Localization of acyl ghrelin- and des-acyl ghrelin-immunoreactive cells in the rat stomach and their responses to intragastric pH

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Mizutani M, Atsuchi K, Asakawa A, Matsuda N, Fujimura M, Inui A, Kato I, Fujimiya M. Localization of acyl ghrelin- and des-acyl ghrelin-immunoreactive cells in the rat stomach and their responses to intragastric pH. Am J Physiol Gastrointest Liver Physiol. 297: G974–G980, 2009. First published August 20, 2009; doi:10.1152/ajpgi.00147.2009.—Acyl ghrelin has a 28-amino acid sequence with O-n-octanoyl acid modification at the serine 3 position, whereas des-acyl ghrelin has no octanoyl acid modification. Although these peptides exert different physiological functions, no previous studies have shown the different localization of acyl ghrelin and des-acyl ghrelin in the stomach. Here we have developed an antibody specific for des-acyl ghrelin that does not crossreact with acyl ghrelin. Both acyl ghrelin- and des-acyl ghrelin-immunoreactive cells were distributed in the oxyntic and antral mucosa of the rat stomach, with higher density in the antral mucosa than oxyntic mucosa. Immunofluorescence double staining showed that acyl ghrelin- and des-acyl ghrelin-positive reactions overlapped in closed-type round cells, whereas des-acyl ghrelin-positive reaction was found in open-type cells in which acyl ghrelin was negative. Acyl ghrelin-/des-acyl ghrelin-positive closed-type cells contain somatostatin; on the other hand, des-acyl ghrelin-positive open-type cells contain somatostatin. We measured the release of acyl ghrelin and des-acyl ghrelin in vascularly perfused rat stomach by ELISA, and the effects of different intragastric pH levels on the release of each peptide were examined. The release of des-acyl ghrelin from the perfused stomach was greater at pH 2 than at pH 4; however, the release of acyl ghrelin was not affected by intragastric pH. The present study demonstrated the differential localization of acyl ghrelin and des-acyl ghrelin in the rat stomach and their different responses to the intragastric pH.

Ghrelin is a newly identified orexigenic peptide from rat and human stomach that functions as an endogenous ligand for growth hormone secretagog receptor (19). The peptide has a 28-amino acid sequence with O-n-octanoyl acid modification at the serine 3 position (19). Des-acyl ghrelin, on the other hand, has the same amino acid sequence with no octanoyl acid modification (19). Although they are derived from the same precursor (proghrelin), the unique posttranslational modification of ghrelin (acyl ghrelin) serves different effects from des-acyl ghrelin (1, 15). Therefore, it seems necessary to identify the different localization of acyl ghrelin and des-acyl ghrelin in the stomach and measure separately the plasma concentration of each peptide in response to the physiological stimulation. In fact, a number of previous studies have measured the plasma ghrelin levels by radioimmunoassay (RIA) or ELISA and examined the localization of ghrelin in the gastrointestinal tract. However, confusion exists in previous studies because the commercially available RIA kit often recognized total ghrelin (acyl ghrelin + des-acyl ghrelin) rather than being specific for acyl ghrelin (3, 5, 6, 10, 17, 20, 26). This problem has been overcome by development of antibodies against the NH2 terminus (1–11) of ghrelin and COOH terminus (13–28) of ghrelin (4, 13). The antibody for ghrelin-(1–11) includes n-octanoylated serine 3; therefore, it is acyl ghrelin-specific (4, 25, 28), whereas the antibody for ghrelin-(13–28) recognizes both acyl ghrelin and des-acyl ghrelin (19, 27). By using these antibodies, the plasma level of acyl ghrelin has been separately measured from total and/or des-acyl ghrelin by RIA (14, 22) or ELISA (18, 12, 21, 23).

Concerning immunohistochemical study, the localization of acyl ghrelin in the stomach has been studied in various animals by using the antibody for ghrelin-(1–11) (4, 25, 28); however, no previous studies have shown the localization of des-acyl ghrelin in the stomach. In the present study, we developed polyclonal antibodies specific for acyl ghrelin [anti-rat octanoyl ghrelin (1–15)-cys-keyhole limpet hemocyanin (KLH) serum] and for des-acyl ghrelin [anti-rat des-octanoyl ghrelin (1–15)-cis-KLH serum]. By using these antibodies, we successfully detected the different localization of acyl ghrelin and des-acyl ghrelin in the rat stomach.

