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Xenin delays gastric emptying rate and activates the brainstem in mice

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ABSTRACT

Xenin, a 25-amino acid gastrointestinal peptide, inhibits feeding by acting through the central nervous system. Gastrointestinal hormones reduce food intake partly by activating the brainstem and inhibiting gastric emptying. Therefore, we hypothesized that xenin delays gastric emptying through the activation of the brainstem cells. To address this hypothesis, we examined the effect of intraperitoneal (i.p.) injection of xenin on gastric emptying rate and brainstem Fos expression in mice. Gastric emptying rate was reduced by about 93% in xenin-treated mice compared to saline-treated control mice. The i.p. xenin injection significantly increased Fos-immunoreactive cells in the nucleus of the solitary tract (NTS) of the brainstem, but not area postrema (AP) and dorsal motor nucleus of the vagus (DMV). These findings support the hypothesis that xenin-induced anorexia is at least partly due to delayed gastric emptying and the activation of the NTS cells.

Xenin is a 25-amino acid peptide that was initially identified in human gastric mucosa and subsequently found in other tissues and in other species [13,14]. Xenin is produced by a subpopulation of chromogranin A-positive endocrine cells in the duodenal and jejunal mucosa [2,14]. Similar to other anorectic gastrointestinal peptides, levels of circulating xenin increase after a meal, suggesting that xenin also regulates food intake by acting as a satiety factor [13]. Consistent with this hypothesis, it has been demonstrated that intracerebroventricular (i.c.v.) or intrahypothalamic administration of xenin reduces food intake [1,8,9,21,27]. Intraperitoneal (i.p.) injection of xenin also reduces food intake and increases Fos expression in a number of hypothalamic regions that are involved in the regulation of energy homeostasis [8,21]. In addition, xenin reduces food intake in animal models of obesity [21]. More recently, it was also reported that i.p. injection of xenin enhances glucose-dependent insulinotropic polypeptide (GIP)-mediated insulin secretion and improves hyperglycemia in mouse models with impaired insulinotropic action of GIP [38]. Xenin exerts these effects through the activation of non-ganglionic cholinergic neurons that innervate the pancreatic islet [38]. Taken together, these findings suggest that peripherally produced xenin reduces food intake at least partly by acting through the central nervous system (CNS) including the hypothalamus, and that enhancement of xenin action is a potential strategy to ameliorate obesity and type 2 diabetes. However, the mechanism by which xenin regulates food intake is not well understood.

Gastric emptying rate affects food intake and the anorectic effects of a number of gastrointestinal peptides such as cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) are associated with delayed gastric emptying [25,26,28]. Conversely, feeding-stimulatory effect of ghrelin is associated with accelerated gastric emptying rate [3]. Xenin injected i.c.v. at low doses increases gastrointestinal transit time in chicks, suggesting that the delayed gastric emptying contributes to the anorectic effect of centrally administered xenin [1,8,9,21,27]. However, it is unknown whether the same is true in mice when xenin is administered peripherally.

The dorsal vagal complex (DVC) of the brainstem includes area postrema (AP), nucleus of the solitary tract (NTS), and dorsal motor nucleus of the vagus (DMV), and participates in the mediation of gastrointestinal peptides-induced satiation [26,33]. Gastrointestinal signals including gastric distension and gastrointestinal hormones activate the DVC as indicated by the induction of Fos expression [26,33]. These findings led us to hypothesize that peripherally administered xenin reduces food intake partly by slowing gastric emptying through the activation of the cells in the DVC of the brainstem. In the present study, we addressed this hypothesis by examining the effect of intraperitoneal (i.p.) injection of xenin at a dose which can cause feeding suppression on gastric emptying rate and brainstem Fos expression as a marker for cell activation.

Male C57BL/6 mice were obtained from Charles River Laboratories (Montreal, QC) or from our animal facility. Mice were individually housed with free access to food and water under 12 h-light and 12 h-dark cycle (lights on at 06:00) throughout the experiment except during fasting. The University of Mani-

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Table 1
Estimation of gastric distention before i.p. injection of saline, xenin, or urocortin.

	Saline (n = 6)	Xenin (n = 6)	Urocortin (n = 4)	P^*
1-h food intake (g)	0.85 ± 0.06	0.72 ± 0.08	0.80 ± 0.04	0.39
Stomach weight (g)	0.163 ± 0.005	0.148 ± 0.006	0.155 ± 0.006	0.24
1-h food intake (g)/stomach weight (g)	5.20 ± 0.31	4.84 ± 0.52	5.22 ± 0.45	0.89

Values are means ± SEM (n = 4–6/group).
* P values by one-way ANOVA.

toba Protocol Management and Review Committee approved all procedures.

In the first study, we examine the effect of xenin on gastric emptying rate. The rate of gastric emptying was measured according to the method described previously [29]. Briefly, mice were fasted for 16 h and re-fed with pre-weighed food pellets for 1 h. After measuring 1-h food intake, mice were injected i.p. with saline, xenin (50 μg/g b.w., American Peptide Co., Sunnyvale, CA), or urocortin (3 nmol/mouse, Phoenix Pharmaceuticals, Belmont, CA). We chose 50 μg/g b.w. dose of xenin, because we found that the i.p. injection of xenin at this specific dose consistently reduced food intake in both *ad libitum* fed and 16-h fasted mice [8,21]. Urocortin was injected as a positive control, because this dose of urocortin is known to delay gastric emptying in mice [4]. Mice did not have access to food after the injection and were sacrificed by exposing to carbon dioxide 2 h after injection. The stomach was quickly exposed by laparotomy, ligated at both the pylorus and cardia, and removed. The weight of the stomach and the wet content of the stomach were immediately weighed. The rate of gastric emptying (%) was calculated by the following formula: Gastric emptying (%) = {1 − (wet weight of food recovered from the stomach/wet weight of food intake)} × 100. The wet weight of food intake was calculated by the following formula: Wet weight of food intake = $A \times (B/C)$, A = dry weight of food intake, B = average wet weight of gastric content after 1-h feeding, C = average dry weight of food intake after 1-h feeding. B and C were determined in control mice by measuring both wet and dry weights of gastric contents which were collected 1 h after re-feeding. To estimate gastric distension before i.p. injection of drugs, food intake (g) during the 1-h feeding period was normalized to stomach weight (g).

