Xenin, a Gastrointestinal Peptide, Regulates Feeding Independent of the Melanocortin Signaling Pathway

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OBJECTIVE—Xenin, a 25–amino acid peptide, was initially isolated from human gastric mucosa. Plasma levels of xenin rise after a meal in humans, and administration of xenin inhibits feeding in rats and chicks. However, little is known about the mechanism by which xenin regulates food intake. Signaling pathways including leptin and melanocortins play a pivotal role in the regulation of energy balance. Therefore, we addressed the hypothesis that xenin functions as a satiety factor by acting through the melanocortin system or by interacting with leptin.

RESEARCH DESIGN AND METHODS—The effect of intracerebroventricular and intraperitoneal administration of xenin on food intake was examined in wild-type, agouti, and ob/ob mice. The effect of intracerebroventricular injection of SHU9119, a melanocortin receptor antagonist, on xenin-induced anorexia was also examined in wild-type mice. To determine whether the hypothalamus mediates the anorectic effect of xenin, we examined the effect of intraperitoneal xenin on hypothalamic Fos expression.

RESULTS—Both intracerebroventricular and intraperitoneal administration of xenin inhibited fasting-induced hyperphagia in wild-type mice in a dose-dependent manner. The intraperitoneal injection of xenin also reduced nocturnal intake in ad libitum–fed wild-type mice. The intraperitoneal injection of xenin increased Fos immunoreactivity in hypothalamic nuclei, including the paraventricular nucleus and the arcuate nucleus. Xenin reduced food intake in agouti and ob/ob mice. SHU9119 did not block xenin-induced anorexia.

CONCLUSIONS—Our data suggest that xenin reduces food intake partly by acting through the hypothalamus but via signaling pathways that are independent of those used by leptin or melanocortins. Diabetes 58:87–94, 2009

Obesity is associated with an increased risk of various disorders, including diabetes, dyslipidemia, cardiovascular diseases, and some forms of cancer. Obesity is now epidemic and recognized as a global health problem. Several peptides produced in the gastrointestinal tract have been shown to be involved in the regulation of energy homeostasis by acting through the central nervous system (1).

Xenin is a 25–amino acid peptide that was initially isolated from human gastric mucosa (2). Xenin is produced in the gastrointestinal tract have been shown to be involved in the regulation of energy homeostasis by acting through the central nervous system (1). Xenin is a 25–amino acid peptide that was initially isolated from human gastric mucosa (2). Xenin is produced in the gastrointestinal tract and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0 for details.

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μg) or intraperitoneally (0.5, 5, 15, or 50 μg/g body wt) at 100 h. To compare the feeding-suppressing effect between xenin and neurotensin, equimolar amounts (16.5 nmol) of xenin (50 μg/g body wt) or neurotensin (25 μg/g body wt; Sigma-Aldrich, St. Louis, MO) were injected intraperitoneally after an overnight fast. Control mice received either intracerebroventricular injection of aCSF or intraperitoneal saline injection. Preweighed food was provided to mice immediately after the injection. Cumulative food intake was measured at time points indicated in each figure up to 24 h after injection.

To determine whether xenin can reduce normal food intake, the effect of xenin on food intake was examined in ad libitum-fed wild-type mice. Mice were injected intraperitoneally with xenin (50 μg/g body wt) or saline just before lights out, and cumulative food intake was measured 1, 2, 4, 6, 8, 12, 18, and 24 h after injection.

To determine whether the central melanocortin system mediates the anorectic effect of xenin, the effect of SHU9119, a melanocortin receptor 3 and 4 (MC3-R/MC4-R) antagonist, on xenin-induced anorexia was examined. Wild-type mice were fasted overnight and injected intracerebroventricularly with SHU9119 (0.5 mmol; Bachem, King of Prussia, PA) or aCSF immediately before the intracerebroventricular injection of xenin (5 μg) or aCSF at 1000 h. The anorectic effect of intraperitoneal xenin (50 μg/g body wt) was also examined in wild-type (a/a) and agouti (A/a) mice after overnight fasting. Cumulative food intake was measured hourly up to 4 h after injection. Furthermore, to determine whether xenin alters mRNA levels of proopiomelanocortin (POMC) and agouti-related protein (AGRP) in the hypothalamus, wild-type mice received daily intracerebroventricular xenin (5 μg) or aCSF injection (two bottles of water for 30 min after 24 h after the last injection by exposure to carbon dioxide. The brain was quickly removed, and the hypothalamus was dissected, frozen on dry ice, and stored at −80°C for RNA analysis.

