Peripheral and Central Administration of Xenin and Neurotensin Suppress Food Intake in Rodents

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Xenin is a 25-amino acid peptide highly homologous to neurotensin. Xenin and neurotensin are reported to have similar biological effects. Both reduce food intake when administered centrally to fasted rats. We aimed to clarify and compare the effects of these peptides on food intake and behavior. We confirm that intracerebroventricular (ICV) administration of xenin or neurotensin reduces food intake in fasted rats, and demonstrate that both reduce food intake in satiated rats during the dark phase. Xenin reduced food intake more potently than neurotensin following ICV administration. ICV injection of either peptide in the dark phase increased resting behavior. Xenin and neurotensin stimulated the release of corticotrophin-releasing hormone (CRH) from *ex vivo* hypothalamic explants, and administration of α-helical CRH attenuated their effects on food intake. Intraperitoneal (IP) administration of xenin or neurotensin acutely reduced food intake in fasted mice and *ad libitum* fed mice in the dark phase. However, chronic continuous or twice daily peripheral administration of xenin or neurotensin to mice had no significant effect on daily food intake or body weight. These studies confirm that ICV xenin or neurotensin can acutely reduce food intake and demonstrate that peripheral administration of xenin and neurotensin also reduces food intake. This may be partly mediated by changes in hypothalamic CRH release. The lack of chronic effects on body weight observed in our experiments suggests that xenin and neurotensin are unlikely to be useful as obesity therapies.

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INTRODUCTION

Xenin is a 25-amino acid peptide originally identified as the human counterpart of xenopsin, an octapeptide present in the skin of *Xenopus laevis* (1). Xenin is expressed in peripheral and central tissues, but its biological functions are as yet poorly characterized. Xenin shows 100% homology with the N-terminus of human coat protein-α (2), and it has been suggested that xenin is produced by post-translational cleavage of coat protein-α.

Xenin is highly homologous with the biologically active C-terminal region of neurotensin. Neurotensin is a 13-amino acid neuropeptide, first identified in calf hypothalamus (3), with a number of central and peripheral functions (4). Three neurotensin receptors have been identified to date: NTS1, NTS2, and NTS3 (5,6). Xenin is believed to bind to the neurotensin receptor, NTS1 (7).

Xenin is abundantly expressed in gastric, duodenal, and jejunal mucosa, and is found at lower levels in the pancreas. Within the duodenal and jejunal mucosa, it is expressed in specific endocrine cells (8). Xenin is released into the circulation postprandially (9) and has been reported to stimulate pancreatic endocrine and exocrine secretion, inhibit gastrin secretion, and influence gastrointestinal motility (10–12).

The anorectic effects of neurotensin have been known for some time, and there is growing evidence that xenin plays a similar role. Intracerebroventricular (ICV) injection of neurotensin or xenin reduces food intake in rats (13–15). Additionally, neurotensin has also been shown to reduce food intake when administered into the paraventricular nucleus (16) and when administered peripherally, although peripheral administration was carried out before a period of access to alcohol to assess the effects of neurotensin on both food intake and alcohol consumption (17). However, the relative potencies of xenin and neurotensin have not been directly compared following central or peripheral administration. We aimed to compare the effects of centrally administered xenin and neurotensin, and investigate potential hypothalamic mechanisms for their anorexigenic actions. The peripheral effects of xenin and neurotensin were also investigated as both peptides are present in the circulation and plasma concentrations rise after a meal, suggesting they may act as endogenous circulating satiety factors.

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METHODS AND PROCEDURES

Rats

Male Wistar rats (Charles River, Margate, UK) (200–250 g) were maintained in individual cages under controlled temperature (21–23 °C) and light (12:12 light–dark cycle, lights on at 0700 hours) with *ad libitum* access to food (RM1 diet; SDS, Witham, UK) and water unless otherwise stated.

Mice

Male C57Bl/6 mice (Harlan, Bicester, UK) (20–25 g) were housed in single individually ventilated cages. Conditions as described above for rats.

For all studies, animals were randomized into groups with approximately equal mean body weight (within 2 g for rats and within 1 g for mice), and food was weighed on balances accurate to 0.01 g (Adam Equipment, Milton Keynes, UK).

ICV cannulation and injection

Rats were implanted with a permanent 22-gauge stainless steel cannula projecting to the third ventricle, as described previously (18), according to the co-ordinates of Paxinos and Watson (19). A total volume of 5 µl was injected over 1 min. Xenin was obtained from the Advanced Biotechnology Centre, Imperial College London, and neurotensin from Bachem, St Helens, UK. In all feeding studies, food intake was measured at 1, 2, 4, 8, and 24 h postinjection and body weight was measured at 0 and 24 h.

Study 1: Central administration of xenin and neurotensin to rats

ICV administration of xenin and neurotensin to fasted rats in the early light phase. Overnight fasted male Wistar rats (n = 8–10 per group) received a single ICV injection of either saline 0.3, 1 or 3 nmol xenin or 6 nmol neurotensin between 0900 and 1000 hours.