In the second study, we examined the effect of xenin on the activity of the brainstem cells using Fos-immunoreactivity as a marker for cell activation. Because both feeding and prolonged fasting increase the levels of Fos protein in the brain areas which are involved in the regulation of food intake, we fasted mice for 6 h to minimize the possible effect of spontaneous feeding and prolonged fasting on Fos expression [6,35]. After 6-h fasting, mice were injected i.p. with saline or xenin (50 μg/g b.w.) at 1400 h and perfused with 4% paraformaldehyde 2 h later under avertin (5 mg/g b.w., i.p.) anesthesia. Brains were removed and post-fixed in 4% paraformaldehyde solution at room temperature and coronal sections (30 μm) were cut on a cryostat.

For immunohistochemical visualization of Fos-immunoreactive cells, tissue sections were washed in PBS followed by overnight incubation in a polyclonal rabbit antibody specific for c-Fos (1:20,000, Ab-5 Calbiochem, La Jolla, CA) in 0.3% Triton X-100 in PBS. Sections were washed in PBS followed by 1-h incubation in biotinylated goat anti-rabbit IgG antibody (1:200, Vector Laboratories, Burlingame, CA) in 0.3% Triton X-100 in PBS. After rinsing with PBS, sections were incubated in a solution of avidin and biotinylated peroxidase (Vector Laboratories). After washing in PBS, sections were developed for 5 min in a solution of 0.1% 3,3′-diaminobenzidine tetrahydrochloride (DAB) in 0.1 M Tris, pH 7.4, with 0.0025% H_2O_2. After rinsing in PBS, sections were mounted on slides followed by drying overnight and dehydration and coverslipping with VectaMount Permanent Mounting Medium (Vector Laboratories). All incubations were performed at room temperature.

Two sections at different anterior–posterior levels of the brainstem (approximately 7.3 mm and 7.5 mm posterior from the bregma) from each animal were processed for immunohistochemistry, according to the mouse brain atlas [30]. Photomicrographs were produced by capturing images using a digital camera under a 4× objective. We counted the number of Fos-immunoreactive cells in the AP, NTS, and DMV in the captured images in a blind fashion. These brainstem areas were identified according to the mouse brain atlas [30]. For each area, the sum of the number of Fos-immunoreactive cells on both sides of the two sections were calculated in each animal and used for the statistical analysis.

In the gastric emptying study, data were analyzed by a one-way ANOVA followed by Tukey–Kramer post hoc test. Immunohistochemistry data were analyzed by Student's t-test. Data represent means ± SEM. A P-value of less than 0.05 was considered significant.

To test the hypothesis that xenin reduces gastric emptying rate, we compared 2-h gastric emptying rate between xenin-injected mice and saline-injected control mice. Mice ate similar amounts of food during the 1-h re-feeding period prior to i.p. injection in all 3 groups (Table 1). Stomach weight was not distinguishable between the groups (Table 1). The i.p. injection of xenin significantly reduced the rate of gastric emptying by about 93% compared to the saline-treated group (Fig. 1). Urocortin significantly delayed gastric emptying compared to saline treatment. There was no statistical difference in gastric emptying rate between xenin-treated mice and urocortin-treated mice (Fig. 1).

It has been well demonstrated that ingestion of food affects gastrointestinal motility and the rate of gastric emptying, in turn, affects food intake [10]. The rate of gastric emptying is accelerated in animal models of obesity, indicating that rapid gastric emptying contributes to hyperphagia and increased body weight gain [4,5]. The anorectic effects of a number of gastrointestinal peptides are

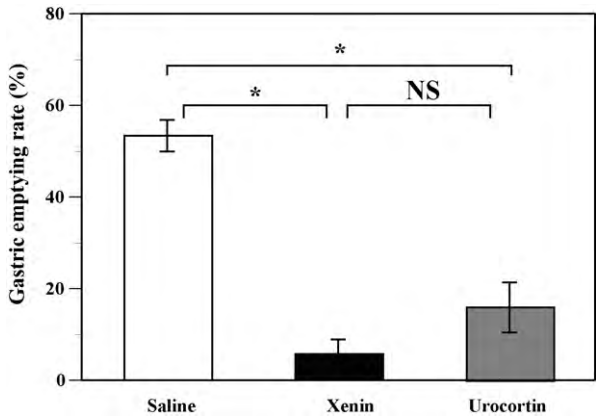

Fig. 1. Effect of i.p. administration of xenin on gastric emptying in mice. Mice were fasted overnight, refed for 1 h, and injected i.p. with saline, xenin (50 μg/g b.w.), or urocortin (3 nmol/mouse). Gastric emptying rates were measured 2 h after injection. Data are means ± SEM (n = 4–6/group). *P < 0.05 by Tukey–Kramer test. NS: not significantly different.