reaction was terminated by transferring the sections to prewarmed stop/wash buffer for 30 mins at 37°C. The sections were then covered with a few drops of rabbit serum and incubated for 20 mins at room temperature, after which they were incubated with 55 ml of antidigoxigenin peroxidase for 30 mins at room temperature. Next, the sections were soaked in Tris buffer that contained 0.02% diaminobenzidine and 0.02% hydrogen peroxide for 1 min to induce color development. Finally, the specimens were counterstained by immersion in hematoxylin. A minimum of 50 crypts were randomly selected for the analysis of the apoptotic index, and the number of apoptotic cells was calculated. The apoptotic index was determined by dividing the number of positively stained cells by the total number of cells in the villus column and multiplying by 100.

Mucosal cell proliferation was assessed by using 5-bromo-2-deoxyuridine (5-BrdU) incorporation to identify cells in the S phase of the cell cycle (29). Rats were given ip injections of 120 mg/kg 5-BrdU (40 g/L 5-BrdU and 4 g/L 5-fluorodeoxyuridine) at 90 mins before sacrifice. BrdU was detected with a monoclonal antibody and a streptavidin–biotin staining system. The number of labeled cells in at least 10 well-oriented longitudinal crypts in each sample was determined under a light microscope by an examiner blinded to the study groups, and the result was reported as the number of 5-BrdU–labeled cells among the total crypt cells.