ICV administration of xenin and neurotensin to rats during the dark phase. Male Wistar rats (n = 8–10 per group) received a single ICV injection of either saline 0.3, 1, or 3 nmol xenin or 1, 3, or 9 nmol neurotensin between 1900 and 2000 hours and were immediately placed into the dark.

Behavior analysis following ICV administration of xenin and neurotensin to rats during the dark phase. Male Wistar rats (n = 8–10 per group) received a single ICV injection of either saline, 3 nmol xenin, 9 nmol neurotensin, or 3 nmol neuromedin U (NMU) between 1900 and 2000 hours and were immediately placed into the dark. Behavior was analyzed for 1 h postinjection by observers blinded to the treatment group using a method adapted from Scott *et al.* (20). Observers remained in the room throughout the observation period and behaviors were recorded without disturbance to the animals. Behavior was classified into four categories: activity, feeding, grooming, and resting. Each animal was observed for 15 s every 5 min during the test period; three separate behaviors were recorded during the 15 s observation with a total of 36 observations per hour.

Study 2: Potential hypothalamic mechanisms behind the anorexigenic effects of xenin and neurotensin

The effect of xenin and neurotensin on release of anorectic neuropeptides from hypothalamic explants. The static hypothalamic explant incubation system was used as described previously (21,22). Following a 2 h equilibration period in artificial cerebrospinal fluid, hypothalami were incubated in either 600 µl artificial cerebrospinal fluid, 1,000 nmol/l xenin or 1,000 nmol/l neurotensin for 45 min. Viability of the tissue was confirmed by a final exposure to 56 mmol/l KCl in artificial cerebrospinal fluid. Samples were analyzed by radioimmunoassay for cocaine- and amphetamine-regulated transcript (CART), α-melanocyte-stimulating hormone (α-MSH) and corticotrophin-releasing hormone (CRH).

Radioimmunoassays: CART, α-MSH, and CRH were measured using established RIAs developed in this laboratory as described previously (22–24). Inter- and intra-assay variation was <10% for all assays performed.

ICV administration of xenin and neurotensin following pretreatment with a CRH antagonist. Male Wistar rats were cannulated and injected as described. Rats received an ICV injection of 1 µg α-helical CRH (Sigma-Aldrich, Gillingham, UK) or saline at 1800 hours, followed 60 min later by a second ICV injection of either 3 nmol xenin, 9 nmol neurotensin, or saline before being placed into the dark (25).

Study 3: Acute peripheral administration of xenin and neurotensin to mice

Peripheral administration of xenin and neurotensin to fasted mice. Overnight fasted male C57Bl/6 mice (n = 8–10 per group) received a single intraperitoneal (IP) injection of either saline, 150, 400 or 1,200 nmol/kg xenin, or 1,200 nmol/kg neurotensin in a total volume 0.1 ml.

Peripheral administration of xenin and neurotensin to mice during the dark phase. Male C57Bl/6 mice (n = 8–10 per group) received a single IP injection of either saline, 1,200 or 3,600 nmol/kg xenin, or 1,200 or 3,600 nmol/kg neurotensin just before the onset of the dark phase.

Study 4: Chronic peripheral administration of xenin and neurotensin to mice

Chronic subcutaneous administration of xenin and neurotensin via ALZET minipumps. Male C57Bl/6 mice (n = 10 per group) received continuous infusion of either saline, xenin (1,200 nmol/kg/day or 3,600 nmol/kg/day) or neurotensin (1,200 nmol/kg/day or 3,600 nmol/kg/day) for 6 days. Animals were anesthetized under deep inhalation anesthesia with isoflurane (Abbott Animal Health, Kent, UK) and implanted in the intrascapular region with preloaded primed ALZET minipumps, model 1007D (Vet Tech Solutions, Cheshire, UK). Body weight and food intake were measured each morning between 0900 and 1000 hours. On day 6, animals were killed by CO_2 asphyxiation and pumps removed. Pumps were checked to ensure contents had been expelled. Peptide remaining in the pumps was removed and the bioactivity compared to freshly prepared peptide. Fasted mice received an IP injection of saline, 1,200 nmol/kg freshly prepared xenin or neurotensin or 1,200 nmol/kg xenin or neurotensin from the pumps.

Twice daily IP administration of xenin and neurotensin. Male C57Bl/6 mice (n = 10 per group) received twice daily IP injections of either saline, 1,200 nmol/kg or 3,600 nmol/kg xenin, or 1,200 nmol/kg or 3,600 nmol/kg neurotensin. Injections were carried out at 0900 and 1830 hours. Food was weighed at 1300 and 1930 hours. Body weight was measured just before injection. Injections were carried out for 5 days (on day 5 only the 0900 hours injection was performed). Following 0900 hours injections on days 1 and 5, behavior was observed for 1 h postinjection. Behavioral analysis was carried out as described above under Study 1.

