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Competing interests statement

The authors declare that they have no competing financial interests.

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Gut hormone PYY_{3-36} physiologically inhibits food intake

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Food intake is regulated by the hypothalamus, including the melanocortin and neuropeptide Y (NPY) systems in the arcuate nucleus[1]. The NPY Y2 receptor (Y2R), a putative inhibitory presynaptic receptor, is highly expressed on NPY neurons[2] in the arcuate nucleus, which is accessible to peripheral hormones[3]. Peptide YY_{3-36} (PYY_{3-36}), a Y2R agonist[4], is released from the gastrointestinal tract postprandially in proportion to the calorie content of a meal[5–7]. Here we show that peripheral injection of PYY_{3-36} in rats inhibits food intake and reduces weight gain. PYY_{3-36} also inhibits food intake in mice but not in *Y2r*-null mice, which suggests that the anorectic effect requires the Y2R. Peripheral administration of PYY_{3-36} increases c-Fos immunoreactivity in the arcuate nucleus and decreases hypothalamic *Npy* messenger RNA. Intra-arcuate injection of PYY_{3-36} inhibits food intake. PYY_{3-36} also inhibits electrical activity of NPY nerve terminals, thus activating adjacent pro-opiomelanocortin (POMC) neurons[8]. In humans, infusion of normal postprandial concentrations of PYY_{3-36} significantly decreases appetite and reduces food intake by 33% over 24 h. Thus, postprandial elevation of PYY_{3-36} may act through the arcuate nucleus Y2R to inhibit feeding in a gut–hypothalamic pathway.

The orexigenic NPY and the anorectic alpha melanocyte-stimulating hormone (α-MSH) systems of the hypothalamic arcuate nucleus are involved in the central regulation of appetite[1]. However, the potential mechanisms that signal meal ingestion directly to these hypothalamic-feeding circuits are unclear. PYY_{3-36} is a gut-derived hormone that is released postprandially in proportion to the calories ingested[5]. We therefore investigated the effects of peripheral administration of PYY_{3-36} on feeding.

An intraperitoneal (i.p.) injection of PYY_{3-36} to freely feeding rats before the onset of the dark phase significantly decreased subsequent food intake (Fig. 1a). A similar inhibition of feeding was seen after i.p. injection in rats fasted for 24 h (Supplementary Information Fig. 1). A time course of the plasma PYY_{3-36} concentrations after i.p. injection of PYY_{3-36} showed a peak at 15 min after injection, which was within the normal postprandial range (peak PYY_{3-36} 15 min after i.p. injection of 0.3 μg per 100 g (body weight), 99.3 ± 10.4 pmol l^{-1}; peak postprandial PYY_{3-36}, 112.1 ± 7.8 pmol l^{-1}; $n = 8$–10 per group), suggesting that physiological concentrations of PYY_{3-36} inhibit feeding. PYY_{3-36} did not affect gastric emptying (percentage of food ingested remaining in the stomach at 3 h (ref. 9): PYY_{3-36}, $36 \pm 1.9\%$; saline, $37.4 \pm 1.0\%$; $n = 12$). PYY_{3-36} that was administered i.p. twice daily for 7 d reduced cumulative food intake (7-d cumulative food intake: PYY_{3-36}, 187.6 ± 2.7 g; saline, 206.8 ± 2.3 g; $n = 8$ per group, $P < 0.0001$) and decreased body weight gain (PYY_{3-36}, 48.2 ± 1.3 g; saline, 58.7 ± 1.9 g; $n = 8$ per group, $P < 0.002$; Fig. 1b).

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To investigate whether this inhibition of food intake involved a hypothalamic pathway, we examined c-Fos expression in the arcuate nucleus, which is an important centre of feeding control[1,8], after a single i.p. injection of PYY_{3-36}. There was a twofold increase in the number of cells positive for c-Fos in the lateral arcuate of the rat (PYY_{3-36}, 168 ± 2; saline, 82.7 ± 5; $n = 3$, $P < 0.0001$). Similarly, in *Pomc–EGFP* transgenic mice[8] i.p. administration of PYY_{3-36} resulted in a 1.8-fold increase in the number of arcuate cells positive for c-Fos (Fig. 1c, d), as compared with saline control animals (PYY_{3-36}, 250 ± 40; saline, 137 ± 15; $n = 5$, $P < 0.05$). Injection of i.p. PYY_{3-36} caused a 2.6-fold increase in the proportion of POMC neurons that expressed c-Fos (PYY_{3-36}, 20.4 ± 2.9%; saline, 8 ± 1.4%; $n = 5$, $P < 0.006$; Fig. 1e, f).