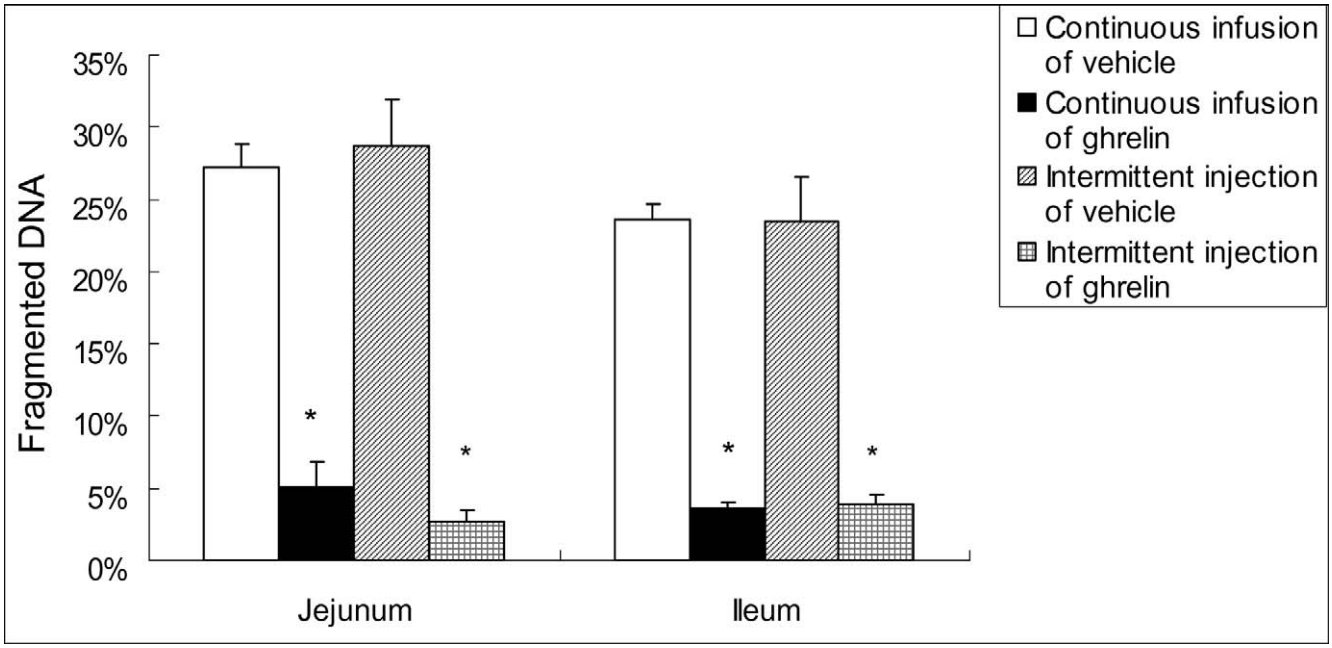

Figure 2. Percentages of fragmented DNA in relation to the delivery method of ghrelin. The percentage of fragmented DNA was determined in mucosal scrapings in the jejunal and ileal mucosa after 48 hrs of fasting. The increase in apoptosis induced by fasting was attenuated by continuous infusion of ghrelin (250 μg/kg per 48 hrs). This attenuation effect was observed in rats given ghrelin at 12-hr intervals over the 48-hr fast (each 62.5 μg/kg, four times). Each bar represents the mean ± SE of six rats. *$P < 0.01$ vs. the corresponding control group.

Western Blot Analysis of Caspase-3. Mucosal scrapings were immediately homogenized in 10 volumes of lysis buffer that consisted of 50 m*M* Tris-HCl (pH 7.6), 300 (or 150) m*M* NaCl, 0.5% (or 1%) Triton X-100, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 m*M* serine peptidase inhibitor, and 1.8 mg/ml iodoacetamide and lysed at 4°C. Insoluble material was removed by centrifugation at 14,000 *g* for 15 mins at 4°C, and the protein concentration of the soluble fraction was determined. Equal quantities (20 μg) of the lysates were resolved by 15% SDS–PAGE and electroblotted onto a nitrocellulose membrane (Trans-Blot; Bio-Rad, Hercules, CA). After blocking with PBS that contained 0.1% polyoxyethylene sorbitan monolaurate and 5% dry milk at 4°C overnight, the membrane was incubated for 2 hrs with a polyclonal rabbit anti–caspase-3 antibody (1:1000; Santa Cruz Biotechnology Inc., Santa Cruz, CA), which recognizes cleaved caspase-3 (the active form) (30). Antigen-antibody complexes were detected with horseradish peroxidase–conjugated anti–rabbit IgG (1:1000; Santa Cruz Biotechnology Inc.). Chemiluminescence was detected with ECL Western blotting detection reagents (Amersham Pharmacia Biotech, Buckinghamshire, UK) according to the manufacturer's recommendations. Densitometric assessment of bands from the autoradiogram was done by using Image Gauge VDS (Fujifilm, Tokyo, Japan). Band intensities were quantified by measuring the absolute integrated optical intensity, which estimates the band in the lane profile. Results were expressed as ratios to β-actin densitometry units.

Statistical Analysis. Results are presented as means ± standard errors (SE). Data were evaluated with Student's *t* test. Differences were considered significant if the probability of the difference occurring by chance was less than 5 in 100 ($P < 0.05$).

Results

DNA Fragmentation in Intestinal Mucosa. The percentages of fragmented DNA in the jejunal and ileal mucosa in the control rats increased after 48 hrs of fasting (Fig. 2). This increase in apoptosis induced by fasting was dramatically attenuated by continuous ip infusion of ghrelin at a dosage of 250 μg/kg per 48 hrs ($P < 0.01$). This attenuation effect of ghrelin on the increased apoptosis in the intestinal mucosa after fasting was also observed in rats given ghrelin at 12-hr intervals over the 48-hr fast ($P < 0.01$). The degree of suppression of intestinal mucosal apoptosis did not differ between the group that received a continuous infusion (250 μg/kg per 48 hrs) and the group that received injections of ghrelin at 12-hr intervals at a dosage of 62.5 μg/kg over the 48-hr fast. With a continuous infusion of ghrelin at 25 μg/kg per 48 hrs, one tenth of the initial dose attenuated the increased apoptosis in the intestinal mucosa of 48-hr fasting rats (jejunum: 15.2% ± 1.4%; ileum: 17.0% ± 2.2%; $P < 0.05$ for each). This attenuation induced by ghrelin on mucosal apoptosis was not observed at a dosage of 2.5 μg/kg per 48 hrs, which was 1% of the initial dose. In contrast to ip infusion, continuous infusion of ghrelin into the third cerebroventricle at a dosage of 2.5 μg/kg per 48 hrs attenuated intestinal apoptosis (jejunum: 1.99% ± 0.36%; ileum: 3.70% ± 0.78%; $P <$