Statistics

All data are presented as mean ± s.e.m. other than data from behavioral studies. All food intake data were analyzed using one-way ANOVA with Newman-Keul's *post hoc* test (GraphPad Prism version 4.00 for Windows; GraphPad Software, San Diego, CA). Data from the hypothalamic explant release study were compared by paired t test between the basal period and the test period (GraphPad Prism). Behavior data were analyzed using the Kruskall–Wallis test (Systat, Evanston, IL),

as data were nonparametric. Cumulative daily food intake and body weight data from chronic studies were compared using the generalized estimating equation (Stata 9, StataCorp LP, College Station, TX). In all cases, $P < 0.05$ was considered to be statistically significant.

RESULTS

Study 1: Central administration of xenin and neurotensin to rats

A significant reduction in food intake was seen in the first hour following ICV administration of 3 nmol xenin to fasted rats in the early light phase (**Figure 1a**) ($P < 0.05$, $F = 3.49$ by ANOVA). During the dark phase, ICV administration of 1 nmol xenin, 3 nmol xenin, or 9 nmol neurotensin significantly reduced food intake in the first hour postinjection (**Figure 1b**) ($P < 0.01$, $F = 3.88$ by ANOVA). No significant differences in food intake were detected at any other time points measured. No significant differences in body weight were detected at 24 h. Behavioral analysis 0–1 h postinjection, following ICV administration of 3 nmol xenin at the beginning of the dark phase revealed significantly increased resting behavior and significantly decreased grooming and activity compared to animals receiving saline (**Figure 1c**). ICV administration of 9 nmol neurotensin significantly increased resting and decreased grooming behavior compared to animals administered saline (time spent resting 7.9% (saline), 50.5% (3 nmol xenin), $P < 0.001$, 46.9% (9 nmol neurotensin), $P < 0.05$, time spent grooming 18.5% (saline), 6.5% (3 nmol xenin), $P < 0.05$, 2.2% (9 nmol neurotensin), $P < 0.01$, time spent active 55.6% (saline), 29.9% (3 nmol xenin), $P < 0.01$) (**Figure 1c**).

NMU was used as a positive control to ensure the observation system used was sensitive enough to detect behavioral changes, as it is known to increase grooming behavior (26). ICV administration of 3 nmol NMU increased grooming behavior between 0 and 1 h postinjection (time spent grooming: 18.5% (saline), 39.4% (3 nmol NMU)) (**Figure 1c**).

Study 2: Potential hypothalamic mechanisms behind the anorexigenic effects of xenin and neurotensin

A hypothalamic explant system was used to assess the effect of xenin and neurotensin on release of anorectic hypothalamic neuropeptides previously shown to mediate the effects of central and circulating anorexigenic agents (21,27–30).

The effect of xenin and neurotensin on release of anorectic neuropeptides from hypothalamic explants. Incubation of hypothalamic explants in 1,000 nmol/l xenin significantly increased release of CRH (**Figure 2a**), and a trend toward an increase in CRH release was seen when explants were incubated in 1,000 nmol/l neurotensin (**Figure 2b**). Potassium-stimulated release was not included in the statistical analysis. No significant changes in the release of CART or α-MSH were detected following treatment with xenin or neurotensin (data not shown).