Mechanisms that regulate the release of acyl ghrelin have been actively investigated. These include fasting (5, 22, 27), the blood glucose (11) and insulin levels (6), gut peptides (14, 17, 18, 20, 26), and the autonomic nervous system (14). However, only a relatively small number of studies have examined the mechanism regulating the release of des-acyl ghrelin from the stomach (20). In general, acyl ghrelin is an orexigenic peptide (15) and des-acyl ghrelin is an anorexigenic peptide (1); therefore the former is stimulated in the fasted state, whereas the latter is stimulated in the fed state. Although intragastric pH is an important mediator in the fed and fasted states, to the best of our knowledge, no previous studies have investigated the effects of intragastric pH on the release of acyl ghrelin and des-acyl ghrelin from the stomach. In the present study, we examined the effects of different intragastric pHs on the release of acyl ghrelin and des-acyl ghrelin by ELISA using specific antibodies against these two peptides.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 200–300 g were used. Care of animals was conducted in accordance with the ILAR Guide for Care...
and Use of Laboratory Animals. Animals were housed in a light-controlled room with free access to laboratory food and water but were fasted overnight (16–18 h) before the experiments.

Development of antibodies against acyl ghrelin and des-acyl ghrelin and the specificity of the antibodies. N-(6-maleimidocaproyloxy)-sulfosuccinimidyl sodium salt (107 mg; Dojindo Laboratories, Kumamoto, Japan) and KLH (75 mg; Wako Pure Chemical, Osaka, Japan) were dissolved in 0.1 M HEPES buffer (6 ml, pH 8.1). The mixture was stirred for 2 h at room temperature and purified on an Econo-Pac 1- DG column (Bio-Rad Laboratories, Hercules, CA). The purified maleimido-activated KLH in 0.1 M phosphate buffer (PB) (6 ml, pH 7.2) was combined with synthetic rat acyl ghrelin (1–15)-Cys (25 mg) or synthetic rat des-acyl ghrelin (1–15)-Cys (25.0 mg) in 0.1 M PB (2 ml, pH 7.2), and the mixture was stirred for 18 h at 4°C. The ensuing conjugate (2 ml) was emulsified with Freund’s complete adjuvant (2 ml; Calbiochem-Behring, San Diego, CA) with a mixer for 45 min in an ice bath. The emulsion was injected intradermally into multiple sites of 10 Japanese white female rabbits (weight ~2.0–2.5 kg, 5 for acyl ghrelin and 5 for des-acyl ghrelin). For primary immunization, each rabbit received a peritoneal injection containing ~1.25 mg of peptide. Immunization was performed at 2-wk intervals using a half dose of the antigen used for the primary immunization. The rabbits were bled from the marginal ear vein 10 days after each immunization. After the sixth immunization, one of the five rabbits had a high titer antiserum against acyl ghrelin (RY1601) and against des-acyl ghrelin (RY1595).

We also developed the antibody against obstatin by using the same methods as described above. The polyclonal anti-obestatin antiserum (RY1682) recognized the COOH terminus (11–23) of amino acid sequences of obstatin.

The specificities of the antisera used in this study were characterized with peptide-specific ELISA.

ELISA for rat acyl ghrelin and des-acyl ghrelin. Goat anti-rabbit-IgG (Fc) (CAPPEL; MP Biomedical, Solon, OH) (diluted 1:200 with 0.1 M NaHCO3, pH 8.3) was added to wells of a microtiter plate (Maxisorp; NUNC, Roskilde, Denmark) (0.1 ml/well). The plate was kept for 18 h at room temperature, and the solution in each well was removed by decantation followed by treatment of the wells with 25% (v/v) ethanol for 30 min. The plates were then incubated for 3 h at room temperature. For decantation of the BlockAce solution, the plate was dried for 3 days, then sealed and stored at 4°C until use. Synthetic rat acyl ghrelin as the standard antigen and [Lys(biotin)24]-rat acyl ghrelin as the labeled antigen (0.5 ng/ml) were dissolved in standard diluents, respectively. Standard solution (50 μl), labeled antigen (50 μl), and rabbit anti-rat acyl ghrelin serum RY1601 (1:32,000 dilution) (50 μl) were added in duplicate to wells, and the wells were incubated for 18 h at 4°C. On the other hand, synthetic rat des-acyl ghrelin as the standard antigen, des-acyl-[Lys(biotin)24] rat ghrelin as the labeled antigen, and diluted anti-des-acyl ghrelin serum RY1595 (1:16,000 dilution) were used. Streptavidin-horseradish peroxidase solution (1:10,000 dilution, Calbiochem) (100 μl) was added to the wells. The wells were then incubated for 2 h at room temperature. o-Phenylenediamine (Sigma Chemical, St. Louis, MO) solution (1 mg/ml, 0.1 M citrate-PB containing 0.015% hydrogen peroxide) was added (100 μl/well). After 30 min, the absorbance of each well at 492 nm was measured using a microplate reader (Labsystems Multiskan MS, Dainippon Pharmaceutical).