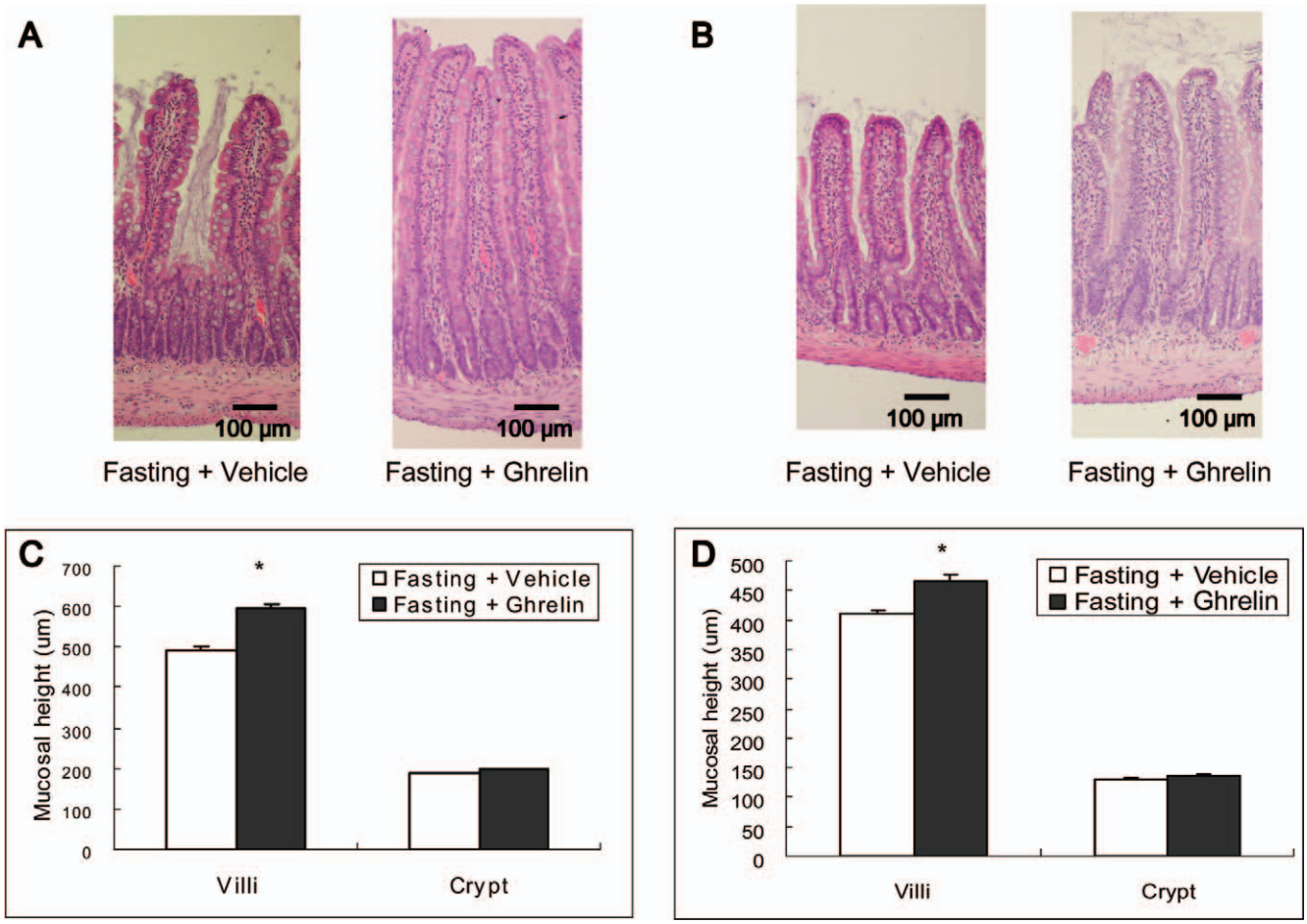

Figure 3. Effect of ghrelin on intestinal mucosal height in 48-hr fasting rats. (A and B) Light micrographs of the jejunum and ileum stained by hematoxylin and eosin (magnification, ×100). Jejunal and ileal mucosal height was significantly longer in ghrelin-treated rats than in control rats. (C and D) Mucosal height of the jejunum and ileum. The increase in jejunal and ileal mucosal height after ghrelin infusion was due to an increase in villus height. Each bar represents the mean ± SE of six rats. Fasting + vehicle, 48-hr fasting with vehicle treatment. Fasting + ghrelin, 48-hr fasting with ghrelin treatment. *$P < 0.05$ vs. the corresponding control group. A color figure is available in the online version of this article.