As these observations suggested that PYY_{3-36} might act through the arcuate nucleus, we studied the effects of PYY_{3-36} on NPY and POMC circuits in the hypothalamus. In view of the sustained inhibition of food intake and the effects on weight gain after peripheral administration of PYY_{3-36}, we measured both *Pomc* and *Npy* hypothalamic messenger RNA (mRNA) using RNase protection assays. A significant decrease in *Npy* mRNA in response to PYY_{3-36} was observed 6 h after i.p. injection, as compared with saline-treated animals (relative optical density units: saline, 17.3 ± 2.0; PYY_{3-36}, 8.8 ± 1.0; $P < 0.02$). A nonsignificant increase occurred in *Pomc* mRNA.

PYY_{3-36} shares 70% amino-acid sequence identity with NPY and acts through NPY receptors[10]. The Y2R is a putative inhibitory presynaptic receptor and is highly expressed on the arcuate NPY neurons[2], although not on the neighbouring POMC neurons. PYY_{3-36} is a high affinity agonist at the Y2 receptor[7]. We thought that peripheral PYY_{3-36} might inhibit food intake through the Y2R in the arcuate nucleus, an area that is directly accessible to circulating hormones[3].

To investigate this hypothesis, we injected PYY_{3-36} directly into the arcuate nucleus[11]. In rats fasted for 24 h, food intake was significantly decreased by doses as low as 100 fmol (Fig. 2a), which resulted in a similar inhibition to that seen after i.p. administration. To establish whether these effects occurred through the Y2R we used a Y2R selective agonist[12], Y2A (*N*-acetyl [Leu^{28}, Leu^{31}] NPY(24–36)). Its affinity was confirmed using receptor-binding studies[13] on cell lines that expressed the NPY Y1, Y2 and Y5 receptors (inhibitor concentration for half-maximum response (IC_{50}): Y2, 1.3 ± 0.2 nM; Y1, >5,000 nM; Y5, >5,000 nM). Intra-arcuate nucleus injection of Y2A in rats previously fasted for 24 h dose-dependently inhibited (100 fmol to 1 nmol) food intake (chow ingested 2 h after injection: 0.1 nmol of Y2A, 6.2 ± 0.5 g; saline, 8.2 ± 0.6 g, $n = 8$ per group, $P < 0.05$). To confirm the anatomical specificity of this effect we injected Y2A (100 fmol to 1 nmol) into the paraventricular nucleus (PVN)[14] of rats fasted for 24 h and found no alteration of food intake (2 h after injection: saline, 8.3 ± 0.4 g; 0.1 nmol of Y2A, 8.0 ± 0.6 g; $n = 8$ per group). To define further the role of the Y2R in the feeding inhibition caused by peripheral PYY_{3-36}, we examined the effect of PYY_{3-36} on *Y2r*-null mice and littermate controls. PYY_{3-36} inhibited daytime feeding in a dose-responsive manner in fasted male wild-type mice but did not inhibit food intake in fasted male *Y2r*-null mice (Fig. 2b, c).

We examined the electrophysiological response of hypothalamic POMC neurons to administration of both PYY_{3-36} and Y2A. These neurons were identified using mice with targeted expression of green fluorescent protein in POMC neurons[8]. PYY_{3-36} disinhibited the POMC neurons, resulting in a significant depolarization of 19 of the 22 POMC neurons tested (Fig. 3a, inset; 10.3 ± 2.1 mV depolar-