Immunohistochemistry for acyl ghrelin and des-acyl ghrelin. Rats were anesthetized with an injection of pentobarbital sodium (100 mg/kg ip; Nembutal, Abbott Laboratories, North Chicago, IL), perfused for 10 min via the left ventricle with 0.01 M PBS to wash out the blood, and then perfused with a fixative containing 4% paraformaldehyde, 0.5% glutaraldehyde, and 0.2% picric acid in 0.1 M PB at 4°C for 10 min at the speed of 30 ml/min. The stomach was taken, immersed in a postfixative containing 4% paraformaldehyde and 0.2% picric acid in 0.1 M PB at 4°C, and washed for 4 days with several changes of 0.1 M PB containing 15% sucrose. The stomach was cut into 20-μm longitudinal sections in a cryostat and collected in 0.1 M PBS containing 0.3% Triton X-100 (PBST). The sections were incubated with the acyl ghrelin antibody (RY1601) or des-acyl ghrelin antibody (RY1595) diluted 1:5,000 in PBST for 2 days at 4°C. The sections were then processed for immunohistochemistry by the avidin-biotin-peroxidase complex (ABC) method and colorized with Nickel diaminobenzidine reactions as described previously (8). The sections were counterstained with 0.1% neutral red solution.

Immunoadsorption study. The specificity of the positive staining in the gastrointestinal tract was determined by preabsorption study. The acyl ghrelin antibody (RY1601) was substituted for by the acyl ghrelin antibody (diluted 1:5,000) preabsorbed with 10 μM synthetic rat acyl ghrelin or 10 μM synthetic rat des-acyl ghrelin. The des-acyl ghrelin antibody (RY1595) was substituted for by the des-acyl ghrelin antibody (diluted 1:5,000) preabsorbed with 10 μM synthetic rat des-acyl ghrelin or 10 μM synthetic rat acyl ghrelin. The antigen and antibody mixture was incubated for 48 h at 4°C before application to the sections. Sections were processed for immunohistochemical staining as described above.

Immunofluorescence overlap staining. Twenty-micrometer-thick sections were incubated with a mixture of the Texas red-labeled acyl ghrelin antibody (RY1601) diluted 1:1,000 in PBST and FITC-labeled des-acyl ghrelin antibody (RY1595) diluted 1:1,000 in PBST for 48 h at 4°C. After being washed for 30 min with PBST, the stained sections were mounted on glass slides, dried, coveslipped with Vectorshield (Vector Laboratories, Burlingame, CA), and then observed under a confocal laser scanning microscope (LSM510META, Carl Zeiss, Jena, Germany).

For overlap staining of des-acyl ghrelin and somatostatin, the frozen sections were incubated with a mixture of FITC-labeled des-acyl ghrelin antibody (RY1595) and purified somatostatin antibody (mouse monoclonal, Ref. 7) diluted 1:1,000 in PBST for 48 h at 4°C. After being washed, the sections were incubated with Cy3-labeled anti-mouse IgG (Chemicon, Temecula, CA) diluted 1:1,000 in PBST for 2 h at room temperature. The stained sections were mounted on glass slides and observed under a confocal laser scanning microscope.

For triple staining of acyl ghrelin, des-acyl ghrelin, and obstatin, the frozen sections were incubated with a mixture of the Texas red-labeled acyl ghrelin antibody (RY1601), FITC-labeled des-acyl ghrelin antibody (RY1595), and Alexa Fluor 647-labeled obstatin antibody (RY1682) diluted 1:1,000 in PBST for 48 h at 4°C. After being washed for 30 min with PBST, the stained sections were mounted on glass slides and observed under a confocal laser scanning microscope.

Quantitative analysis for distribution of acyl ghrelin- and des-acyl ghrelin-positive cells in the rat stomach. Twenty-micrometer-thick longitudinal sections of stomach from three rats were processed for immunofluorescence overlap staining of acyl ghrelin and des-acyl ghrelin. The density of acyl ghrelin-positive cells, des-acyl ghrelin-positive cells, and acyl ghrelin/des-acyl ghrelin double positive cells in either oxyntic mucosa or antral mucosa was measured. The density was calculated from the mean number of positive cells per unit area in ten randomly selected fields at ×40 objective under a confocal laser scanning microscope.