0.01 for each). We also evaluated the influence of truncal vagotomy on the effects of ip ghrelin infusion. Truncal vagotomy had no effect on ghrelin's antiapoptotic effect in 48-hr fasting rats. The percentages of fragmented DNA in the jejunal and ileal mucosa of vagotomized fasted rats with continuous ip infusion of vehicle were 23.9% ± 1.1% and 26.7 ± 2.7%, respectively, whereas the corresponding percentages in the jejunal and ileal mucosa of vagotomized ghrelin-treated rats were 1.1% ± 0.8% and 2.6% ± 0.5%, respectively.

Measurement of Mucosal Height. The decrease in the jejunal villus height in 48-hr fasting rats was significantly recovered in 48-hr fasting rats with a continuous ip infusion of ghrelin at a dosage of 250 μg/kg per 48 hrs ($P < 0.05$; Fig. 3). The ileal villus height in 48-hr fasting rats treated with ghrelin was higher than that in 48-hr fasting rats treated with vehicle (vehicle, 467 ± 9 μm; ghrelin, 411 ± 6 μm; $P < 0.05$ for each). In contrast to the villus height, the crypt heights in the jejunal and ileal mucosa were not influenced by continuous infusion of ghrelin (Fig. 3).

***In Situ* Detection of Apoptosis by Immunohistochemical Staining.** TUNEL staining of the jejunum and ileum revealed an increase in the number of apoptotic cells in 48-hr fasting rats, with significant localization of the apoptotic cells in the upper third of the intestinal villus (Fig. 4). This increase in apoptotic cells in the intestinal mucosa was significantly suppressed in rats receiving continuous infusion of ghrelin at a dosage of 250 μg/kg per 48 hrs. This effect of ghrelin in the fasted intestinal mucosa was measured by calculation of the apoptotic index. As shown in Figure 4, the apoptotic index increased in 48-hr fasting rats, and this increase was attenuated in the jejunum and ileum of 48-hr fasting rats treated with ghrelin ($P < 0.01$ for each).

Western Blot Analysis of Apoptotic Proteins. The results of Western blot analysis of 48-hr fasting rats treated with ghrelin are shown in Figure 5. In the cytosolic fractions, we evaluated the cleavage of caspase-3,