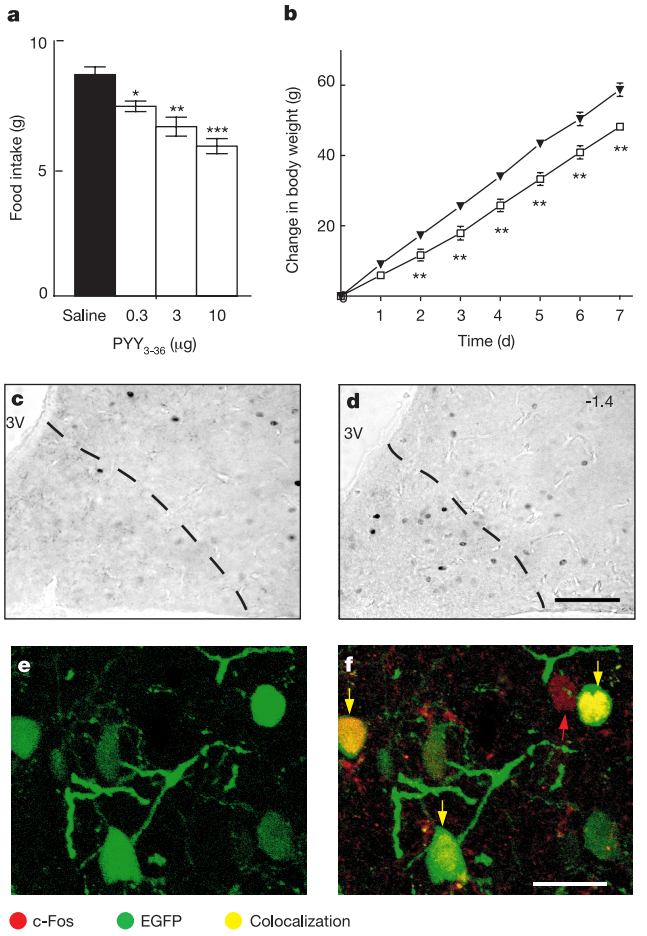

Figure 1 Feeding response to PYY_{3-36} in rats and c-Fos expression in *Pomc–EGFP* mice. **a**, Dark-phase feeding: food intake after i.p. injection of PYY_{3-36}. Freely feeding rats were injected with PYY_{3-36} at the doses indicated (μg per 100 g) or saline, just before 'lights off', and 4-h cumulative food intake was measured. Results are means ± s.e.m. ($n = 8$ per group); asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$ versus saline. **b**, Body weight gain during chronic treatment with PYY_{3-36}. Rats were injected i.p. with PYY_{3-36} (5 μg per 100 g; open squares) or saline (filled inverted triangles) twice daily for 7 d. Body weight gain was calculated each day. Results are expressed as means ± s.e.m. ($n = 12$ per group); double asterisk, $P < 0.01$ versus saline. **c**, **d**, Representative example (Bregma, 1.4 mm)[22] of c-Fos expression in the arcuate nucleus of *Pomc–EGFP* mice in response to i.p. injection of saline (**c**) or PYY_{3-36} (5 μg per 100 g; **d**). Scale bar, 100 μm. 3V, third ventricle. **e**, **f**, Example of POMC–EGFP neurons (**e**), and c-Fos immunoreactivity (**f**) either colocalizing (yellow arrows) or alone (red arrow). Scale bar, 25 μm.

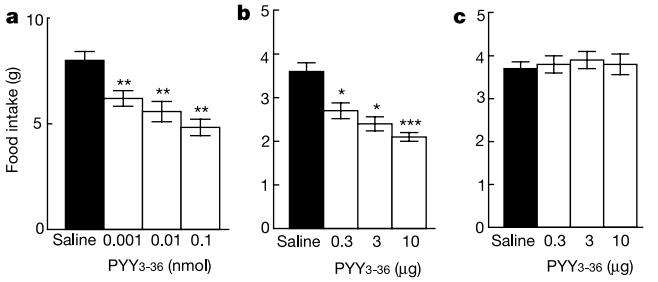

Figure 2 Feeding effects of intra-arcuate injection of PYY_{3-36} in rats and of i.p. PYY_{3-36} in *Y2r*-null mice. **a**, Food intake after intra-arcuate injection of PYY_{3-36}. Fasted rats were injected with saline or PYY_{3-36} into the arcuate nucleus at the doses indicated, and 2-h cumulative food intake was measured; double asterisk, $P < 0.01$ versus saline. **b**, **c**, Feeding response to PYY_{3-36} in *Y2r*-null mice after i.p. administration. Wild-type littermates (**b**) and *Y2r*-null mice (**c**), fasted for 24 h, were injected with PYY_{3-36} at the doses indicated (μg per 100 g) or saline, and 4-h cumulative food intake was measured. Results are the mean ± s.e.m. ($n = 5$ per group); asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$ versus saline.