Ex vivo vascularly perfused rat stomach. Rats were anesthetized with an injection of pentobarbital sodium (60 mg/kg ip). Arterial perfusion was achieved through an aortic cannula with the tip lying adjacent to the celiac artery, and the effluent perfuse was collected through a portal vein cannula. All vasculature apart from that leading into the stomach was cut between double ligatures. The duodenum, jejunum, ileum, colon, pancreas, and spleen were removed. The vascular perfuse consisted of Krebs solution containing 3% dextan, 0.2% bovine serum albumin (RIA grade; Sigma Chemical), and 5 mM glucose, and the perfusate was saturated with 95% O2-5% CO2 gas to maintain a pH of 7.4. Luminal perfuses were citrate-PB at pH 2 and pH 4 to examine the effects of different pHs on the release of acylated
ghrelin and des-acyl ghrelin. Vascular and luminal perfusates and the preparation were kept at 37°C throughout the experiment by a thermostatically controlled heating apparatus. The flow rate for vascular perfusion was maintained at 3 ml/min. After a 25-min equilibration period for citrate-PB at pH 4 in luminal perfusates, vascular effluents were collected for 10 min. After a 5-min equilibration period for citrate-PB at pH 2 in luminal perfusates, vascular effluents were collected for 10 min. Vascular perfusates were mixed with aprotinin/EDTA and 1 mol/l of HCl.

The vascular perfusates were loaded onto an Oasis HLB cartridge (Waters, Milford, MA) preequilibrated with H2O. The cartridge was washed with 0.1% trifluoroacetic acid (TFA) and then eluted with 80% CH3CN in 0.1% TFA. The eluate was lyophilized and stored at −80°C. The lyophilized sample was dissolved in assay buffer and the acyl ghrelin and des-acyl ghrelin levels were measured by ELISA.

**Statistical analysis.** Results were expressed as means ± SE. Statistical analysis of the data shown in Fig. 5 was performed by use of single-factor ANOVA for repeated measures followed by Scheffé’s F-test.

**RESULTS**

Crossreactivity of the anti-acyl ghrelin antibody and des-acyl ghrelin antibody was examined by rat acyl ghrelin- and des-acyl ghrelin-specific ELISA, and the results are shown in Fig. 1. The acyl ghrelin antibody crossreacted 0% with rat des-acyl ghrelin (Fig. 1A), whereas the des-acyl ghrelin antibody crossreacted 2–3% with rat acyl ghrelin (Fig. 1B).

Immunohistochemical localization of acyl ghrelin and des-acyl ghrelin was examined in the rat stomach. Both acyl ghrelin- and des-acyl ghrelin-positive cells were found in the epithelium of both oxyntic mucosa and antral mucosa of the stomach (Fig. 2Aa and Ba). The specificity of the positive staining was examined by immunoabsorption study. The acyl ghrelin-positive reaction completely disappeared when the antibody was adsorbed with acyl ghrelin (Fig. 2Ab); however, the acyl ghrelin-positive reaction was not affected when the antibody was adsorbed with des-acyl ghrelin (Fig. 2Ac). The des-acyl ghrelin-positive reaction completely disappeared when the antibody was adsorbed with des-acyl ghrelin (Fig. 2Bc). Bars = 50 μm.

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**Fig. 1.** Crossreactivity of the anti-acyl ghrelin antibody (RY1601) and anti-des-acyl ghrelin antibody (RY1595) measured by ELISA. Anti-acyl ghrelin antibody (RY1601) crossreacts 0% with rat des-acyl ghrelin (A). Anti-des-acyl ghrelin antibody crossreacts 2–3% with rat acyl ghrelin (B).

**Fig. 2.** Immunohistochemistry for acyl ghrelin- and des-acyl ghrelin-positive cells in the oxyntic mucosa of rat stomach and immunoabsorption study for specificity of the antibody (ab). Acyl ghrelin-positive reaction (Aa) completely disappears when the antibody is adsorbed with acyl ghrelin (Ab); however, it is not affected when the antibody is adsorbed with des-acyl ghrelin (Ac). Des-acyl ghrelin-positive reaction (Ba) is completely adsorbed when the antibody is adsorbed with des-acyl ghrelin (Bb); however, it is not affected when the antibody is adsorbed with acyl ghrelin (Bc). Bars = 50 μm.
though the des-acyl ghrelin-positive reaction was not affected when the antibody was adsorbed with acyl ghrelin (Fig. 2Bc).

