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Meal-Related Ghrelin Suppression Requires Postgastric Feedback

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Plasma ghrelin levels are rapidly suppressed by ingestion or gastric delivery of nutrients. Given that the majority of circulating ghrelin appears to be of gastric origin, we addressed the contribution of gastric distention or nutrient sensitivity to this response. Awake, unrestrained rats received intragastric infusions of glucose or water (1 ml/min for 12 min) with gastric emptying either proceeding normally or prevented by inflation of a pyloric cuff. When emptying was permitted, glucose infusion reduced ghrelin level by approximately 50%, and, in agreement with previous data, water infusions were without effect. Ghrelin level was not affected by either infusate when gastric emptying was prevented, thereby discounting a role for gastric distention in the meal-related ghrelin response. That glucose and water infusions were similarly ineffective when the pylorus was occluded shows, further, that gastric chemosensation is not a sufficient trigger for the ghrelin response. We conclude that the meal-related suppression of plasma ghrelin requires postgastric (pre- or postabsorptive) stimulation. (*Endocrinology* 144: 2765–2767, 2003)

GHRELIN, A RECENTLY DISCOVERED ligand for the GH secretagogue receptor, is an orexigenic hormone produced primarily by cells in the oxyntic gland of the stomach (1–3). The hypothesis that ghrelin plays a physiological role in the regulation of food intake is supported by the finding that plasma ghrelin levels rise shortly before meals (4) and are rapidly suppressed by food consumption in humans and rodents (3–5). The source of the consumption-related feedback that decreases ghrelin level has not been determined and provides the focus of the present study. Oral or gastric administration of glucose causes a decline in plasma ghrelin level that is of greater magnitude and longer duration than that seen after iv infusion of glucose (5), suggesting that stimulation of the gut is important for this response. Given that the majority of circulating ghrelin appears to be of gastric origin (6, 7), it is reasonable to hypothesize that the feedback relevant to feeding-induced ghrelin reduction arises from the stomach itself. Here we address the contribution of gastric mechanical and chemical sensation to the meal-related inhibition of circulating ghrelin.

Intragastric infusion of glucose reduces plasma ghrelin, whereas equivolemic infusion of saline or water has no effect (3, 5). Taken at face value, this finding appears to discount gastric distention as a mechanism for ghrelin reduction. Glucose infusion, however, results in significantly greater (and more prolonged) distention than an infusion of the same volume of saline because of greater, nutrient-based feedback inhibition of gastric emptying (8). This increased distention may contribute, at least in part, to the ghrelin response to the nutrient load.

Various gastric secretory and hormonal functions are sensitive to the chemical properties of food within the stomach (9, 10). Gastric ghrelin-producing cells, however, are primarily of the closed type, situated at the base of the mucosal layer and not in direct contact with the gastric lumen (2, 11, 12). The possibility that they are nevertheless directly responsive to the chemical properties of gastric chyme has not been evaluated. It is also possible that the cells are influenced indirectly, via neurocrine or paracrine signals arising from gastric nutrient sensation, in a manner that may contribute to the nutrient-related suppression of ghrelin level.

We evaluated the contribution of the stomach to prandial reduction in plasma ghrelin in the rat bearing a chronically implanted gastric fistula and an inflatable pyloric cuff. By allowing gastric emptying to proceed normally, or preventing it via pyloric cuff inflation as rats received equivolemic infusions of water or glucose, we addressed both gastric distention and chemosensation as possible triggers for the suppression of ghrelin level. When gastric emptying is prevented, water and glucose infusions cause equivalent gastric distention. If distention mediates the ghrelin response, therefore, plasma levels of the hormone should be reduced comparably in both of these conditions. Evidence for gastric chemosensory mediation of the ghrelin response would be provided if a plasma ghrelin reduction were achieved with glucose, but not water, when both stimuli were confined to the stomach. If ghrelin level were not affected by water or glucose infusions under restricted emptying conditions, attention would be focused on intestinal or postabsorptive mechanisms as necessary for the expression of the nutrient-related response.

Materials and Methods

Animals

Six naive male Sprague Dawley rats (Charles River, Wilmington, MA; weight, 300–400 g) were individually housed in hanging stainless steel cages on a 12-h light, 12-h dark cycle. Pelleted food (Purina 5001, St. Louis, MO) and water were available *ad libitum* unless otherwise noted. All experimental protocols used conform to institutional standards of animal care and the Guide for the Care and Use of Laboratory Animals (National Research Council 1996).