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ization, $n = 22$, $P < 0.0003$). A similar depolarization was seen with Y2A (8.7 ± 1.8 mV depolarization, $n = 9$, $P < 0.002$). The depolarization caused by PYY_{3-36} stimulated a significant increase in the frequency of action potentials in POMC neurons (Fig. 3a; 93% increase over control, $P < 0.05$, $n = 22$). In the whole-cell mode, the effect of PYY_{3-36} was sometimes reversed on wash-out, but only after a long latency (30 min). We have observed a similar effect on wash-out of leptin in these neurons.

To exclude effects of cellular rundown or seal deterioration, we examined the effects of PYY_{3-36} in the 'loose cell-attached' (or extracellular) configuration. PYY_{3-36} caused a reversible fivefold increase in the frequency of action potentials in loose cell-attached recordings of POMC neurons (Fig. 3b). This increase in firing rate occurred with the same latency, because PYY_{3-36} reduced the frequency of inhibitory postsynaptic currents (IPSCs) onto all 13 POMC neurons tested (Fig. 3c; 51.9 ± 9.2% reduction, $n = 13$, $P < 0.0001$), which indicated a reduced frequency of GABA (γ-aminobutyric acid) release onto POMC neurons. Notably, the firing rate of POMC neurons returned to basal levels, in spite of continued inhibition of IPSCs. A similar effect on IPSC frequency was seen with Y2A (44.4 ± 9.3% reduction, $n = 8$, $P < 0.004$), which suggests that this effect occurs through Y2R. PYY_{3-36} (25 nM) caused a hyperpolarization (5.2 ± 1.16 mV; $P < 0.004$, $n = 5$) of unidentified, but presumably NPY-containing, non-POMC neurons in the arcuate nucleus (data not shown). There is a tonic GABA-mediated inhibition of POMC neurons by NPY neurons[8], and these results suggest that PYY_{3-36} acts by inhibiting NPY neurons, thus decreasing this GABA-mediated tone and consequentially disinhibiting POMC neurons. The effect of Y2A on peptide secretion was also examined using hypothalamic explants[14]. Y2A significantly decreased NPY release, with a concomitant increase in α-MSH release from hypothalamic explants (Fig. 3d, e). Together, these observations suggest that PYY_{3-36} modulates both the NPY and melanocortin systems in the arcuate nucleus.

Because of the known importance of the melanocortin system in man[15], and the profound effects of PYY_{3-36} on both feeding and weight change observed in rodents, we investigated the effects of PYY_{3-36} on appetite and food intake in man. Twelve healthy fasted, non-obese volunteers (six men and six women: mean age, 26.7 ± 0.7 years; BMI, 24.6 ± 0.94 kg m^{-2}) were infused with PYY_{3-36} (0.8 pmol per kg (body weight) per min) or saline for 90 min in a double-blind placebo-controlled crossover study. PYY_{3-36} plasma increased from a mean basal concentration of 8.3 ± 1.0 pM to 43.5 ± 3 pM during the PYY_{3-36} infusion and mimicked postprandial concentrations[5,6]. After infusion, PYY_{3-36} concentrations returned to basal levels within 30 min. PYY_{3-36} infusion resulted in a significant decrease in hunger scores[16] (Fig. 4c). Calorie intake during a free-choice buffet meal[17] 2 h after the termination of the infusion was reduced by more than a third as compared with saline controls (36 ± 7.4%, $P < 0.0001$; Fig. 4a). There was no effect on fluid intake and no difference in sensations of fullness or nausea reported by the volunteers (data not shown). PYY_{3-36} administration had no effect on gastric emptying, as estimated by the paracetamol absorption method[17,18], or on plasma glucose, plasma leptin or insulin (data not shown). Analysis of the food diaries showed a significant inhibition of food intake in the 12-h period after the PYY_{3-36} infusion (saline, 2,205 ± 243 kcal; PYY_{3-36}, 1,474 ± 207 kcal). But food intake between the two groups during the 12-h to 24-h period was virtually identical. Overall