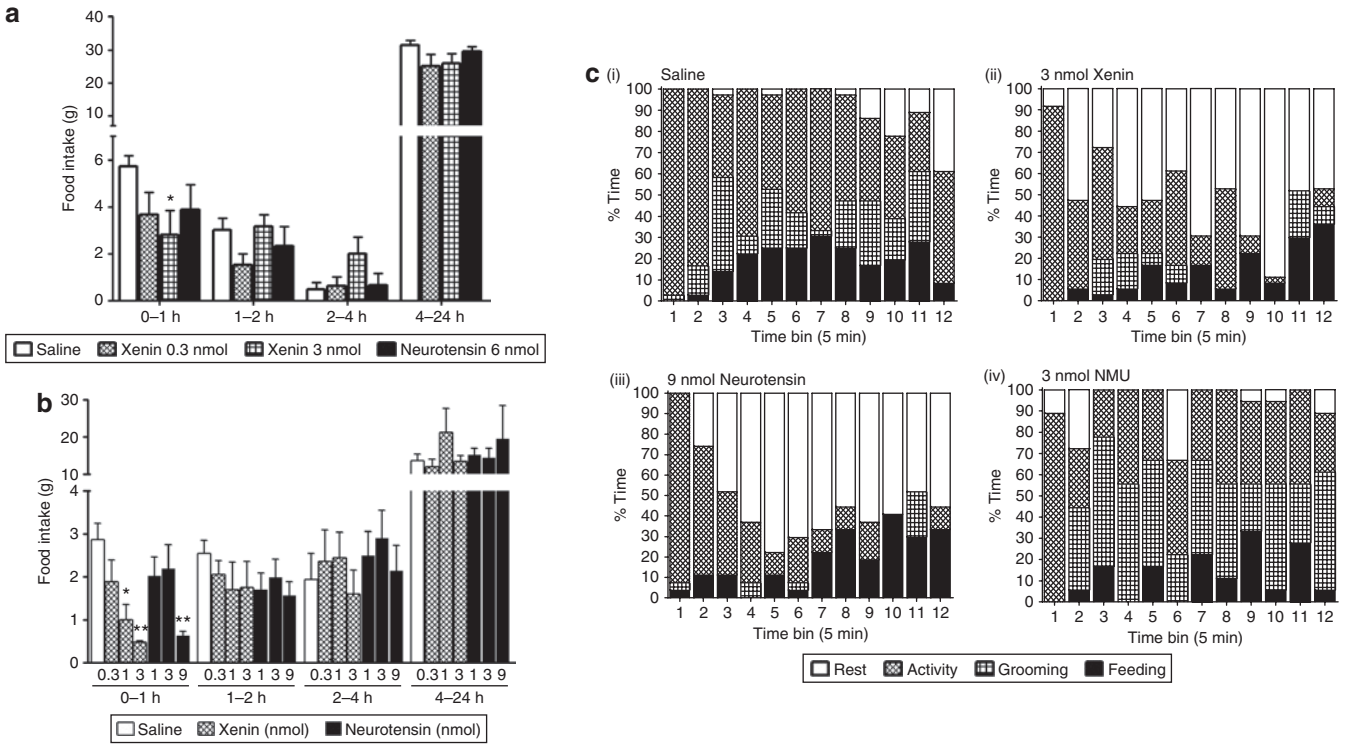

Figure 1 Effects of central administration of xenin and neurotensin on food intake and behavior. Food intake (mean grams per rat ± s.e.m.) 0–1, 1–2, 2–4, and 4–24 h after ICV injection of saline, xenin, or neurotensin in (**a**) fasted rats in the early light phase and (**b**) *ad libitum* fed rats during the dark phase ($n = 8$–10 per group). $^*P < 0.05$ vs. saline, $^{**}P < 0.01$ vs. saline by ANOVA with Newman-Keuls *post hoc* test. (**c**) Effect of ICV administration of xenin, neurotensin, and neuromedin U (NMU) at the beginning of the dark phase on the behavior of rats, (i) saline, (ii), 3 nmol xenin, (iii) 9 nmol neurotensin, (iv) 3 nmol NMU. Behavior was categorized as activity, feeding, grooming, or resting and monitored for 15 s every 5 min for 60 min postinjection. Data are shown in 5-min time bins as a percentage of total time spent in a particular activity ($n = 8$–10 per group). ICV, intracerebroventricular.

ICV administration of xenin and neurotensin following pretreatment with a CRH antagonist. ICV administration of xenin and neurotensin following pretreatment with saline significantly reduced food intake 0–1 h postinjection compared to animals administered saline then saline and compared to animals administered α-helical CRH then saline ($P < 0.001$, $F = 5.43$ by ANOVA). However, administration of xenin and neurotensin following pretreatment with α-helical CRH did not significantly reduce food intake compared to controls (Figure 2c). No significant differences in food intake were observed between any of the groups at any other time points measured. No significant differences in body weight were observed at 24 h.

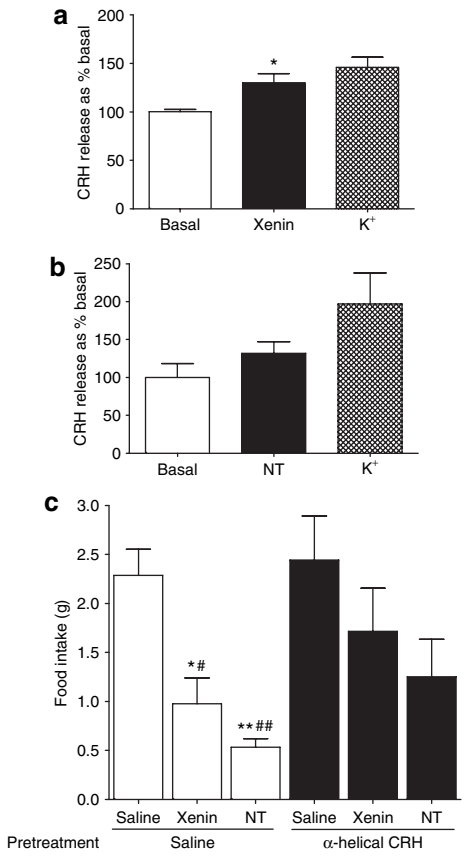

Figure 2 The interactions of xenin and neurotensin with the hypothalamic CRH system. Release of CRH from hypothalamic explants following treatment with artificial cerebrospinal fluid (aCSF) (basal) and (**a**) 1,000 nmol/l xenin, or (**b**) 1,000 nmol/l neurotensin, or with 56 mmol/l KCl (*P< 0.05 vs. aCSF control, n = 15–22 per group). (**c**) Food intake (mean in grams per rat ± s.e.m.) following pretreatment with 1 µg α-helical CRH or saline followed by ICV administration of saline, 3 nmol xenin or 9 nmol neurotensin in *ad libitum* fed rats during the dark phase (n = 8–10 per group). *P < 0.05, **P < 0.01 vs. saline then saline, #P < 0.05, ##P < 0.01 vs. α-helical CRH then saline by ANOVA with Newman-Keuls *post hoc* test. CRH, corticotrophin-releasing hormone; ICV, intracerebroventricular.