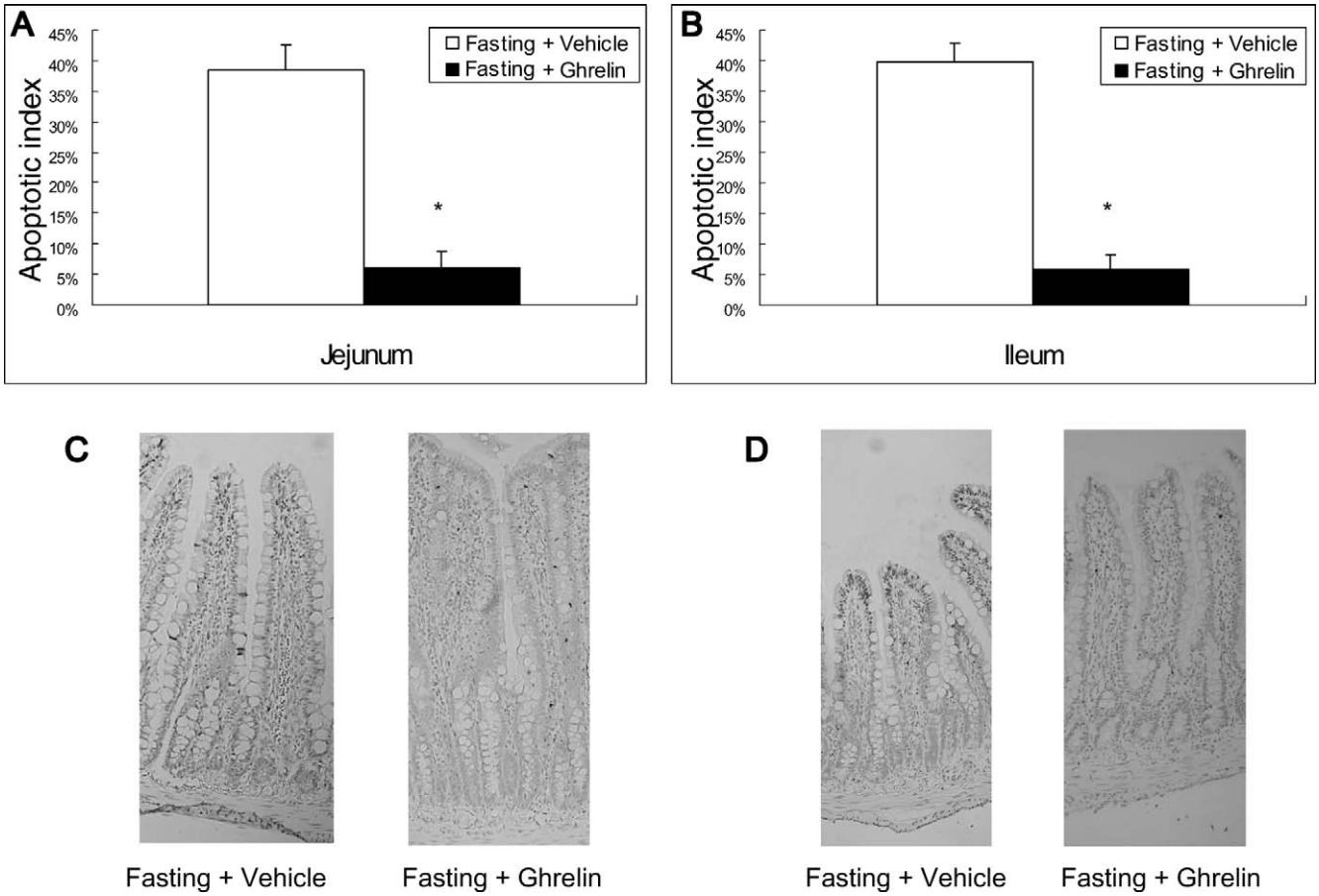

Figure 4. Apoptotic index and light micrographs of the small intestine stained by using the TUNEL method. (A and B) Apoptotic index of the jejunal and ileal mucosa after 48 hrs of fasting. TUNEL staining revealed an increase in the number of apoptotic cells in 48-hr fasting rats. This increase in the apoptotic cells was attenuated by ghrelin. (C and D) Light micrographs of the jejunum and ileum, respectively (magnification, ×100). Apoptotic nuclei were stained dark brown, and nonapoptotic nuclei were stained blue. An increase in the number of apoptotic cells in 48-hr fasting rats was observed with significant localization of apoptotic cells in the upper third of the jejunal and ileal villi. Fasting + vehicle, 48 hrs of fasting with vehicle treatment. Fasting + ghrelin, 48 hrs of fasting with ghrelin treatment. Each bar represents the mean ± SE of six rats. *$P <$ 0.01 vs. the corresponding control group.

which activates the final execution pathway of apoptosis. The increased expression of cleaved caspase-3 (17 and 12 kDa) following 48 hrs of fasting was significantly attenuated by continuous ip infusion of ghrelin ($P < 0.05$ for each).

Cell Proliferation Assay. Crypt cell proliferation was evaluated in 48-hr fasting rats treated with ghrelin to assess its contribution to the maintenance of mucosal morphologic changes. Compared with vehicle in 48-hr fasting rats, continuous infusion of ghrelin at a dosage of 250 μg/kg per 48 hrs increased the number of BrdU-positive cells in rats fasting for 48 hrs (Fig. 6). This result indicates that ghrelin enhanced cell proliferation in 48-hr fasting rats.