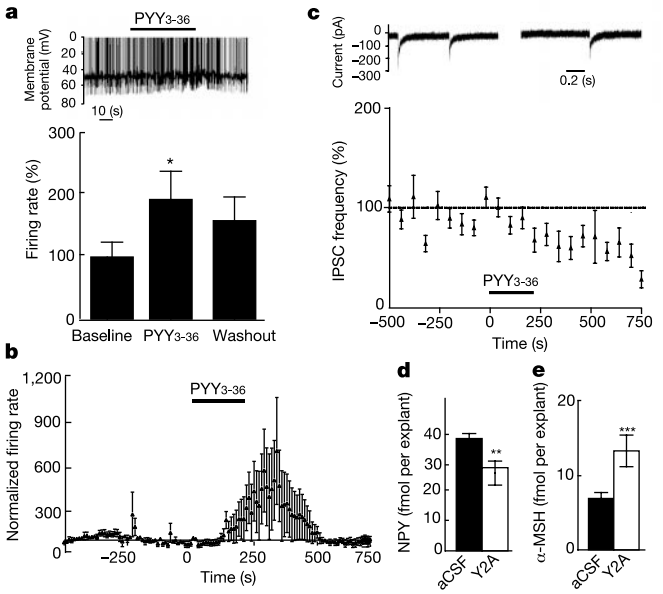

Figure 3 Electrophysiological and neuropeptide responses to PYY_{3-36} and Y2A. **a**, Effect of PYY_{3-36} (10 nM) on the frequency of action potentials in POMC neurons (whole-cell configuration recordings; $n = 22$); asterisk, $P < 0.05$. PYY_{3-36} was administered at time 0 for 3 min; baseline, −3 to 0 min; PYY_{3-36}, 2–5 min; and wash-out, 8–11 min. The top panel shows a representative recording of membrane potential and action potential frequency. **b**, Effect of PYY_{3-36} (10 nM) on the frequency of action potentials in loose cell-attached patch recordings ($n = 8$). Data from individual cells were normalized to the firing rate for the 200 s before PYY_{3-36} addition. **c**, Effect of PYY_{3-36} (50 nM) on spontaneous IPSCs onto POMC neurons ($n = 13$). The top panel shows a representative recording of IPSCs before and after PYY_{3-36} (50 nM), respectively. Results in **a**–**c** are expressed as means ± s.e.m. **d**, **e**, NPY (**d**) and α-MSH (**e**) released from hypothalamic explants in response to Y2A. Hypothalamic slices were incubated with artificial CSF (aCSF), with or without 50 nM Y2A, for 45 min. Results are expressed as means ± s.e.m. ($n = 40$); double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$ versus saline.

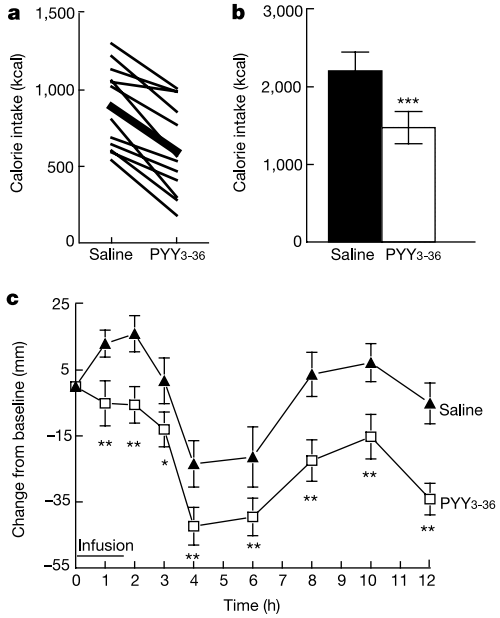

Figure 4 Effect of PYY_{3-36} infusion on appetite and food intake in humans. **a**, Calorie intake from free-choice buffet meal 2 h after infusion with saline or PYY_{3-36}. The thin lines indicate individual changes in calorie intake for each subject between saline and PYY_{3-36} administration. The thick line represents mean change between the two infusions ($n = 12$). **b**, Total calorie intake, as assessed by food diaries, for the 24-h period after either saline or PYY_{3-36} infusion. Data are expressed means ± s.e.m. ($n = 12$); triple asterisk, $P < 0.0001$ versus saline. **c**, Appetite score (relative scale). Visual analogue scores[16] show perceived hunger during and after infusions. The results are presented as change from baseline scores and are the mean ± s.e.m. for all 12 subjects. Asterisk, $P < 0.05$; double asterisk, $P < 0.01$ versus saline.

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there was a 33% decrease in cumulative total calorie consumption in the 24-h period after the PYY_{3-36} infusion (Fig. 4b). These findings show that infusing amounts of PYY_{3-36} that match postprandial concentrations cause a marked inhibition of both appetite and food intake in man.