Surgery

Under ketamine (90 mg/kg) and xylazine (15 mg/kg im) anesthesia, rats were implanted with stainless steel gastric cannulas as described previously (13). Pyloric cuffs (40 mm long and 6 mm wide) were constructed of silicone sheeting (Novatech, Grasse, France), tubing (VWR, West Chester, PA), and adhesive (Dow Corning Corp., Midland, MI), according to the method of Young and Deutsch (14) and were implanted as follows. The pylorus was exposed and the cuff gently drawn around it, then sutured together at the ends to form a loose ring. The cuff was inflated with distilled water to a volume sufficient to blanch the underlying tissue. This volume was noted and used in the subsequent experimental conditions requiring cuff inflation. The silicone tubing attached to the cuff was then tunneled sc to the top of the skull, where it was press-fit to a 1-cm length of stainless steel tubing and anchored to the skull with four jewelers' screws and dental cement. After 1 wk of recovery, one or two intragastric glucose infusions (see below) were delivered to adapt the animals to this procedure and to verify that the inflation volume was sufficient to prevent gastric emptying.

Procedures

For the 24 h before each test, rats were food restricted to 75% of their preexperiment average daily intakes to ensure high preinfusion ghrelin levels. One hour before each session, the gastric fistula was opened and gastric contents were removed by gentle lavage with warm normal saline. All rats were tested under each of four experimental conditions, presented in counterbalanced order with 2 d between tests: 1) water infusion with pylorus open; 2) glucose infusion with pylorus open; 3) water infusion with pylorus closed; 4) glucose infusion with pylorus closed. For the closed-pylorus conditions, their pyloric cuffs were inflated with distilled water before infusions. Approximately 5 min before infusions, 5 μl of tail blood was taken for glucose analysis (Accu-Chek Complete blood glucose monitor, Roche Diagnostics, Basel, Switzerland), and an additional 200 μl was collected in EDTA tubes for ghrelin measurement. Gastric fistulas were then opened and fitted with tubes connected to an infusion pump (Harvard Apparatus, Holliston, MA). The pump was engaged to infuse 12 ml of either distilled water or 25% glucose at 1 ml/min (chosen to approximate the rate and duration of ingestion when rats lick glucose solution from a spout). Immediately after the infusion, a second blood glucose measure was made. Twenty minutes later (32 min from the onset of infusion), a third blood glucose sample was taken and a second blood sample was collected for ghrelin assay. After this blood sample, gastric contents were aspirated and volumes recorded. In the two closed-pylorus conditions, cuffs remained inflated until this time. Aspirate volumes and blood glucose measurements were used to verify proper function of the pyloric cuffs.

Ghrelin assay

Total immunoreactive ghrelin levels were measured in EDTA-containing plasma with an RIA that uses a polyclonal antibody raised against acylated, human ghrelin, and I^{131}-labeled ghrelin as the tracer (Phoenix Pharmaceuticals, Inc., Belmont, CA). This assay detects both acylated and des-acyl ghrelin. Although only acylated ghrelin is bioactive (1), levels of total ghrelin are a good surrogate for those of acylated ghrelin because the ratio of the two remains constant under a wide variety of physiological manipulations (15, 16).

Statistical analysis

Change in ghrelin level was assessed by three-way ANOVA, with pyloric cuff condition, infusate (water or glucose), and time as factors. Differences in volume of gastric aspirate were evaluated with two-way ANOVA, with pyloric cuff condition and infusate as factors. *Post hoc* comparisons were made with Tukey's honestly significant difference method.

Results

As shown in Fig. 1, intragastric infusion of glucose in the open-pylorus condition significantly suppressed plasma ghrelin level ($P < 0.05$). This was the only condition in which circulating ghrelin was affected by the intragastric infusion. An equivalent nutrient infusion had no effect when the pylorus was closed, and infusion of water did not affect ghrelin in either the open- or closed-pylorus conditions. The reduction in ghrelin level with glucose infusion when the pylorus was open accounts for the significant two- and three-way interactions obtained with the overall ANOVA (*e.g.* three-way interaction: $F(1,5) = 21.10$, $P < 0.01$).