Colocalization of acyl ghrelin and des-acyl ghrelin in the rat stomach was examined by immunofluorescence double staining (Fig. 3). The results showed that acyl ghrelin-positive reactions were always observed in the closed-type round and oval cells, and acyl ghrelin-positive reactions and des-acyl ghrelin-positive reactions overlapped within the closed-typed cells (Fig. 3 arrows). Des-acyl ghrelin-positive reactions were found in open-type cells, which were spindle-shaped and extended cytoplasmic processes toward the gastric lumen (Fig. 3, arrowheads). In these open-type cells, des-acyl ghrelin was positive, but acyl ghrelin was negative (Fig. 3).

To identify des-acyl ghrelin-positive open-type cells, we performed overlap staining for des-acyl ghrelin and somatostatin. The results showed that des-acyl ghrelin-positive open-type cells were also positive for somatostatin (Fig. 4A, arrows). However, des-acyl ghrelin-positive closed-type cells were negative for somatostatin (Fig. 4A). We further performed the triple staining of acyl ghrelin, des-acyl ghrelin, and obestatin. The results showed that obestatin-positive reaction was always detected in the closed-type cells that contain acyl ghrelin and des-acyl ghrelin (Fig. 4B, arrows).

The density of acyl ghrelin-positive cells, des-acyl ghrelin-positive cells, and acyl ghrelin/des-acyl ghrelin double-positive cells in either oxyntic or antral mucosa was measured. The density of acyl ghrelin-positive cells in the oxyntic and antral mucosa was 28 ± 4/mm² and 58 ± 9/mm², respectively. The density of des-acyl ghrelin-positive cells in the oxyntic and antral mucosa was 33 ± 4/mm² and 70 ± 6/mm², respectively. The density of acyl ghrelin/des-acyl ghrelin double-positive cells in the oxyntic and antral mucosa was 28 ± 4/mm² and 55 ± 8/mm², respectively.

Fig. 3. Immunofluorescence double staining for acyl ghrelin- (red) and des-acyl ghrelin-positive (green) reaction in the antral mucosa of rat stomach. Acyl ghrelin-positive reaction and des-acyl ghrelin-positive reaction are colocalized in closed-type cells (arrows), whereas des-acyl ghrelin-positive reaction is localized in open-type cells (arrowheads). Bars = 10 μm.
The release of acyl ghrelin and des-acyl ghrelin in response to different intragastric pH levels was examined in the ex vivo perfused rat stomach. The gastric lumen was perfused with buffers having different pHs and contents of acyl ghrelin and des-acyl ghrelin in the vascular effluent were measured. The results showed that basal release of acyl ghrelin (14.5 ± 6.1 fmol/ml) at pH 4 was not changed from that at pH 2 (14.8 ± 5.9 fmol/ml) (Fig. 5A). However, the release of des-acyl ghrelin at pH 2 (34.4 ± 3.1 fmol/min) was significantly increased compared with that at pH 4 (25.0 ± 9.3 fmol/min) (Fig. 5B).

**DISCUSSION**

The present study demonstrated the localization of des-acyl ghrelin-immunoreactive cells in the rat stomach for the first time by using a des-acyl ghrelin-specific antibody that did not cross-react with an acyl ghrelin-specific antibody. The localization of acyl ghrelin-immunoreactive cells in the gastrointestinal tract has been widely investigated in various animals by using acyl ghrelin-specific antibodies (4, 16, 24, 25). In the rat stomach, it has been reported that acyl ghrelin-immunoreactive cells are densely distributed in the oxyntic gland, infrequent in the pyloric gland, and very rare in small intestine (4). Characteristically, these acyl ghrelin-positive cells are round, closed-type cells in the rat stomach (4, 24), but both closed-type and open-type cells (cytoplasm reaching the lumen) were found in the stomach of the rainbow trout (25). The present results showed that both acyl ghrelin-positive cells and des-acyl ghrelin-positive cells were located in the epithelium of both oxyntic mucosa and antral mucosa of the stomach in rats. We measured the density of acyl ghrelin- and des-acyl ghrelin-positive cells in oxyntic mucosa and antral mucosa. The results showed that the density of both ghrelin-positive as well as des-acyl ghrelin-positive cells in the antral mucosa was higher than that in the oxyntic mucosa. The value of distribution density in acyl ghrelin-positive cells in the oxyntic mucosa was consistent between present and previous (24) studies. However, the density of acyl ghrelin-positive cells in the antral mucosa was quite different between them. Acyl ghrelin-immunoreactive cells were adsorbed with a high concentration of acyl ghrelin but not with des-acyl ghrelin, and des-acyl ghrelin-immunoreactive cells were adsorbed with a high concentration of des-acyl ghrelin but not acyl ghrelin; therefore, the specificity of the positive staining of each peptide was confirmed. Most des-acyl ghrelin-positive reactions overlapped acyl ghrelin-positive reactions in closed-type cells; however, most characteristically des-acyl ghrelin-positive reactions were found in open-type cells, in which no acyl ghrelin reaction was found. The present results demonstrated that des-acyl ghrelin-positive open-type cells contain somatostatin and that acyl ghrelin-positive closed-type cells contain obestatin. Previous study has shown that acyl ghrelin and obestatin are colocalized in A-like cells in the rat stomach, which does not contain histamine or serotonin (29). Therefore the closed-type cells detected in the present study might be identified as A-like cells (29).

