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Synergistic action of gastrin and ghrelin on gastric acid secretion in rats

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ABSTRACT

Gastrin and ghrelin are secreted from G cells and X/A-like cells in the stomach, respectively, and respective hormones stimulate gastric acid secretion by acting through histamine and the vagus nerve. In this study, we examined the relationship between gastrin, ghrelin and gastric acid secretion in rats. Intravenous (iv) administration of 3 and 10 nmol of gastrin induced transient increases of ghrelin levels within 10 min in a dose-dependent manner. Double immunostaining for ghrelin and gastrin receptor revealed that a proportion of ghrelin cells possess gastrin receptors. Although (iv) administration of gastrin or ghrelin induced significant gastric acid secretion, simultaneous treatment with both hormones resulted in a synergistic, rather than additive, increase of gastric acid secretion. This synergistic increase was not observed in vagotomized rats.

These results suggest that gastrin may directly stimulate ghrelin release from the stomach, and that both hormones may increase gastric acid secretion synergistically.

Ghrelin, recently purified from rat and human stomachs as an endogenous ligand for the growth hormone (GH) secretagogue receptor (GHS-R), is a 28-amino-acid peptide with an *n*-octanoylation modification at the Ser3 residue [1]. This octanoylation is essential for stimulation of GH secretion from the pituitary gland [1]. Although cells showing immunostaining for ghrelin are distributed widely in the stomach, hypothalamus, pituitary gland, liver, kidney, pancreas and placenta, the main source of circulating ghrelin has been considered to be the gastrointestinal tract, especially in X/A-like cells of fundic glands [2–6]. Several studies on the physiological function of ghrelin have demonstrated that, in addition to stimulating GH secretion, ghrelin also stimulates food intake, body weight gain, stomach motility, insulin release, cell proliferation and gastric acid secretion [7–13].

It is well known that ghrelin secretion is stimulated by starvation, and that subsequently this increase of ghrelin induces food intake [7–9,14]. Certainly, central and peripheral treatment with ghrelin causes initiation of food intake within 5 min in rats [9,14]. Therefore, ghrelin has been considered an appetite-promoting hormone. In addition to regulation of ghrelin secretion by the autonomic nervous system and nutrients, some previous studies have demonstrated that ghrelin secretion is influenced by many gastrointestinal hormones, such as glucagon, insulin, cholecystokinin, glucagon-like peptide, gastrin and somatostatin [15–21]. However, the relationship between ghrelin secretion influenced by these gastrointestinal hormones and the physiological role of secreted ghrelin is still largely unknown. Circulating ghrelin levels exhibit a diurnal pattern, with bimodal peaks occurring before the dark and the light periods, respectively [14]. These two peaks are consistent with the minimum (emptiness) and maximum (satiety) volumes of the gastric content, respectively. This indicates that ghrelin secretion increases not only when the stomach is empty but also when it is full. Therefore, it has been assumed that the function of the ghrelin peak observed at stomach emptiness is to stimulate food intake, whereas that of the satiety peak is to stimulate gastric acid secretion [14]. If this hypothesis is correct, it is important to clarify what stimulates ghrelin secretion under satiety conditions. We have suspected that gastrin may be involved in this ghrelin increase at satiety, since gastrin is released at this time and stimulates gastric acid secretion, whereas somatostatin and cholecystokinin inhibit gastric acid secretion [22].

In this study using rats, therefore, we examined whether intravenous injection of gastrin affects ghrelin release from the stomach, or whether ghrelin and gastrin stimulate gastric acid secretion independently or cooperatively.

Materials and methods

Animals. Male Wistar rats weighing 400–500 g were used in all experiments. The rats were maintained under a 12:12-h light/dark

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cycle (lights on at 07.00 h) and a room temperature of 23 ± 1 °C with standard laboratory food and water provided *ad libitum*. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care.

Gastrin administration. Rats were anesthetized with an intraperitoneal (ip) injection of pentobarbital. To exclude the possibility that several treatments, such as implantation of a polyethylene tube into a stomach vein, or injection of saline and gastrin, might affect ghrelin secretion by stimulating the endings of sensory neurons in the stomach or vagus nerve, rats were firstly vagotomized at the subphrenic esophagus level using the method described previously [23] and were then maintained at a body temperature at 37 °C by a small-animal heat controller while monitoring the rectal temperature (Unique Medical Co., Ltd., Tokyo, Japan). Next, to analyze ghrelin secretion into the gastric vein, a heparin-treated PE10 tube (inner diameter 0.28 mm: Becton–Dickinson, NJ, USA) was inserted and fixed to the gastric vein [20]. Blood samples were collected at 10-min intervals. After the third blood sample collection, 0.2 ml of 3 or 10 nmol gastrin (Peptide Institute, Osaka, Japan), or saline as a control, was injected into the femoral vein. Thereafter, blood sampling was continued for 60 min.

Measurement of plasma ghrelin. Ghrelin concentration was measured by the method described previously [12]. Briefly, blood samples were collected into chilled polypropylene tubes containing a protease inhibitor, aprotinin (Sigma–Aldrich, St. Louis, USA), and EDTA-2Na, and immediately centrifuged at 14,000 rpm for 3 min. Plasma samples were acidified with a 10% volume of 1 N HCl and stored at −80 °C until assay. Acyl-ghrelin was measured using an active ghrelin ELISA kit (Mitsubishi Kagaku Iatron, Tokyo, Japan).