Discussion

The results of the present study indicate that the increase in intestinal mucosal apoptosis in 48-hr fasting rats can be attenuated in a dose-dependent manner by the ip administration of ghrelin. This effect of ghrelin was also induced by an intracerebroventricular infusion. The reasons why we used 48-hr fasting rats in this study were as follows: ghrelin enhances appetite and increases food intake (1), and the effect of ghrelin on apoptosis can be clearly evaluated in fasting rats, in which intestinal apoptosis is enhanced, whereas intestinal mucosal apoptosis is low in rats fed *ad libitum* (15, 16). This suppressive effect of ghrelin on intestinal apoptosis is opposite of the effect of leptin, which induces apoptosis after its administration (17, 23). The plasma ghrelin concentration increases with fasting, and this increase is reversed by food intake, particularly by high-calorie and/or high-carbohydrate meals (31–33). It has been suggested that ghrelin signals the hypothalamus when an increase in metabolic efficiency is required, whereas the leptin concentration is reduced in this state (34). In the present study, ghrelin treatment reduced the elevated casepase-3 activity, attenuated the reduction of villus height, and lowered DNA fragmentation of mucosal cells, all of which were induced by fasting. These results indicate that ghrelin may be involved in metabolic efficiency partly through the suppression of intestinal apoptosis.