Study 3: Peripheral administration of xenin and neurotensin in mice

Following confirmation of the anorexigenic effects of ICV administration of xenin and neurotensin, the effects of

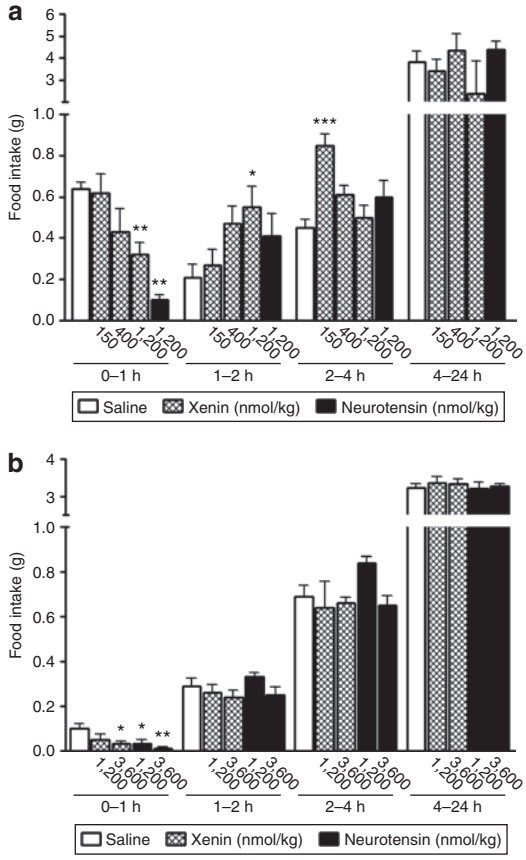

Figure 3 Effects of acute peripheral administration of xenin and neurotensin in mice. Food intake (mean grams per mouse ± s.e.m.) 0–1, 1–2, 2–4, and 4–24 h after acute IP injection of saline, xenin, or neurotensin in (**a**) fasted C57Bl/6 mice in the early light phase or (**b**) *ad libitum* fed C57Bl/6 mice at the beginning of the dark phase (n = 8–10 per group). *P < 0.05, **P < 0.01, ***P < 0.001 (vs. saline) by ANOVA with Newman-Keuls *post hoc* test. IP, intraperitoneal.

Table 1 Daily food intake (mean in grams per mouse ± s.e.m.) following subcutaneous implantation of Alzet minipumps

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Saline	3.63 ± 0.24	4.10 ± 0.22	4.71 ± 0.10	4.83 ± 0.12	4.22 ± 0.11	4.43 ± 0.14
Xenin 1,200 nmol/kg/day	3.81 ± 0.16	4.53 ± 0.30	5.11 ± 0.19	5.48 ± 0.17	4.30 ± 0.17	4.91 ± 0.22
Xenin 3,600 nmol/kg/day	4.10 ± 0.18	4.58 ± 0.21	5.11 ± 0.20	5.37 ± 0.17	4.42 ± 0.23	4.7 ± 0.17
Neurotensin 1,200 nmol/kg/day	2.82 ± 0.47	4.12 ± 0.22	4.87 ± 0.25	5.42± 0.18	4.69 ± 0.19	4.95 ± 0.20
Neurotensin 3,600 nmol/kg/day	3.37 ± 0.23	4.33 ± 0.20	4.82 ± 0.19	5.67 ± 0.31	4.40 ± 0.39	4.74 ± 0.12

Pumps were implanted on day 0, continuously dispensing saline, 1,200 nmol/kg/day xenin or neurotensin, or 3,600 nmol/kg/day xenin or neurotensin (n = 10 per group).

peripheral administration of both peptides were investigated and compared. Both xenin and neurotensin circulate and plasma levels increase following a meal (1,31,32).

In fasted mice, IP administration of 1,200 nmol/kg xenin during the early light phase significantly reduced food intake in the first hour with a trend toward a reduction in food intake seen in the 400 nmol/kg group as well. Administration of 1,200 nmol/kg neurotensin significantly reduced food intake in the first hour postinjection ($P < 0.0001$, $F = 10.45$ by ANOVA) (Figure 3a). Food intake was significantly increased between 1 and 2 h postinjection following administration of 1,200 nmol/kg xenin (P = NS, $F = 2.66$ by ANOVA), and between 2 and 4 h postinjection following administration of 150 nmol/kg xenin ($P < 0.001$, $F = 7.60$ by ANOVA) (Figure 3a).