It has been proposed that the cells in the arcuate nucleus detect circulating peripheral satiety signals and relay these signals to other brain regions[19]. This is supported by the observation that leptin modifies the activity of both the POMC and NPY arcuate neurons[8]. We have now shown, through a combination of electrophysiological and hypothalamic explant studies, that the gut hormone, PYY_{3-36}, can directly influence hypothalamic circuits, which results in coordinate changes in POMC and NPY action. In addition, PYY_{3-36} administered directly into this brain region reduces food intake. Our data show that postprandial concentrations of PYY_{3-36} inhibit food intake in both rodents and man for up to 12 h, which suggests that PYY_{3-36} has a role in 'longer term' regulation of food intake. This contrasts with previously characterized gut-derived 'short-term' satiety signals such as cholecystokinin[1,20], the effects of which are relatively short-lived. The failure of PYY_{3-36} to inhibit food intake in the *Y2r*-null mice provides further evidence that PYY_{3-36} reduces food intake through a Y2R-dependent mechanism. Our results suggest that a gut–hypothalamic pathway that involves postprandial PYY_{3-36} acting at the arcuate Y2R has a role in regulating feeding. Thus, the PYY_{3-36} system may provide a therapeutic target for the treatment of obesity. □

Methods

Animals

We maintained male Wistar rats (200–250 g), aged 7–8 weeks (Charles River Laboratories), under controlled temperature (21–23 °C) and light conditions (lights on 7:00–19:00) with *ad libitum* access to water and food (RM1 diet; SDS) except where stated. We carried out arcuate and paraventricular nuclei cannulations and injections as described[11,13,14]. Correct intranuclear cannula placement was confirmed histologically at the end of each study period[11,13,14]. All animal procedures were approved under the British Home Office Animals (Scientific Procedures) Act, 1986. All injection studies on fasting animals were done in the early light phase (8:00 to 9:00). All dark-phase feeding studies injections were done just before lights off.

Male *Pomc–EGFP* mice[8] were studied at 5–6 weeks of age. *Y2r*-null mice were generated using Cre–loxP mediated recombination, which results in germline deletion of the whole coding region of the Y2 receptor. We maintained all *Y2r*-null mice on a mixed C57BL/6-129SvJ background. Male mice aged 8–12 weeks with 20–30 g body weight were kept under controlled temperature (21–23 °C) and light conditions (lights on 6:00–18:00) with *ad libitum* access to water and food (Gordon's Speciality Stock feeds) except where stated. All studies were carried out in the early light phase (7:00–8:00).

Intraperitoneal injections

Rats were accustomed to i.p. injection by injections of 0.5 ml of saline for 2 d before the study. For all studies, animals received an i.p. injection of either PYY_{3-36} or saline in 500 μl (for rats) or 100 μl (for mice).

Electrophysiology

Whole-cell patch-clamp recordings were made from POMC neurons in the hypothalamus of 180-μm thick coronal slices from *Pomc–EGFP* mice, as described[8]. Loose cell-attached recordings were made by using extracellular buffer in the electrode solution and by maintaining seal resistance between 3 and 5 MΩ throughout the recording. Firing rates were analysed using mini-analysis protocols (MiniAnalysis, Jaejin Software). Vehicle controls were used in this system that had been validated for the electrophysiological actions of neuropeptides[8]. We analysed data by analysis of variance (ANOVA), Neuman–Keuls posthoc comparison and Wilcoxon signed rank test.

Hypothalamic explants

Male Wistar rats were killed by decapitation. The whole brain was removed immediately, mounted with the ventral surface uppermost and placed in a vibrating microtome (Biorad, Microfield Scientific Ltd). A 1.7-mm slice was taken from the base of the brain to include the PVN and the ARC, and immediately transferred to 1 ml of artificial CSF (aCSF)[14] equilibrated with 95% O_2/5% CO_2 and maintained at 37 °C. After an initial 2-h equilibration period, with aCSF replaced every 60 min, the hypothalami were incubated for 45 min in 600 μl of aCSF (basal period) before being exposed to the Y2A (50 nM) in 600 μl of aCSF. We verified the viability of the tissue by a 45-min exposure to 56 mM KCl; isotonicity was maintained by substituting K^+ for Na^+. At the end of each period, the aCSF was removed and frozen at −20 °C until being assayed for NPY and α-MSH by radioimmunoassay.

c-Fos expression

c-Fos expression was measured in adult Wistar rats and *Pomc–EGFP* mice 2 h after i.p. administration of saline or PYY_{3-36} (5 μg per 100 g (body weight)) using standard immunohistochemical techniques[21]. We obtained data from three rats and five mice in each group. For the *Pomc–EGFP* mice, five anatomically matched arcuate nucleus sections[22] were counted from each animal, and images were acquired using a Leica TSC confocal microscope[23].