Conditioned taste aversion. Mice were accustomed to having access to water from two water bottles for 7 h (0000–1630 h) per day for 2 weeks. Daily food intake and body weight were stable after the 4th day of training. On the day of conditioning, mice were given two bottles of a novel 0.15% saccharin solution for the 30-min period (0930–1000 h) instead of water. Mice were injected intraperitoneally with xenin (50 μg/g body wt) or saline at the end of the 30-min period. LiCl (0.3 mmol/l, 2% body wt i.p.) was used as a positive control. Mice were then given two bottles of water for the remaining 6.5 h (1000–1630 h). On the next day, mice were given a choice of two bottles containing either 0.15% saccharin solution or water for 30 min (0000–1000 h). Consumption of saccharin solution and water was measured. Total fluid intake was the sum of the water and saccharin solution.

c-fos expression study. Mice were fasted overnight and injected intraperitoneally with xenin (50 μg/g body wt) or saline at 1000 h. Mice were not fed after injection and killed 30 min later by exposure to carbon dioxide. The brain was quickly removed, and the hypothalamus was dissected, frozen on dry ice, and stored at −80°C for RNA analysis.

RNA analysis. Total RNA was extracted in TRIzol reagent (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized from 5 μg of total RNA using SuperScriptII RNaseH reverse transcriptase and random primer (Invitrogen) and diluted 1:20–1:150. Hypthalamic gene expression levels were measured by real-time PCR as described previously (18). All primers (Supplemental Table 2) were designed using Primer Express software (ver. 3.0; Applied Biosystems, Foster City, CA). Data were analyzed by the ΔΔCt method using an ABI 7500 Fast System SDS software package (ver. 1.3.1; Applied Biosystems), and mRNA levels were normalized to cyclophilin mRNA levels. Data are expressed as means (% of the control group) ± SE. All reactions were performed in triplicate, and the coefficient of variation was <2% for each triplicate.

Immunohistochemistry. Mice were adapted to the injection procedure by intraperitoneal saline injection every 24 h for 8 days. On the last day of the adaptation, mice were fasted for 6 h and injected intraperitoneally with saline or xenin (50 μg/g body wt) at 1400 h. Mice were deeply anesthetized with an intraperitoneal injection of avertin (5 mg/g body wt) and perfused with 1 ml/30 g body weight followed by a fixative (4% paraformaldehyde in 0.1 mol/l phosphate buffer) 2 h after injection. Brains were removed and incu- bated in fixative for 5 h at room temperature and stored in 10% sucrose at 4°C at least overnight. Coronal sections (30 μm) were cut on a cryostat and stored in cryoprotectant (30% sucrose, 1% polyvinylpyrrolidone, and 30% ethylene glycol in 0.1 mol/l phosphate buffer) at −20°C until tissue sections were processed for immunohistochemistry. Immunohistochemical visualization of Fos was performed as described previously (19). Immunohistochemistry was performed on 20-μm-thick tissue sections throughout the anterior-posterior length of the hypothalamus covering the paraventricular nucleus (PVN), the ventromedial nucleus (VMH), the arcuate nucleus (ARC), the lateral hypothalamic area (LHA), and the dorsomedial nucleus (DMH). We counted the number of Fos-immunoreactive cells on both sides of the brain. The sum of the number of Fos-immunoreactive cells on both sides of the brain. The sum of the number
inhibitory effect of xenin on cumulative food intake remained significant up to 24 h after injection (Fig. 4).

**Effect of intraperitoneal administration of xenin on hypothalamic activation.** The intraperitoneal injection of xenin (50 µg/g body wt) significantly increased hypothalamic c-fos mRNA by ~170% compared with saline injection (Fig. 5A). In contrast, c-fos mRNA in the cortex was not different between the two groups (Fig. 5A). By immunohistochemical examination, the intraperitoneal injection of xenin significantly increased the number of Fos-immunoreactive cells in the PVH, ARC, VMH, and DMH by ~300–400% compared with control saline injection (Fig. 5B–F). A small number of Fos-immunoreactive cells were present in the LHA, and the number of Fos-immunoreactive cells in the LHA was not different between the xenin-treated group (8 ± 1 cells) and the saline-treated group (11 ± 3 cells, P = 0.24, Student’s t test).

**Effect of intraperitoneal administration of xenin on food intake in leptin-deficient mice.** To determine whether xenin can reduce food intake in hyperphagic obese animals independent of the action of leptin, we examined the effect of xenin (15 µg/g body wt, i.p.) on food intake in leptin-deficient ob/ob mice. Xenin significantly inhibited the fasting-induced hyperphagic response for the first 4 h after injection compared with saline injection (Fig. 6). The effect of xenin on cumulative food intake was no longer significant at the 6-h time point.