Administration of 1,200 nmol/kg or 3,600 nmol/kg neurotensin or 3, 600 nmol/kg xenin to *ad libitum* fed mice just before

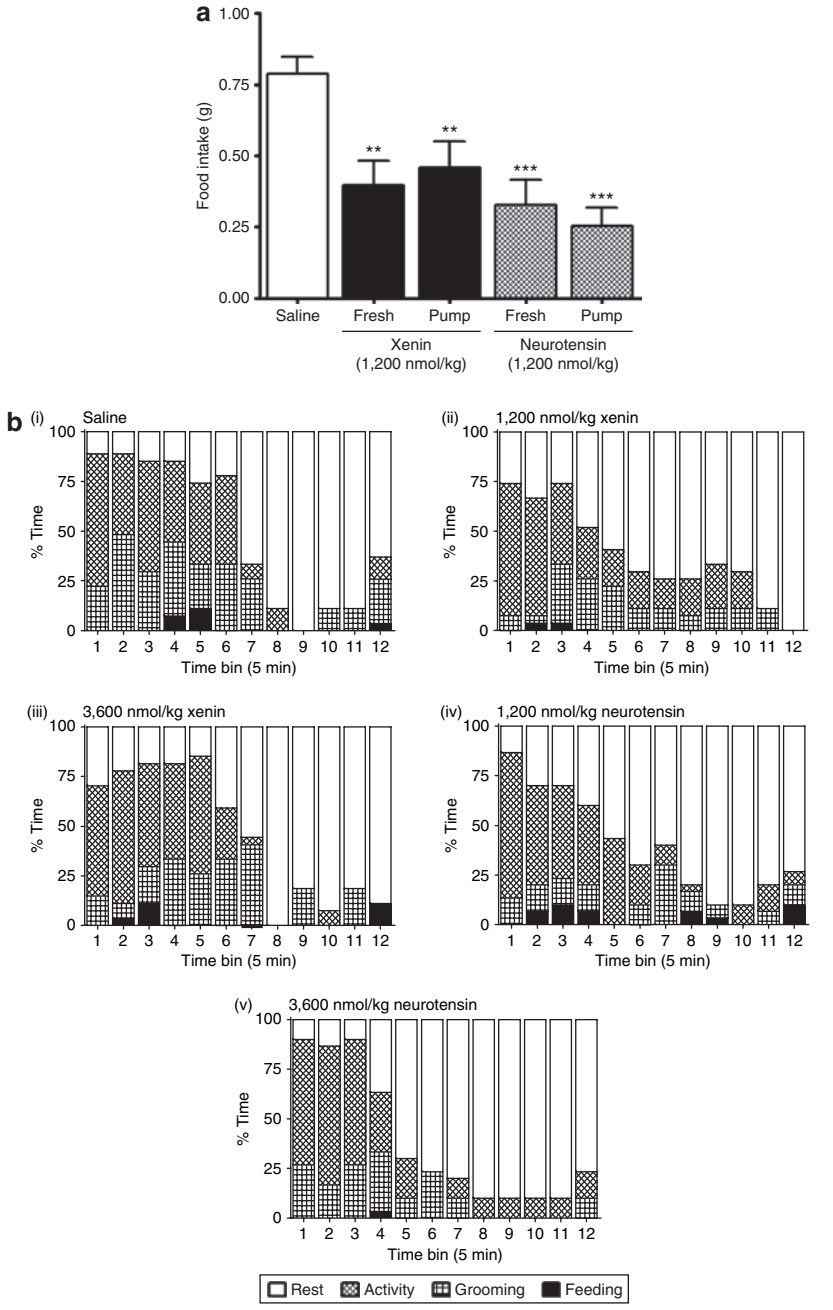

Figure 4 Effects of chronic peripheral administration of xenin and neurotensin in mice. (**a**) Food intake (mean grams per mouse ± s.e.m.) 0–1 h after IP injection of saline, 1,200 nmol/kg fresh xenin, 1,200 nmol/kg xenin removed from minipumps, 1,200 nmol/kg fresh neurotensin, or 1,200 nmol/kg neurotensin removed from minipumps, in fasted mice in the early light phase (n = 10–15 per group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (vs. saline) by ANOVA with Newman-Keuls *post hoc* test. (**b**) Behavior analysis for 1 h following IP injection of saline, xenin, or neurotensin to male C57Bl/6 mice in the early light phase. Analysis was carried out on day 1 of study (i) saline, (ii) 1,200 nmol/kg xenin, (iii) 3,600 nmol/kg xenin, (iv) 1,200 nmol/kg neurotensin, (v) 3,600 nmol/kg neurotensin. Behavior was categorized as activity, feeding, grooming, or resting and monitored for 15 s every 5 min for 60 min postinjection. Data are shown in 5-min time bins as a percentage of total time spent in a particular activity (n = 10 per group). IP, intraperitoneal.

Table 2 Effect of twice daily IP injection of xenin or neurotensin on daily food intake (mean in grams per mouse ± s.e.m.)

	Day 1	Day 2	Day 3	Day 4
Saline	4.41 ± 0.1	5.04 ± 0.28	4.65 ± 0.17	4.56 ± 0.17
Xenin 1,200 nmol/kg/day	4.31 ± 0.16	4.70 ± 0.12	4.92 ± 0.25	5.10 ± 0.48
Xenin 3,600 nmol/kg/day	4.27 ± 0.14	4.70 ± 0.11	4.84 ± 0.08	4.75 ± 0.13
Neurotensin 1,200 nmol/kg/day	4.06 ± 0.12	4.7 ± 0.12	4.84 ± 0.08	4.75 ± 0.12
Neurotensin 3,600 nmol/kg/day	3.94 ± 0.15	4.49 ± 0.13	4.72 ± 0.18	4.70 ± 0.15

IP, intraperitoneal.Mice received a twice daily IP injection of saline, 1,200 nmol/kg xenin or neurotensin, or 3,600 nmol/kg xenin or neurotensin (n = 10 per group).

the onset of the dark phase (1830 h) significantly reduced food intake in the first hour postinjection. A trend toward reduced food intake was seen in the first hour following administration of 1,200 nmol/kg xenin (Figure 3b) ($P < 0.01$, $F = 4.076$ by ANOVA). No significant differences in food intake were detected at any other time points measured. No differences in body weight were detected at 24 h.