There are several potential mechanisms for the effect of

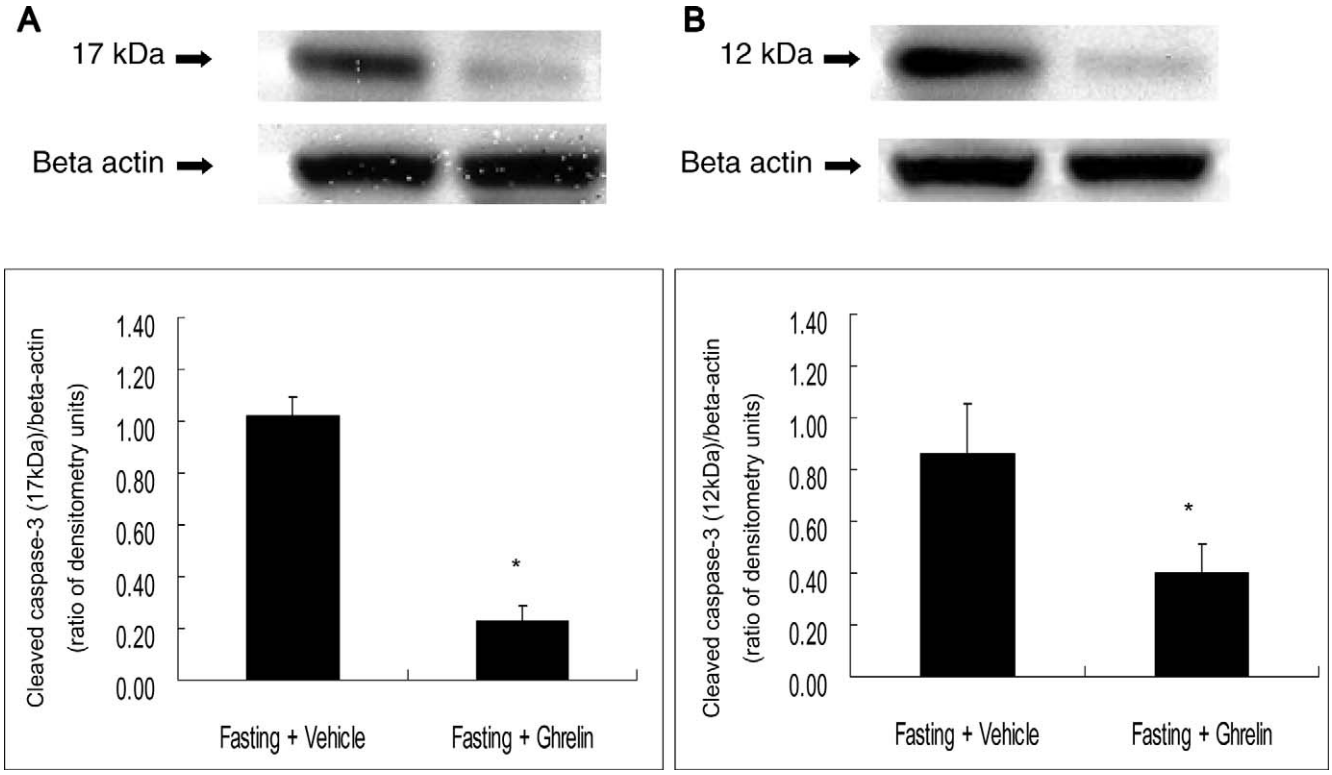

Figure 5. Effects of a 48-hr fast and ghrelin on the expression of apoptotic proteins. Levels of cleaved caspase-3 (A, 17 kDa; B, 12 kDa) were assessed by densitometric analysis, and results are expressed as ratios of caspase to β-actin densitometry units. The increase in cleaved caspase-3 (17 and 12 kDa) in 48-hr fasting rats was significantly attenuated by ip infusion of ghrelin. Each value represents the mean ± SE for six rats. Fasting + vehicle, 48 hrs of fasting with vehicle treatment. Fasting + ghrelin, 48 hrs of fasting with ghrelin treatment. *$P < 0.05$ vs. the corresponding control group.

ghrelin on the attenuation of intestinal mucosal apoptosis. Whether the effect is direct and/or indirect remains unclear. The antiapoptotic effect of ghrelin could be mediated by an increase in GH secretion, because ghrelin was originally detected as a natural ligand for the GHS-R (1). In animal models, GH increases intestinal growth by stimulating crypt cell production (35, 36). Another study indicated that ghrelin may have the potential to improve nutrient absorption in patients with short bowel syndrome (37). The antiapoptotic effect of ghrelin may be induced directly by the activation of peripheral ghrelin receptors, because these receptors are expressed in many peripheral organs (3), including the small and large intestines, suggesting a potential direct action of ghrelin on the small intestine.

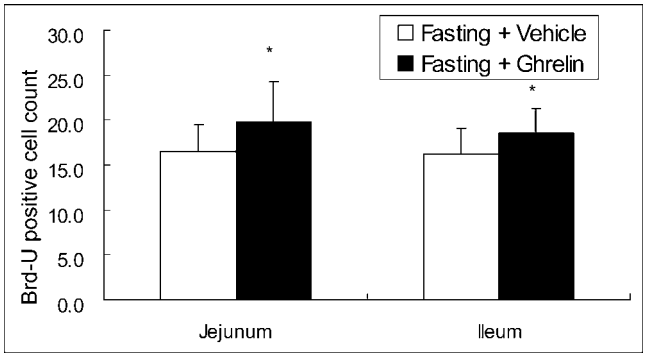

Figure 6. BrdU-positive cells in 48-hr fasting rats. The mean BrdU-positive cell counts in jejunal and ileal crypts were significantly higher in ghrelin-infused rats than in control rats. Each value represents the mean ± SE for six rats. Fasting + vehicle, 48 hrs of fasting with vehicle treatment. Fasting + ghrelin, 48 hrs of fasting with ghrelin treatment. *$P < 0.05$ vs. the corresponding control group.

Peripheral ghrelin administration affects the activity of vagal afferents in rats, and ghrelin has no stimulatory effect on patients after vagotomy (38–40). Another study indicated that an acute eating-stimulatory effect of ip ghrelin does not require vagal afferent signaling in rats (41). In the present study, truncal vagotomy had no effect on the decrease in intestinal mucosal apoptosis induced by ghrelin; this result suggested that the vagal nerve is not important for the attenuation of intestinal apoptosis by ghrelin. We did not evaluate the role of the sympathetic nerves in intestinal apoptosis. Further experiments are required to clarify the precise mechanism of the effect of ghrelin on intestinal apoptosis.

The results of this study indicate that ghrelin enhances the proliferation of crypt cells in the small intestine in addition to attenuation of intestinal apoptosis. Previous studies have indicated that ghrelin has anti-inflammatory activity in the gastrointestinal tract (20, 42). GH may be a relieving agent for chemotherapy-induced gastrointestinal damage (43). These results suggest that ghrelin, in addition

to other growth factors such as insulin-like growth factor 1, epidermal growth factor, and GH, could be a candidate for use in treating injured intestinal mucosa in malabsorption syndrome and inflammatory bowel syndrome (44–47).

In conclusion, ghrelin has a role in the physiologic control of energy balance, not only by stimulating eating but also by affecting intestinal mucosal growth with a decrease in apoptosis during the fasting state in rats.

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