**Effect of melanocortin blockade on xenin-induced anorexia.** To address the hypothesis that the inhibitory effect of xenin on food intake is mediated through melanocortin signaling, we assessed xenin-induced anorexia in agouti mice. Some of the metabolic abnormalities in agouti mice are evident before the animals become overtly obese (20). To determine whether the effect of xenin on food intake is independent of obesity, we examined the effect of xenin on food intake in young (7 weeks old) preobese agouti mice. There was no significant difference in body weight between wild-type and agouti mice at this age (wild-type: 21.3 ± 0.3 g [n = 12]; agouti: 21.5 ± 0.3 [n = 12], P = 0.61, Student’s t test). Xenin was effective in reducing food intake in both young wild-type and agouti mice (data not shown). To determine whether the anorectic effect of xenin is attenuated after developing obesity, we examined the effect of xenin on food intake in obese agouti mice. At 10 weeks of age, agouti mice were signifi-
icantly heavier by ~25% compared with wild-type mice (agouti: 27.8 ± 0.5 g [n = 20], wild-type: 22.1 ± 0.5 [n = 16]; P < 0.0001, Student’s t test). Xenin (50 μg/g body wt i.p.) significantly reduced 2-h food intake compared with saline injection in both wild-type and agouti mice (Fig. 7, upper panel). Similar results were observed for 1-, 3-, and 4-h cumulative food intake (data not shown).

To further determine whether the feeding-suppressing effect of xenin is mediated through the central melanocortin system, SHU9119 (0.5 nmol) was injected intracerebroventricularly immediately before intracerebroventricular injection of xenin (5 μg) in overnight-fasted wild-type mice. The intracerebroventricular administration of xenin significantly reduced 2-h food intake compared with aCSF administration (Fig. 7, lower panel). Injection of SHU9119 alone did not alter food intake. Xenin-induced anorexia was not attenuated by SHU9119 (Fig. 7, lower panel). Similar results were observed for 1-, 3-, and 4-h cumulative food intake (data not shown). The intracerebroventricular injection of the same dose (0.5 nmol) of SHU9119 blocked
the anorectic effect of intracerebroventricular MTII (melanotan II), a MC3-R/MC4-R agonist (Supplemental Fig. 1).

To examine whether xenin alters expression levels of genes associated with the melanocortin system, hypothalamic POMC and AGRP mRNA levels were measured in xenin-treated mice. A daily intracerebroventricular injection of xenin (5 μg/injection for 11 days) did not cause significant changes in hypothalamic POMC and AGRP mRNA levels compared with control daily intracerebroventricular aCSF treatment (Fig. 8). In a separate study, the intracerebroventricular leptin treatment (1 μg/injection) significantly increased hypothalamic POMC mRNA levels, with a trend of reduced AGRP mRNA levels (Fig. 8).

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DISCUSSION

Xenin, a gastrointestinal peptide, was identified almost 15 years ago (2). Its anorectic effect in fasted rats was reported >10 years ago, but little research has been conducted to investigate the role of xenin in the regulation of energy homeostasis since then (14). Recently, it was demonstrated that xenin also reduced food intake in chicks, suggesting that this function is conserved across species (15). However, thus far there have been no studies reporting the effect of xenin on food intake in mice. To generalize the anorectic action of xenin across species, we examined the effect of xenin on food intake in mice in the current study. We have now confirmed that both intracerebroventricular and intraperitoneal administration of xenin reduced food intake in mice. We have also demonstrated that the intraperitoneal injection of xenin increases Fos immunoreactivity in specific hypothalamic nuclei, suggesting that the anorectic effect of xenin is mediated at least partly through the activation of the hypothalamus. However, our study demonstrated that xenin-induced anorexia in obese mouse models is independent of both leptin and melanocortin actions.

Plasma levels of xenin rise after a meal in humans, and intracerebroventricular administration of xenin reduces food intake (Fig. 1) (2,13,14). Although xenin is detected in the hypothalamus, the level of xenin in the hypothalamus is considerably lower than that in the gastrointestinal tract (5). These data suggest that gut-derived xenin may function as a satiety factor by acting through the central nervous system. We therefore investigated the effects of peripheral administration of xenin on feeding. The intraperitoneal administration of xenin dose-dependently reduced food intake in mice, consistent with the previous observation in chicks (15). The kinetics were similar to those seen with intracerebroventricular administration, with potent inhibition of feeding for the first 2 h. Thus, the robust anorectic effect was observed during the 1st and 2nd hour after intraperitoneal injection. This is consistent with a short half-life of exogenously administered xenin (21). It is often observed that the short-lasting anorectic effect is reversed by a rebound hyperphagia. Interestingly, cumulative food intake after xenin injection remained significantly lower than that of control mice up to 24 h.
after injection (Figs. 1, upper panel, and 4). These data strongly suggest that xenin has an ability to maintain lower energy intake even after the initial robust anorectic effect has disappeared. However, the intraperitoneal injection of xenin did not reduce 24-h cumulative food intake after overnight fasting (Fig. 2A), suggesting the possibility that xenin delays the initiation of feeding behavior after fasting.