Study 4: Chronic peripheral administration of xenin and neurotensin in mice

Following confirmation of the anorexigenic effects of peripheral administration of xenin and neurotensin, chronic peripheral administration studies were carried out to establish their effects on long-term food intake and body weight regulation.

Chronic subcutaneous administration of xenin and neurotensin via ALZET minipumps. Chronic administration of 1,200 nmol/kg or 3,600 nmol/kg/day xenin or neurotensin through a subcutaneous minipump had no significant effect on body weight or food intake compared to animals administered saline over the 6 days studied (Table 1).

Peptide remaining in the pumps at the end of the study period was removed and administered to fasted mice. Administration of xenin or neurotensin at 1,200 nmol/kg, either freshly prepared or removed from pumps, significantly reduced food intake relative to saline controls in the first hour postinjection (Figure 4a). There were no significant differences between the reductions in food intake observed following administration of peptide remaining in the pumps and freshly prepared peptide for either xenin or neurotensin.

Twice daily IP administration of xenin or neurotensin. IP administration of xenin and neurotensin significantly reduced food intake 1 h postinjection at 1830 h on study days 1 and 2 (data not shown). However, there were no significant changes in daily food intake (Table 2) or body weight (data not shown). On study days 3 and 4, IP administration of xenin or neurotensin at 1830 h did not significantly reduce food intake 1 h postinjection (data not shown). No significant effects on cumulative food intake or body weight were observed.

Behavior analysis

Behavior was analyzed on days 1 and 5 for 1 h after 0900 hours injection.

On day 1, a significant decrease in grooming was seen following administration of 1,200 nmol/kg xenin, 1,200 nmol/kg, and 3,600 nmol/kg neurotensin compared to animals administered saline (Figure 4b). There were no significant differences detected between the groups on day 5 of the study (data not shown).

DISCUSSION

Our results confirm that ICV administration of xenin or neurotensin reduces food intake in fasted rats, demonstrate that ICV administration of xenin and neurotensin also reduces food intake in *ad libitum* fed rats during the dark phase, and show for the first time that peripheral administration of xenin acutely reduces food intake, as does neurotensin.

As described in previous studies, the effects of ICV administration of xenin and neurotensin on food intake were relatively short-lived, with significant reductions only occurring in the first hour postinjection (13,15). Xenin appears to reduce food intake more potently than neurotensin when administered into the third ventricle; a 3 nmol dose of xenin causes a significant reduction in food intake similar in magnitude to the reduction seen following administration of 9 mol neurotensin. Xenin is thought to bind to NTS1, (7,11) a neurotensin receptor present in the hypothalamus which is suggested to mediate the anorexigenic effects of neurotensin (33,34). Unfortunately, the poor solubility of specific NTS1 antagonists (4) makes them difficult to administer centrally to verify that they block the anorexigenic effects of xenin and neurotensin. In the intestine, xenin activates NTS1 with a similar potency to neurotensin (7). Thus, if xenin does reduce food intake via NTS1, it seems unlikely that simple receptor binding affinities account for the difference in potencies. It is possible that xenin has a longer half-life or better tissue penetration than neurotensin when administered ICV.

A hypothalamic explant system was used to investigate possible hypothalamic mechanisms by which xenin and neurotensin might reduce food intake. Xenin and neurotensin did not influence the release of the anorexigenic neuropeptides α-MSH and CART. These peptides are thought to comprise neuropeptide circuits in the arcuate nucleus that at least partially mediate the anorectic actions of specific circulating hormones, including leptin and insulin (27). Our results suggest that xenin and neurotensin may not target these arcuate nucleus circuits. However, xenin significantly stimulated CRH release from *ex vivo* hypothalamic explants and a trend toward increased CRH release was seen following neurotensin treatment. CRH is a known anorexigenic agent (27,35) which has previously been shown to mediate the anorectic effects of other

regulators of appetite, including NMU (36). The stronger stimulation of CRH release by xenin corresponds with our ICV feeding studies which suggested that within the hypothalamus xenin is more potent than neurotensin. Pretreatment with a CRH antagonist, α-helical CRH attenuated the anorexigenic effects of xenin and neurotensin, suggesting their anorectic effects are partially mediated by CRH. However, it is likely that other systems are also involved in mediating the anorexigenic effects of xenin and neurotensin. For example, the central effects of neurotensin on food intake have been suggested to occur via effects on dopamine release (37).