Double immunostaining for ghrelin and gastrin receptor. A trimmed part of the glandular stomach was washed using saline and placed in 0.34% formalin for 4 days at 4 °C, and then transferred to 0.1 M phosphate buffer containing 20% sucrose. Sections were cut at a thickness of 18 μm with a cryostat at a temperature of −20 °C. The sections were fixed with 40% paraformaldehyde for 20 min and blocked for 1 h in 5% normal donkey serum in PBST and then incubated overnight at 4 °C with rabbit antiserum against rat ghrelin together with goat antiserum against CCKB-R C18 (Santa Cruz Biotechnology, Santa Cruz, CA). This anti-rat ghrelin antibody (antiserum #G606) specifically recognizes ghrelin with *n*-octanoylated Ser-3 and does not recognize des-acyl-ghrelin. After washing, the sections were incubated with a second antibody solution of Alexa-488-labeled anti-rabbit IgG antibody and Alexa-555-labeled donkey anti-goat IgG antibody solution for 30 min. The samples were observed using a fluorescence microscope (Axioskop 2plus: Zeiss, MA, USA). Digital images were contrasted and color-adjusted using Adobe Photoshop 7.0 for Windows.

Measurement of gastric acid secretion. Intact and vagotomized rats were used in this study. Vagotomy was performed at the subphrenic esophagus level under pentobarbital anesthesia as mentioned above. Four days after the operation, the rats were used for the following experiment. After anesthesia with urethane, two gastric perfusion cannulae were implanted in the pyloric and cardiac parts of the stomach by insertion from the duodenum and esophagus, respectively. The body temperature was maintained at 37 °C until the end of sampling. Five milliliters of water warmed at 37 °C was perfused from the cardiac side to the pyloric part side with an infusion pump at a flow rate of 2.5 ml/min at 10-min intervals. Acidity of the perfusate was recorded by the method reported previously [24]. After acidity had been stabilized at between pH5 and pH7, 3 nmol of either gastrin or ghrelin, or 3 nmol of both gastrin and ghrelin was injected into the femoral vein. Injection of saline was used as a control. After treatment with the agents, the perfusate was collected at 10-min intervals for 60 min.

Data analysis. Data are expressed as means ± SEM for the groups. Comparisons between groups were performed using unpaired *t*-test or ANOVA followed by the Tukey–Kramer test for multiple comparisons. Changes in plasma ghrelin levels over time were analyzed using repeated measures ANOVA followed by Dunnett's test. Differences at $P < 0.05$ were considered significant.

Result and discussion

The effect of intravenous injection of gastrin on ghrelin secretion from the stomach in vagotomized rats is shown in Fig. 1C. The plasma concentration of ghrelin in the gastric vein at the start of the experimental period, at 0 min in Fig. 1C, was 44.7 ± 5 fmol/ml. Saline administration did not cause significant changes in plasma ghrelin levels after treatment. On the other hand, gastrin induced a significant dose-dependent increase in ghrelin levels. The peak level of ghrelin was observed 10 min after treatment with 3 and 10 nmol of gastrin, thereafter falling rapidly to below the control level (Fig. 1C). This decrease of the ghrelin level to below the baseline may be due to exhaustion of stored ghrelin after secretion in quantity. These results indicate that gastrin may play an important role in ghrelin secretion, and that the effect is probably limited to stimulation of secretion, and not synthesis.

Double-staining immunohistochemistry for ghrelin and gastrin receptor in the lamina propria mucosae of the lower gastric corpus was performed to examine the possible presence of gastrin receptors on ghrelin cells. Cells immunoreactive for ghrelin and gastrin

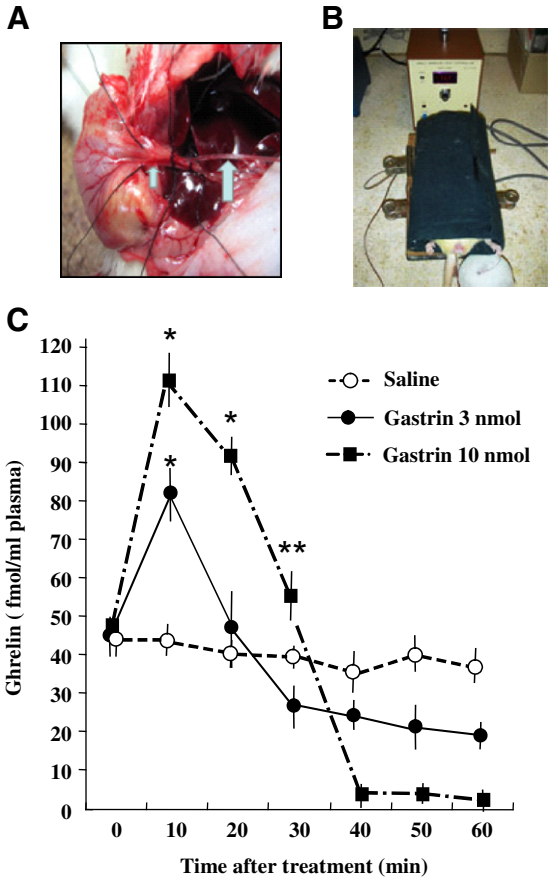

Fig. 1. Effect of peripheral injection of gastrin on plasma ghrelin levels. (A) Photograph showing cannulation of stomach vein (small arrow) by a PE10 tube (large arrow). (B) Body temperature was maintained constantly using a thermostat. (C) Ghrelin levels after injection of saline, and with 3 nmol and 10 nmol gastrin into the femoral vein. Each symbol and vertical bar represent means ± SEM ($n = 6$). Asterisks indicate significant differences ($^{*}P < 0.01$, $^{**}P < 0.05$ vs saline).