The characteristic features of open-type cells that contain des-acyl ghrelin and closed-type cells that contain acyl ghrelin indicate that they may respond differently to intraluminal factors. It is highly possible that open-type cells may react to luminal stimuli more than closed-type cells. Therefore, we investigated the effects of different intragastric pH levels on the release of acyl ghrelin and that of des-acyl ghrelin from the ex vivo perfused rat stomach. In a preliminary study, we measured the intragastric pH levels in the fasting and fed states of rats and found that intragastric pH in the fasting state was pH 4, whereas that in the fed state was pH 2 (9). The present results showed that the release of acyl ghrelin was not affected by different intragastric pH levels (A). The release of des-acyl ghrelin at pH 2 is significantly higher than that at pH 4 (B).

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**Fig. 4.** A: immunofluorescence double staining for des-acyl ghrelin-positive (green) and somatostatin-positive (red) reaction in the antral mucosa of rat stomach. Des-acyl ghrelin-positive closed-type cells are not positive for somatostatin; however, des-acyl ghrelin-positive open-type cells are positive for somatostatin (arrows). B: immunofluorescence triple staining for des-acyl ghrelin (green), acyl ghrelin (red), and obestatin (blue) in the antral mucosa of rat stomach. Three peptides are colocalized in the closed-type cells (arrows). Bars = 10 μm.

**Fig. 5.** Release of acyl ghrelin and des-acyl ghrelin from ex vivo perfused rat stomach. Concentrations of acyl ghrelin and des-acyl ghrelin are measured in the vascular effluent collected for 10 min. The release of acyl ghrelin is not affected by different intragastric pH levels (A). The release of des-acyl ghrelin at pH 2 is significantly higher than that at pH 4 (B).
fmol/ml and 13–41 fmol/min, respectively. Previous studies that measured the release of acyl ghrelin and des-acyl ghrelin in rats showed that the circulating plasma level of acyl ghrelin was 50–150 fmol/ml (14) and the levels of acyl ghrelin and des-acyl ghrelin in the gastric vein were 78.7 fmol/ml and 3,593 fmol/min (18), respectively. The discrepancy between the present and previous data, especially in the level of des-acyl ghrelin, seems to be due to the difference in the sample collection. In the present study, samples were collected from ex vivo vascular perfusion, whereas, in the previous study, the samples were collected directly from the gastric vein (18). The present results suggest that des-acyl ghrelin-containing cells may sense the intragastric pH via their cytoplasmic processes and release the peptide in accordance with the lower intragastric pH. Because, however, des-acyl ghrelin is also contained in closed-type cells in the stomach, the contribution of closed-type des-acyl ghrelin-containing cells to intragastric pH is not known at present. The fact that the release of des-acyl ghrelin is stimulated by lower intragastric pH seems reasonable because des-acyl ghrelin may act as a satiety signal (1, 2) in the fed state of animals.

In conclusion, in the present study, we developed antibodies that recognized acyl ghrelin and des-acyl ghrelin separately. By using these antibodies we demonstrated the localization of des-acyl ghrelin-positive cells in the rat stomach for the first time and compared it with that of acyl ghrelin-positive cells. The release of acyl ghrelin and des-acyl ghrelin from the ex vivo vascularly perfused rat stomach in response to different intragastric pH levels was measured by ELISA.

REFERENCES