RNase protection assay

We extracted total RNA from hypothalami in Trizol (Gibco-BRL). Assays were done with an RPAIII kit (Ambion) using 5 μg of RNA and probes specific for NPY, α-MSH and β-actin (internal standard). For each neuropeptide, the ratio of the optical density of the band of neuropeptide mRNA to that of β-actin was calculated. Neuropeptide mRNA is expressed relative to that of saline controls (means ± s.e.m, $n = 4$ per group). For statistical analysis, we used ANOVA with Bonferroni posthoc analysis.

Plasma assays

Human leptin was measured using a commercially available radioimmunoassay (Linco Research). All other plasma hormones were measured using established in-house radioimmunoassays[18]. Glucose concentrations were measured using a YSI 2300STAT analyser (Yellow Springs Instruments). We measured concentrations of plasma paracetamol by an enzymatic colorimetric assay (Olympus AU600 analyser).

Human studies

We purchased PYY_{3-36} from Bachem. The Limulus amoebocyte lysate assay test for pyrogen was negative and the peptide was sterile on culture. Ethical approval was obtained from the Local Research Ethics Committee (project registration 2001/6094) and the study was carried out in accordance with the principles of the Declaration of Helsinki. Subjects gave informed written consent. Each subject was studied on two occasions with at least 1 week between each study. Volunteers filled out a food diary for 3 d before each infusion and for the following 24 h. All subjects fasted and drank only water from 20:00 on the evening before each study. Subjects arrived at 8:30 on each study day, were cannulated and then allowed to relax for 30 min before the onset of the study protocol. We collected blood samples every 30 min into heparin-coated tubes containing 5,000 Kallikrein inhibitor units (0.2 ml) of aprotinin (Bayer). Plasma was separated by centrifugation and stored at −70 °C until analysis. Subjects were infused with either saline or 0.8 pmol per kg (body weight) per min PYY_{3-36} for 90 min, in a double-blind randomized crossover design. Two hours after terminating the infusion, subjects were offered an excess free-choice buffet meal[17], such that all appetites could be satisfied. Food and water were weighed pre- and postprandially, and caloric intake was calculated. Appetite ratings were made on 100-mm visual analogue scores with the text expressing the most positive and the negative rating anchored at each end[16]. Visual analogue score was used to assess hunger, satiety, fullness, prospective food consumption and nausea. Caloric intake after saline and PYY_{3-36} was compared using a paired *t*-test. The postprandial response curves were compared by ANOVA using repeated paired measures, with time and treatment as factors.

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Competing interests statement

The authors declare that they have no competing financial interests.

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A naturally occurring MTA1 variant sequesters oestrogen receptor-α in the cytoplasm

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Oestrogen receptor (ER) is a good prognostic marker for the treatment of breast cancers. Upregulation of metastatic tumour antigen 1 (MTA1) is associated with the invasiveness and metastatic potential of several human cancers[1,2] and acts as a co-repressor of nuclear ER-α[3]. Here we identify a naturally occurring short form of MTA1 (MTA1s) that contains a previously unknown sequence of 33 amino acids with an ER-binding motif, Leu-Arg-Ile-Leu-Leu (LRILL). MTA1s localizes in the cytoplasm, sequesters ER in the cytoplasm, and enhances non-genomic responses of ER. Deleting the LRILL motif in MTA1s abolishes its co-repressor function and its interaction with ER, and restores nuclear localization of ER. Dysregulation of human epidermal growth factor receptor-2 in breast cancer cells enhances the expression of MTA1s and the cytoplasmic sequestration of ER. Expression of MTA1s in breast cancer cells prevents ligand-induced nuclear translocation of ER and stimulates malignant phenotypes. MTA1s expression is increased in human breast tumours with no or low nuclear ER. The regulation of the cellular localization of ER by MTA1s represents a mechanism for redirecting nuclear receptor signalling by nuclear exclusion.