The volume of aspirated stomach contents (see Fig. 2) differed as a function of cuff inflation and infusate (two-way interaction: $F(1,4) = 19.86$, $P < 0.05$). A comparison between the volume of stomach contents retrieved under open-pylorus conditions for water and glucose infusions confirms the significant difference in gastric distention for those two stimuli ($P < 0.01$). The high volume of aspirate obtained after infusions under closed-cuff conditions ($P < 0.05$), in addition to stable blood glucose levels over the course of those test

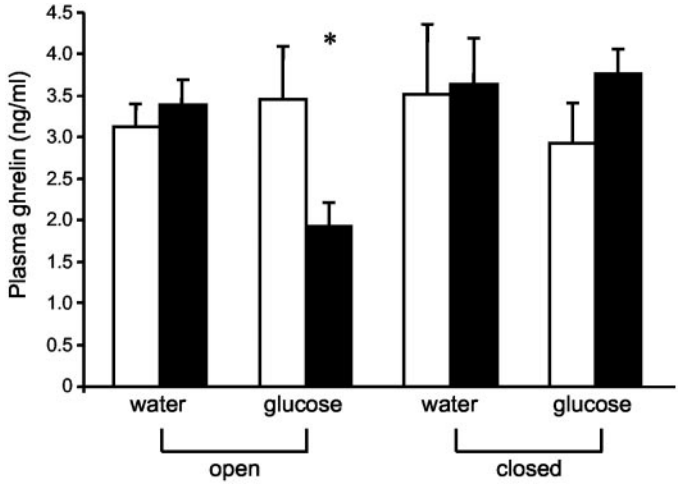

FIG. 1. Plasma ghrelin (mean ± SEM) before (*white bars*) and 32 min after (*black bars*) the onset of intragastric infusions of water or glucose, under open- and closed-pylorus conditions. *, $P < 0.05$.

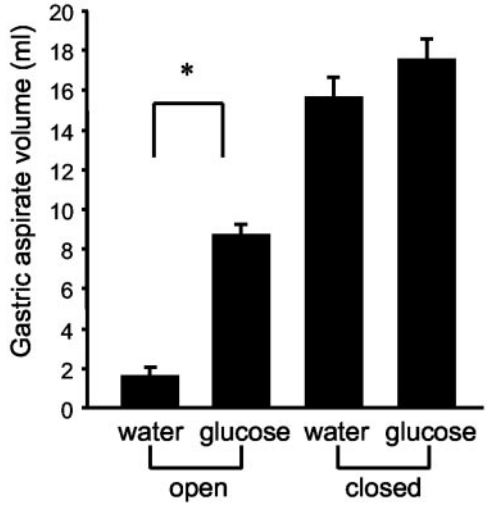

FIG. 2. Volume (mean ± SEM) of gastric contents aspirated 32 min after the onset of intragastric infusions of water or glucose under open- and closed-pylorus conditions. *, $P < 0.05$.

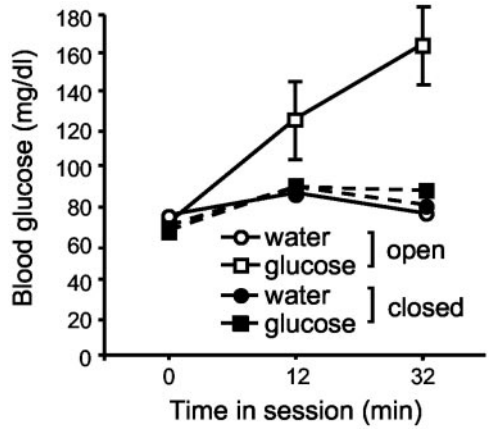

FIG. 3. Mean blood glucose before, 12 min after, and 32 min after intragastric infusion onset. (SE was too small to represent for most sample points, with the exception of those taken after glucose infusion in the open-pylorus condition.)

sessions (see Fig. 3), confirm that cuff inflation completely occluded the pylorus.

Discussion

We have demonstrated with this simple pyloric occlusion experiment that gastric sensation alone is not sufficient for the meal-related regulation of circulating ghrelin. This negative judgment applies to distensive, chemical, and osmotic properties of the infusates, because all three parameters varied substantially across the present testing conditions. There was only one condition of the four tested in which ghrelin was suppressed; levels fell by approximately 50% when glucose was infused and the stomach was allowed to empty normally. This effect, and the lack of effect when water was allowed to empty normally, replicate previous findings (3, 5). Importantly, neither glucose nor water infusions affected ghrelin level when gastric emptying was prevented. Gastric distention was pronounced under these conditions, given the appreciable volume delivered, the restraint of the portion that would normally empty during infusion (13), and the addition of gastric secretions (see Fig. 2). A role for gastric distention in the meal-related ghrelin response is therefore discounted by the lack of ghrelin response under the closed-pylorus conditions. The fact that glucose and water infusions were similarly ineffective when emptying was restrained shows, further, that gastric chemosensation is not sufficient for the ghrelin response under the present conditions. Our data do not rule out the possibility that gastric sensation contributes to the reduction in ghrelin level provided that postgastric sites are concurrently stimulated. We can conclude firmly, however, that the ghrelin response to glucose delivery requires feedback arising from postgastric (pre- and/or postabsorptive) sources.

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