Analysis of behavior following ICV administration of xenin and neurotensin to rats at the beginning of the dark phase revealed a significant increase in the amount of time spent resting during the first hour postinjection. Previous studies have reported intra-paraventricular nucleus administration of neurotensin does not significantly alter resting behavior (16). It is possible that neurotensin does not increase resting behavior via the paraventricular nucleus; different behavioral effects have been reported following administration of hypothalamic neuropeptides into different regions of the hypothalamus (38,39). In addition, the intra-paraventricular nucleus administration study was carried out in fasted animals in the light phase. In contrast, our study used *ad libitum* fed animals in the dark phase, when different behavioral patterns might be expected (40,41). Comparison of these studies is thus difficult. Further studies are required to establish which hypothalamic nucleus or nuclei mediate the behavioral effects of xenin and neurotensin. The well-characterized behavioral satiety sequence describes how a feeding period is followed by grooming and finally resting (20,41,42). Our studies were carried out during the dark phase to increase their physiological relevance. However, during this period the final resting phase of this sequence was not clearly observed in saline-treated animals. The observed increase in resting observed following administration of xenin or neurotensin is consistent with an accelerated onset of satiety. However, increased resting behavior can also occur as a result of sickness. Although the behavioral satiety sequence did not appear to be disrupted, and no obvious adverse behaviors such as hunched posture or tremors were observed, the possibility of reduced food intake due to malaise cannot be excluded. Conditioned taste aversion studies might be used to determine whether the anorectic effects of xenin or neurotensin are due to aversive effects (43).

Circulating levels of xenin and neurotensin are increased postprandially, in accord with a role in satiety (1,31,32). The effects of peripherally administered xenin and neurotensin on food intake were thus investigated. Previous studies in rats have shown IP injection of neurotensin reduces food intake following a 30-min period of access to 5 or 10% ethanol (17). In our studies, IP injection of either xenin or neurotensin reduced food intake for 1 h in fasted mice and in *ad libitum* fed mice during the dark phase. The behavioral pattern of mice administered xenin or neurotensin was different to that observed in animals administered saline. Mice treated with xenin or neurotensin showed a less intensive grooming period followed by an earlier onset of predominantly resting behavior. Intravenous administration of neurotensin to rats has been reported to reduce food intake only at doses which also significantly altered behavior in a manner considered to adversely effect the animals (16). We did not observe any adverse behaviors but observed a behavioral pattern consistent with an accelerated onset of satiety, suggesting behavioral changes may be an effect rather than a cause of the anorexigenic actions of xenin and neurotensin. Peripheral injections were carried out in mice as inconsistent effects on food intake have been reported following peripheral administration of gut hormones to rats. This is suggested to be due to stress resulting from the injection procedure (44). The mechanisms by which peripherally administered xenin and neurotensin reduce appetite are unclear. A number of circulating factors are thought to act on the arcuate nucleus, which is imperfectly isolated from the general circulation by the blood–brain barrier, to influence appetite (27). However, results from our hypothalamic explant studies suggest xenin and neurotensin do not act via the CART or α-MSH in the arcuate nucleus (45). It is possible that the reduction in food intake observed following peripheral administration of xenin and neurotensin occurs via the vagus nerve or is secondary to direct effects on the gut. NTS1 is expressed in the gut (46) and both xenin and neurotensin affect gastric and gut motility (7,47,48). Further studies are required to clarify the mechanisms by which peripheral administration of these peptides influences appetite.

Once the acute anorexigenic effects of peripheral xenin and neurotensin administration had been established, chronic administration studies were carried out to establish their potential as agents for long-term reduction in food intake and body weight. Administration of 1,200 nmol/kg day or 3,600 nmol/kg/day xenin and neurotensin via subcutaneous minipumps had no significant effect on daily food intake or body weight. This may be due to the relatively low doses used. Although these doses were effective when administered acutely, release over 24 h may not achieve equivalently high circulating levels. Higher doses were not feasible to use as they proved impossible to dissolve in the volume required for pump administration.

An alternative chronic study was carried out in which peptide was administered by twice daily IP injection. For the first two study days, acute reductions in food intake were observed; however, no significant effects on daily food intake or body weight were detected. The effects of xenin and neurotensin therefore appear short-lived and any short-term reductions in food intake are too small to influence daily food intake, or are compensated for by increased food consumption during the rest of the day. This is similar to the reported effects of cholecystokinin, which reduces food intake acutely but has little chronic effect on food intake or body weight (49). The acute effects of xenin and neurotensin only persisted for 2 days, presumably due to tachyphylaxis. The potential of xenin or neurotensin as antiobesity agents thus appears limited.

In summary, xenin and neurotensin acutely reduce food intake when administered ICV in rats or peripherally in mice. These effects are most pronounced in the first hour after administration and long-term continuous administration via minipump or twice daily injection does not reduce daily food intake or influence body weight in mice. Both peptides increase resting behavior, but do not cause obvious adverse effects. Xenin and neurotensin may act as short-term regulators of food intake, and their anorectic effects may be mediated in part via CRH.

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DISCLOSURE

The authors declared no conflict of interest.

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