Many appetite-suppressing substances, including gut-derived peptides, inhibit feeding partly by causing nausea and taste aversion (1). The intraperitoneal injection of xenin at a dose that produces the anorectic effect did not cause a significant taste aversion, whereas intraperitoneal LiCl caused severe taste aversion. These data suggest that xenin-induced anorexia is not attributable to an aversive response to xenin.

How does peripherally injected xenin reduce food intake? The effects of gastrointestinal hormones on metabolism are mediated through the central nervous system, including the hypothalamus (1). The intracerebroventricular injection of xenin was effective in reducing food intake, and hypothalamic c-fos mRNA was significantly increased after intraperitoneal injection of xenin in the current study. In contrast, xenin did not cause significant changes in c-fos mRNA levels in the cortex. Furthermore, the intraperitoneal injection of xenin increased the number of Fos-immunoreactive cells in the PVN, ARC, VMH, and DMH but not in the LHA. These data support our hypothesis that xenin inhibits feeding at least partly through the activation of specific cells in these hypothalamic regions. It should be noted that the brainstem and the vagus nerve also play a role in mediating the satiety effect of a variety of gastrointestinal peptides (1). Thus, it is possible that the anorectic effect of xenin is also partially mediated through the brainstem and vagus nerve.

Leptin, secreted by adipocytes, regulates a variety of physiological functions, including feeding, by acting through several different signaling pathways in the hypothalamus. Leptin also regulates metabolism by interacting with other nutritional signals, including gut hormones (22–24). In the current study, the intraperitoneal injection of xenin significantly reduced food intake in leptin-deficient ob/ob mice. We did not have wild-type mice in the same experiment, and therefore we cannot directly compare the magnitude of xenin-induced anorexia between wild-type and ob/ob mice in the current study. However, by comparing the anorectic effect of xenin between wild-type (Fig. 2A) and ob/ob mice (Fig. 6) in two independent studies, the 15-µg dose of xenin significantly reduced food intake in both wild-type and ob/ob mice with similar kinetics. These data suggest that xenin reduces food intake at least partly through a mechanism that is independent of leptin. Because human obesity is generally characterized by reduced sensitivity or resistance to leptin instead of leptin deficiency, it is possible that treatment with xenin may be effective in reversing metabolic impairments in obese subjects with impaired leptin sensitivity. This possibility was further supported by our findings that leptin-resistant agouti mice reduced food intake in response to xenin administration.

Hypothalamic melanocortin signaling plays a critical role in the regulation of metabolism by integrating signals from the gastrointestinal tract (16). Anorectic effects of some of the gut-derived hormones are mediated through MC4-R. For example, the feeding-suppressing effect of cholecystokinin is abolished in MC4-R-deficient mice and attenuated by a MC3-R/MC4-R antagonist, SHU9119 (25). Ghrelin stimulates food intake by interacting with melanocortins, and the orexigenic effect of ghrelin is abolished in mice lacking both AGRP and neuropeptide Y (24,26). In contrast, the anorectic effects of glucagon-like peptide-1 and peptide YY3-36 are independent of the melanocortin signaling pathway (27–29). Because xenin activated the hypothalamus, as measured by c-fos mRNA expression and Fos immunoreactivity, we wished to determine whether xenin-induced anorexia involves central melanocortin signaling. The anorectic effect of xenin was intact in both preobese and obese agouti mice. Furthermore, the intracerebroventricular injection of SHU9119 at a dose that is effective in blocking the anorectic effect of the MC3-R/MC4-R agonist failed to block the anorectic effect of xenin. If the anorectic effect of xenin is mediated through the central melanocortin system, expression of genes associated with the melanocortin system may be regulated by xenin. However, the intracerebroventricular injection of xenin did not cause significant changes in hypothalamic POMC and AGRP mRNA levels. Taken together, our data suggest that xenin reduces food intake through a mechanism independent of the melanocortin signaling pathway.

In summary, the current study demonstrated that both central and peripheral administration of xenin reduces food intake in mice and suggested that the anorectic effect of xenin is mediated at least partly through hypothalamic activation. In addition to the generalization of the anorectic effect of xenin across species, the current study opens up the possibility of the use of mouse models to investigate the mechanism of xenin-induced anorexia. Using two well-characterized mouse models of obesity, the ob/ob and agouti mice, we have shown that xenin can alter feeding independent of leptin or melanocortin action. These data suggest the possibility that xenin provides a novel mechanism of satiety control. It remains to be shown whether chronic enhancement of xenin action is a viable long-term obesity therapy.

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No potential conflicts of interest relevant to this article were reported.

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