By screening a human mammary gland complementary DNA library with a MTA1 cDNA probe, we isolated MTA1 cDNAs of varying lengths. We sequenced and subcloned the MTA1 cDNAs into the pcDNA3.1 expression vector using the MTA1 cDNA open reading frames (Fig. 1a). To assess the functionality of these clones, we translated MTA1 cDNAs *in vitro* and resolved the resulting protein products on an SDS polyacrylamide gel (Fig. 1b). All of the MTA1 clones except the MTA1-S2 clone were translated into proteins of the expected sizes. The MTA1-S2 clone, which lacks the amino-terminal 58 amino acids and an internal sequence of 47 base pairs (bp), was translated into a protein with a much lower relative molecular mass (M_r) of 44,000 (44K) than that expected on the basis of the MTA1-S2 cDNA length (M_r 78K). We called this much shorter MTA1 variant MTA1s. By re-screening the human mammary gland cDNA library and using recombination methods, we isolated a full-length MTA1s. The T7-tagged MTA1s was translated *in vivo* into a protein of M_r 54K (Fig. 1c).

To determine whether the full-length MTA1s cDNA existed

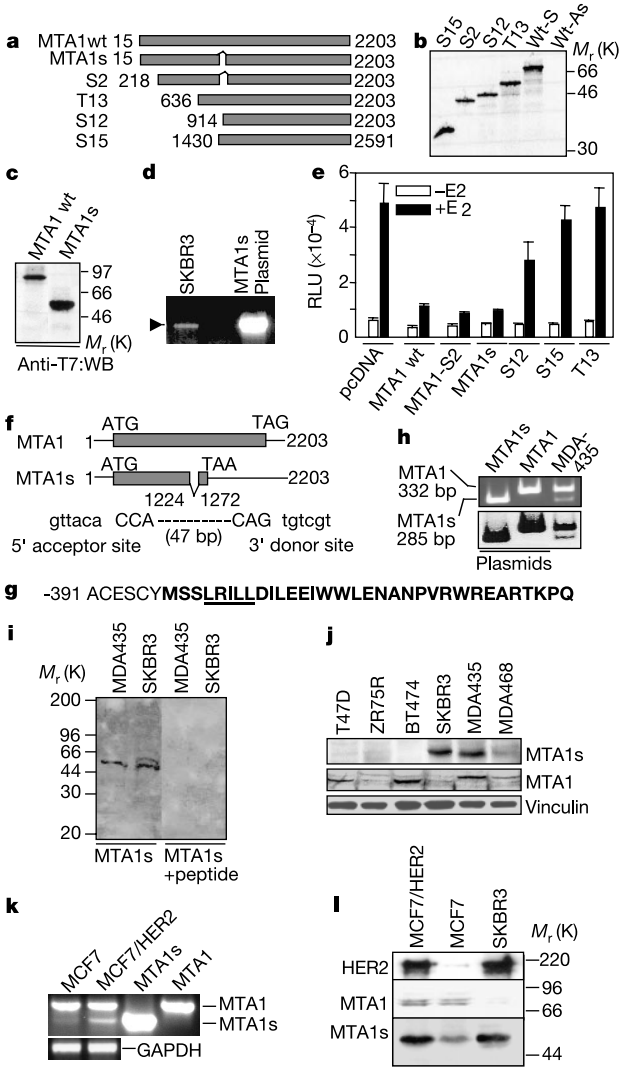

Figure 1 MTA1s is a variant of MTA1. **a**, Representation of the MTA1 clones. **b**, Translation of T7-tagged MTA1 clones. **c**, Expression of T7–MTA1 and T7–MTA1s cDNA. **d**, RT–PCR amplification of the MTA1s open reading frame. **e**, Repression of ERE–luc activity by MTA1 clones. **f**, Human MTA1s transcript generated by alternative splicing. **g**, MTA1s sequence showing the unique 33 amino acids (bold). **h**, RT–PCR (top) and Southern blot (bottom) analysis of RNA from MDA-MB435 cells. **i**, Characterization of MTA1s antibody. **j**, **k**, Expression of MTA1s protein and MTA1s mRNA by immunoblotting and RT–PCR. **l**, Expression of HER2, MTA1 and MTA1s in indicated cell lines.

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